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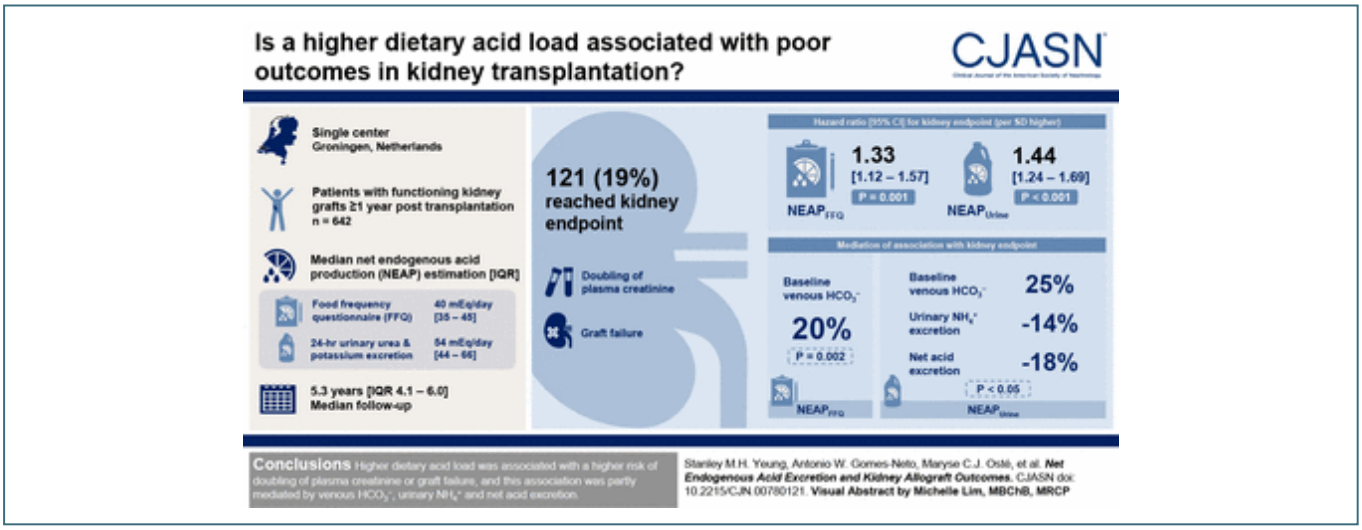
Visual Abstract



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Abstract

Background and objectives High dietary acid load may accelerate a decline in kidney function. We prospectively investigated whether dietary acid load is associated with graft outcomes in kidney transplant recipients, and whether venous bicarbonate mediates this association.

Design, setting, participants, & measurements We used data from 642 kidney transplant recipients with a functioning graft ≥1 year after transplantation. Net endogenous acid production was estimated using food frequency questionnaires and, alternatively, 24-hour urinary urea and potassium excretion to estimate net endogenous acid production. We defined the composite kidney end point as a doubling of plasma creatinine or graft failure. Multivariable Cox regression analyses, adjusted for potential confounders, were used to study the

associations of dietary acid load with the kidney end point. We evaluated potential mediation effects of venous bicarbonate, urinary bicarbonate excretion, urinary ammonium excretion, titratable acid excretion, and net acid excretion on the association between net endogenous acid production and the kidney end point.

Results The median net endogenous acid production using food frequency questionnaires and net endogenous acid production using urinary excretion were 40 (interquartile range, 35–45) and 54 (interquartile range, 44–66) mEq/day, respectively. During a median follow-up of 5.3 years (interquartile range, 4.1–6.0), 121 (19%) participants reached the kidney end point. After multivariable adjustment, net endogenous acid production using food frequency questionnaires and net endogenous acid production using urinary excretion (per SD higher) were independently associated with higher risk for kidney end point (hazard ratio, 1.33; 95% confidence interval, 1.12 to 1.57, P=0.001 and hazard ratio, 1.44; 95% confidence interval, 1.24 to 1.69, P<0.001, respectively). Baseline venous bicarbonate mediated 20% of the association between net endogenous acid production using food frequency questionnaires and the kidney end point. Baseline venous bicarbonate, urinary ammonium excretion, and net acid excretion mediated 25%, −14%, and −18%, respectively, of the association between net endogenous acid production using urinary excretion and the kidney end point.

