Prediction and monitoring of chronic kidney disease

Schutte, Elise

Chapter 2

Serum bicarbonate and kidney and cardiovascular outcomes in patients with diabetic nephropathy; A post hoc analysis of the RENAAL (Reduction in End Points in Non-Insulin-dependent diabetes with the Angiotensin II Antagonist Losartan) and IDNT (Irbesartan Diabetic Nephropathy Trial) trials

Elise Schutte
Hiddo J. Lambers Heerspink
Helen L. Lutgers
Stephan J.L. Bakker MD
Priya Vart
Bruce H.R. Wolffenbuttel
Kausik Umanath
Julia B. Lewis
Dick de Zeeuw
Ron T. Gansevoort

American Journal of Kidney Diseases 66(3): 450-458, 2015.

ABSTRACT

Background: Low serum bicarbonate has been reported to be an independent predictor of renal function decline and mortality in patients with chronic kidney disease. Mechanisms underlying low levels of serum bicarbonate may differ in patients with and without diabetes. We aimed to specifically investigate the association of serum bicarbonate with renal and cardiovascular (CV) outcome in a cohort of patients with type 2 diabetes and nephropathy.

Study design: Post hoc analysis of 2 multicenter randomized controlled trials.

Setting and participants: 2628 Adults with type 2 diabetes and nephropathy.

Factor: Serum bicarbonate level.

Outcomes: Incidence of (1) end-stage renal disease (ESRD), (2) ESRD or doubling of serum creatinine (DSCR), (3) all cause mortality, (4) cardiovascular (CV) events (fatal/non-fatal stroke/myocardial infarction), and (5) heart failure

Measurements: Serum bicarbonate was measured at baseline as total carbondioxide. Associations of baseline serum bicarbonate with endpoints were investigated using Cox regression models. Serum bicarbonate levels were studied as continuous variable and stratified in quartiles. Follow-up time was 2.8±1.0 years.

Results: Cox regression analyses showed that serum bicarbonate had inverse associations with incident ESRD (HR 0.91 (95% CI 0.89-0.93), P<0.001) and with incidence of the combined endpoint of ESRD or DSCR (HR 0.94 (0.92-0.96), P<0.001). These associations were independent of age, gender and cardiovascular risk factors, but disappeared after adjustment for baseline eGFR (all P>0.05). Analysis of bicarbonate quartiles showed similar results for the quartile with lowest bicarbonate (<21 mEq/L) versus the quartile with normal bicarbonate levels (24-26 mEq/L). There was no association of bicarbonate with cardiovascular events and heart failure.

Limitations: Post hoc analysis and single measurement of serum bicarbonate.

Conclusion: In this cohort of type 2 DM patients with nephropathy, serum bicarbonate associations with kidney disease endpoints were not retained after adjustment for eGFR, which contrasts results of earlier studies in non-diabetic populations.

Introduction

One of the major complications of diabetes mellitus is diabetic nephropathy (DN), which currently accounts for 45% of the prevalence of chronic kidney disease (CKD) cases in the US.[1] Presence of DN is associated with an increased risk for end stage renal disease (ESRD) and death. To predict which CKD patients are at highest risk of developing ESRD or mortality various biomarkers have been examined, among which serum bicarbonate. Low serum bicarbonate is not only a symptom of impaired renal function, but has also been reported an independent predictor of decline in renal function and death. [2-6] The alleged pathogenesis of renal function decline due to metabolic acidosis is complex. Three possible pathways have been suggested. First, metabolic acidosis increases production of ammonia in the kidney, which in turn activates the complement system, leading to interstitial fibrosis.[7,8] Second, metabolic acidosis activates the renin-angiotensin system and persistent activation causes hyperfiltration, proteinuria, and progressive CKD .[7,8] Third, endothelin-1 levels are increased in metabolic acidosis, leading to stimulation of endothelin-A receptors, also causing interstitial fibrosis.[7,8] The studies that investigated serum bicarbonate as risk predictor for renal or cardiovascular endpoints and mortality included predominantly CKD patients without diabetes.[2-6] Interestingly, several studies have indicated that levels of serum bicarbonate in patients with diabetes and CKD differ from non-diabetic patients.[4-6,9,10] Some of these studies showed that CKD patients with lower serum bicarbonate have lower diabetes prevalence rates, and others revealed that patients with CKD and diabetes had higher serum bicarbonate levels compared to CKD patients without diabetes.[4-6,9,10]

Since few researchers have investigated the predictive value of serum bicarbonate in specifically patients with DN, we investigated the association of serum bicarbonate with renal, cardiovascular and mortality outcomes in this population.

Methods

Clinical trials

We conducted a post hoc analysis on the combined RENAAL/IDNT database. Both studies were large, multicentre, randomized controlled trials investigating the effect of angiotensin receptor blockers on renal outcomes compared with placebo in patients with type 2 diabetes and nephropathy. The rationale, study design and outcome of these trials have been published previously. [11,12] In RENAAL, losartan was the studied drug. In IDNT, irbesartan was investigated along with an amlodipine treatment arm. Inclusion criteria in both trials were similar; patients aged 30 to 70 years with type 2 diabetes, hypertension and diabetic nephropathy were included. Hypertension was defined as having blood pressure >135/85 mmHg or the use of antihypertensive medication. Serum creatinine ranged between 1.0 and 3.0 mg/dL. All subjects had proteinuria, defined as urinary albumin-to-creatinine ratio (UACR) of >300 mg/g (or proteinuria of >500 mg/24h in RENAAL and >900 mg/24h in IDNT). Main exclusion criteria were type 1 diabetes, non-diabetic renal disease, myocardial infarction or coronary artery bypass grafting (CABG) within the previous month, cerebrovascular accident or

percutaneous transluminal coronary angioplasty (PCI) within the previous six months, or a history of heart failure before enrollment.

Study participants

A total of 3228 patients were enrolled in the RENAAL (1513 patients) and IDNT trials (1715 patients). Baseline bicarbonate measurements were available in all RENAAL participants. In IDNT, bicarbonate was not recorded in 600 patients. These patients were excluded from the present analysis. There were no important differences in characteristics between patients with missing bicarbonate measurements and the remainder of the IDNT cohort (Supplemental Table 1).

Measurement of serum bicarbonate and renal function

Serum bicarbonate was measured at baseline as total carbondioxide, a method commonly used by chemical analyzers. Fresh serum samples were shipped to a central laboratory and analyzed within 48 hours after obtaining the samples. Creatinine was measured with the Jaffe reaction method at baseline and every three months thereafter. The 4-variable Modification of Diet in Renal Disease (MDRD) study equation was used to estimate GFR.[13]

Outcome measures

The present study had five endpoints:

1. ESRD, defined as the need for chronic dialysis or renal transplantation. The IDNT trial had a confirmed serum creatinine ≥6 mg/dl (≥530 µmol/l) as an additional definition of ESRD.
2. A combined endpoint of ESRD or confirmed doubling of serum creatinine (DSCR) from baseline.
3. All cause mortality.
4. Fatal or non-fatal cardiovascular events, defined as incidence of stroke, myocardial infarction, coronary revascularization (PCI, CABG) or cardiovascular death.
5. Hospitalization for heart failure.

