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Dietary acid reduction with fruits and vegetables or bicarbonate attenuates kidney injury in patients with a moderately reduced glomerular filtration rate due to hypertensive nephropathy

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The neutralization of dietary acid with sodium bicarbonate decreases kidney injury and slows the decline of the glomerular filtration rate (GFR) in animals and patients with chronic kidney disease. The sodium intake, however, could be problematic in patients with reduced GFR. As alkali-induced dietary protein decreased kidney injury in animals, we compared the efficacy of alkali-inducing fruits and vegetables with oral sodium bicarbonate to diminish kidney injury in patients with hypertensive nephropathy at stage 1 or 2 estimated GFR. All patients were evaluated 30 days after no intervention; daily oral sodium bicarbonate; or fruits and vegetables in amounts calculated to reduce dietary acid by half. All patients had 6 months of antihypertensive control by angiotensin-converting enzyme inhibition before and during these studies, and otherwise ate *ad lib*. Indices of kidney injury were not changed in the stage 1 group. By contrast, each treatment of stage 2 patients decreased urinary albumin, *N*-acetyl β-D-glucosaminidase, and transforming growth factor β from the controls to a similar extent. Thus, a reduction in dietary acid decreased kidney injury in patients with moderately reduced eGFR due to hypertensive nephropathy and that with fruits and vegetables was comparable to sodium bicarbonate. Fruits and vegetables appear to be an effective kidney protective adjunct to blood pressure reduction and angiotensin-converting enzyme inhibition in hypertensive and possibly other nephropathies.

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Developing safe and effective kidney-protective interventions to slow or stop the progression of established nephropathy is an important strategy in reducing the incidence of complete kidney failure. Such interventions will likely have the greatest benefit in those with no or moderately reduced glomerular filtration rate (GFR) who comprise the largest cadre of subjects with chronic kidney disease (CKD).[1] Hypertension-associated nephropathy is the second leading cause of complete kidney failure in the United States,[2] and most of them with moderately reduced GFR have progressive GFR decline despite blood pressure (BP) reduction with renin–angiotensin system inhibition.[3,4] Because acid-inducing diets increased and strategies to reduce dietary acid with oral alkali- or base-inducing dietary protein decreased kidney injury in animals with normal GFR,[5] and additionally slowed GFR decline in animals with moderately reduced GFR,[6,7] the largely acid-inducing diets of industrialized societies[8] might mediate progressive GFR decline in those with moderately reduced GFR due to hypertensive nephropathy. Supporting this hypothesis, adding oral $NaHCO_3$ to renin–angiotensin system inhibition ameliorated kidney injury and slowed estimated GFR (eGFR) decline in subjects with moderately reduced GFR due to hypertensive nephropathy without metabolic acidosis.[4] Together, these data support the fact that dietary acid reduction is kidney protective in subjects with moderately reduced GFR due to hypertensive nephropathy.

Despite its apparent benefits, dietary acid reduction with $NaHCO_3$ obligates added dietary Na^+ that might worsen hypertension and/or volume control in subjects with reduced GFR. Other strategies to reduce dietary acid might be equally or more kidney protective while limiting added Na^+. Industrialized society diets are acid-inducing largely because of a high ratio of acid-inducing to base-inducing proteins, the latter being mostly fruits and vegetables (F + V).[8] Consequently, decreasing dietary acid by adding base-inducing F + V might decrease kidney injury as effectively as $NaHCO_3$, but with less added Na^+. We compared urine indices of kidney injury in subjects with CKD due to

hypertensive nephropathy with normal or moderately reduced eGFR after 30 days of dietary acid reduction with F + V or oral $NaHCO_3$ (HCO_3) to compare the kidney-protective efficacy of F + V.

RESULTS

Table 1 shows general subject characteristics by group at baseline. There was no significant difference in age, weight, systolic BP (Sys BP), or gender proportion among groups. Subjects with metabolic acidosis were excluded (Materials and Methods), but *P*-value for venous plasma total CO_2 (TCO_2), across all groups, supports slightly, but significantly, lower plasma TCO_2 in CKD 2 than in CKD 1. Nevertheless, there were no differences in potential renal acid load, a measure of dietary acid intake,[9] or in urine 8 h net acid excretion (8 h NAE) between CKD 1 and CKD 2. Urine albumin excretion (Ualb), a general index of the course and level of kidney injury,[10] was not significantly different among groups. Urine *N*-acetyl β-D-glucosaminidase excretion (UNAG), an index of tubulo-interstitial injury,[11] was not different among groups, but CKD 2 had higher urine excretion of transforming growth factor-β (UTGF), a possible mediator of hypertensive nephropathy activity,[12] than CKD 1.

Table 2 shows net changes in acid–base parameters at 30 days after HCO_3, F + V, or no intervention (Time Control). Venous plasma TCO_2 did not change significantly in any group. Although potential renal acid load did not decrease in either HCO_3 group, potential renal acid load decreased in CKD 1 F + V and CKD 2 F + V. Each intervention to reduce dietary acid decreased 8 h NAE in both CKD 1 and CKD 2, and the net decrease was not significantly different between respective F + V and HCO_3 groups of CKD 1 and CKD 2.

Figure 1 shows box plots for net Ualb change (post–pre) in the three CKD 1 and CKD 2 groups. Net Ualb change was not different among the three CKD 1 groups ($P = 0.201$). Net Ualb for CKD 2 Time Control (9.0 ± 29 mg/g Creatinine (Cr)) was not significantly different from zero ($P = 0.0564$), but Ualb significantly decreased in CKD 2 HCO_3 (-14.7 ± 22.2 mg/g Cr, $P < 0.001$ versus zero) and CKD 2 F + V (-34.3 ± 46.9 mg/g Cr, $P < 0.001$ versus zero). Figure 1 also shows that the net Ualb decrease was significantly more than Time Control in CKD 2 HCO_3 ($P = 0.003$) and CKD 2 F + V ($P < 0.001$), and that the net decrease in CKD 2 F + V was significantly greater than CKD 2 HCO_3 ($P = 0.012$).

