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Urinary ammonia and long-term outcomes in chronic kidney disease

Marion Vallet[1,2,13], Marie Metzger[3,4,13], Jean-Philippe Haymann[5,6,7], Martin Flamant[8,9,10], Cédric Gauci[2,3], Eric Thervet[2,11], Jean-Jacques Boffa[6,7], François Vrtovsnik[8,9,10], Marc Froissart[3], Bénédicte Stengel[3,4], and Pascal Houillier[2,12] on behalf of the NephroTest Cohort Study group

[1]Université Paris-Descartes, Paris, France; [2]Departments of Physiology and Nephrology, Hopital Européen Georges Pompidou, Assistance Publique-Hopitaux de Paris, Paris, France; [3]INSERM U1018, Centre for Research in Epidemiology and Population Health (CESP), Diabetes, Obesity, and Chronic Kidney Disease Epidemiology Team, Villejuif, France; [4]Université Paris-Sud, UMRS 1018, Villejuif, France; [5]Departments of Physiology and Nephrology, Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, Paris, France; [6]Université Pierre et Marie Curie, Paris, France; [7]INSERM UMR 1155, Paris, France; [8]Departments of Physiology and Nephrology, Hôpital Bichat, Assistance Publique-Hôpitaux de Paris, Paris, France; [9]Université Paris Diderot, Paris, France; [10]INSERM UMR S 1149, Paris, France; [11]INSERM UMR S 970, Paris, France and [12]Sorbonne Universités, UPMC Univ Paris 06, INSERM, Université Paris Descartes, Sorbonne Paris Cité, UMR_S 1138, Centre de Recherche des Cordeliers, CNRS ERL_8228, Paris, France

Recent studies suggest that alkalinizing treatments improve the course of chronic kidney disease (CKD), even in patients without overt metabolic acidosis. Here, we tested whether a decreased ability in excreting urinary acid rather than overt metabolic acidosis may be deleterious to the course of CKD. We studied the associations between baseline venous total CO_2 concentration or urinary ammonia excretion and long-term CKD outcomes in 1065 patients of the NephroTest cohort with CKD stages 1–4. All patients had measured glomerular filtration rate (mGFR) by ^{51}Cr-EDTA renal clearance. Median mGFR at baseline was 37.6 ml/min per 1.73 m^2. Urinary ammonia excretion decreased with GFR, whereas net endogenous acid production did not. After a median follow-up of 4.3 years, 201 patients reached end-stage renal disease (ESRD) and 114 died before ESRD. Twenty-six percent of the patients had mGFR decline rate greater than 10% per year. Compared with patients in the highest tertile of urinary ammonia excretion, those in the lowest tertile had a significantly increased hazard ratio for ESRD, 1.82 (95% CI, 1.06–3.13), and a higher odds ratio of fast mGFR decline, 1.84 (0.98–3.48), independent of mGFR and other confounders. Patients in the lowest tertile of venous total CO_2 had significantly increased risk of fast mGFR decline but not of ESRD. None of these biomarkers was associated with mortality. Thus, these results suggest that the inability to excrete the daily acid load is deleterious to renal outcomes.

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Correspondence: *Pascal Houillier, Département de Physiologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, Paris 75015, France. E-mail: pascal.houillier@inserm.fr*

[13]MV and MM share first authorship.

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In chronic kidney disease (CKD), the ability of the kidney to excrete the daily acid load declines[1–3] and the prevalence of metabolic acidosis increases with the severity of the disease.[4,5] The results of prospective studies suggest that alkali-based therapy efficiently delays CKD progression in patients with[6,7] or without overt metabolic acidosis.[8,9] In cohort studies, a low plasma bicarbonate concentration is associated with increased mortality.[10–12] However, whether a low plasma bicarbonate level is a risk factor for a faster decline in the glomerular filtration rate (GFR) remains uncertain.[11,13–15] Scialla *et al.*[16] have recently studied in the AASK trial the association between net endogenous acid production (NEAP), the balance between fixed acid and alkali precursors in diet, and CKD progression. A higher NEAP was associated with a significantly faster decline in GFR. This could indicate that the daily acid load itself and/or the inability of the kidney to cope with the acid load is a risk factor for disease worsening in CKD patients. So far, most of the published studies have focused on the link between plasma bicarbonate level, a surrogate marker of the status of acid balance, and the decline in GFR. None has tested whether the impaired ability of the kidney to excrete the daily net acid load is an independent risk factor for the progression of CKD.

Kidneys excrete the daily acid load mainly by generating and excreting ammonia (ammonia refers to the sum of NH_4^+ and NH_3; as NH_3 concentration is negligible in urine, ammonia concentration virtually equals that of NH_4^+) and to a lesser extent by excreting hydrogen ions as titratable acid (TA). Urinary excretion of ammonia decreases in parallel to GFR, whereas TA excretion is maintained until very advanced CKD stages.[2,3,17]

We made the assumption that a low urinary ammonia excretion might be an earlier marker of the propensity to develop a positive acid balance (and hence metabolic acidosis) compared with plasma bicarbonate concentration. Therefore, we calculated the balance of fixed acid and compared the associations between plasma total CO_2 (tCO_2) level or urinary ammonia excretion and long-term outcomes including the rate of decline in GFR, the incidence of end-stage renal disease (ESRD), and mortality, in the NephroTest study cohort.

