Title
Association of Uremic Solutes With Cardiovascular Death in Diabetic Kidney Disease.

Journal
American Journal of Kidney Diseases, 80(4)

Authors
Katz, Ronit
Ix, Joachim
Sarnak, Mark
et al.

Publication Date
2022-10-01

DOI
10.1053/j.ajkd.2022.02.016

Peer reviewed

HHS Public Access

Author manuscript

Am J Kidney Dis. Author manuscript; available in PMC 2023 October 01.

Published in final edited form as:

Am J Kidney Dis. 2022 October ; 80(4): 502–512.e1. doi:10.1053/j.ajkd.2022.02.016.

Association of Uremic Solutes With Cardiovascular Death in Diabetic Kidney Disease

Hima Sapa, PhD[1], **Orlando M. Gutiérrez, MD, MMSc**[2], **Michael G. Shlipak, MD, MPH**[3], **Ronit Katz, DPhil**[4], **Joachim H. Ix, MD, MAS**[5], **Mark J. Sarnak, MD, MS**[6], **Mary Cushman, MD**[7], **Eugene P. Rhee, MD**[8], **Paul L. Kimmel, MD**[9], **Ramachandran S. Vasan, MD**[10], **Sarah J. Schrauben, MD, MSCE**[11,12], **Harold I. Feldman, MD, MSCE**[11,12], **Jesse Seegmiller, PhD**[13], **Henri Brunengraber, MD, PhD**[14], **Thomas H. Hostetter, MD**[1,15], **Jeffrey R. Schelling, MD**[16]
CKD Biomarkers Consortium

[1]Division of Nephrology, Department of Internal Medicine, University Hospitals Cleveland, Case Western Reserve University School of Medicine, Cleveland, OH

[2]Departments of Medicine and Epidemiology, University of Alabama at Birmingham, Birmingham, AL

[3]Kidney Health Research Collaborative and Department of Medicine, San Francisco VA Medical Center and University of California, San Francisco, San Francisco, CA

[4]Kidney Research Institute, University of Washington, Seattle, WA

[5]Division of Nephrology and Hypertension, Department of Medicine, University of California San Diego, San Diego, CA

[6]Division of Nephrology, Department of Medicine, Tufts Medical Center, Boston, MA

Correspondence: Jeffrey R. Schelling, M.D. Division of Nephrology, MetroHealth Medical Center, 2500 MetroHealth Drive, R425, Cleveland, OH 44109, jeffrey.schelling@case.edu.

Authors' Contributions:

Designed study: OMG, MGS, MJS, MC, EPR, SJS, HIF, PLK, RSV, THH, JRS; conducted assays: HS, JS, HB, THH, JRS; analyzed data: RK, MGS, JHI, MJS. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

Article Information

CKD Biomarkers Consortium:

In addition to authors Gutiérrez*, Shlipak*, Ix*, Sarnak*, Rhee*, Kimmel, Vasan, Feldman*, Seegmiller, Hostetter*, and Schelling*, the collaborators in phase II of the CKD Biomarkers Consortium are (asterisk indicates Principal Investigator [PI]): Joseph Massaro PhD (Boston University School of Medicine); Clary Clish PhD (Broad Institute); Michelle Denburg, MD, MSCE*, Susan Furth, MD, PhD* (The Children's Hospital of Philadelphia); Bradley Warady, MD (Children's Mercy Hospital); Joseph Bonventre, MD, PhD*, Sushrut Waikar, MD, MPH*, Gearoid McMahon MB, BCH, Venkata Sabbisetti PhD (Brigham and Women's Hospital, Harvard University); Josef Coresh, MD, PhD*, Morgan Grams MD, Casey Rebholz, PhD, Alison Abraham, PhD, Adriene Tin PhD, Chirag Parikh, MD, PhD* (Johns Hopkins University); Jon Klein, MD, PhD (University of Louisville); Steven Coca, DO, MS*, Bart S. Ferket, MD, PhD, Girish N. Nadkarni, MD, MPH, CPH (Icahn School of Medicine at Mount Sinai); Daniel Gossett, PhD (NIDDK/NIH); Brad Rovin, MD* (Ohio State University); Andrew S. Levey, MD, Lesley A. Inker, MD, MS, Meredith Foster, PhD (Tufts Medical Center); Ruth Dubin, MD* (University of California San Francisco); Rajat Deo, MD* (University of Pennsylvania); Amanda Anderson, PhD, Theodore Mifflin, PhD, DABCC, Dawei Xie, PhD, Haochang Shou, PhD, Shawn Ballard, MS, Krista Whitehead, MS, Heather Collins, PhD (University of Pennsylvania Coordinating Center); Jason Greenberg, MD (Yale School of Medicine); Peter Ganz, MD* (Zuckerberg San Francisco General Hospital). Dr Vasan is Chair of the Steering Committee.