Statistics

Baseline characteristics are presented for the overall study population as well as serum bicarbonate quartiles: <21, 22-23, 24-26 and >27 mEq/L. Serum bicarbonate measures were rounded to one decimal and whole numbers in respectively IDNT and RENAAL, which led to a difference in the number of patients in each quartile. Continuous data are presented as mean with standard deviation (SD) or as median and interquartile range (IQR) in case of skewed distribution. Categorical data are presented as percentiles. We tested for differences in baseline characteristics between quartiles using analysis of variance (ANOVA) and chi-squared tests for continuous and categorical variables, respectively. Univariate and multivariable linear regression was used to examine factors associated with serum bicarbonate. Covariates were chosen a priori based on associations previously shown or assumed to be related to serum bicarbonate and mortality. These include age, gender, race (white or non-white), smoking, body mass index (BMI), systolic blood pressure (SBP), total cholesterol, Hba1c, eGFR, potassium, chloride, phosphate, UACR, renin-angiontensin-aldosteron-system (RAAS) inhibitor, alpha blocker, beta blocker, and diuretic use at baseline. UACR was log transformed to achieve normal distribution.

We used five Cox proportional hazard regression models to evaluate the association of serum bicarbonate levels with endpoints. Model 1: adjusted for age and gender. Model 2: adjusted for variables in model 1 and additionally for smoking status, BMI, systolic blood pressure, Hba1c and total cholesterol. Model 3: as model 2 plus covariates that had significant association with serum bicarbonate in both univariate and multivariable regression analysis (i.e. trial, diuretics, potassium, chloride and phosphate), Model 4: as model 3 plus UACR. Model 5: as model 4 plus eGFR. Bicarbonate quartile 24-26 mEq/L was used as reference group for the analysis of bicarbonate quartiles because this quartile contains the normal values for serum bicarbonate. In addition, spline analyses were performed to assess possible non-linear associations of serum bicarbonate with the five endpoints.

Serum bicarbonate was examined as a possible mediator between eGFR and endpoints. Preacher and Hayes procedures were used to test significance and magnitude of mediation.[14,15] First, the total effect of eGFR on endpoints was estimated by performing regression analysis of eGFR with endpoints. Second, the indirect effect of eGFR on endpoints via bicarbonate was obtained by computing the product of two coefficients that were obtained after regression analysis of bicarbonate i) with eGFR and ii) with endpoints (Supplemental Figure 1). Third, significance of the indirect effect (product-of-coefficients) was tested by computing bias-corrected bootstrap confidence intervals with 2000 repetitions. Finally, the magnitude of mediation was calculated by dividing the coefficient of the indirect effect by the total effect. Significance of mediation was proven with $P<0.05$ if zero was not between the lower and upper bound of the 95% confidence interval of the indirect effect.

To test the robustness of our results three sensitivity analyses were performed. First, all analyses were repeated with the CKD-EPI equation instead of the MDRD study equation to estimate GFR. Second, analyses were stratified for treatment allocation (placebo/active treatment). Third, in case of significant results of the association of bicarbonate (as continuous variable) with endpoints in models 4 and 5, an interaction term for cohort (RENAAL/IDNT) was included in the fully adjusted Cox regression model. When interaction was significant, separate analyses were performed for both cohorts. Finally,competing risk proportional hazards regression analysis was applied according to the method of Fine and Gray for the endpoint ESRD with death as competing risk.[16] All statistical analyses were performed using STATA (Stata Corp, College Station, TX, USA), and a P value of ≤0.05 was adopted to indicate statistical significance.

Results

Baseline characteristics

Baseline characteristics of the participants included in the present study are listed in Table 1. Bicarbonate data was available in 2628 patients in the two trials (81.4% of total cohort). Mean age was 60±8 (SD) years, 65% were male and 60% were white. Serum bicarbonate level was 24.3±3.7 mEq/L and 602 patients had serum bicarbonate level <21 mEq/L. Baseline eGFR was 44±16 mL/min/1.73m^2. After stratifying by baseline bicarbonate quartiles, there were significant differences between these quartiles in trial. For instance, patients in the lowest bicarbonate quartile were more often from the IDNT trial, younger, less often male, used less alpha blockers at baseline, had lower eGFR and

Table 1 Baseline characteristics

		Bicarbonate Quartile (mEq/L)				
	All	<21	22-23	24-26	>27	P
N	2628	602	497	841	688	
Mean bicarbonate (mEq/L)	24.3±3.7	19.3±1.9	22.6±0.5	25.0±0.8	28.8±2.0	
RENAAL (n(%))	1513 (58)	318 (53)	285 (57)	496 (59)	414 (60)	**0.05**
IDNT (n(%))	1115 (42)	284 (47)	212 (43)	345 (41)	274 (40)	
Age (years)	59.6±7.6	58.7±8.1	59.8±7.3	59.7±7.7	60.1±7.3	**0.007**
Male (n(%))	1701 (65)	360 (60)	307 (62)	556 (66)	478 (69)	**0.001**
White (n(%))	1572 (60)	368 (61)	284 (57)	498 (59)	422 (61)	0.4
Smoker (n(%))	466 (18)	119 (20)	89 (18)	154 (19)	104 (15)	0.2
BMI (kg/m^2)	30.2±6.1	29.8±6.1	30.1±6.1	30.1±6.0	30.5±6.3	0.3
SBP (mmHg)	155±20	156±21	156±20	155±19	155±19	0.4
DBP (mmHg)	84±11	85±11	84±11	84±11	85±11	0.4
Baseline antihypertensive medication use						
RAAS inhibitor use (n(%))	1266 (48)	276 (46)	227 (46)	405 (48)	358 (52)	0.08
Alpha Blocker use (n(%))	464 (18)	90 (15)	82 (17)	145 (17)	147 (21)	**0.02**
Beta Blocker use (n(%))	487 (19)	113 (19)	95 (19)	161 (19)	118 (17)	0.8
CCB use (n(%))	1513 (58)	341 (57)	285 (57)	497 (59)	390 (57)	0.7
Diuretic use (n(%))	1412 (54)	312 (52)	253 (51)	451 (54)	396 (58)	0.09
Total Cholesterol (mg/dL)	227±56	233±62	229±59	226±53	223±53	**0.03**
Glucose (mg/dL)	182±81	183±88	180±81	182±80	181±75	0.9
HbA1c (%)	8.3±1.7	8.2±1.7	8.3±1.7	8.4±1.7	8.4±1.7	0.3
Serum Creatinine (mg/dL)	1.8±0.5	2.0±0.6	1.8±0.5	1.7±0.5	1.7±0.5	**<0.001**
eGFR (ml/min/1.73m^2)	44±16	39±16	43±16	44±15	46±15	**<0.001**

Table 1 *(Continued)*

	All	Bicarbonate Quartile (mEq/L)				P
		<21	22-23	24-26	>27	
Potassium (mEq/L)	4.6±0.5	4.7±0.5	4.6±0.5	4.6±0.5	4.5±0.5	**<0.001**
Chloride (mEq/L)	105±4	107±4	106±4	105±3	103±4	**<0.001**
Phosphate (mg/ml)	3.8±0.6	4.0±0.7	3.8±0.6	3.9±0.6	3.7±0.6	**<0.001**
UACR (mg/g)	1366 (682 – 2653)	1636 (728 – 2993)	1480 (709 – 2809)	1256 (607 – 2585)	1155 (584 – 2204)	<0.001*

Continuous variables are presented as mean± standard deviation or median (interquartile range). Categorical variables are presented as count (percentage).

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RAAS, renin-angiotensin-aldosteron system; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio. *p for lnUACR

Conversion factors for units: Total Cholesterol mg/dL to mmol/L, x0.02586; Glucose mg/dL to mmol/L, x0.05551; Serum Creatinine mg/dL to µmol/L, x88.4.

higher total cholesterol, serum creatinine, potassium, chloride, phosphate and UACR, compared to the highest bicarbonate quartile.