RESULTS

Baseline characteristics of the study cohort

Patients' mean age was 60 ± 15 years and most were men (70.6%) (Table 1). Eighty per cent were on angiotensin-converting enzyme inhibitor/angiotensin receptor blockers treatment at baseline. Median measured glomerular filtration rate (mGFR) was 37.6 ml/min per 1.73 m^2 (interquartile range (IQR) 27.6–51.4). Fifty-four percent of the patients had CKD stage 3 and 31% had stage 4. Mean venous tCO_2 concentration was 26 ± 3 mmol/l, and only 8.3% of the patients had overt metabolic acidosis (defined by a venous tCO_2 concentration below 22 mmol/l). Median NEAP was 56.3 mEq/24 h (IQR 44.4, 73.7). Median urinary 24-h ammonia excretion was 1.70 mEq/mmol creatinine (IQR 1.17–2.42) and median fasting ammonia concentration was 12.3 mEq/l (IQR 7.5–20.0). NEAP was not associated with mGFR, whereas 24-h ammonia excretion and fasting urinary ammonia concentration significantly decreased with decreasing baseline mGFR (Figure 1). Fasting urinary pH was inversely related to urinary ammonia excretion. No relation between body mass index (BMI), urinary phosphate or urinary urea nitrogen, and 24-h urinary ammonia excretion was observed in our study population.

Cross-sectional analysis of acid balance in CKD patients

In a subset of our study population ($n = 160$, Table 1), we measured 24-h net acid excretion (NAE) and calculated acid balance (Figure 2). Both urinary ammonia and TA excretions decreased with mGFR ($r = 0.30$, $P < 0.0001$ and $r = 0.16$, $P = 0.05$, respectively). NAE also significantly decreased with mGFR ($r = 0.22$, $P = 0.004$), mainly because of the decrease in ammonia excretion. Acid balance was not

Table 1 | Population characteristics, mean ± s.d., median (Q1–Q3), or % (*n*)

	All patients	Sub-group of patients with ≥ 2 visits	Sub-group of patients with 1 visit	Sub-group of patients with acid balance
N	1065	711	354	160
Demographic				
Men	70.6 (752)	69.9 (497)	72.0 (255)	70.6 (113)
Age, year	60.1 ± 14.9	58.9 ± 14.8	62.4 ± 14.7	62.2 ± 13.9
Sub-Saharan African origin	11.5 (118)	11.4 (80)	11.5 (38)	9.4 (15)
BMI	26.5 ± 5.0	26.2 ± 4.9	27.0 ± 5.0	26.1 ± 4.1
Systolic/Diastolic BP, mm Hg	138 ± 21/75 ± 12	137 ± 21/75 ± 11	139 ± 21/75 ± 12	139 ± 21/76 ± 11
Diabetes	29.0 (309)	27.0 (192)	33.1 (117)	18.1 (29)
History of cardiovascular disease	20.5 (216)	18.7 (131)	24.1 (85)	14.5 (23)
History of smoking, past/current	35.4 (377)/16.0 (170)	36.1 (257)/17.0 (121)	33.9 (120)/13.8 (49)	40.6 (65)/13.1 (21)
Renal function				
mGFR, ml/min per 1.73 m^2	37.6 (27.6–51.4)	37.7 (27.6–50.6)	37.4 (27.5–53.4)	39.1 (26.7–49.5)
≥60	15.4(164)	14.3 (102)	17.5 (62)	13.8 (22)
45–59	20.8(221)	21.7 (154)	18.9 (67)	21.3 (34)
30–44	32.7(348)	33.9 (241)	30.2 (107)	31.9 (51)
15–29	31.2(332)	30.1 (214)	33.3 (118)	33.1 (53)
ACR, mg/mmol	9.0 (1.8–47.9)	9.8 (2.0–44.0)	7.5 (1.7–60.4)	7.2 (1.4– 31.7)
PCR, mg/mmol	30.0 (11.1–110.0)	30.0 (11.8–102.3)	29.0 (10.0–120.0)	18.3 (9.9–60.2)
Acid-base homeostasis				
Fasting urinary NH_4 concentration, mEq/l	12.3 (7.5–20.0)	12.1 (7.3–19.8)	13.0 (7.6–20.7)	13.6 (8.7–18.9)
Fasting urinary osmolality, mOsm/kgH_2O	487 ± 139	489 ± 138	483 ± 143	472 ± 134
24-h urinary NH_4 excretion, mEq/24 h	18.5 (12.1–27.2)	18.2 (12.0–26.6)	18.9 (12.4–27.7)	20.7 (15.7–29.1)
24-h urinary creatinine excretion, mmol/24 h	11.6 ± 4.3	11.8 ± 4.2	11.1 ± 4.4	12.9 ± 5.4
24-h urinary NH_4/creatinine mEq/mmol	1.70 (1.17–2.42)	1.66 (1.11–2.33)	1.80 (1.28–2.53)	1.74 (1.32–2.37)
Venous total CO_2, mmol/l	26.0 ± 3.0	26.0 ± 2.9	26.1 ± 3.1	25.9 ± 3.0
Acidosis, $tCO_2 < 22$ mmol/l	8.3 (88)	7.5 (53)	9.9 (35)	8.8 (14)
Treatments				
Loop diuretics treatment	33.9 (361)	32.9 (234)	35.9 (127)	18.8 (30)
Phosphate binders treatment	1.0 (11)	1.1(8)	0.8(3)	0.6 (1)
ACEi or ARB treatment	80.2 (854)	82.7 (588)	75.1 (266)	91.3 (146)

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-creatinine ratio; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; mGFR, measured glomerular filtration rate; PCR, urinary protein-creatinine ratio; tCO_2, total CO_2.