Conclusions Higher dietary acid load was associated with a higher risk of doubling of plasma creatinine or graft failure, and this association was partly mediated by venous bicarbonate, urinary ammonium, and net acid excretion.

nutrition **kidney transplantation** **outcomes** **transplant outcomes** **chronic allograft failure** **chronic metabolic acidosis** **clinical epidemiology** **dietary acid load** **net endogenous acid production**

Introduction

Long-term kidney graft survival in kidney transplant recipients has improved greatly in the last few decades, but graft failure still occurs in approximately 22% of patients 5 years and approximately 50% 10 years after transplantation (**1**). Preservation of transplanted kidneys is vital to delay premature mortality and to lower costs associated with returning to dialysis and retransplantation (**2**,**3**). Identifying modifiable risk factors, such as diet, may help preserve kidney function in kidney transplant recipients (**4**).

Diet contributes to the net acid load on the basis of the intake of acid- or base-producing foods (**5**,**6**). Acid precursors, which are phosphates, and sulfuric acid are produced from metabolizing methionine and cysteine, and are mostly found in animal proteins. Base precursors are anionic alkali salts, and together with potassium as the cation, are mainly found in fruits and vegetables (**5**,**6**). The current human dietary pattern contains reduced base-producing products and is usually associated with a net acidifying effect (**6** ⇓–**8**). Previous studies have shown that a higher dietary acid load is associated with kidney function decline and albuminuria in patients with CKD (**9**,**10**). Also, dietary acid load has been linked to low-grade metabolic acidosis and a decreased level of venous bicarbonate (HCO_3^-) (**11** ⇓–**13**). Furthermore, multiple studies have shown that metabolic acidosis reflected by lower plasma HCO_3^- is associated with the progression of CKD, the development of kidney failure and of graft failure in patients with CKD and kidney transplant recipients, respectively (**14**,**15**). However, whether

dietary acid load is associated with kidney function decline in kidney transplant recipients, and whether venous HCO_3^- explains this, is unknown.

Our aim was to study the association between dietary acid load, expressed as net endogenous acid production (NEAP), and a higher risk of kidney function decline or graft failure in kidney transplant recipients, and to evaluate whether venous HCO_3^- levels would explain (mediate) such an association.

Materials and Methods

Study Population

We used data from the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT02811835), which included stable kidney transplant recipients with a functioning graft for ≥1 year who were visiting the outpatient clinic of the University Medical Center of Groningen (UMCG) between 2008 and 2011, as described earlier (**16**). Of the 817 initially invited patients, 707 (87%) provided informed consent to participate. For this study, we excluded participants with missing data of NEAP on the basis of the food frequency questionnaire (FFQ, see Supplemental Material), resulting in 642 participants eligible for analyses. No differences in demographic factors were observed between participants with missing data and participants with NEAP by FFQ. The study was conducted according to the guidelines in the Declaration of Helsinki and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. The Institutional Review Board of the UMCG approved the study protocol (METc 2008/186).

Baseline Measurements and Kidney Transplant Characteristics

Measurements were performed once at baseline, during a visit to the outpatient clinic. Blood pressure was measured by using a semiautomatic device (Dinamap 1846; Critikon, Tampa, FL). Information on dietary intake was assessed using a semiquantitative FFQ that inquired about intake of 177 food items during the last month (**17**). Dietary data were converted into energy and nutrient intake by research dietitians using the Dutch Food Composition Table (2006) (**18**). Smoking behavior was inquired about using a questionnaire. Transplantation characteristics were retrieved from the local UMCG Renal Transplantation Database.

Sample Collection and Biochemistry

Blood samples were collected after an 8–12-hour overnight fasting period. Venous pH and PCO_2 were assessed potentiometrically on a ABL800 Flex Blood Gas Analyzer (Radiometer Medical ApS, København, Denmark) and HCO_3^- was calculated on the basis of pH and PCO_2 levels. Plasma creatinine was measured by using an enzymatic assay, traceable to isoptope dilution mass spectrometry, on a Roche Modular P/Cobas C702 platform (Roche Diagnostics, Basel, Switzerland), and kidney function was assessed by using the CKD Epidemiology Collaboration formula to calculate eGFR. To ensure adequate 24-hour urine samples, all participants were instructed to discard the morning urine specimen and collect all subsequent urine for the next 24 hours, including the next morning urine specimen. Urine was collected under oil, as recommended, and commonly applied in the field of urinary acid load studies (**19**). Chlorhexidine was added as an antiseptic agent. Urine pH and titratable acid were measured with an automated titrator (855 Robotic Titrosampler; Metrohm, Herisau, Switzerland).