Cross-sectional analysis of variables independently associated with serum bicarbonate

Factors possibly associated with serum bicarbonate levels are listed in Table 2. Univariate and multivariable analyses showed significant associations of serum bicarbonate with several covariates. Lower age, less diuretic use at baseline and lower eGFR were independently associated with lower bicarbonate levels whereas higher total cholesterol, potassium, chloride and phosphate were independently associated with lower bicarbonate levels.

Table 2 Regression analysis of baseline serum bicarbonate levels with covariates

	Univariate			Multivariable		
	Beta	95% CI	P	Beta	95% CI	P
RENAAL/IDNT	-0.285	-0.575 to 0.004	0.05	-0.832	-1.125 to -0.538	<0.001
Age (years)	0.061	0.022 to 0.099	0.002	0.064	0.026 to 0.101	0.001
Female vs male	-0.702	-1.000 to -0.404	<0.001	-0.099	-0.405 to 0.207	0.5
White vs non-white	0.048	-0.244 to 0.339	0.7	-	-	-
Smoker vs non-smoker	0.368	-0.743 to 0.006	0.05	0.334	-0.688 to 0.199	0.06
BMI (kg/m^2)	0.055	0.017 to 0.093	0.005	0.006	-0.312 to 0.043	0.7
SBP (mmHg)	-0.030	-0.068 to 0.009	0.13	-	-	-
RAAS inhibitor use vs no use	0.411	0.125 to 0.697	0.005	0.208	-0.058 to 0.475	0.1
Alpha Blocker use vs no use	0.565	0.191 to 0.939	0.003	0.316	-0.367 to 0.669	0.08
Beta Blocker use vs no use	0.084	-0.452 to 0.284	0.7	-	-	-
Diuretic use vs no use	0.424	0.137 to 0.710	0.004	0.455	0.172 to 0.739	0.002
Total Cholesterol (mg/dL)	-0.063	-0.1027 to -0.023	0.002	-0.059	-0.098 to -0.020	0.003
HbA1c (%)	0.027	-0.013 to 0.066	0.18	-	-	-
eGFR (ml/min/1.73)	0.192	0.154 to 0.231	<0.001	0.136	0.093 to 0.179	<0.001
Potassium (mEq/L)	-0.200	-0.237 to -0.161	<0.001	-0.076	-0.113 to -0.038	<0.001
Chloride (mEq/L)	-0.402	-0.437 to -0.367	<0.001	-0.367	-0.405 to -0.328	<0.001
Phosphate (mg/ml)	-0.141	-0.180 to -0.103	<0.001	-0.053	-0.093 to -0.013	0.009
lnUACR (ln(mg/g)	-0.114	-0.152 to -0.076	<0.001	0.035	-0.006 to 0.075	0.09

Beta is expressed per 1SD increase for continuous variables and versus the reference category for dichotomous variables

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RAAS, renin-angiotensin-aldosteron system; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; lnUACR, natural log urinary albumin-to-creatinine ratio.

Prospective study

During median follow-up for 2.8±1.0 years, 576 (22%) patients died, 491 (19%) patients developed ESRD, 731 (28%) patients had doubling of serum creatinine (DSCR) or ESRD, 457 (17%) patients had a cardiovascular event, and 343 (13%) patients developed heart failure.

Kaplan Meier figures for endpoints ESRD, death and cardiovascular events are presented in Figure 1. The figures show clear differences in event-free survival between the bicarbonate quartiles for ESRD (log rank P<0.0001) and death (log rank P=0.03). Patients in the lowest bicarbonate quartile had more ESRD and mortality compared to the highest bicarbonate quartile: 156 (26%) vs. 78 (11%) patients developed ESRD and 118 (20%) vs. 116 (17%) died. There were no differences for cardiovascular events (log rank P=0.5).

The hazard ratios of the Cox regression models for all endpoints are listed in Table 3. When analyzing serum bicarbonate as a continuous variable, there was an inverse association of bicarbonate with ESRD (HR 0.91 per 1 mEq/L increase in serum bicarbonate level (95% CI 0.89-0.93), P<0.001). The association remained after cumulative adjustments performed in models 1 to 4. However, after adjusting for eGFR (model 5), the association with ESRD was no longer present. The association with combined endpoint of ESRD or DSCR (HR 0.94 (0.92-0.96), P<0.001) was only present in models 1 and 2. Associations between serum bicarbonate and mortality became insignificant when adjustment was performed for covariates included in models 3 to 5. Serum bicarbonate was not associated with cardiovascular events in any of the models.

Analysis of the bicarbonate quartiles was performed to allow assessment of non-linear associations (Figure 2). This analysis showed similar associations of the lowest bicarbonate quartile (<21 mEq/L) versus the reference quartile (24-26 mEq/L) with increased risk for: ESRD, the combined endpoint of ESRD or DSCR and mortality in unadjusted models (HR 1.51 (1.21-1.88), 1.37 (1.14-1.66), 1.38 (1.07-1.77) respectively). This association disappeared in adjusted models. The highest bicarbonate quartile (>27 mEq/L) showed a lower risk of ESRD and combined endpoint ESRD or DSCR, unadjusted HR 0.56 (95% CI 0.43-0.74) and 0.72 (95% CI 0.59-0.89), respectively. This association also disappeared in adjusted models. Patients in the highest bicarbonate quartile appeared to have a higher risk of developing heart failure, even in the fully adjusted model (HR 1.36 (95% CI 1.01-1.83)). In addition, spline analysis showed that the associations of serum bicarbonate with all endpoints were linear and, as in the main Cox regression analyses, significance was lost in the fully adjusted model for all endpoints (Supplemental Figure 2).

Of note, sensitivity analyses showed no interaction of treatment allocation with bicarbonate versus risk of any of the five outcomes (P for interaction >0.05 for all outcomes). The interaction term for bicarbonate versus trial (RENAAL or IDNT) was not significant. Furthermore, results did not differ when the CKD-EPI formula was used instead of the MDRD study equation to estimate GFR (Supplemental Tables 2 and 3). Lastly, a competing risk proportional hazards regression analysis weakened the association of serum bicarbonate with ESRD. The inverse association of serum bicarbonate with ESRD remained significant in the unadjusted model, albeit weaker than in the regular Cox regression analysis (HR 0.97 (95% CI 0.95-0.98) in the competing risk analysis versus HR 0.91 (95% CI 0.89-0.93) in the regular Cox regression analysis). However, as in the main Cox regression analysis the association was lost in the fully adjusted model containing eGFR (HR 1.01 (95% CI 0.99-1.03)).