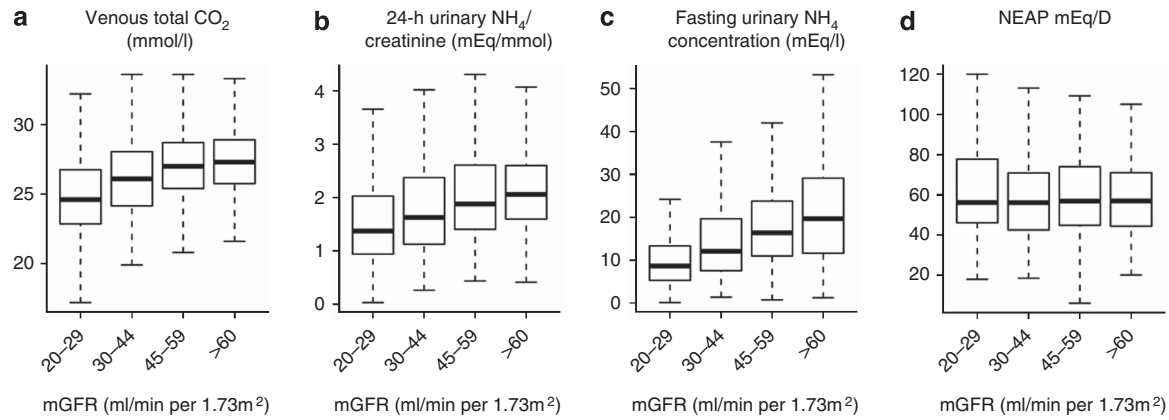

Figure 1 | Baseline acid base status in 1065 chronic kidney disease (CKD) patients. Total venous CO_2 (**a**), 24-h urinary NH_4/creatinine (**b**), fasting urinary NH_4 concentration (**c**), and net endogenous acid production (NEAP) (**d**) according to the measured glomerular filtration rate (mGFR). Median concentrations (interquartile range, IQR) are displayed. Whiskers extend to 1.5 times the IQR.

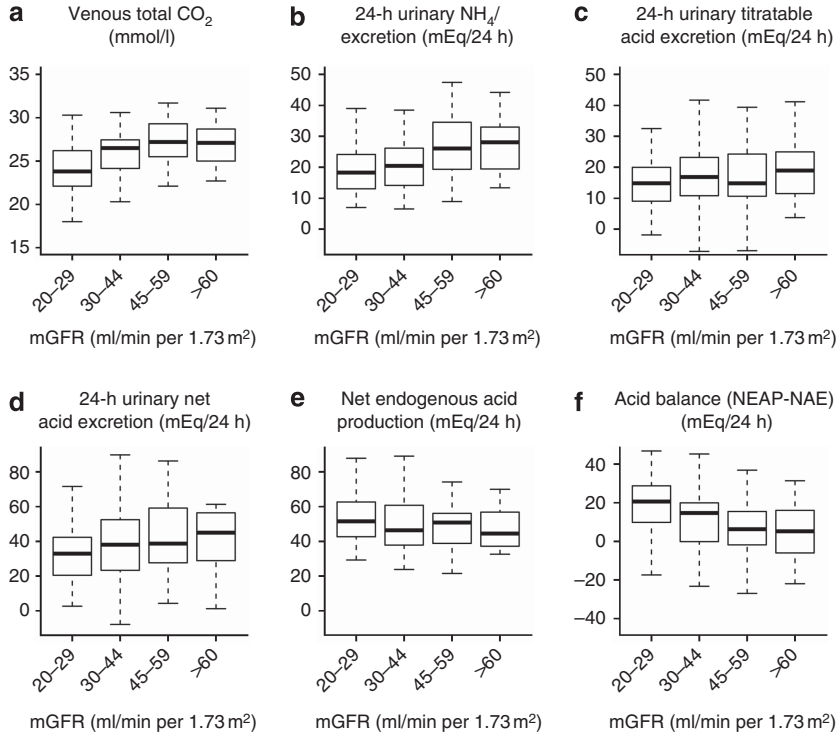

Figure 2 | Acid balance and its determinants in 160 consecutive chronic kidney disease (CKD) patients. Venous total CO_2 (**a**), 24-h urinary NH_4 excretion (**b**), 24-h titratable acid excretion (**c**), 24-h net acid excretion (NAE) (**d**), net endogenous acid production (NEAP) (**e**), and net acid balance (**f**) according to the measured glomerular filtration rate (mGFR). Median concentrations (interquartile range, IQR) are displayed. Whiskers extend to 1.5 times the IQR.

different from zero in patients with CKD stages 1 and 2 ($P=0.2$); it increased as mGFR declined ($r=-0.28$, $P=0.0003$), eventually being significantly positive in patients with CKD stage 4 ($P<0.001$) when most patients still had a normal blood tCO_2 concentration. Acid balance was inversely and significantly related to urinary ammonia excretion ($r=-0.38$, $P<0.0001$) but not to venous tCO_2 concentration ($r=-0.12$, $P=0.13$).

Associations with the risk of ESRD

During a median follow-up of 4.3 years (IQR 2.37, 6.53), 201 patients (18.9%) reached ESRD. A lower venous tCO_2 level at baseline was associated with a higher risk of ESRD before and after stratification on mGFR, but this association was no longer significant after adjustment for other covariates (Figure 3; Table 2). Patients with overt metabolic acidosis were not at higher risk for ESRD after stratification on mGFR (Table 2).