Figure 2 shows box plots of the net UNAG change (post–pre) in the three groups of CKD 1 and CKD 2. After 30 days, net UNAG change was not significantly different among the three CKD 1 groups ($P = 0.994$). On the other hand, net UNAG significantly increased for CKD 2 Time Control (0.062 ± 0.136 U/g Cr, $P = 0.006$) but significantly decreased for CKD 2 HCO_3 (-0.088 ± 0.134 U/g Cr, $P < 0.001$) and CKD F + V (-0.080 ± 0.080 U/g Cr, $P < 0.001$). Figure 2 shows that the net UNAG decrease was significantly greater than CKD 2 Time Control in CKD 2 HCO_3 ($P < 0.001$) and CKD 2 F + V ($P < 0.001$), but the net decrease was not significantly different between CKD 2 HCO_3 and CKD 2 F + V ($P = 0.081$).

Figure 3 shows box plots of the net change (post–pre) of UTGF. After 30 days, UTGF significantly decreased in CKD 1 Time Control (-1.819 ± 3.106 ng/g Cr, $P = 0.005$),

Table 1 | General patient characteristics by group at baseline

	CKD1			CKD2			
	Time Control (*N*=27)	HCO_3 (*N*=26)	F+V (*N*=26)	Time Control (*N*=40)	HCO_3 (*N*=40)	F+V (*N*=40)	*P*-value
Males (%)	44.4	50	46.2	47.5	47.5	47.5	0.999
Black/White/Hispanic (%)	29.6/22.2/48.2	34.6/23.1/42.3	34.6/19.2/46.2	62.5/22.5/15.0	62.5/20.0/17.5	62.5/25.0/12.5	0.005
Variable	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**	
Age (years)	49.3 ± 9.1	49.9 ± 8.6	49.4 ± 9.6	51.5 ± 8.3	51.2 ± 8.2	51.3 ± 8.5	0.836
Wt (kg)	83.5 ± 2.9	83.4 ± 2.6	83.7 ± 2.2	82.7 ± 2.1	82.8 ± 1.9	82.7 ± 2.8	0.329
Systolic BP (mm Hg)	133.9 ± 4.6	133.0 ± 5.7	133.5 ± 5.4	134.3 ± 8.3	134.1 ± 5.8	133.7 ± 8.6	0.985
eGFR (ml/min)	100.3 ± 6.7	101.6 ± 7.3	100.8 ± 8.5	75.6 ± 6.5	75.3 ± 6.1	75.6 ± 6.2	<0.001
Plasma total CO_2 (mmol/l)	26.4 ± 1.0	26.4 ± 0.6	26.4 ± 0.8	26.0 ± 0.8	25.9 ± 0.6	25.9 ± 0.8	0.004
Potential renal acid load (mmol/day)	63.9 ± 8.9	62.0 ± 8.8	62.9 ± 14.5	59.3 ± 21.1	64.3 ± 17.7	60.4 ± 19.4	0.756
8-Hour urine net acid excretion (mEq)	24.4 ± 2.5	25.6 ± 4.2	24.7 ± 2.9	24.6 ± 5.7	24.8 ± 5.6	24.6 ± 5.0	0.948
PNa (mEq/l)	139.2 ± 1.7	139.2 ± 1.8	139.1 ± 1.6	139.7 ± 1.7	139.7 ± 1.7	139.6 ± 1.3	0.526
PK (mEq/l)	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	0.960
Paldo (pg/ml)	60.7 ± 12.2	61.2 ± 12.6	59.6 ± 9.5	93.7 ± 19.8	90.7 ± 6.8	92.5 ± 10.2	<0.001
PET-1 (pg/ml)	3.0 ± 0.3	3.1 ± 0.2	3.0 ± 0.2	4.8 ± 1.1	4.8 ± 0.5	4.8 ± 0.4	<0.001
Ualb (mg/g Cr)	402.1 ± 71.4	396.8 ± 76.8	414.9 ± 78.7	413.6 ± 147.9	419.3 ± 150.8	422.2 ± 151.6	0.966
UNAG (U/g Cr)	2.6 ± 0.3	2.6 ± 0.3	2.5 ± 0.5	2.7 ± 0.4	2.7 ± 0.4	2.7 ± 0.7	0.476
UTGF (ng/g Cr)	42.5 ± 11.5	44.6 ± 12.2	42.5 ± 17.9	63.6 ± 18.0	60.7 ± 6.8	63.8 ± 14.5	<0.001
UET-1 (ng/g Cr)	3.5 ± 0.6	3.7 ± 0.8	3.5 ± 1.7	5.5 ± 1.1	5.7 ± 1.0	5.5 ± 1.2	<0.001
Ualdo (μg/g Cr)	13.3 ± 2.7	13.9 ± 2.4	13.1 ± 2.8	37.4 ± 8.7	32.7 ± 8.4	36.7 ± 8.7	<0.001
UNaV (mmol/g Cr)	74.2 ± 11.5	75.0 ± 11.4	77.4 ± 8.2	71.6 ± 7.9	70.9 ± 10.2	73.0 ± 9.5	0.103
UKV (mmol/g Cr)	35.9 ± 6.3	38.1 ± 6.6	37.6 ± 4.4	38.6 ± 5.5	41.1 ± 6.1	39.5 ± 6.6	0.019

Abbreviations: BP, blood pressure; CKD, chronic kidney disease; Cr, creatinine; eGFR, estimated GFR by the MDRD formula; F+V, 30 days after dietary intervention with fruits + vegetables; HCO_3, 30 days after oral intervention with oral $NaHCO_3$, 0.5 mEq/kg bw/day; Paldo, plasma aldosterone excretion; PET, plasma endothelin excretion; PK, plasma potassium excretion; PNa, plasma sodium excretion; Ualb, urine albumin excretion; Ualdo, urine aldosterone excretion; UET, urine endothelin excretion; UKV, urine excretion of K^+; UNAG, urine *N*-acetyl β-D-glucosaminidase excretion; UNaV, urine excretion of Na^+; UTGF, urine excretion of transforming growth factor-β.
P-values for overall (six groups) comparison were presented.