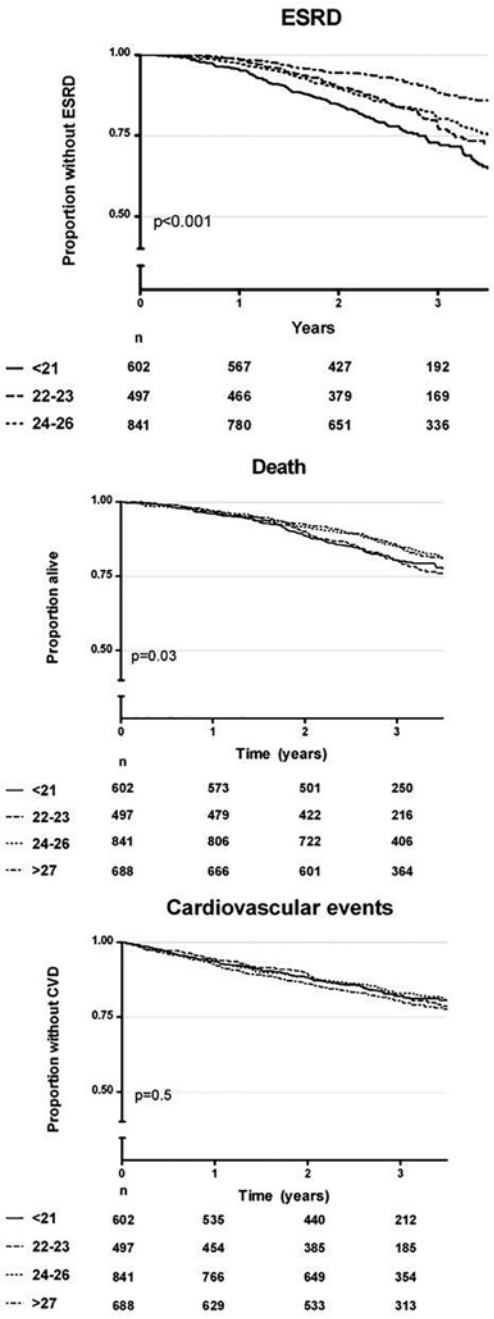


Figure 1 Kaplan Meier curves showing per bicarbonate quartile (in mEq/L) the proportion of participants without ESRD (upper panel), death (middle panel) and cardiovascular events (lower panel)

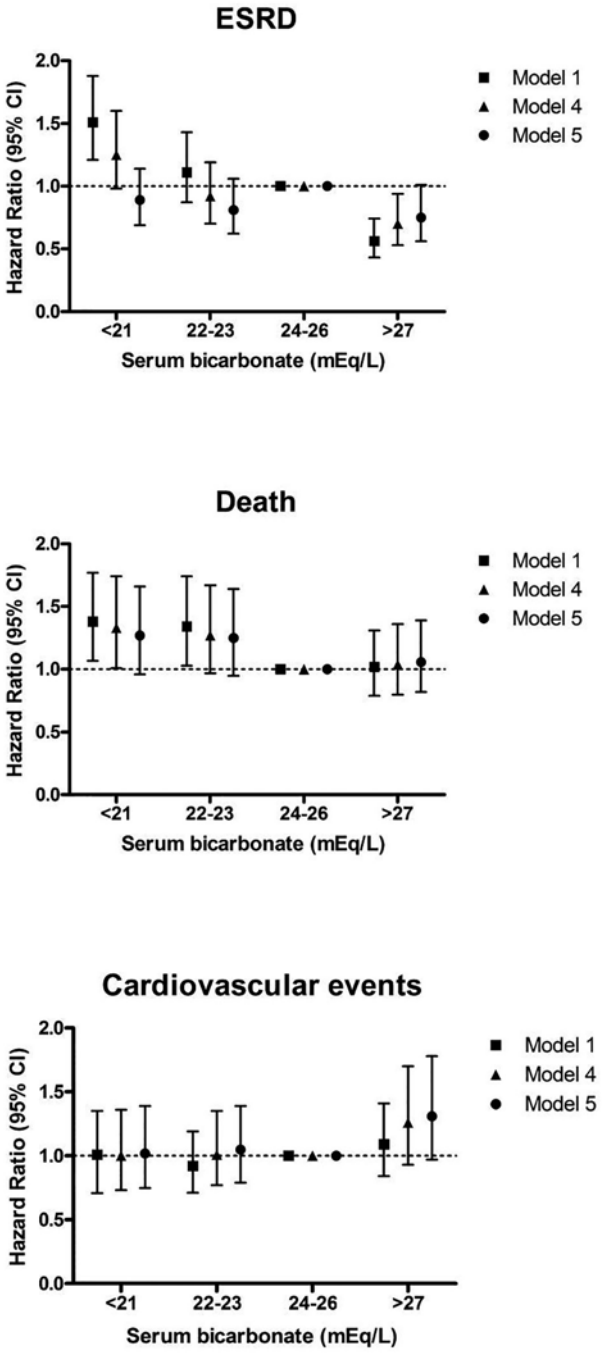


Figure 2 Hazard ratio and 95% confidence interval for endpoints ESRD, death and cardiovascular events per bicarbonate quartile. Models are: 1, adjusted for age, gender; 4, adjusted for 1 + smoking, body mass index, systolic blood pressure, Hba1c, total cholesterol, trial, diuretics, potassium, chloride and phosphate; 5, adjusted for 4 + eGFR.

Table 3 Cox regression analysis of baseline serum bicarbonate levels and endpoints at follow-up, hazard ratio + 95% confidence interval

	Bicarbonate		Bicarbonate quartiles (mEq/L)			
	Continuous	P	<21	22-23	24-26	>27
ESRD						
Model 1	0.91 (0.89 – 0.93)	<0.001	1.51 (1.21 – 1.88)*	1.11 (0.87 – 1.43)	1.00 (reference)	0.56 (0.43 – 0.74)*
Model 2	0.92 (0.90 – 0.95)	<0.001	1.42 (1.13 – 1.79)*	1.10 (0.85 – 1.43)	1.00 (reference)	0.59 (0.45 – 0.78)*
Model 3	0.96 (0.93 – 0.98)	0.002	1.19 (0.94 – 1.52)	1.00 (0.77 – 1.31)	1.00 (reference)	0.69 (0.52 – 0.92)*
Model 4	0.95 (0.93 – 0.98)	0.001	1.25 (0.98 – 1.60)	0.92 (0.70 – 1.19)	1.00 (reference)	0.70 (0.53 – 0.94)*
Model 5	1.00 (0.97 – 1.03)	0.9	0.89 (0.69 – 1.14)	0.81 (0.62 – 1.06)	1.00 (reference)	0.75 (0.56 – 1.01)
ESRD or DSCR						
Model 1	0.94 (0.92 – 0.96)	<0.001	1.37 (1.14 – 1.66)*	1.19 (0.97 – 1.46)	1.00 (reference)	0.72 (0.59 – 0.89)*
Model 2	0.94 (0.93 – 0.96)	<0.001	1.30 (1.07 – 1.58)*	1.18 (0.96 – 1.46)	1.00 (reference)	0.73 (0.59 – 0.91)*
Model 3	0.98 (0.96 – 1.00)	0.1	1.09 (0.89 – 1.34)	1.10 (0.89 – 1.36)	1.00 (reference)	0.87 (0.70 – 1.08)
Model 4	0.98 (0.95 – 1.00)	0.054	1.13 (0.92 – 1.38)	1.02 (0.82 – 1.26)	1.00 (reference)	0.87 (0.69 – 1.08)
Model 5	1.00 (0.98 – 1.03)	0.9	0.94 (0.76 – 1.16)	0.96 (0.77 – 1.19)	1.00 (reference)	0.92 (0.73 – 1.15)
Death						
Model 1	0.97 (0.94 – 0.99)	0.008	1.38 (1.07 – 1.77)*	1.34 (1.03 – 1.74)*	1.00 (reference)	1.02 (0.79 – 1.31)
Model 2	0.97 (0.94 – 1.00)	0.02	1.37 (1.06 – 1.78)*	1.31 (1.00 – 1.72)*	1.00 (reference)	1.04 (0.80 – 1.34)
Model 3	0.97 (0.95 – 1.00)	0.08	1.33 (1.01 – 1.74)*	1.34 (1.04 – 1.75)*	1.00 (reference)	1.07 (0.82 – 1.39)
Model 4	0.97 (0.94 – 1.00)	0.06	1.33 (1.01 – 1.74)*	1.27 (0.97 – 1.67)	1.00 (reference)	1.04 (0.80 – 1.36)
Model 5	0.98 (0.95 – 1.01)	0.2	1.27 (0.96 – 1.66)	1.25 (0.95 – 1.64)	1.00 (reference)	1.06 (0.82 – 1.39)