Directly after collection, additional urine samples were stored at −80°C for a maximum of 2 years. Ammonium (NH_4^+) and HCO_3^- were measured *via* chromatography in freshly thawed 24-hour urine samples (Alliance HT 2795, Waters, Milford, MA; type 861, Metrohm, respectively). Stability of both variables over repeated freeze-thaw cycles was analyzed in advance of the study; differences after thawing compared with the original mean values were insignificant. Total urinary protein was analyzed using an enzymatic assay, traceable to isoptope dilution mass spectrometry, on a Roche Modular P/Cobas C702 platform (Roche Diagnostics, Basel, Switzerland). Other laboratory measurements were taken according to routine laboratory methods.

Dietary Acid Load

Dietary acid load was assessed by using the equation by Frassetto to calculate "estimated NEAP" (**20**) (54.5*protein [g/d]/potassium [mEq/d]–10.2). We used the FFQ to estimate protein and potassium intake to calculate $NEAP_{FFQ}$. We estimated protein intake from urinary urea excretion by the Maroni formula (**21**) and we used urinary potassium excretion to calculate $NEAP_{Urine}$. Net acid excretion (NAE) was calculated as follows: NAE = urinary titratable acid + urinary NH_4^+– urinary HCO_3^- excretion.

Study End Point

The study end point was a composite kidney end point, consisting of doubling of plasma creatinine or graft failure, defined as return to dialysis or retransplantation. Participants were censored at death. Data on graft failure and death were retrieved from medical records and verified with the corresponding nephrologist or the Municipal Personal Records Database in case of death. End points were recorded until September 30, 2015. No participants were lost to follow-up.

Statistical Analyses

Normally distributed data are presented as mean±SD, whereas skewed data are presented as median (interquartile range, IQR) and percentages are used for categorical data. Some participants had missing values for baseline variables. Exclusion of participants with missing values could result in bias, and therefore, we used multiple imputation (fully conditional specification [Markov chain Monte Carlo] to obtain five imputed datasets) (**22**,**23**). Rubin's rules were used to obtain pooled estimates of the regression coefficients and their standard errors across imputed datasets (**24**). Significant baseline variables within $NEAP_{FFQ}$ tertiles were cumulatively added in the multivariable regression analyses to the main model (age, sex, time since transplantation, and eGFR) to identify independent associations with $NEAP_{FFQ}$.

Mediation Analyses

For mediation analysis, we added each parameter, venous HCO_3^-, urinary HCO_3^-, NH_4^+, titratable acid, and NAE to Cox regression model 3 to observe possible point estimate changes. Furthermore, we analyzed mediation according to the method described by Preacher and Hayes (**25**) as follows: First, we estimated the total effect of $NEAP_{FFQ}$ or $NEAP_{Urine}$ on kidney function decline (c) using accelerated failure time. Subsequently, we estimated the effect of NEAP on the acid parameters (a). The effect of potential mediators on kidney function decline was estimated with accelerated failure time (b). The indirect effect of a potential mediating effect was then calculated by computing the product of the two coefficients of the potential mediator on NEAP and kidney

function decline (a*b). The magnitude of indirect effects was calculated by dividing the coefficient of the indirect effects by the total effect. All mediation analyses were adjusted for age, sex, primary kidney disease, time between transplantation and study baseline, eGFR, and proteinuria. Significance of mediation was tested by computing bias-corrected bootstrap 95% confidence intervals (95% CIs) with 1000 repetitions.

Sensitivity Analyses

Methods for sensitivity analyses are included in the Supplemental Methods.

Data were analyzed with SPSS version 23.0 (IBM Corp., Armonk, NY), R version 3.2.3 (The R Foundation for Statistical Computing, Vienna, Austria). A *P* value <0.1 was considered statistically significant to detect effect modification. Otherwise, a *P* value <0.05 was considered statistically significant.

Results

A total of 642 participants were included at a median of 5.5 (IQR 1.8–12.1) years after transplantation in our study. Mean eGFR was 52±20 ml/min per 1.73 m^2. Median $NEAP_{FFQ}$ and median $NEAP_{Urine}$ levels at baseline were 40 (IQR 35–45) mEq/day and 54 (IQR 44–66) mEq/day, respectively. Baseline characteristics according to tertiles of $NEAP_{FFQ}$ and $NEAP_{Urine}$ are summarized in **Tables 1** and **2** and Supplemental Tables 1 and 2. Numbers of missing baseline variables can be found in Supplemental Table 3. Multivariable linear regression analyses showed that venous HCO_3^-, plasma chloride, urinary sodium, urea and NH_4^+ excretion, and NAE were independently associated with $NEAP_{FFQ}$ (Supplemental Table 4).