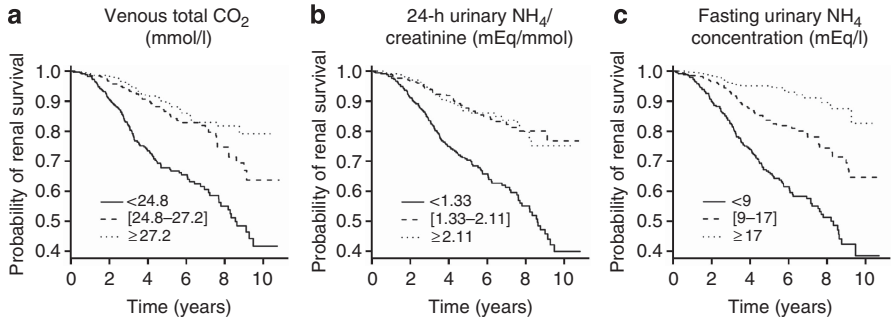

Figure 3 | Risk of ESRD and acid base status in chronic kidney disease (CKD) patients. Kaplan–Meier survival curves for end-stage renal disease (ESRD) outcome according to baseline venous total CO_2 (tCO_2) (**a**), 24-h urinary NH_4/creatinine (**b**), and fasting urinary NH_4 concentration (**c**).

Table 2 | Hazard-ratios of ESRD according to venous total CO_2 levels, 24-h urinary NH_4/creatinine ratio, or fasting urinary NH_4 concentration in all patients

	N	*n* events	Model 0	Model 1	Model 2	Model 3
Venous total CO_2, mmol/l						
≥27.2	379	40	0.70 (0.46, 1.06)	1.10 (0.72, 1.68)	1.09 (0.71, 1.69)	
[24.8–27.2]	336	51	1 (ref)	1 (ref)	1 (ref)	
<24.8	350	110	2.32 (1.67, 3.24)	1.50 (1.08, 2.11)	1.26 (0.89, 1.79)	
P for trend			<0.0001	0.04	0.3	
P Wald test			<0.0001	0.04	0.4	
Acidosis[a]						
No	977	168	1 (ref)	1 (ref)	1 (ref)	
Yes	88	33	2.56 (1.76, 3.73)	1.37 (0.93, 2.01)	1.08 (0.72, 1.61)	
24-h NH_4/creatinine ratio, mEq/mmol						
≥2.11	356	38	1 (ref)	1 (ref)	1 (ref)	
[1.33, 2.11]	355	44	0.98 (0.63, 1.51)	0.90 (0.58, 1.40)	0.80 (0.51, 1.26)	
<1.33	354	119	2.78 (1.93, 4.01)	1.52 (1.05, 2.20)	1.41 (0.96, 2.08)	
P for trend			<0.0001	0.007	0.02	
Fasting NH_4 concentration, mEq/l						
≥17.0	357	22	1 (ref)	1 (ref)	1 (ref)	1 (ref)
[9.0–17.0]	357	56	2.66 (1.62, 4.35)	1.53 (0.93, 2.53)	1.48 (0.88, 2.47)	1.37 (0.80, 2.34)
<9.0	351	123	6.19 (3.93, 9.74)	2.27 (1.42, 3.62)	2.07 (1.27, 3.36)	1.82 (1.06, 3.13)
P for trend			<0.0001	0.0002	0.001	0.02

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-creatinine ratio; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; mGFR, measured glomerular filtration rate.
Model 0: crude hazard ratio, **Model 1**: stratified for mGFR (15–20, 20–30, 30–40, 40–50, 50–60, >60 ml/min per 1.73 m^2), **Model 2**: Model 1 + adjustment for covariates: age, gender, sub-Saharan African origin, BMI (<25, 25–30, >30 kg/m^2), BP>140/90 mm Hg (0,1), diabetes, history of cardiovascular disease, smoking (never, past, current), ACEi or ARB treatment, ACR (<3, 3–30, >30 mg/mmol), center. **Model 3:** Model 2 + fasting urine osmolality.
[a]Acidosis: venous total CO_2 < 22 mmol/l.

The Kaplan–Meier survival curves showed a higher risk of ESRD in patients with urinary ammonia in the lowest tertile (Figure 3). Crude hazard ratio (HR) of ESRD was significantly higher in patients in the lowest 24-h urinary ammonia/creatinine ratio tertile as compared with patients in the first tertile (Table 2). This association remained statistically significant after taking mGFR and other covariates into account. The risk of ESRD associated with urinary ammonia/creatinine ratio was not linear, with the highest risk for patients with the lowest values of the ratio (Figure 4). Similar trend was observed when ammonia excretion was expressed in mEq/24 h.

Regarding fasting urinary ammonia concentration, we observed a strong graded negative relationship with the risk of ESRD that remained statistically significant after stratification on mGFR and adjustment for the same covariates and fasting urine osmolality. Every 10 mEq/L decrease in fasting NH_4 concentration was associated with a 43% increase in the risk of ESRD after stratification on mGFR and adjustment (HR 1.43, 95% confidence interval (CI) 1.09–1.86). In contrast with urinary 24-h ammonia/creatinine ratio, the linearity of the relationship between fasting urinary NH_4 and ESRD risk was confirmed using regression splines (Figure 5). The HRs of ESRD and pre-ESRD mortality according to fasting NH_4 concentration and adjustment covariates are shown in Supplementary Table 2. Further adjustment for nephropathy type did not significantly change the adjusted HR of ESRD.

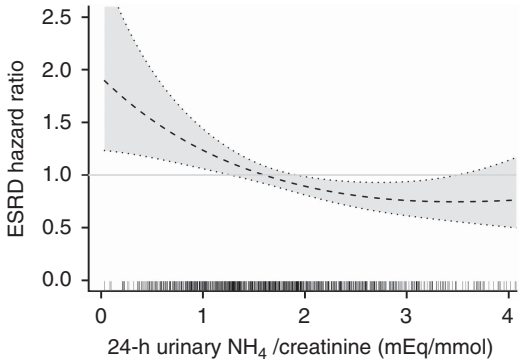

Figure 4 | Estimated adjusted hazard ratio with 95% confidence intervals for the association of 24-h urinary NH_4/creatinine with end-stage renal disease (ESRD) using penalized-splines estimator. Ticks represent distribution of 24-h urinary NH_4/creatinine values, and hazard ratio (HR) were plotted only for values below 95th percentile.