Table 3 *(Continued)*

	Bicarbonate		Bicarbonate quartiles (mEq/L)			
	Continuous	P	<21	22-23	24-26	>27
Cardiovascular Events						
Model 1	1.01 (0.98 – 1.03)	0.6	1.01 (0.75 – 1.35)	0.92 (0.71 – 1.19)	1.00 (reference)	1.09 (0.84 – 1.41)
Model 2	1.01 (0.99 – 1.04)	0.3	1.01 (0.74 – 1.37)	0.98 (0.75 – 1.29)	1.00 (reference)	1.18 (0.89 – 1.55)
Model 3	1.02 (0.99 – 1.05)	0.2	1.02 (0.75 – 1.39)	1.01 (0.76 – 1.33)	1.00 (reference)	1.24 (0.92 – 1.67)
Model 4	1.02 (0.99 – 1.05)	0.2	1.00 (0.73 – 1.36)	1.01 (0.77 – 1.35)	1.00 (reference)	1.26 (0.93 – 1.70)
Model 5	1.03 (1.00 – 1.06)	0.1	1.02 (0.75 – 1.39)	1.05 (0.79 – 1.39)	1.00 (reference)	1.31 (0.97 – 1.78)
Heart failure						
Model 1	1.02 (0.99 – 1.05)	0.2	0.90 (0.66 – 1.24)	1.20 (0.89 – 1.63)	1.00 (reference)	1.28 (0.97 – 1.68)
Model 2	1.02 (0.99 – 1.05)	0.2	0.90 (0.65 – 1.26)	1.18 (0.86 – 1.62)	1.00 (reference)	1.33 (1.00 – 1.76)*
Model 3	1.03 (0.99 – 1.06)	0.1	0.86 (0.61 – 1.21)	1.17 (0.85 – 1.61)	1.00 (reference)	1.38 (1.03 – 1.83)*
Model 4	1.02 (0.99 – 1.06)	0.2	0.84 (0.60 – 1.19)	1.10 (0.80 – 1.52)	1.00 (reference)	1.32 (0.99 – 1.77)
Model 5	1.03 (0.99 – 1.07)	0.1	0.82 (0.58 – 1.16)	1.10 (0.80 – 1.53)	1.00 (reference)	1.36 (1.01 – 1.83)*

*=P<0.05 Hazard ratios are presented per 1 mEq/L increase in bicarbonate for bicarbonate as continuous variable, and versus the reference category for bicarbonate quartiles

Abbreviations: ESRD, end stage renal disease; DSCR, doubling of serum creatinine

Models are: 1, adjusted for age, gender; 2, adjusted for 1 + smoking, body mass index, systolic blood pressure, Hba1c, total cholesterol; 3, adjusted for 2 + variables with P<0.05 in multivariable regression analysis (with exception of eGFR and UACR); 4, adjusted for 3 + UACR; 5, adjusted for 4 + eGFR

Mediation analysis

As the association between serum bicarbonate and endpoints was not significant after adjusting for eGFR, serum bicarbonate was examined as a possible mediator in the association between eGFR and endpoints (Table 4). Mediation analysis showed that serum bicarbonate indeed was a mediator in the association of eGFR with ESRD and with the combined endpoint ESRD or DSCR. The magnitude of mediation was, however, small: 1.9% and 2.1% for ESRD and for ESRD or DSCR, respectively. There was no significant mediator effect of bicarbonate with mortality. Mediation analysis was not performed for the endpoints cardiovascular events and heart failure, as no association of serum bicarbonate with these endpoints was found.

Table 4 Mediating effect of bicarbonate on the association of eGFR with endpoints

Endpoint		Coefficient (95% CI, bc)	Proportion mediated (%)
ESRD	Indirect effect	-0.011 (-0.021 to -0.003)	1.9%
	Total effect	-0.612 (-0.656 to -0.564)	
DSCR or ESRD	Indirect effect	-0.010 (-0.018 to -0.002)	2.1%
	Total effect	-0.447 (-0.493 to -0.396)	
Death	Indirect effect	-0.001 (-0.011 to 0.010)	NM
	Total effect	-0.233 (-0.300 to -0.173)	

Abbreviations: ESRD, end stage renal disease; DSCR, doubling of serum creatinine; CI, confidence interval; bc, bias corrected; NM, not mediated

Discussion

In this large cohort of type 2 DM patients with nephropathy we showed that low serum bicarbonate (<21 mEq/L) was associated with a 51% higher risk of developing ESRD and a 38% higher risk of mortality compared to the reference group of 24-26 mEq/L in unadjusted models. However, these associations were not present when adjusting for confounders, especially baseline eGFR. No association was found between serum bicarbonate with cardiovascular events incidence.

The association of serum bicarbonate with renal and cardiovascular endpoints and mortality has been investigated in several other studies.[2-6]All included mixed populations, mostly patients without diabetes. In general an eGFR independent association of serum bicarbonate with renal outcomes and mortality was found. Although these findings seem to contradict our results, it should be noted that, first, one other study that included 6% patients with diabetes, also found an eGFR dependent association of low serum bicarbonate with kidney disease endpoints and mortality.[3] Second, in two other studies subgroup analysis stratified for diabetes status was performed.[5,6,8] Interestingly, no significant association was found between serum bicarbonate and mortality in the subgroup of patients with diabetes, whereas this association was significant for patients without diabetes. These data, in combination with our findings, lead us to hypothesize that the consequences of metabolic acidosis may differ between patients with and without diabetes.

We pose three possible explanations for our findings. First, regardless of diabetes history, serum bicarbonate could be an indirect measure of kidney function. Therefore adjusting for eGFR (as proxy for renal function) may leave no significant effect to serum bicarbonate. We analyzed this possibility by mediation analysis, which showed that bicarbonate was indeed a mediator between eGFR and kidney disease endpoints. However, the magnitude of mediation was small, as bicarbonate explained only 1.9% of the association of eGFR with ESRD, and bicarbonate was not a mediator in the association of eGFR with mortality. Moreover, this would not explain the different results that are obtained in subjects with and without diabetes. A second, more appealing possibility is therefore that specifically in diabetes acid-base balance may be different. It has been suggested that keto-acid anions, which are often present in patients with diabetes, can be oxidized to bicarbonate, thus serving as an extrarenal source for bicarbonate.[10] The same authors also found that patients with diabetes have a lower prevalence and a less severe degree of metabolic acidosis compared to patients without diabetes. On the other hand, patients with diabetes are more prone to developing type IV renal tubular acidosis due to hyporeninemic hypoaldosteronism.[17] We therefore have multiple angles to explain differences in acid-base balance between subjects with and without diabetes. Third, presence of diabetes may put patients at such high risk for cardiovascular events and mortality by established cardiovascular and CKD risk factors (e.g. obesity, hypertension, high cholesterol) that it is difficult to show the importance of additional risk factors, such as metabolic acidosis.

Interestingly, we found that patients with serum bicarbonate >27 mEq/L had a higher risk of heart failure than the reference group, and this association was independent of renal and cardiovascular risk factors, including eGFR. Since we found this association only in adjusted categorical models (and not in the unadjusted model) and that this finding was not evident in our spline analyses (Supplementary Figure 2), it is possible that this finding is merely coincidental. However, an association of high serum bicarbonate with heart failure was recently also found in another study.[2] The authors hypothesized that alkalosis may affect regulatory proteins related to cell survival in the myocardium. An additional hypothesis may be that patients with subclinical congestive heart failure at baseline already had (mild) respiratory acidosis and as compensation secondary metabolic alkalosis. The underlying subclinical heart failure may explain why these patients have a higher likelihood to be admitted for overt heart failure during follow-up.