Table 1.

View inline

Baseline characteristics of 642 kidney transplant recipients in the TransplantLines Food and Nutrition Biobank and Cohort Study according to tertiles of net endogenous acid production using the food frequency questionnaire

Table 2.

Collapse inline

Lifestyle characteristics according to tertiles of net endogenous acid production using a food frequency questionnaire in 642 kidney transplant recipients

	Tertiles of Net Endogenous Acid Production Using a Food Frequency Questionnaire		
Characteristics	**I**	**II**	**III**
Net endogenous acid production using food frequency questionnaire, (mEq/day)	33 (30–35)	40 (38–41)	47 (45–52)
Lifestyle parameters			
Smoking, ever (%)	97 (47)	95 (46)	91 (46)
Smoking, current (%)	27 (13)	32 (16)	19 (10)
Current alcohol consumption, yes (%)	144 (67)	154 (72)	136 (64)
Total energy intake, kcal/day	2163±610	2224±667	2119±639
Total energy intake, kJ/day	9063±2540	9318±2794	8872±2671

	Tertiles of Net Endogenous Acid Production Using a Food Frequency Questionnaire		
Characteristics	**I**	**II**	**III**
Total protein intake, gram/day[a]	77±19	83±19	86±21
Normalized energy intake, kcal/kg per day	28±9	29±10	27±11
Normalized energy intake, kJ/kg per day	115±36	121±41	115±47
Normalized protein intake, gram/kg per day[a]	1.1±0.3	1.1±0.3	1.1±0.3
Plant-derived protein intake, gram/day	32±10	31±10	29±10
Animal-derived protein intake, gram/day[a]	48±14	52±15	54±16
Total protein intake, % of total kcal/day[a]	14±2	15±2	17±3
Fat intake, gram/day	87±30	90±36	87±37
Total fat intake, % of total kcal/day[a]	35±6	36±5	38±6
Carbohydrate intake, gram/day[a]	260±78	252±77	233±76
Total carbohydrate intake, % of total kcal/day[a]	48±6	46±6	44±6
Dairy, gram/day	330 (195–477)	338 (212–510)	329 (211–462)
Meat, gram/day[a]	85 (61–107)	95 (75–118)	103 (80–127)
Fruits, gram/day[a]	166 (70–261)	132 (77–232)	99 (50–182)
Vegetables, gram/day[a]	125 (86–168)	98 (66–149)	91 (64–121)
Legumes and nuts, gram/day[a]	11 (4–21)	12 (4–25)	10 (2–20)
Fish, gram/day[a]	8 (0–17)	12 (5–18)	12 (4–22)

[a]Represents significant (P<0.05) difference between the tertiles analyzed by independent t test for normally distributed data, Kruskal-Wallis test for skewed distributed variables, and chi-squared test for categorical data.

$NEAP_{FFQ}$ and $NEAP_{Urine}$ and Risk of Kidney Function Decline

The median time interval for the measure of doubling of plasma creatinine was 5.2 years (IQR, 4.0–5.9). During a median follow-up time of 5.3 (IQR, 4.1–6.0) years, 121 (19%) out of 642 participants reached the kidney end point. $NEAP_{FFQ}$ per SD was independently associated with the kidney end point (**Table 3**, model 5: hazard ratio [HR], 1.33; 95% CI, 1.12 to 1.57; P=0.001). The third tertile of $NEAP_{FFQ}$ had the highest risk of kidney function decline compared with the participants in the first tertile (**Table 3**, model 5: HR, 1.71; 95% CI, 1.08 to 2.70, P = 0.02). No differences were found when urinary albumin excretion was substituted for urinary protein excretion in adjustments. An adjusted restricted cubic spline for the association between $NEAP_{FFQ}$ and kidney end point on the basis of model 5 is shown in **Figure 1A**.

Table 3.