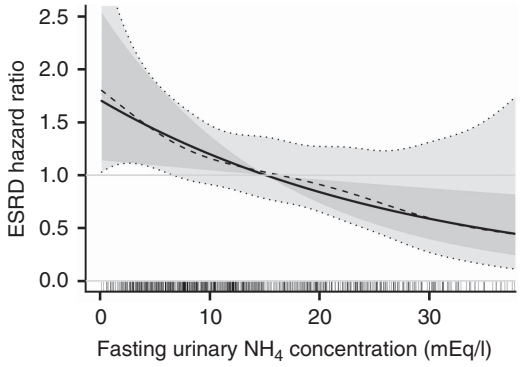

Figure 5 | Estimated adjusted hazard (solid lines) with 95% confidence intervals (CI 95%: dark gray) for the association of fasting urinary NH_4 concentration with end-stage renal disease (ESRD). Dashed lines give prediction (CI 95%: dotted lines, light gray) with p-spline method. Ticks represent distribution of fasting urinary NH_4 concentration, and hazard ratio (HR) were plotted only for values below 95th percentile.

Survival analysis

During follow-up, 160 patients (15%) died of whom 114 died before reaching ESRD. There was no association between venous tCO_2 concentration and overall mortality before (HR 1.31, 95% CI 0.80–2.14) or after stratification on mGFR and adjustment for covariates (HR 0.96, 95% CI 0.80–2.14). Pre-ESRD mortality was significantly higher in patients with metabolic acidosis (HR 1.83, 95% CI 1.04–3.21), but this association was no longer significant after adjustment (HR 1.29, 95% CI 0.71–2.34).

We did not find any association between urinary ammonia/creatinine ratio or fasting urinary ammonia concentration with mortality (data not shown).

CKD progression analysis

In patients with at least two mGFR measurements, mean time between the first and last mGFR was 3.9 ± 2.0 years and the mean number of measures was 4.4 ± 2.4. They were slightly younger and had less often diabetes mellitus and cardiovascular disease than those with one single mGFR measurement, but the two groups did not significantly differ with respect to baseline mGFR or acid-base status (Table 1). The median follow-up to events or census date was significantly higher in the former group than in the latter, 4.92 (2.95–7.00) vs. 2.92 years (1.49–5.33). Median slope of mGFR over time was -1.79 ml/min/year (IQR -3.82, 0.14), and median percent change was -4.39% per year (IQR -10.47, 0.25). One hundred and eighty-nine patients (26.6%) had a rate of decline in mGFR higher than 10% per year (fast rate of decline in mGFR).

The odds ratio (OR) of fast mGFR decline was significantly higher in the lowest venous tCO_2 tertile as compared with the middle tertile both before and after adjusting for mGFR and other covariates (Table 3). Regarding fasting ammonia concentration, there was a strong graded association with fast rate of decline in mGFR, similar to that observed for ESRD risk, which was also attenuated, but remained statistically significant after adjusting for covariates and further adjustment for fasting urinary osmolality (Table 3). It is worth noting that adding venous tCO_2 in model 3 strengthened the association of both venous tCO_2 and fasting ammonia concentration with fast mGFR decline: adjusted ORs for fasting ammonia concentration middle and first vs. last tertile, 1.85 (1.05–3.27) and 2.00 (1.05–3.82), respectively, and for venous tCO_2 first and last vs. middle tertile, 2.02 (1.25–3.28) and 1.52 (0.91–2.56), respectively. Repeating the analysis using an absolute cutoff value of 4 ml/min/year instead of 10% per year yielded similar associations with slightly reduced OR for model 2: 1.52 (0.94, 2.47) for middle vs. last tertile of urinary fasting ammonium and 1.73 (1.03, 2.91) for first vs. last tertile.

DISCUSSION

In our cohort of adult patients with CKD stages 1–4, urinary ammonia and NAE decrease with GFR, whereas NEAP does not. Therefore, patients with CKD develop a positive acid balance as CKD worsens. A lower urinary ammonia excretion or concentration is associated with a significantly higher risk of progression toward ESRD, and a lower concentration is also associated with fast mGFR decline. By contrast, a lower plasma tCO_2 concentration is related with higher risk of mGFR decline but not with ESRD risk. We did not observe a relationship between the risk of death and venous tCO_2 concentration or urinary ammonia excretion or concentration.