Some limitations of this study need to be addressed. As this was a post hoc analysis, the results can only be interpreted as hypothesis generating. Furthermore, there was no full acid-base status available, and we had no information on use of acid-base balance altering medication or comorbidity, such as bicarbonate suppletion or pulmonary diseases. Finally, three possible sources of bias lie in our measurement of serum bicarbonate. First, we used a single measurement of baseline serum bicarbonate, which may have lead to misclassification in the bicarbonate categories. Second, serum bicarbonate was measured as total CO_2, which can lead to an overestimation of serum bicarbonate 1-2 mEq/L.[18] Third, the transport time of serum samples to the lab may have caused a small diffusional loss of CO_2 may have led to an underestimaton of serum bicarbonate (1 mEq/L after 2 days).[19] However, we find it unlikely that both possible under- and overestimation of serum bicarbonate concentration may have biased our results towards a null hypothesis, as all unadjusted Cox regression models found that low serum bicarbonate is associated with a higher risk of renal endpoints, cardiovascular events as well as mortality.

Strengths of the present study are that it includes a large, homogenous population of exclusively type 2 DM patients with rapidly declining renal function. Such a population has not been investigated before with regard to serum bicarbonate status. Furthermore, the results obtained were largely similar in both cohorts that were combined in this study, emphasizing the robustness of our findings.

Differences in the association between bicarbonate and endpoints in CKD patients with and without diabetes may have therapeutic consequences. The current KDIGO guideline for the management of chronic kidney disease recommends bicarbonate suppletion for patients with serum bicarbonate levels <22 mEq/L to prevent or treat complications of metabolic acidosis, including bone disease, muscle degradation, reduced albumin synthesis and increased inflammation.[9,20] In line, a recent meta-analysis and a review showed potential benefit of alkali therapy in chronic kidney disease for kidney-related endpoints.[21,22] However, neither the KDIGO guideline nor the meta-analysis and review specifically address patients with diabetes. Our data, in combination with previous findings in non-diabetic populations, suggest that it may be prudent to have to different cut-off levels for serum bicarbonate in diabetic and non-diabetic chronic kidney disease to start alkali suppletion. A definitive answer to this question should be provided by randomized clinical trials. At present four randomized controlled trials are being conducted to assess the benefits and risks of bicarbonate suppletion in patients with chronic kidney disease.[23-26] Two trials specifically address patients with diabetes: one group aims for specific subgroup analyses of patients with diabetes, a second group will analyze the effect of bicarbonate suppletion on urinary markers for kidney disease in veterans with type 2 diabetes.[25,26] Hopefully the results of these trials will lead to the development of future evidence-based guidelines for correction of acidosis in CKD patients with diabetes.

To conclude, in this study of type 2 diabetes patients with nephropathy, low serum bicarbonate was not an independent risk factor for kidney disease progression, cardiovascular events or mortality.

References

1 KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. *Am J Kidney Dis* 49(2, Supplement 2): S12-S154, 2007.

2 Dobre M, Yang W, Chen J, Drawz P, Hamm LL, Horwitz E, Hostetter T, Jaar B, Lora CM, Nessel L, Ojo A, Scialla J, Steigerwalt S, Teal V, Wolf M, Rahman M. Association of serum bicarbonate with risk of renal and cardiovascular outcomes in CKD: A report from the chronic renal insufficiency cohort (CRIC) study. *Am J Kidney Dis* 62(4): 670-678, 2013.

3 Menon V, Tighiouart H, Vaughn NS, Beck GJ, Kusek JW, Collins AJ, Greene T, Sarnak MJ. Serum bicarbonate and long-term outcomes in CKD. *Am J Kidney Dis* 56(5): 907-914, 2010.

4 Shah SN, Abramowitz M, Hostetter TH, Melamed ML. Serum bicarbonate levels and the progression of kidney disease: A cohort study. *Am J Kidney Dis* 54(2): 270-277, 2009.

5 Kovesdy CP, Anderson JE, Kalantar-Zadeh K. Association of serum bicarbonate levels with mortality in patients with non-dialysis-dependent CKD. *Nephrol Dial Transplant* 24(4): 1232-1237, 2009.

6 Navaneethan SD, Schold JD, Arrigain S, Jolly SE, Wehbe E, Raina R, Simon JF, Srinivas TR, Jain A, Schreiber MJ, Nally JV. Serum bicarbonate and mortality in stage 3 and stage 4 chronic kidney disease. *Clin J Am Soc Nephrol* 6(10): 2395-2402, 2011.

7 Goraya N, & Wesson DE. Does correction of metabolic acidosis slow chronic kidney disease progression? *Curr Opin Nephrol Hypertens* 22(2): 193-197, 2013.

8 Kovesdy CP. Metabolic acidosis and kidney disease: Does bicarbonate therapy slow the progression of CKD? *Nephrol Dial Transplant* 27(8): 3056-3062, 2012.

9 Kraut JA, & Kurtz I. Metabolic acidosis of CKD: Diagnosis, clinical characteristics, and treatment. *Am J Kidney Dis* 45(6): 978-993, 2005.

10 Caravaca F, Arrobas M, Pizarro JL, Espárrago JF. Metabolic acidosis in advanced renal failure: Differences between diabetic and nondiabetic patients. *Am J Kidney Dis* 33(5): 892-898, 1999.

11 Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving H, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345(12): 861-869, 2001.

12 Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345(12): 851-860, 2001.

13 Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. modification of diet in renal disease study group. *Ann Intern Med* 130(6): 461-470, 1999.

14 Hayes AF. Beyond baron and kenny: Statistical mediation analysis in the new millennium. *Commun Monogr* 76(4): 408-420, 2009.

15 Preacher KJ, & Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Meth Ins* C 36(4): 717-731, 2004.

16 Fine JP, & Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association* 94(446): 496, 1999.

17 DeFronzo RA. Hyperkalemia and hyporeninemic hypoaldosteronism. *Kidney Int* 17(1): 118-134, 1980.

18 Kalantar-Zadeh K, Mehrotra R, Fouque D, Kopple JD. Poor Nutritional Status and Inflammation: Metabolic acidosis and malnutrition-inflammation complex syndrome in chronic renal failure. *Semin Dial* 17(6): 455-465, 2004.

19 Devgun MS. Delay in centrifugation and measurement of serum constituents in normal subjects. *Clin Physiol Biochem* 7(3-4): 189-197, 1989.

20 KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 3(1)2013.

21 Susantitaphong P, Sewaralthahab K, Balk EM, Jaber BL, Madias NE. Short- and long-term effects of alkali therapy in chronic kidney disease: A systematic review. *Am J Nephrol* 35(6): 540-547, 2012.