Table 2 | Net change in acid-base data pre- and post-dietary intervention (post–pre), expressed as mean ± s.d.

	CKD 1			CKD 2		
	Time Control mean ± s.d.	HCO_3 mean ± s.d.	F+V mean ± s.d.	Time Control mean ± s.d.	HCO_3 mean ± s.d.	F+V mean ± s.d.
Plasma total CO_2 (mmol/l)	0.0 ± 1.2	0.0 ± 0.7	−0.1 ± 1.1	0.0 ± 0.5	0.1 ± 0.6	0.0 ± 0.4
P-value, pre versus post	0.886	0.935	0.687	0.742	0.387	0.604
Potential renal acid load (mmol/day)	0.1 ± 2.5	−0.1 ± 2.7	−20.9 ± 10.9*,**	−0.2 ± 2.6*	0.0 ± 2.5	−21.7 ± 11.9*,**
P-value, pre versus post	0.809	0.865	<0.001	0.688	0.914	<0.001
8-Hour urine net acid excretion (mEq)	0.1 ± 1.1	−6.0 ± 4.8*	−7.9 ± 5.2*,**	0.3 ± 1.7	−7.2 ± 6.0*	−8.1 ± 4.6*
P-value, pre versus post	0.751	<0.001	<0.001	0.333	<0.001	<0.001

Abbreviations: CKD, chronic kidney disease; F+V, 30 days after dietary intervention with fruits + vegetables; HCO_3, 30 days after oral intervention with oral $NaHCO_3$.
*$P<0.05$ versus Time Control in change (Post-Pre).
**$P<0.05$, versus HCO_3 in change.

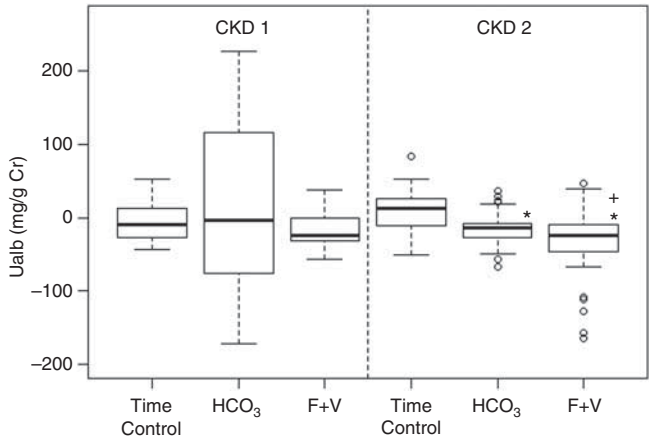

Figure 1 | Box plots of change of urine albumin (mg)-to-creatinine (g) ratio (Ualb) for the three groups of subjects with estimated glomerular filtration rate (eGFR) >90 ml/min (CKD 1) and eGFR 60–90 ml/min (CKD 2). CKD, chronic kidney disease; F + V, subjects given fruits + vegetables for 30 days; HCO_3, subjects given oral $NaHCO_3$ daily for 30 days; Time Control, subjects followed up for 30 days with no further intervention. *$P<0.05$ versus Time Control; $^{+}P<0.05$ versus HCO_3.

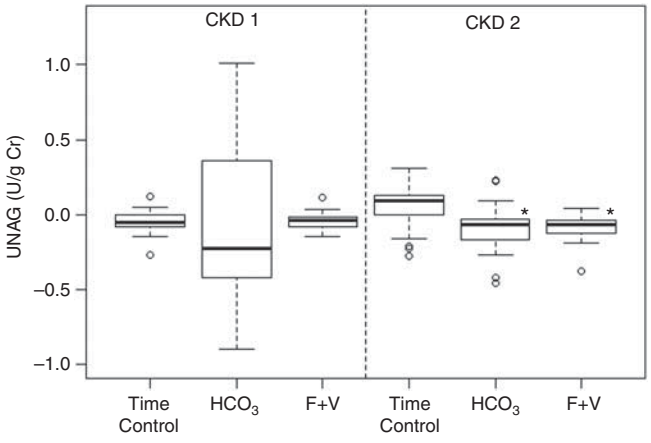

Figure 2 | Box plots of change of urine *N*-acetyl-β-D-glucosaminidase (U)-to-creatinine (g) ratio (UNAG) for the three groups of subjects with eGFR >90 ml/min (CKD 1) and eGFR 60–90 ml/min (CKD 2). CKD, chronic kidney disease; F + V, subjects given fruits + vegetables for 30 days; HCO_3, subjects given oral $NaHCO_3$ daily for 30 days; Time Control, subjects followed up for 30 days with no further intervention. *$P<0.05$ versus Time Control.