The question whether patients with a low level of plasma tCO_2 have a higher risk of CKD progression is still discussed. Several cohort studies found a significant association between low plasma bicarbonate levels and poorer renal outcomes.[10,13,15,18,19] Menon *et al.*[14] in the Modification of Diet in Renal Disease cohort found an association that was, however, not independent of baseline mGFR value. In our study, the increased risk of ESRD associated with low venous

Table 3 | Odds ratio (95% CI) of fast mGFR decline (>10%/year) according to venous total CO_2 levels, 24-h urinary NH_4/creatinine ratio, or fasting urinary NH_4 concentration in the subgroup of 711 patients with ≥2 visits

	N	*n* events	Model 0	Model 1	Model 2	Model 3
Venous total CO_2, mmol/l						
≥27.2	247	51	0.98 (0.63, 1.52)	1.53 (0.94, 2.47)	1.55 (0.93, 2.59)	
[24.8–27.2]	233	49	1 (ref)	1 (ref)	1 (ref)	
<24.8	231	89	2.35 (1.56, 3.55)	1.74 (1.12, 2.70)	1.88 (1.17, 3.03)	
P for trend			<0.0001	0.4	0.3	
P Wald test			<0.0001	0.04	0.03	
Acidosis[a]						
No	658	162	1 (ref)	1 (ref)	1 (ref)	
Yes	53	27	3.18 (1.80, 5.61)	1.71 (0.93, 3.14)	1.71 (0.89, 3.31)	
24-h NH_4/creatinine ratio, mEq/mmol						
≥2.11	224	54	1 (ref)	1 (ref)	1 (ref)	
[1.33, 2.11]	229	49	0.86 (0.55, 1.33)	0.67 (0.42, 1.09)	0.62 (0.37, 1.03)	
< 1.33	258	86	1.57 (1.05, 2.35)	0.87 (0.56, 1.36)	0.78 (0.48, 1.29)	
P for trend			0.02	0.7	0.4	
Fasting NH_4 concentration, mEq/l						
≥17.0	234	29	1 (ref)	1 (ref)	1 (ref)	1 (ref)
[9.0–17.0]	239	65	2.64 (1.63, 4.28)	1.79 (1.07, 2.98)	1.89 (1.10, 3.23)	1.76 (1.00, 3.08)
<9.0	238	95	4.70 (2.94, 7.49)	2.01 (1.20, 3.36)	2.13 (1.23, 3.70)	1.84 (0.98, 3.48)
P for trend			<0.0001	0.01	0.01	0.08

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-creatinine ratio; ARBs, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; mGFR, measured glomerular filtration rate.
Model 0: crude odd ratio. **Model 1**: adjusted for mean mGFR. **Model 2**: Model 1 + adjustment for covariates: age, gender, sub-Saharan African origin, BMI, mean blood pressure, diabetes, history of cardiovascular disease, smoking (never, past, current), ACEi or ARB treatment, ACR (<3, 3–30, >30 mg/mmol), center. **Model 3:** Model 2 + fasting urine osmolality.
[a]Acidosis: venous total CO_2 <22 mmol/l.

tCO_2 was independent of mGFR, but strongly attenuated by other confounders, whereas the risk for fast mGFR decline was independent of these factors. Within the last years, several interventional studies have tested the effect of alkali therapy (sodium bicarbonate or sodium citrate) or fruit and vegetable-enriched diet on the rate of decline in GFR.[6–9] Most studies have been conducted on small groups of patients and have used estimated rather than measured GFR; in addition, some of them were not randomized controlled trials. Every study found a protective effect of alkali therapy on the course of kidney function. The renal protective effect of alkali therapy was also observed in patients with early hypertensive nephropathy without overt metabolic acidosis.[9] This protective effect of alkali therapy could be due to the attendant increase in plasma bicarbonate concentration or to the decrease in net acid load and acid balance. In accordance with the latter, higher NEAP associated with a faster rate of decline in GFR in patients with CKD[16] and higher dietary acid load associated with reduced eGFR in the NHANES cohort.[20] Moreover, lowering dietary acid load, by bicarbonate therapy or by fruit and vegetable-enriched diet, reduced the urinary indices of kidney injury in patients with hypertensive nephropathy stage 3[8] or early stages of CKD.[21] Taken together, these data suggest that the reduction in daily acid production improves CKD progression through the reduction in acid retention.

In our study, there was no significant association between venous tCO_2 concentration or urinary ammonia and mortality. This is seemingly in contradiction with the results of some recent cohort studies.[10–12] However, others did not find a significant association with mortality either.[18,19] In the Modification of Diet in Renal Disease study, the association between tCO_2 levels and mortality was not independent of the baseline mGFR value.[14] One potential reason for this discrepancy is that some studies included all stages and all causes of CKD,[18] including the present one, whereas others included more selected populations.[11,12,14]

In healthy subjects, an increase in acid load elicits an increase in urinary NAE, mainly due to an increase in urinary ammonia excretion. In patients with CKD, the ability to adapt urinary ammonia excretion to increased or even normal acid load is impaired.[1,3,22] Consequently, the development of a positive acid balance is achieved more easily than in normal subjects, favoring a decrease in plasma bicarbonate concentration but also the occurrence of bone disease, muscle wasting, and reduced albumin synthesis.[23–25] Likely, plasma bicarbonate concentration is not the best index for assessing the status of acid balance (see Figure 2), as it depends not only on the acid balance itself but also on intracellular and bone buffering of the excess in acid. In this study, the decrease in ammonia and acid excretion is likely due to an impaired ability of the kidney to excrete ammonia and not a consequence of decreased acid load, as NEAP remains the same at any mGFR level.

We show, for the first time to our knowledge, that a low urinary ammonia excretion is associated with a higher risk of

ESRD. Moreover, a low fasting urinary ammonia concentration is associated with a higher risk of fast rate of decline in mGFR. The relationship between 24-h ammonia/creatinine ratio and renal outcomes is slightly different from that observed for fasting ammonia concentration: the risk of ESRD associated with 24-h ammonia/creatinine ratio is not linear, with the lowest risk for patients being in the mid tertile and no association found with a fast rate of decline in mGFR. At variance with fasting ammonia, 24-h ammonia excretion does not only depend on basal acid production but is also affected by dietary acid load and, conceivably, by changes in plasma potassium concentration. The fact that the association with renal outcomes is strongest with fasting ammonia concentration supports our hypothesis that the inability of the kidney to excrete the acid load rather than the daily acid load itself is deleterious to the course of renal disease. This may seem in contradiction with the study by Scialla *et al.*,[16] who showed that higher NEAP is associated with faster decline in GFR. However, the population studied by Scialla *et al.* (patients in the AASK trial) is notably different from the NephroTest cohort study population, particularly regarding the range of NEAP (72.8 mEq/24 h (IQR 57.2–89.5) in the AASK population vs. 56.3 mEq/24 h (44.4–73.7) in the NephroTest cohort study population). We did not assess whether a higher NEAP is associated with a poorer renal outcome in patients with CKD, which was not the aim of this study; however, it is quite possible that a high acid load would promote acid retention and its potentially deleterious effects, given the compromised ability in increasing urinary excretion demonstrated in the present study.