22 Dobre M, Rahman M, Hostetter TH. Current status of bicarbonate in CKD. Journal of the American Society of Nephrology 2014

23 Di Iorio. Correction of Metabolic Acidosis in End Stage Renal Disease (ESRD). [Internet][Updated 2012 July 12; Cited 2014March 6] Available from: http://www.clinicaltrials.gov/ct2/show/NCT01640119?term=NCT01640119&rank=1

24 Melamed. Alkali Therapy in Chronic Kidney Disease. [Internet][Updated 2011 October 12; Cited 2014March 6] Available from: http://www.clinicaltrials.gov/ct2/show/NCT01452412?term=NCT01452412&rank=1

25 Gaggl M, Cejka D, Plischke M, Heinze G, Fraunschiel M, Schmidt A, Horl WH, Sunder-Plassmann G. Effect of oral sodium bicarbonate supplementation on progression of chronic kidney disease in patients with chronic metabolic acidosis: Study protocol for a randomized controlled trial (SoBic-study). *Trials* 14(1): 196, 2013

26 Raphael. Investigations of the Optimum Serum Bicarbonate Level in Renal Disease. [Internet] [Updated 17 Jul 2014; Cited 2014Sept/01] Available from: https://clinicaltrials.gov/ct2/show/NCT01574157?term=NCT01574157&rank=1

Supplemental Figures and Tables

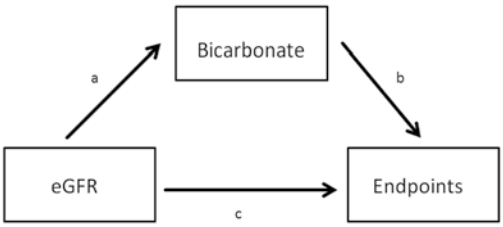
Supplemental Table 1 Baseline characteristics of IDNT patients with measured serum bicarbonate vs patients without measured bicarbonate

	Bicarbonate measured	Bicarbonate not measured	P
N	1115	600	
Age (years)	58.8±7.8	59.0±7.8	0.7
Male (%)	67	66	0.7
White (%)	75	68	0.001
Smoker (%)	17	18	0.8
BMI (kg/m^2)	30.8±5.8	30.8±5.7	0.9
SBP (mmHg)	159±20	160±20	0.2
DBP (mmHg)	87±11	87±11	0.3
RAAS inhibitor use (%)	44	52	0.002
Alpha Blocker use (%)	9	7	0.2
Beta Blocker use (%)	19	17	0.4
CCB use (%)	39	38	0.7
Diuretic use (%)	48	46	0.3
Glucose	188±80	185±79	0.4
HbA1c (%)	8.1±1.8	8.2±1.7	0.2
Serum Creatinine (mg/dL)	1.7±0.6	1.7±0.5	0.3
eGFR (ml/min/1.73m^2)	48±18	46±17	0.06
Potassium (mEq/L)	4.6±0.5	4.6±0.5	0.5
Chloride	104±4	105±4	0.4
Phosphate (mg/ml)	3.76±0.6	3.90±0.7	<0.001
Total Cholesterol (mg/dL)	226±58	231±59	0.1
UACR	1331 (656-2602)	2071 (829-2819)	0.3*

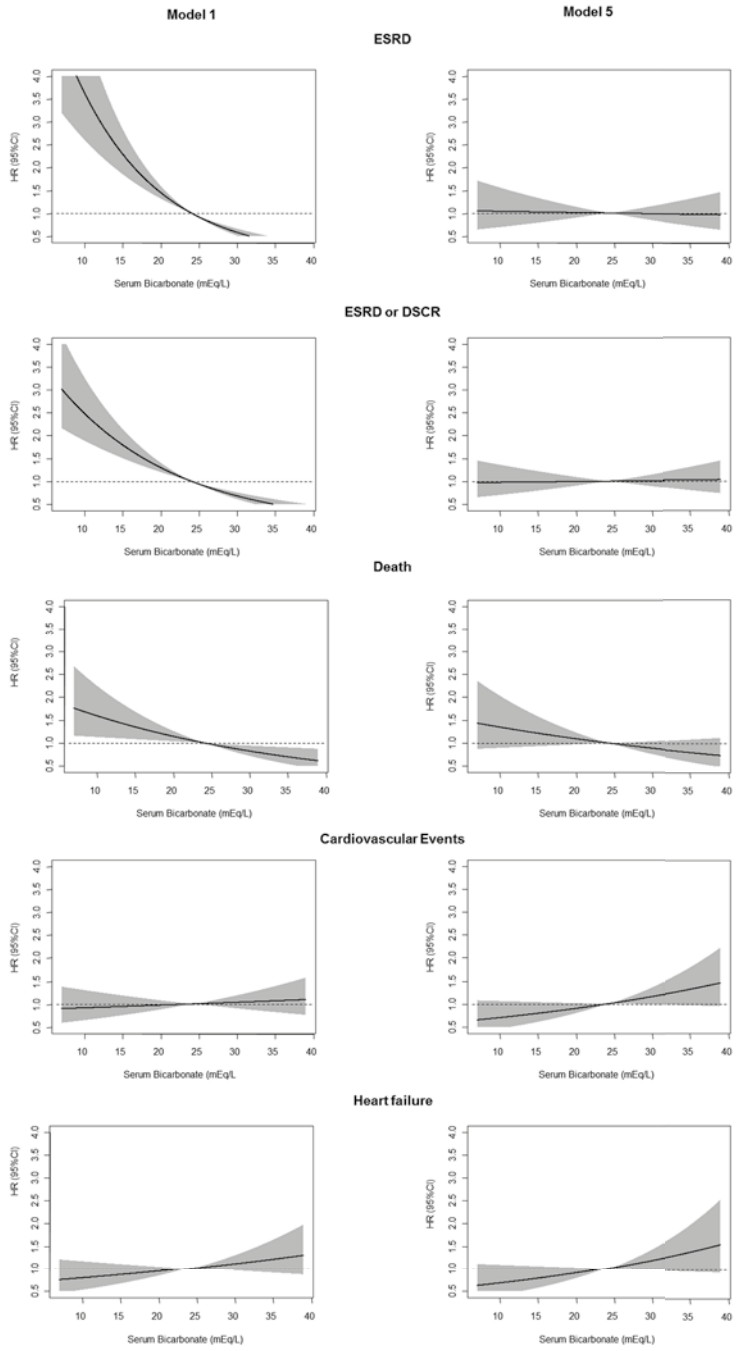
Continuous variables are presented as mean±standard deviation or median (interquartile range). Categorical variables are presented as count (percentage).

Abbreviations are: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEi, angiotensine converting enzyme inhibitor; ATIIa, angiotensine 2 antagonist; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; UACR, urinary albumin-to-creatinine ratio. *P for lnUACR

Conversion factors for units: Total Cholesterol mg/dL to mmol/L, x0.02586; Glucose mg/dL to mmol/L, x0.05551; Serum Creatinine mg/dL to µmol/L, x88.4.



Supplemental Figure 1 Mediation analysis on the relationship of eGFR with endpoints. a, b and c are the standardized regression coefficients between variables. The indirect effect (through bicarbonate) is calculated as a*b. Total effect is a*b + c. Magnitude of mediation is calculated as indirect effect divided by total effect.



Supplemental Figure 2 Association of serum bicarbonate level with ESRD (a), ESRD or DSCR (b), death (c), cardiovascular events (d) and heart failure (e). Spline model for crude (left) and fully adjusted (right) analyses. The hazard ratio is represented by the solid line with the 95% confidence interval in grey.