HCO_3 (−2.223 ± 2.721 ng/g Cr, $P<0.001$), and F + V (−2.397 ± 3.403 n/g Cr, $P=0.001$), but net UTGF decrease was not significantly different among the three CKD 1 groups ($P=0.783$). By contrast, UTGF significantly increased in CKD 2 Time Control (2.298 ± 6.994 ng/g Cr, $P=0.044$) but decreased in HCO_3 (−6.888 ± 4.953 ng/g Cr, $P<0.001$) and F + V (−6.483 ± 4.908 ng/g Cr, $P<0.001$). Figure 3 shows that net UTGF decrease was significantly greater than CKD 2 Time Control in CKD 2 HCO_3 ($P<0.001$) and CKD 2 F + V ($P<0.001$), but the net decrease was not significantly different between HCO_3 and F + V ($P=0.751$).

Table 3 shows the net change in body weight, Sys BP, and selected plasma and urine parameters. Body weight did not change significantly in Time Control and HCO_3 groups of both CKD 1 and CKD 2, but it decreased significantly in both CKD 1 and CKD 2 groups given F + V. The net body weight decrease was not significantly different between CKD 1 and CKD 2 groups given F + V ($P=0.062$). Similarly, Sys BP did not change significantly in any Time Control or HCO_3 group, but decreased significantly in both CKD 1 and CKD 2 given F + V. In contrast to body weight, the net Sys BP decrease was significantly greater in CKD 2 F + V than in CKD 1 F + V ($P=0.001$).

In addition, Table 3 shows the net change in plasma levels and urine excretion of Na^+ (UNaV) and K^+ (UKV). Plasma Na^+ did not change significantly in any group and plasma K^+ did not change significantly in any CKD 1 group. Although plasma K^+ did not significantly change in CKD 2 Time Control and CKD 2 F + V, plasma K^+ decreased slightly but significantly in CKD 2 group given HCO_3, and the net decrease was greater than CKD 2 Time Control and F + V. Both UNaV and UKV increased significantly in CKD 1 and CKD 2 groups given HCO_3, and the net increase in CKD 1 and CKD 2 was significantly greater than their respective Time Control. UNaV decreased significantly and UKV increased significantly in both CKD 1 and CKD 2 groups given F + V, and the net UNaV decrease and the net UKV increase in both CKD 1 and CKD 2 groups given F + V were significantly

different from their respective Time Control. The UKV increase was greater in the respective F + V than in the HCO_3 group for CKD 1 ($P<0.001$) and CKD 2 ($P<0.001$).

Table 3 also shows the net change in plasma levels and urine excretion of endothelin (ET)-1 and aldosterone. Urine ET-1 excretion (UET), a surrogate of kidney ET-1 levels,[13] did not decrease in any CKD 1 group or in CKD Time Control. By contrast, UET decreased significantly in both intervention groups of CKD 2, and the net decrease was significantly greater than CKD 2 Time Control in both CKD 2 intervention groups. Urine aldosterone excretion (Ualdo), a surrogate of kidney aldosterone levels,[7] significantly decreased in both the intervention groups of CKD 1 and CKD 2. The net decrease in Ualdo was significantly greater in both the intervention groups than the respective Time Controls in both CKD 1 and CKD 2. Although the net Ualdo decrease was not significantly different between the two CKD 1 intervention groups, the net Ualdo decrease was greater in CKD 2 HCO_3 than in CKD 2 F + V.

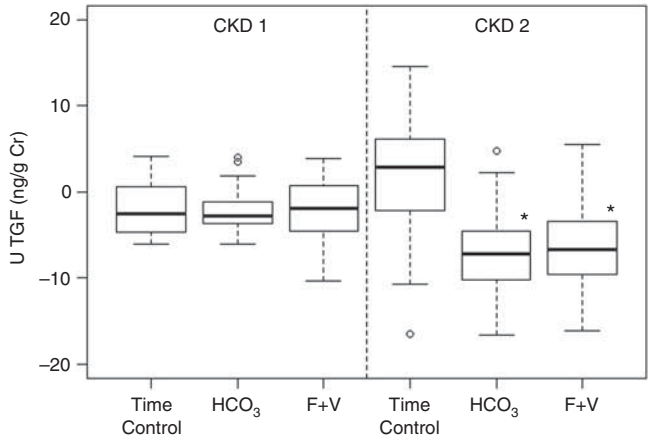

Figure 3 | Box plots of change of urine transforming growth factor-β (ng)-to-creatinine (g) ratio (UTGF) for the three groups of subjects with estimated glomerular filtration rate (eGFR) > 90 ml/min (CKD 1) and eGFR 60–90 ml/min (CKD 2). CKD, chronic kidney disease; F + V, subjects given fruits + vegetables for 30 days; HCO_3, subjects given oral $NaHCO_3$ daily for 30 days; Time Control, subjects followed up for 30 days with no further intervention. *$P<0.05$ versus Time Control.

DISCUSSION

Dietary acid reduction with oral $NaHCO_3$ for 5 years ameliorated kidney injury and slowed GFR decline in CKD subjects with moderately reduced eGFR due to hypertensive nephropathy.[4] The present studies extend these observations by showing that (1) 30 days of HCO_3 or F + V comparably decreased kidney injury in subjects with CKD 2 but not with CKD 1 due to hypertensive nephropathy; and (2) kidney injury reduction was more evident in CKD 2 than in CKD 1. The data support the fact that both F + V and $NaHCO_3$ are kidney-protective adjuncts to reduced Sys BP and angiotensin-converting enzyme inhibition in hypertensive and possibly other nephropathies.

Kidney injury did not change significantly after 30 days of HCO_3 or F + V in CKD 1. By contrast, F + V and $NaHCO_3$

Table 3 | Change in systolic blood pressure and urine and plasma variables, pre- and post-dietary intervention (Post–Pre), expressed as means ± s.d.