Collapse inline

Associations of net endogenous acid production using a food frequency questionnaire (either as a continuous covariate per SD or as tertiles) with the kidney end point in 642 kidney transplant recipients

	Net Endogenous Acid Production Using Food Frequency Questionnaire per SD	Tertiles of Net Endogenous Acid Production Using a Food Frequency Questionnaire		
Model	Hazard Ratio (95% Confidence Interval)	I	II	III
1	1.26 (1.06 to 1.49)**	1.0 (ref)	0.99 (0.62–1.58)	1.58 (1.02–2.42)*
2	1.25 (1.06 to 1.48)*	1.0 (ref)	1.00 (0.63–1.60)	1.56 (1.01–2.40)*
3	1.29 (1.10 to 1.53)**	1.0 (ref)	1.16 (0.71–1.89)	1.67 (1.06–2.61)*
4	1.29 (1.10 to 1.52)**	1.0 (ref)	1.18 (0.72–1.92)	1.71 (1.09–2.69)*
5	1.33 (1.12 to 1.57)**	1.0 (ref)	1.16 (0.73–1.83)	1.71 (1.08–2.70)*

Data are presented as HR, hazard ratio; 95% CI, confidence interval; *P* value is shown as: *<0.05, **< 0.01.

Model 1=Crude value of $NEAP_{FFQ}$. Model 2=as model 1 and additionally adjusted for age, sex, and body mass index.

Model 3=as model 2 and additionally adjusted for time after transplantation, primary kidney disease, eGFR, and total urinary protein excretion. Model 4=as model 3 and additionally adjusted for living donor status, pre-emptive transplantation, and calcineurin inhibitors. Model 5=as model 3 and additionally adjusted for systolic blood pressure, urinary sodium excretion, cholesterol, and diabetes.

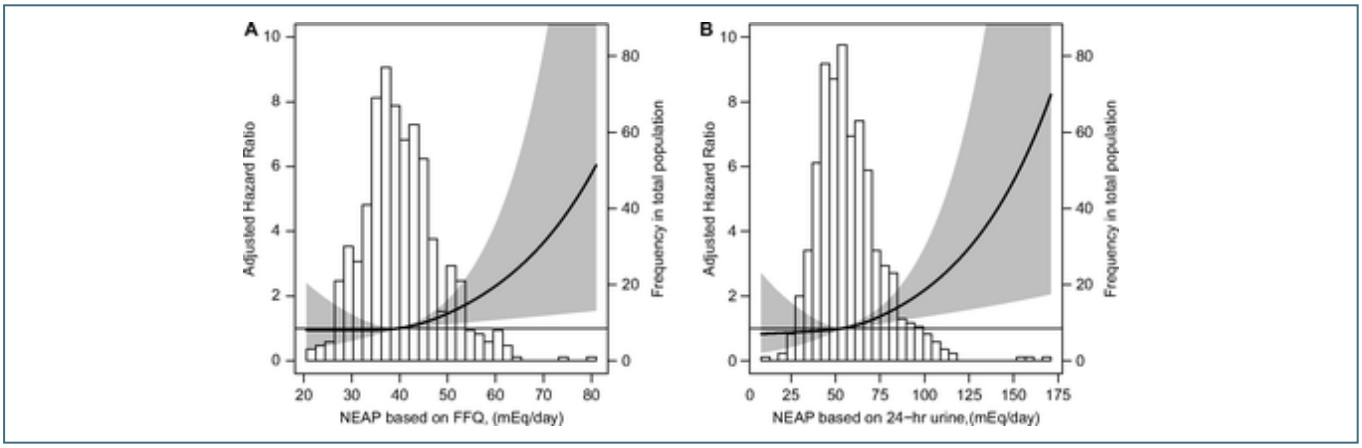

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Figure 1.

High net endogenous acid production assessed with food frequency questionnaire or urine excretion is associated with a higher risk of doubling of plasma creatinine or graft failure. Associations between net endogenous acid production using food frequency questionnaire (A) and net endogenous acid production using urine excretion (B) and kidney function decline in 642 kidney transplant recipients. Data were fit by a Cox proportional-hazard regression model on the basis of restricted cubic splines and adjusted for age, sex, body mass index, primary kidney disease, time after transplantation, eGFR, proteinuria (<0.5 g/day), history of diabetes, plasma cholesterol, and systolic blood pressure. The gray area represents the 95% confidence interval. NEAP, net endogenous acid production; FFQ, food frequency questionnaire.

We observed heterogeneity by age on the association of $NEAP_{FFQ}$ and kidney end point. This association was stronger in participants younger than 50 years old (HR, 1.55; 95% CI, 1.19 to 2.02 versus HR, 1.11; 95% CI, 0.97 to 1.27). No effect modification by sex, eGFR, body mass index, proteinuria, and time after transplantation was

observed (Supplemental Table 5). Younger participants had lower NEAP, venous HCO_3^-, and dietary intake of fruits and vegetables compared with older participants (Supplemental Table 6).