The major strength of this study is that it includes a large number of patients, with various causes of CKD and a wide range of CKD levels, who were extensively phenotyped with a gold-standard method of GFR measurement. However, our study has also several limitations. First, only fasting ammonia concentration (and not excretion rate) was available and we were unable to factor it by urinary creatinine concentration. Therefore, patients with urine concentration defect could have lower fasting ammonia for this sole reason. However, adjusting for fasting osmolality did not alter the results, suggesting that the level of urine dilution is not a concern. Second, we were able to measure TA and NAEs only in a subset of study patients, in whom we were, however, able to confirm that changes in ammonia were the main determinants of changes in NAE. Third, we cannot rule out that normal GFR fluctuations and measurement errors may impact the determination of the mGFR slopes, especially in patients who only had two measurements. This could explain why the link between the risk of fast rate decline in mGFR and urinary ammonia was weaker compared with the link observed for ESRD. Finally, the NephroTest cohort included few patients with CKD stages 1–2. Therefore, our results mainly apply to patients with moderate or advanced CKD.

In summary, our data show that in CKD a defect in fixed acid excretion and a positive acid balance are present before the emergence of overt metabolic acidosis, that a lower urinary ammonia excretion is associated with a higher risk of ESRD, and that a lower urinary ammonia excretion and lower plasma tCO_2 values are associated with a faster rate of GFR decline. Consequently, we propose that the mismatch between daily acid load and urinary acid excretion is deleterious to renal outcomes. The recent interventional studies showing a beneficial effect of alkaline therapies even in patients with normal plasma bicarbonate concentration may encourage physicians to prescribe these therapies earlier than it is presently advised[26] and currently practiced. Detecting patients with a defect in ammonia excretion in early CKD stages may help physicians to select those patients who may benefit from oral alkali supplementation.

MATERIALS AND METHODS

Patients and study design

The NephroTest study is a prospective hospital-based cohort that began in 2000, enrolling adult patients with CKD stages 1–5, not on dialysis or living with a kidney transplant, not pregnant, and referred to any of three nephrology departments for yearly extensive work-up.[4]

All patients signed informed consent at inclusion. Between January 2000 and December 2010, 1835 patients were enrolled. We excluded 145 patients treated by potassium-sparing diuretic and/or alkalinizing therapy, 91 with no information about their treatments, 95 patients with mGFR less than 15 ml/min per 1.73 m^2 at inclusion, and 85 lost to follow-up. Data for the 85 patients lost to follow-up are shown in Supplementary Table 1; they did not significantly differ from the study group regarding baseline acid-base characteristics but had higher mGFR and lower ACR. Baseline measurements of venous tCO_2 or urinary ammonia were missing for 354 patients at inclusion, most of them because one center did not measure fasting urinary ammonia excretion. Thus, analysis included the data from 1065 patients (Figure 6).

NAE was calculated in a subgroup of 160 consecutive patients enrolled ($n = 62$) or followed up ($n = 98$) between July 2008 and June 2009.

Data collection and measurements

As previously reported, 5-h in-person visits for a complete nephrological work-up provided demographic, clinical, and laboratory data.[27] After an overnight fast, we collected blood and second morning urine sample to determine levels of plasma creatinine, venous tCO_2, as well as urinary ammonia and osmolality. A 24-h urine collection was performed to determine excretion of creatinine, albumin, protein, urea, potassium, phosphate, pH, ammonia and, in 160 patients, TA and bicarbonate. ACR was not available for 26% of patients but was estimated from urinary protein creatine ratio in those without urinary infection (235/1065 = 22.7%) as previously reported.[27] GFR was measured using ^{51}Cr-EDTA renal clearance as described previously,[28] and values were standardized to body surface area.

We measured plasma venous tCO_2 with a specific electrode (Beckman SX9, Beckman Coulter France, Villepinte, France), urinary ammonia and TA by titration (DL55 titrator, Mettler Toledo, Mettler Toledo SAS, Viroflay, France), as previously described,[29] and urinary osmolality by cryoscopy. Urinary pH and bicarbonate concentration were measured with a pH/blood-gas

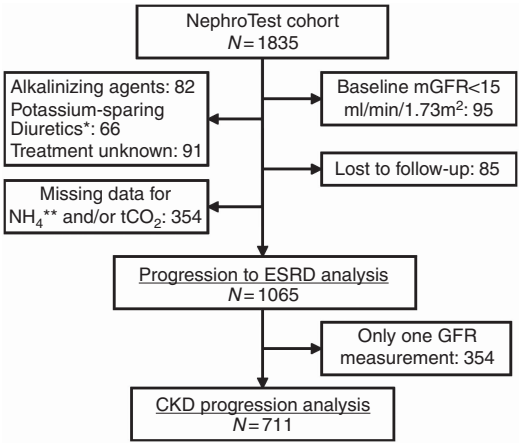

Figure 6 | Study flow chart. *Three patients taking both alkalinizing treatment and potassium-sparing diurectics. **Fasting urinary NH_4 concentration was not measured in one center out of three. CKD, chronic kidney disease; ESRD, end-stage renal disease; mGFR, measured glomerular filtration rate; tCO_2, total CO_2.

analyzer (ABL 705, Radiometer, Radiometer SAS, Neuilly-Plaisance, France). All biological measurements were performed on fresh urine.