	CKD1			CKD2		
	Time Control mean ± s.d.	HCO_3 mean ± s.d.	F+V mean ± s.d.	Time Control mean ± s.d.	HCO_3 mean ± s.d.	F+V mean ± s.d.
Weight (kg)	0.12 ± 0.73	0.12 ± 0.81	−1.82 ± 0.98*,**	0.07 ± 0.81	0.02 ± 0.58	−2.31 ± 1.04*,**
P-value, pre versus post	0.389	0.459	<0.001	0.574	0.807	<0.001
Systolic BP (mm Hg)	0.1 ± 2.6	−0.3 ± 3.0	−2.4 ± 2.3*,**	0.5 ± 4.1	−0.2 ± 2.9	−5.4 ± 4.6*,**
P-value, pre versus post	0.765	0.566	<0.001	0.488	0.749	<0.001
PNa (mEq/l)	0.44 ± 1.34	0.27 ± 1.31	0.54 ± 1.48	0.05 ± 1.36	−0.05 ± 1.24	−0.03 ± 0.92
P-value, pre versus post	0.097	0.306	0.075	0.817	0.800	0.864
PK (mEq/l)	−0.04 ± 0.13	0.02 ± 0.14	0.03 ± 0.14*	0.00 ± 0.10	−0.10 ± 0.09*	0.00 ± 0.11**
P-value, pre versus post	0.076	0.574	0.266	>0.999	<0.001	0.891
PET-1 (pg/ml)	0.02 ± 0.11	−0.16 ± 0.26*	−0.11 ± 0.14*	0.02 ± 0.18	−0.55 ± 0.30*	−0.66 ± 0.34*
P-value, pre versus post	0.323	0.004	<0.001	0.595	<0.001	<0.001
Paldo (pg/ml)	0.04 ± 3.10	−3.58 ± 14.71	−1.81 ± 1.99	0.93 ± 6.41	−18.26 ± 4.48*	−6.17 ± 4.98*,**
P-value, pre versus post	0.951	0.226	<0.001	0.362	<0.001	<0.001
UET-1 (ng/g Cr)	0.04 ± 0.16	−0.33 ± 1.81	−0.23 ± 1.21	0.09 ± 0.45	−0.75 ± 0.50*	−0.54 ± 0.69*
P-value, pre versus post	0.245	0.359	0.342	0.208	<0.001	<0.001
Ualdo (μg/g Cr)	−0.02 ± 0.81	−2.15 ± 2.74*	−1.48 ± 1.55*	0.73 ± 2.80*	−6.96 ± 4.67*	−3.16 ± 2.74*,**
P-value, pre versus post	0.906	0.001	<0.001	0.107	<0.001	<0.001
UNaV (mmol/g Cr)	−0.60 ± 3.60*	12.40 ± 15.87*	−7.52 ± 4.74*,**	1.16 ± 4.83*	5.73 ± 2.65*	−8.00 ± 4.32*,**
P-value, pre versus post	0.391	0.001	<0.001	0.136	<0.001	<0.001
UKV (mmol/g cr)	0.85 ± 3.45*	4.56 ± 7.47*	7.75 ± 4.20*,**	0.93 ± 3.54*	4.13 ± 3.18*	10.01 ± 3.10*,**
P-value, pre versus post	0.213	0.005	<0.001	0.106	<0.001	<0.001

Abbreviations: BP, blood pressure; CKD, chronic kidney disease; F+V, 30 days after dietary intervention with fruits+vegetables; HCO_3, 30 days after oral intervention with oral $NaHCO_3$; Paldo, plasma aldosterone excretion; PET, plasma ET excretion; PK, plasma potassium excretion; PNa, plasma sodium excretion; Ualdo, urine aldosterone excretion; UET, urine endothelin excretion; UKV, urine excretion of K^+; UNaV, urine excretion of Na^+.
*$P<0.05$ versus Time Control in change (post–pre).
**$P<0.05$, versus HCO_3 in change.

each significantly and comparably decreased kidney injury in CKD 2. Animals with 2/3 nephrectomy have reduced GFR without metabolic acidosis, similar to CKD 2, and reduced dietary acid with base-inducing protein, or with $NaHCO_3$ each decreased kidney injury and slowed GFR decline.[6,7] The 2/3 nephrectomy animals had acid retention by microdialysis, which mediated kidney injury and progressive GFR decline despite the absence of metabolic acidosis.[6,7] Amelioration of acid retention associated with reduced GFR with either dietary intervention decreased kidney injury and slowed GFR decline.[6,7] Because CKD 2 subjects without metabolic acidosis appear to have acid retention[14] similar to 2/3 nephrectomy animals, we hypothesize that amelioration of acid retention with each strategy decreases kidney injury in CKD 2. Because dietary acid intake and the initial and follow-up 8 h NAE after 30 days of HCO_3 or F + V were not significantly different, each intervention appears to have reduced acid retention similarly. Together, the data support the fact that decreased kidney injury induced by dietary acid reduction was mediated by amelioration of acid retention in CKD 2.

Dietary acid reduction with F + V, but not $NaHCO_3$, in CKD 2 (Table 2) was accompanied by a significant decrease in Sys BP, which might add cardiovascular protection to F + V as a potentially kidney-protective intervention. Reduced Sys BP with F + V was evident in both CKD 1 and CKD 2 in subjects starting a vegetarian diet.[15] Sys BP reduction decreases Ualb in primary hypertension[16] and the statistically greater reduction in Ualb in CKD 2 subjects given F + V compared with $NaHCO_3$ might have been mediated by Sys BP reduction in CKD 2 subjects given F + V but not $NaHCO_3$. In addition, lower UNaV in F + V compared with $NaHCO_3$ and Time Control CKD 2 subjects, consistent with reduced Na^+ intake, might also have contributed to lower Ualb in CKD 2 subjects given F + V.[17] Reduced UNaV and increased UKV in F + V (Table 3), consistent with reduced and increased intake of each, respectively, likely mediates this BP reduction.[18,19] In addition, reduced body weight (Table 3) with F + V might also have contributed.[20] Furthermore, the greater Ualb decrease in CKD 2 than in CKD 1 group given F + V was associated with greater Sys BP reduction in CKD 2 than in CKD 1 group given F + V. Consequently, greater Sys BP reduction might have mediated the greater Ualb decrease in CKD 2 than in CKD 1 group given F + V. Dietary Na^+ reduction decreases Sys BP more in those patients with reduced than normal GFR,[21] and possibly explains greater Sys BP reduction in CKD 2 than in CKD 1.