Supplemental Table 2 Regression analysis of bicarbonate with covariates, eGFR calculated with the CKD-EPI formula

	Univariate			Multivariable		
	Beta	95% CI	P	Beta	95% CI	P
RENAAL/IDNT	-0.285	-0.575 to 0.004	0.05	-0.820	-1.114 to -0.526	<0.001
Age (years)	0.061	0.022 to 0.099	0.002	0.068	0.030 to 0.106	<0.001
Female vs male	-0.702	-1.000 to -0.404	<0.001	-0.141	-0.446 to 0.164	0.4
White vs non-white	0.048	-0.244 to 0.339	0.7	-	-	-
Smoker vs non-smoker	0.368	-0.743 to 0.006	0.05	0.320	0.674 to 0.034	0.08
BMI (kg/m^2)	0.055	0.017 to 0.093	0.005	0.009	-0.028 to 0.047	0.6
SBP (mmHg)	-0.030	-0.068 to 0.009	0.13	-	-	-
RAAS inhibitor use vs no use	0.411	0.125 to 0.697	0.005	0.214	-0.053 to 0.481	0.1
Alpha Blocker use vs no use	0.565	0.191 to 0.939	0.003	0.319	-0.343 to 0.673	0.08
Beta Blocker use vs no use	0.084	-0.452 to 0.284	0.7	-	-	-
Diuretic use vs no use	0.424	0.137 to 0.710	0.004	0.453	0.169 to 0.737	0.002
Total Cholesterol (mg/dL)	-0.063	-0.1027 to -0.023	0.002	-0.058	-0.006 to -0.001	0.003
HbA1c (%)	0.027	-0.013 to 0.066	0.18	-	-	-
eGFR (ml/min/1.73)	0.111	0.009 to 0.014	<0.001	0.084	0.055 to 0.113	<0.001
Potassium (mEq/L)	-0.200	-0.237 to -0.161	<0.001	-0.080	-0.117 to -0.043	<0.001
Chloride (mEq/L)	-0.402	-0.437 to -0.367	<0.001	-0.365	-0.403 to -0.327	<0.001
Phosphate (mg/ml)	-0.141	-0.180 to -0.103	<0.001	-0.054	-0.094 to -0.014	0.008
lnUACR (ln(mg/g)	-0.114	-0.152 to -0.076	<0.001	0.029	-0.011 to 0.069	0.2

Beta is expressed per 1SD increase for continuous variables and versus the reference category for dichotomous variables

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; RAAS, renin-angiotensin-aldosteron system; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; lnUACR, natural log urinary albumin-to-creatinine ratio.

Supplemental Table 3 Cox regression analysis of bicarbonate and endpoints, eGFR calculated with the CKD-EPI formula

	Bicarbonate		Bicarbonate quartiles (mEq/L)			
	Continuous	P	<21	22-23	24-26	>27
ESRD						
Model 1	0.91 (0.89-0.93)	<0.001	1.51 (1.21-1.88)*	1.11 (0.87-1.43)	1.00 (reference)	0.56 (0.43-0.74)*
Model 2	0.92 (0.90-0.95)	<0.001	1.42 (1.13-1.79)*	1.10 (0.85-1.43)	1.00 (reference)	0.59 (0.45-0.78)*
Model 3	0.96 (0.93-0.98)	0.002	1.19 (0.94-1.52)	1.00 (0.77-1.31)	1.00 (reference)	0.69 (0.52-0.92)*
Model 4	0.95 (0.93-0.98)	0.001	1.25 (0.98-1.60)	0.92 (0.70-1.19)	1.00 (reference)	0.70 (0.53-0.94)*
Model 5	1.00 (0.97-1.03)	0.9	0.89 (0.69-1.13)	0.80 (0.62-1.05)	1.00 (reference)	0.75 (0.56-1.01)
ESRD or DSCR						
Model 1	0.94 (0.92-0.96)	<0.001	1.37 (1.14-1.66)*	1.19 (0.97-1.46)	1.00 (reference)	0.72 (0.59-0.89)*
Model 2	0.94 (0.93-0.96)	<0.001	1.30 (1.07-1.58)*	1.18 (0.96-1.46)	1.00 (reference)	0.73 (0.59-0.91)*
Model 3	0.98 (0.96-1.00)	0.1	1.09 (0.89-1.34)	1.10 (0.89-1.36)	1.00 (reference)	0.87 (0.70-1.08)
Model 4	0.98 (0.95-1.00)	0.054	1.13 (0.92-1.38)	1.02 (0.82-1.26)	1.00 (reference)	0.87 (0.69-1.08)
Model 5	1.00 (0.98-1.03)	0.9	0.93 (0.76-1.15)	0.95 (0.77-1.18)	1.00 (reference)	0.92 (0.73-1.15)
Death						
Model 1	0.97 (0.94-0.99)	0.008	1.38 (1.07-1.77)*	1.34 (1.03-1.74)*	1.00 (reference)	1.02 (0.79-1.31)
Model 2	0.97 (0.94-1.00)	0.02	1.37 (1.06-1.78)*	1.31 (1.00-1.72)*	1.00 (reference)	1.04 (0.80-1.34)
Model 3	0.97 (0.95-1.00)	0.08	1.33 (1.01-1.74)*	1.34 (1.04-1.75)*	1.00 (reference)	1.07 (0.82-1.39)
Model 4	0.97 (0.94-1.00)	0.06	1.33 (1.01-1.74)*	1.27 (0.97-1.67)	1.00 (reference)	1.04 (0.80-1.36)
Model 5	0.98 (0.95-1.01)	0.2	1.27 (0.97-1.67)	1.25 (0.95-1.64)	1.00 (reference)	1.06 (0.81-1.38)

Supplemental Table 3 *(Continued)*

	Bicarbonate		Bicarbonate quartiles (mEq/L)			
	Continuous	P	<21	22-23	24-26	>27
Cardiovascular Events						
Model 1	1.01 (0.98-1.03)	0.6	1.01 (0.75-1.35)	0.92 (0.71-1.19)	1.00 (reference)	1.09 (0.84-1.41)
Model 2	1.01 (0.99-1.04)	0.3	1.01 (0.74-1.37)	0.98 (0.75-1.29)	1.00 (reference)	1.18 (0.89-1.55)
Model 3	1.02 (0.99-1.05)	0.2	1.02 (0.75-1.39)	1.01 (0.76-1.33)	1.00 (reference)	1.24 (0.92-1.67)
Model 4	1.02 (0.99-1.05)	0.2	1.00 (0.73-1.36)	1.01 (0.77-1.35)	1.00 (reference)	1.26 (0.93-1.70)
Model 5	1.02 (1.00-1.06)	0.1	0.96 (0.72-1.27)	0.97 (0.73-1.29)	1.00 (reference)	1.25 (0.97-1.60)
Heart failure						
Model 1	1.02 (0.99-1.05)	0.2	0.90 (0.66-1.24)	1.20 (0.89-1.63)	1.00 (reference)	1.28 (0.97-1.68)
Model 2	1.02 (0.99-1.05)	0.2	0.90 (0.65-1.26)	1.18 (0.86-1.62)	1.00 (reference)	1.33 (1.00-1.76)*
Model 3	1.03 (0.99-1.06)	0.1	0.86 (0.61-1.21)	1.17 (0.85-1.61)	1.00 (reference)	1.38 (1.03-1.83)*
Model 4	1.02 (0.99-1.06)	0.2	0.84 (0.60-1.19)	1.10 (0.80-1.52)	1.00 (reference)	1.32 (0.99-1.77)
Model 5	1.03 (0.99-1.06)	0.1	0.82 (0.58-1.15)	1.10 (0.80-1.52)	1.00 (reference)	1.36 (1.01-1.82)*

Hazard ratio + 95% confidence interval; *= P<0.05

Abbreviations are: ESRD, end stage renal disease; DSCR, doubling of serum creatinine

Models are: 1, adjusted for age, gender; 2, adjusted for 1 + smoking, body mass index, systolic blood pressure, Hba1c, total cholesterol; 3, adjusted for 2 + variables with P<0.05 in multivariate regression analysis (with exception of eGFR and UACR); 4, adjusted for 3 + logUACR; 5, adjusted for 4 + eGFR