$NEAP_{Urine}$ per SD was independently associated with the kidney end point (Supplemental Table 7, model 5: HR, 1.44; 95% CI, 1.24 to 1.69; *P*<0.001). The highest $NEAP_{Urine}$ tertile had the highest risk for the kidney end point compared with the lowest tertile in the fully adjusted model (Supplemental Table 8, model 5: HR, 1.97; 95% CI, 1.24 to 3.13, *P*=0.005). An adjusted restricted cubic spline for the association between $NEAP_{Urine}$ and kidney end point on the basis of model 5 is shown in **Figure 1B**. No heterogeneity was observed on the association between $NEAP_{Urine}$ and kidney end point.

Mediation Analysis

The point estimate of the association between continuous $NEAP_{FFQ}$ and the kidney end point became slightly lower after addition of venous HCO_3^- (HR, 1.23; 95% CI, 1.04 to 1.45, *P*=0.02) and the association of $NEAP_{FFQ}$ in tertiles lost significance (Supplemental Table 8). Consequently, we found that venous HCO_3^- mediated 20% (*P*=0.002) of the association between $NEAP_{FFQ}$ and the kidney end point (**Table 4**).

Table 4.

View inline

Mediation analyses investigating mediation by potential mediators in the association of net endogenous acid production using a food frequency questionnaire on the risk of kidney end point

Likewise, venous HCO_3^- reduced the point estimate of the association between $NEAP_{urine}$ and kidney end point, and the highest $NEAP_{Urine}$ tertile became insignificant after adjustment for venous HCO_3^-. The point estimate of the association became slightly higher after adjustment for urinary NH_4^+or NAE (Supplemental Table 9). Furthermore, venous HCO_3^- (25%, *P*<0.001), urinary NH_4^+(−14%, *P*=0.03), and NAE (−18%, *P*=0.04) were significant mediators (Supplemental Table 10).

Sensitivity Analyses

During a median follow-up time of 5.2 (IQR, 4.0–5.9) years, 93 (18%) of 515 participants developed proteinuria. $NEAP_{FFQ}$ per SD (HR, 1.18; 95% CI, 0.97 to 1.43, *P*=0.09), and $NEAP_{Urine}$ per SD was associated with higher risk for proteinuria (HR, 1.17; 95% CI, 0.94 to 1.44, *P*=0.16) (Supplemental Tables 11–15). Other results of sensitivity analyses are included in the Supplemental Results.

Discussion

Values of $NEAP_{FFQ}$ and $NEAP_{Urine}$ (40, IQR, 35–45 and 54, IQR, 44–66 mEq/day, respectively) were comparable with the general population (approximately 50–75 mEq/day) (**8**). Still, our study showed kidney transplant recipients consuming $NEAP_{FFQ}$ >40 mEq/day have a higher risk for reaching the kidney end point.

Expanding on previous studies (**9** ⇓ ⇓ ⇓–**13**), these results support the hypothesis that high dietary acid load might damage the kidneys, partly by decreasing HCO_3^- levels. In our analyses, dietary acid load was not associated with incident proteinuria. Venous pH but not PCO_2 was inversely associated with the kidney end point, suggesting a more alkalotic environment reflected by HCO_3^- is associated with a lower risk of reaching the

kidney end point. It has been consistently described in animal and human studies that a high acid-producing diet increased NEAP, and in response, the kidneys produce higher levels of angiotensin II, endothelin-1, aldosterone, and ammoniagenesis to excrete excessive acid (**26**,**27**). However, prolonged elevated levels of angiotensin II, endothelin-1, aldosterone, and urinary ammonia excretion entails kidney damage and kidney function decline by inflammation, fibrosis, hypertension, and proteinuria (**28**,**29**). However, some observational studies have found that urinary NH_4^+ excretion is associated with a lower risk for kidney outcomes, and that progressive decline of kidney function hampers the excretion of NH_4^+(**30**,**31**). Our results support this theory because urinary NH_4^+ was inversely associated with the kidney end point and urinary NH_4^+ slightly increased, rather than decreased, the point estimate of the association between NEAP and the kidney end point. This may suggest that urinary NH_4^+ excretion is a marker for a better capacity of the kidneys for neutralizing acid excreted in urine. If the capacity of the kidneys to neutralize acid by generation of NH_4^+ excretion becomes impaired, it may put the kidneys at higher risk of further kidney function decline, resulting in greater risk for reaching the kidney end point. Unfortunately, no interventional studies have been performed in kidney transplant recipients.