Because K^+ depletion increases BP,[19] changes in K^+ balance might also have contributed to differences in Sys BP among CKD 2 groups. The increased UK in F + V is consistent with an increase in K^+-containing foods as determined from follow-up 3-day diaries in these CKD 2 subjects, and this dietary change would be expected to promote a decrease in BP.[19] By contrast, 3-day diaries showed no increase in K^+-containing foods in CKD 2 subjects given $NaHCO_3$, yet they had increased UK in response to $NaHCO_3$ as observed by others.[22] Greater UK in CKD 2 patients given $NaHCO_3$ combined with a slight but significant decrease in plasma K^+ (Table 3) might indicate reduced K^+ stores. Consequently, mild K^+ depletion in CKD 2 given $NaHCO_3$ might have limited or precluded Sys BP reduction in these CKD 2 subjects, potentially making this strategy for dietary acid reduction less cardiovascular protective than F + V. Although no CKD 2 group given F + V experienced an increase in plasma K^+, caution is warranted in prescribing F + V to CKD subjects with lower GFRs.

Urine NAG excretion assessed tubulo-interstitial injury,[11] a component of kidney injury that was induced by dietary acid and ameliorated by dietary alkali and by diets containing base-inducing protein in animals with moderately[6,7] and severely[23] reduced GFR. Similarly, dietary alkali ameliorated UNAG in subjects with CKD due to hypertensive nephropathy with moderately[4] and severely[24] reduced eGFR. The UNAG data support that neither F + V nor $NaHCO_3$ decreased tubulo-interstitial injury in CKD 1. By contrast, UNAG data in CKD 2 support that tubulo-interstitial injury worsened after 30 days of no intervention, but that F + V and $NaHCO_3$ each decreased tubulo-interstitial injury after 30 days. Unlike Ualb, net UNAG decrease was not significantly different between CKD 2 given $NaHCO_3$ and F + V. These latter data support that dietary acid reduction was the greater contributor to decreased tubulo-interstitial injury in CKD 2 than Sys BP reduction, and further support the greater role of acid retention in mediating tubulo-interstitial injury.

UTGF, a possible mediator of hypertensive nephropathy,[12] decreased significantly among all three CKD 1 groups and the net decrease was not significantly different among groups. These data suggest that dietary acid reduction had no measurable UTGF effect in CKD 1, and that lower Sys BP in F + V compared with the remaining CKD 1 groups also did not additionally affect UTGF. Because all groups underwent 6 months of aggressive BP reduction before entering the protocol,[25] sustained effects of this BP reduction might have reduced UTGF similarly in all three CKD 1 groups.[26] Similar to UNAG, UTGF increased in CKD 2 without intervention but UTGF decreased significantly in CKD 2 patients given $NaHCO_3$ and F + V, and the net UTGF decrease between the two interventions to reduce dietary acid was not different. These latter data support the fact that reduced dietary acid decreased UTGF and, similar to the data described for UNAG, the lower Sys BP in CKD 2 given F + V compared with $NaHCO_3$ appears not to have been additive after 30 days. Dietary acid reduction decreased Ualdo in humans with moderately reduced eGFR in this and previous studies,[14] and did so in animals with moderately reduced GFR.[7] Because aldosterone increases UTGF in animals,[27] reduced kidney aldosterone might mediate decreased UTGF in response to dietary acid reduction. Furthermore, because there was no significant difference in net UTGF decrease among CKD 1, because UTGF increased without intervention in CKD 2 but not in CKD 1, and because the net decrease in UTGF was not significantly different after dietary acid reduction with F + V

and HCO_3 in CKD 2, despite Sys BP reduction in F + V but not HCO_3, the data support that acid retention present in CKD 2, and not in CKD 1,[14] more importantly mediates increased UTGF in CKD 2 than Sys BP.

Both dietary acid reduction interventions decreased UET and Ualdo in CKD 2 but not in CKD 1. Because UET and Ualdo are respective surrogates of their kidney levels in animals with and without reduced GFR,[7,22] these data support the fact that dietary acid reduction decreased kidney ET-1 and aldosterone levels in CKD 2 but not in CKD 1. Because the increased kidney ET-1 and aldosterone levels in animals with reduced GFR are mediated by retained acid due to decreased GFR[7,13] and CKD 2 subjects appear to have retained acid,[14] these data support the fact that decreased retained acid mediated the decrease in UET and Ualdo induced by dietary acid reduction. Given that endothelins and aldosterone mediate kidney injury in animals with reduced GFR and that decreasing their levels ameliorates kidney injury,[7,22] decreased kidney ET-1 and aldosterone levels likely contributed to the reduction in kidney injury associated with reduced dietary acid.