Twenty-four-hour ammonia excretion was expressed as ammonia/creatinine ratio (expressed in mEq/mmol) to correct for inaccuracy in 24-h urine collection.

The daily non-volatile acid load was estimated using the NEAP as used by others in previous studies[16,30,31]: NEAP (mEq/24 h) = −10.2 +54.5 (protein intake (g/24 h)/potassium intake (mEq/24 h)). Protein intake was calculated using the Maroni equation.[32] NAE was calculated as NH_4^++TA–HCO_3^- and acid balance as NEAP-NAE.

ESRD and mortality outcomes

Patients were followed until 31 December 2010. ESRD defined by dialysis or preemptive kidney transplantation was identified either from medical records or through linkage with the national REIN registry of dialysis and transplantation. Vital status was ascertained by linkage with the RNIPP (National Identification Register of Private Individuals). All survival data were right-censored on 31 December 2010 or for patients not identified in registries using their date of last visit. We analyzed both overall mortality and pre-ESRD mortality.

Statistical analysis

In cross-sectional analysis of acid balance, associations between tCO_2, urinary ammonia, TA, NAE, acid balance, and mGFR were assessed using Pearson's correlation coefficients. In each mGFR level, acid balance observed mean was compared with 0 using a one-sample t-test.

In longitudinal analysis, we first studied the associations of venous tCO_2 or ammonia levels (24-h NH_4/creatinine excretion ratio or fasting urinary NH_4 concentration) with the risk of ESRD using cause-specific Cox models.[33] In these models, the competing events of death were treated as censored observations. Covariates were demographic characteristics (age, gender, ethnic origin, BMI), center, baseline mGFR, and traditional risk factors of CKD progression: high blood pressure (>140/90 mm Hg), diabetes mellitus, history of cardiovascular disease, smoking, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment, and ACR (<3, 3–30, >30 mg/mmol). For fasting urinary NH_4 concentration, we also adjusted for urinary osmolality. We tested the proportional-hazard assumption with log(–log(S)) and Schoenfeld residuals against time for each covariate. Because it was not satisfied for mGFR, we stratified rather than adjusted for baseline mGFR level in the Cox models, using six classes of mGFR 15–20, 20–30, 30–40, 40–50, 50–60, and >60 ml/min per 1.73 m^2. Crude and adjusted HRs of ESRD were given by tertile of venous tCO_2 or 24-h NH_4/creatinine ratio or fasting urinary NH_4 concentration using the highest tertile as the reference category for NH_4 and the middle tertile for venous tCO_2. HR of ESRD according to metabolic acidosis (venous tCO_2 < vs. ≥ 22 mmol/l) was also estimated. Penalized splines were then used in a fully adjusted Cox model to represent the functional relationship between 24-h NH_4/creatinine ratio or fasting urinary NH_4 concentration and the risk of ESRD.

Second, to test the robustness of our findings, we also studied the associations of the same biomarkers with 20% decline in mGFR over 2 years (i.e., 10% per year) in a sub-group of 711 patients with at least two mGFR measurements. This rate of decline has recently shown to be a reliable alternative end point of CKD progression analysis.[20] Individual slopes in ml/min/year were estimated using ordinary linear regression. We then calculated relative mGFR slopes in % per year and categorized patients into two groups of rate of decline in mGFR: ≤10 vs. >10% per year. Logistic regression was used to estimate the crude and adjusted odd ratios of fast progression according to the three markers. Odds ratio were sequentially adjusted for mean mGFR over time and for the same baseline covariates as in the Coxmodels for ESRD.

Finally, crude and adjusted HRs of overall mortality and pre-ESRD mortality associated with venous tCO_2 level or 24-h NH_4/creatinine excretion ratio or fasting urinary NH_4 concentration were estimated using Cox models including the same covariates as for ESRD analysis. For pre-ESRD mortality, time-to-event was censored at the time of ESRD occurrence.

Statistical analyses were performed with SAS 9.2 (SAS Institute, Cary, NC, USA) and R 2.15 (R Foundation for Statistical Computing, Vienna, Austria, 2012).[34]

DISCLOSURE

MFr was employed by Amgen from January 2011 to October 2014 but was a full-time academic associate professor during the time of study conception and data collection. BS has received research funds from Amgen, Baxter, Genzyme (Sanofi), Fresenius, MSD, and GSK. PH has received consulting or lecture fees or research funds from Amgen and Genzyme (Sanofi).

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AUTHOR CONTRIBUTIONS

MV and PH defined the research theme and designed the methods; MFl, JPH, PH, and MFr carried out the laboratory reference measurements; BS and MM performed the statistical analyses; BS, MFl, JPH, JJB, ET, MV, MM, and PH discussed analyses, interpretation, and presentation; CG, MM, BS, and MFr managed the data collection; MV, MM, BS, and PH have been involved in drafting the manuscript; MFr and BS acquired funding; BS and PH revised the manuscript critically.

SUPPLEMENTARY MATERIAL

Table S1. Characteristics of participants lost to follow-up.
Table S2. Adjusted hazard ratios of ESRD and pre-ESRD mortality according to fasting NH_4 concentration and adjustment covariates.
Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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