Several intervention trials showed that supplementation of sodium HCO_3^- could delay progression of kidney function decline in patients with CKD (**27**,**32**). Furthermore, diets high in fruits and vegetables, containing high citrate or malate, will be metabolized into HCO_3^- with a potential kidney function preserving effect (**33**). Our data showed that higher NEAP consumption is associated with more animal protein, fewer fruits and vegetables, high sodium, and low potassium intake, and is typical for a Western diet, which is associated with a high dietary acid load (**34**). We also found that vegetable intake was inversely associated with the kidney end point, but after adjustment for NEAP, this association became weaker and not significant. Additionally, we found that younger participants eat fewer fruits and vegetables and have a lower venous HCO_3^- compared with older participants, which might explain the modification effect by age that we found. Altogether, this suggests that, although vegetable intake is an important contributor to lower dietary acid load and thus may lower the risk for reaching the kidney end point, dietary acid load as a whole is a more important factor than vegetable intake alone. Moreover, high potassium intake has been observed to be inversely associated with kidney outcomes in kidney transplant recipients (**35**). High potassium intake might lower blood pressure by a direct tubular effect and an increase of natriuresis, which may result in a delay of CKD progression (**36**). Dietary patterns, such as the Mediterranean diet or the Dietary Approaches to Stop Hypertension diet that are high in potassium and citrate and low in protein, are associated with lower dietary acid load and lower risk for CKD progression (**4**,**37**).

High dietary protein intake has been associated with CKD progression (**38**), and a low protein diet may slow CKD progression by a decrease of glomerular hyperfiltration and inflammation (**39**). However, we did not find associations between protein intake according to either Maroni formula or FFQ and the kidney end point in our sensitivity analyses. Other studies showed conflicting data (**40**), and, in line with previous work from our group, a low protein intake was associated with higher risk for mortality and graft failure (**41**,**42**). A recent study suggested that intake of essential amino acids in addition to adherence to low dietary protein intake might slow CKD progression (**43**). Some studies showed that plant-based protein intake is associated with a lower risk for adverse outcomes versus animal-based proteins, which are associated with a higher risk for adverse outcomes (**44**). Moreover, a plant-based diet is often high in fiber, complex carbohydrates, essential monounsaturated and

polyunsaturated fatty acids, low in sodium, and high in potassium, which might have a positive influence on the kidneys (**45**).

In a nutritional follow-up of kidney transplant recipients in our center, no other specific recommendations for protein or potassium intake are given. The focus is on food hygiene, and there is a general recommendation of a low fat and low salt intake.

Strengths of this study include a median follow-up period of 5.2 years with a large number of stable kidney transplant recipients with a functioning graft of ≥1 year with dietary data, blood, and urinary measurements. This study was conducted in a single center, comprising mainly European adults, limiting the generalizability of the results. The observational nature of our study precluded conclusions on a causal role for dietary acid load, and an interventional study should be conducted to prove actual causality. Higher NEAP might reflect an unhealthier diet, but not necessarily a less healthy lifestyle. FFQ was performed once, and dietary intake was assumed to be a stable behavior. Although FFQs were obtained between 2008 and 2011, we used the Dutch Food Composition Table from 2006 to generate intake data, which might add to under- or overestimation of nutrients. The Frassetto equation might under- or overestimate true dietary acid load as the organic acid component is not adequately captured in this equation. Both $NEAP_{FFQ}$ and $NEAP_{Urine}$ assume the sulfuric content is similar across proteins. It remains to be debated whether FFQ or urinary excretion is a more reliable source for estimating dietary intake (**46**). Yet, using FFQ and urine, we found similar results, adding robustness to our findings. Decreased HCO_3^- levels might indicate a tubular dysfunction and progressive kidney function decline (**14**,**26**). We had no access to plasma creatinine other than that of the last updated known visit date to the outpatient clinic.

In conclusion, in a moderately large cohort of kidney transplant recipients, we found that higher dietary acid load, assessed as NEAP, was associated with reaching the kidney end point. Mediation analysis revealed that venous HCO_3^-, urinary NH_4^+, and NAE partly explained this association. Interventional studies assessing the effect of lowering dietary acid load on kidney outcomes in kidney transplant recipients should follow.