Table 3 shows that although the net UET decrease was similar between CKD 2 subjects given $NaHCO_3$ and F + V, Ualdo decrease was statistically significantly greater in CKD 2 patients given $NaHCO_3$ than in those given F + V. Although each intervention reduced dietary acid as evidenced by reduced urine NAE, the $NaHCO_3$ intervention was accompanied by increased Na^+ intake without additional K^+ intake. On the other hand, the F + V intervention was accompanied by reduced Na^+ intake and increased K^+ intake. These two factors would be expected to promote greater Ualdo reduction in $NaHCO_3$ than in F + V. The greater Ualdo reduction in $NaHCO_3$ versus F + V subjects might be expected to lead to greater reduction in kidney injury in $NaHCO_3$ versus F + V CKD 2 subjects, given animal studies that support a role for aldosterone, in addition to ET-1, in mediating kidney injury and GFR decline in animals with partial nephrectomy.[7] Nevertheless, and as indicated earlier, changes in the three parameters of kidney injury support comparable reduction of kidney injury in CKD 2 subjects given $NaHCO_3$ and F + V. The observation that greater Ualdo reduction induced by $NaHCO_3$ than F + V was not accompanied by a greater reduction of kidney injury parameters in CKD 2 subjects given $NaHCO_3$ might be explained, at least in part, by stable Sys BP during the 1-month follow-up in the $NaHCO_3$ subjects as compared with a significant reduction in Sys BP in the F + V subjects. Consequently, CKD2 subjects given $NaHCO_3$ might have had a greater decrease in kidney injury parameters if their Sys BP was similarly reduced. On the other hand, greater kidney injury reduction might become evident in the CKD 2 subjects given $NaHCO_3$ with a longer follow-up. Additional and longer-term studies will be needed to address this issue.

In summary, these studies support the fact that kidney injury increased without dietary acid reduction and show that 30 days of dietary acid reduction with $NaHCO_3$ or F + V reduced kidney injury in subjects with CKD stage 2 eGFR due to hypertensive nephropathy. The present studies also show that F + V, but not $NaHCO_3$, reduced Sys BP, suggesting a possible advantage of F + V as a strategy to reduce dietary acid for kidney protection. These studies encourage longer-term studies to determine whether F + V, similar to $NaHCO_3$, is an effective adjunct to Sys BP reduction and angiotensin-converting enzyme inhibition in slowing GFR decline in hypertensive and possibly other nephropathies.

MATERIALS AND METHODS

This interventional study compared the effect of 30 days of reduced dietary acid with added daily oral $NaHCO_3$ (HCO_3) or added F + V on urine parameters of kidney injury in subjects with macro-albuminuric CKD (urine albumin-to-creatinine ratio >200 mg/g Cr) due to hypertensive nephropathy and stage 1 (>90 ml/min, CKD 1) or stage 2 (60–90 ml/min, CKD 2) eGFR by MDRD formula.[28] Primary outcome was the effect of these interventions on urine excretion of the following parameters of kidney injury: Ualb as a general marker of progressive kidney injury,[10] UNAG as a marker of kidney tubulo-interstitial injury[11] and because it reflected kidney injury induced by dietary acid in experimental model of CKD,[5,22] and transforming growth factor β (UTGFV) because it also reflected kidney injury induced by dietary acid in an experimental model of CKD[22] and because it might be a mediator of hypertensive nephropathy.[12] Secondary outcomes were the effects of these interventions on UET and Ualdo, two mediators of dietary acid-induced kidney injury and GFR decline in an animal model of CKD without metabolic acidosis.[7] We excluded subjects with metabolic acidosis (plasma TCO_2 <24.5 mmol/l, the lower limit of normal for our laboratory).

The protocol is outlined in Figure 4. Three groups of CKD1 and CKD 2 were studied. (1) A total of 26 CKD 1 and 40 CKD 2 subjects received oral $NaHCO_3$ at a concentration of 0.5 mEq/kg/day for 30 days (HCO_3) as scored tablets containing 10 mEq $NaHCO_3$ with sucrose. Each subject was prescribed tablets to the nearest one-half tablet by body weight in kg (for example, a 70 kg subject received 3.5 tablets daily); (2) A total of 26 CKD 1 and 40 CKD 2 subjects received an amount of F + V, free of charge to reduce their dietary acid by 50%, focusing primarily on F + V that are particularly base-inducing.[9] Fruits such as apples, apricots, oranges, peaches, pears, raisins, and strawberries were predominantly provided. Vegetables such as carrots, cauliflower, eggplant, lettuce, potatoes, spinach, tomatoes, and zucchini were predominantly provided. Subjects did not receive specific dietary instructions and were allowed to integrate the added F + V into their *ad lib* diets as they wished. Dietary acid was measured before and after interventions using 3-day diaries of food intake and a published formula.[9] The necessary amount of F + V was prescribed by a dietician and distributed from a community center food bank used by all F + V subjects. To assure that each F + V subject received sufficient F + V, we provided the described amount for each member of the household; (3) A total of 27 CKD 1 and 40 CKD 2 subjects had no intervention (Time Control). Otherwise, all subjects were allowed to eat *ad lib*. Other inclusion criteria were as follows: (1) non-malignant hypertension; (2) $60 \geqslant$ eGFR <90 ml/min for CKD 2, >90 ml/min for CKD 1; (3) $\geqslant 2$ primary care physician visits in the preceding year, showing compliance with clinic visits; (4) age $\geqslant 18$ years and able to give consent. Exclusion criteria were as follows: (1) primary kidney disease or findings consistent thereof such as $\geqslant 3$ red blood cells per