Disclosures

G.J. Navis reports serving as chair of the Scientific Board of the Dutch Kidney Foundation, member of Health Council of the Netherlands, and member of Permanent Advisory Board Prevention Ministry of Health. J.J. Carrero reports having consultancy agreements with AstraZeneca and Bayer; reports receiving research funding from Astellas, AstraZeneca, the Swedish Heart and Lung Foundation, the Swedish Research Council, and Vifor Pharma; reports serving on the Advisory Committee for AstraZeneca and Editorial Boards of the *Journal of Nephrology* and *Nephrology Dialysis Transplantation*; reports speakers bureau for Abbott Laboratories, AstraZeneca, Fresenius, and Vifor Pharma; and reports other interests/relationships with European Renal Nutrition working group at the European Renal Association–European Dialysis and Transplant Association and International Society of Renal Nutrition and Metabolism. M.H. De Borst reports consultancy agreements with Kyowa Kirin, Pharmacosmos, Sanofi Genzyme, and Vifor Pharma; reports receiving research funding from Sanofi Genzyme and Vifor Pharma; and serving as an Associate Editor of *Nephrology Dialysis Transplantation*. S.J.L. Bakker reports receiving research funding from Astellas Pharma and Chiesi and reports serving on the Dutch

Health Council and the Scientific Board of the Dutch Kidney Foundation. S.P. Berger reports having consultancy agreements with and receiving honoraria from Novartis; reports receiving research funding from Chiesi and Novartis; and reports serving on Advisory Board of Novartis and the Supervisory Board of Dutch Transplant Foundation. All remaining authors have nothing to disclose.

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The funder had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. A.W. Gomes-Neto and S.M.H. Yeung performed data analyses and wrote the manuscript; E. van den Berg was responsible for data acquisition and contributed to manuscript revisions; S.P. Berger, J.J. Carrero, M.H. De Borst, J.E. Kootstra-Ros, M.C.J. Osté, and J.S.F. Sanders contributed to data interpretation and manuscript revisions. S.J.L. Bakker and G.J. Navis were responsible for the study design and contributed to data interpretation and manuscript revisions.

Supplemental Material

This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.00780121/-/DCSupplemental

Supplemental Methods.

Supplemental Results.

Supplemental Table 1. Additional baseline characteristics according to tertiles of net endogenous acid production using food frequency questionnaire in 642 kidney transplant recipients.

Supplemental Table 2. Baseline characteristics according to tertiles of net endogenous acid production using urinary excretion in 641 kidney transplant recipients.

Supplemental Table 3. Missing values in 642 kidney transplant recipients with net endogenous acid production using the food frequency questionnaire.

Supplemental Table 4. Multivariable associations of net endogenous acid production using the food frequency questionnaire with separate clinical parameters in 642 kidney transplant recipients.

Supplemental Table 5. Effect-modification analyses on the associations of net endogenous acid production using the food frequency questionnaire with risk of kidney end point stratified by selected characteristics.

Supplemental Table 6. Baseline characteristics comparison between young (aged <50 years) and old (aged ≥50 years) kidney transplant recipients.

Supplemental Table 7. Associations of net endogenous acid production using urinary excretion (either as a continuous covariate per SD or as tertiles) with kidney end point in 641 kidney transplant recipients.

Supplemental Table 8. Potential mediators of the association of continuous and in tertiles of net endogenous acid production using the food frequency questionnaire with the kidney end point in 642 stable kidney transplant recipients.

Supplemental Table 9. Potential mediators of the association of continuous net endogenous acid production using urinary excretion, and net endogenous acid production using urinary excretion in tertiles with the kidney end point in 641 stable kidney transplant recipients.

Supplemental Table 10. Mediation analyses investigating mediation by potential mediators in the association of net endogenous acid production using urinary excretion on the risk of kidney end point.

Supplemental Table 11. Associations of net endogenous acid production using food frequency questionnaire or urinary excretion with incidence of proteinuria in 515 kidney transplant recipients.

Supplemental Table 12. Associations of dietary protein intake using Maroni formula and food frequency questionnaire with kidney end point in 642 kidney transplant recipients.

Supplemental Table 13. Associations of vegetable, fruit, legumes, nuts, or meat intake with the kidney end point in 642 kidney transplant recipients.

Supplemental Table 14. Associations of venous PCO_2 and pH with the kidney end point in 593 kidney transplant recipients.

Supplemental Table 15. Associations of urinary protein and albumin excretion with kidney end point in 642 kidney transplant recipients.

Food frequency questionnaire form.

Footnotes

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- See related editorial, “An Apple a Day Keeps Dialysis Away,” on pages 1306–1308.

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