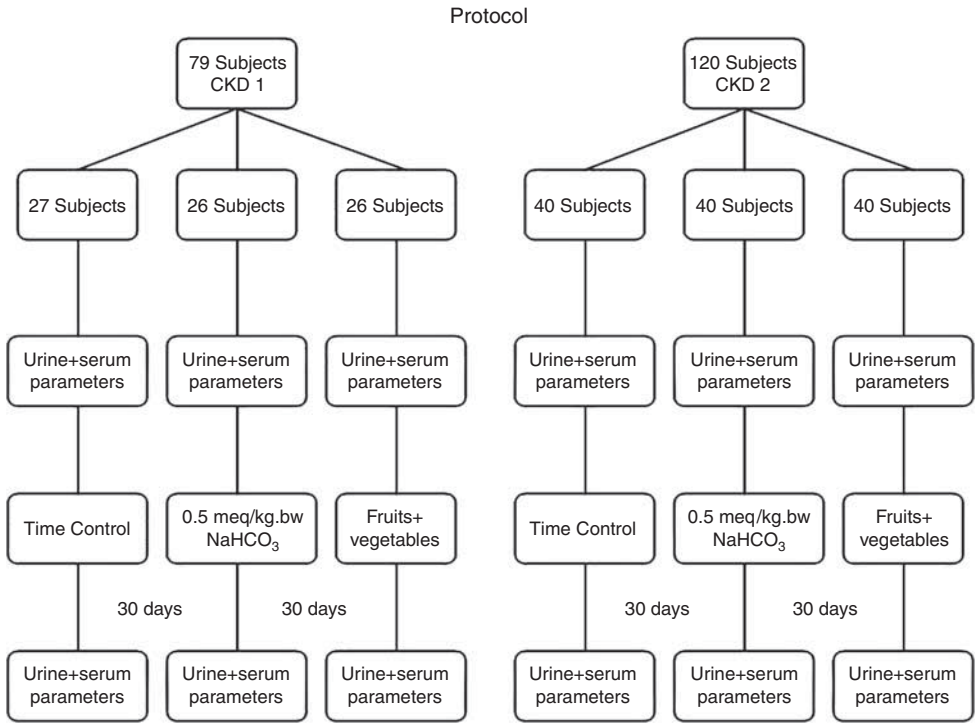

Figure 4 | Outline of the protocol to test the effect after 30 days on parameters of kidney injury of no intervention (Time Control), daily oral $NaHCO_3$ at a concentration of 0.5 mEq/kg bw/day, or fruits + vegetables given in amounts designed to reduce dietary acid intake by 50%.

high-powered field of urine or urine cellular casts; (2) history of diabetes or fasting blood glucose $\geqslant 110$ mg/dl; (3) history of malignances, chronic infections, pregnancy, or clinical evidence of cardiovascular disease; and (4) peripheral edema or diagnoses associated with edema such as heart/liver failure or nephrotic syndrome. The diagnosis of hypertensive nephropathy as the exclusive nephropathy cause was made clinically by excluding subjects with systemic diseases associated with nephropathy, nephrotic-range proteinuria, and urine abnormalities other than albuminuria. None had a kidney biopsy. Secondary causes of hypertension such as renal artery stenosis and hyperaldosteronism were excluded clinically. Kidney Doppler studies and plasma aldosterone-to-renin ratios were not carried out. Subjects were recruited as described.[4] All had had their Sys BP reduced toward a target of <130 mm Hg using a previously described protocol[25] with regimens including angiotensin-converting enzyme inhibitors as recommended for hypertensives with albuminuria.[29]

Eight-hour (0800 to 1600 h) urine NAE (8 h NAE) was measured and calculated from urine titratable acidity, ammonium (NH_4^+), and HCO_3 ($[NH_4^+] + [\text{titratable acidity}] - [HCO_3]$) as described earlier[4] at baseline and 30 days after the intervention. Sys BP and venous plasma acid–base parameters, ET-1, and aldosterone were measured at baseline and after the intervention. Urine excretion of albumin, NAG, TGF, ET-1, aldo, Na^+, and K^+ were each measured before and after intervention in a morning urine specimen and factored per gram of Cr.

Our IRB approved the protocol.

Analytical methods

Plasma and urine Cr and urine albumin were measured using the Sigma Diagnostics Creatinine Kit (Procedure No. 555, Sigma Diagnostics, St Louis, MO).[30] The IRMA SL Series 2000 blood analysis system (Edison, NJ) measured venous plasma/blood pH and plasma CO_2. Urine and plasma TCO_2 were measured using ultrafluorometry.[31] Urine titratable acidity was measured by correction to the ambient plasma pH by NaOH addition, and NH_4^+ by the Formalin titrametric (to ambient plasma pH) method.[32] Plasma ET and urinary ET were measured with an RIA kit (Peninsula Laboratories, Belmont, CA) after extraction using Bound Elut C18 columns (Varian, Harbor City, CA).[13] Plasma and urine aldosterone were measured after extraction with Bound Elut C18 columns (Varian) using a radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA) as performed previously.[7] Urine ET and aldosterone excretion were expressed per gram Cr of a spot AM specimen.

Statistical methods

Patient characteristics at the time of study enrollment were tabulated by percentages or described by mean and standard deviation. We first compared the baseline characteristics among the six subject groups with the χ^2-test or the one-way analysis of variance, as appropriate. The primary outcomes were Ualb, UNAG, and UTGF in response to 30 days of oral F + V or $NaHCO_3$. Secondary outcomes included plasma ET, plasma aldo, Ualdo, urine 8 h NAE, and venous TCO_2. The changes from pre to post for each group were described by mean and s.d., and they were considered with a one-sample *t*-test. The differences among the three groups within CKD cohort were considered with one-way analysis of variance followed by *post-hoc* Tukey's test. In addition, outcomes were compared between a group in CKD 1 and a corresponding CKD 2 group with a two-sample *t*-test. A *P*-value of less than 0.05 indicates a statistical significance. SAS version 9.2 (SAS Institute, Cary, NC)

was used for the statistical analysis and graphs were created using R 2.12.1 (R Core Development Team, 2010).

DISCLOSURE

All the authors declared no competing interests.

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