[7]Department of Medicine, Larner College of Medicine, University of Vermont, Burlington, VT

[8]Division of Nephrology, Department of Medicine, Massachusetts General Hospital and Harvard University, Boston, MA

[9]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

[10]Departments of Medicine and Epidemiology, Boston University School of Medicine and School of Public Health, Boston, MA

[11]Renal Electrolyte and Hypertension Division, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[12]Department of Biostatistics, Epidemiology, and Informatics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

[13]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

[14]Department of Nutrition, Case Western Reserve University School of Medicine, Cleveland, OH

[15]Division of Nephrology, Department of Medicine, University of North Carolina, Chapel Hill, NC

[16]Division of Nephrology, Department of Internal Medicine, MetroHealth Campus, and Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH

Abstract

Rationale and objective: Cardiovascular disease (CVD) is a major cause of mortality among people with diabetic kidney disease (DKD). The pathophysiology is inadequately explained by traditional CVD risk factors. Three uremic solutes, trimethylamine-N-oxide (TMAO), asymmetric and symmetric dimethylarginine (ADMA, SDMA), have been linked to CVD in kidney failure with renal replacement therapy (KFRT), but data are limited in populations with diabetes and less severe kidney disease.

Study design: Observational cohort.

Settings and participants: Random subcohort of 555 REGARDS Study participants with diabetes and eGFR <60 ml/min/1.73m^2 at study entry.

Exposures: ADMA, SDMA and TMAO were assayed by liquid chromatography-mass spectrometry in plasma and urine.

Outcomes: CV mortality (primary outcome), all-cause mortality and incident KFRT (secondary outcomes).

Analytic approach: Plasma and urine:plasma (U/P) ratios of ADMA, SDMA and TMAO were tested for association with outcomes. Adjusted Cox regression models were fitted and hazard ratios (HR) of outcomes presented per standard deviation, $\log_2$ increments and interquartile comparisons.

Results: Mean baseline eGFR was 44 ml/min/1.73 m^2. CV death, overall mortality and KFRT occurred in 120, 285 and 89 participants during mean 6.2 years follow-up. Higher plasma

ADMA, and lower U/P ratios of ADMA, SDMA and TMAO were associated with CV mortality by interquartile (HR 1.9–3.9), $\log_2$ concentration comparisons (HR 1.4–2.1), and per standard deviation (HR 1.2). Higher plasma concentrations (HR 1.1–2.8) and lower U/P ratios (HR 1.3–2.3) of all three solutes were associated with all-cause mortality. Higher plasma SDMA was associated with incident KFRT (HR 1.2–3.0).

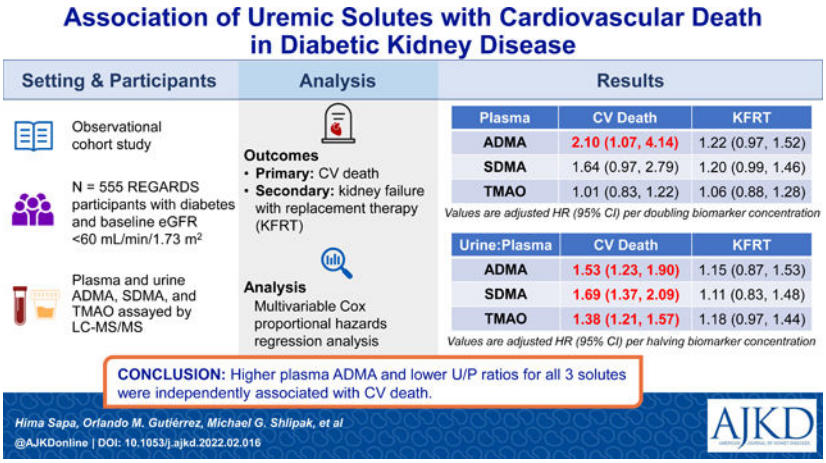
Limitations: Single cohort, restricted to diabetic patients with eGFR <60, potential residual confounding by GFR, no dietary information.

Conclusions: Higher plasma concentrations and lower U/P ratios of uremic solutes were independently associated with CV and all-cause mortality in DKD. Associations of U/P ratios with mortality suggest a connection between renal uremic solute clearance and CVD pathogenesis.

Plain-language summary

For diseases such as CKD, which progress over many years, it would be helpful to identify factors early in the disease course, which are associated with complications or disease progression. These biomarkers could ultimately be relevant to the disease pathophysiology or as prediction tools, to identify patients who deserve the most attention. For CKD patients, premature cardiovascular disease is by far the most common cause of death. In this study of diabetic patients with modest CKD, we investigated the association between three biomarkers that are derived from gut bacteria, which have previously been shown to be elevated in patients on dialysis (ADMA, SDMA and TMAO), and cardiovascular mortality. We find a strong association between ADMA and cardiovascular and all-cause mortality.

Graphical Abstract



Keywords

ADMA; biomarkers; cardiovascular disease; end stage kidney disease; SDMA; TMAO

Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among people with chronic kidney disease (CKD) [1,2], particularly for those with diabetes as the underlying

cause of CKD [3]. Although diabetic kidney disease (DKD) and CVD share some common risk factors, the pathogenesis of CVD in the context of DKD is incompletely understood. This is compounded by the lack of accurate biomarkers associated with these outcomes. Traditional risk factors for CVD (age, sex, diabetes duration, total cholesterol, HDL cholesterol, smoking, systolic blood pressure, hypertensive therapy) [4] are not as strongly associated with CVD in those with CKD compared to the general population [5,6].

A number of low molecular weight organic metabolites accumulate in CKD (collectively termed "uremic solutes") [7–10]. Several uremic solutes have been associated with CVD in people with kidney failure with renal replacement therapy (KFRT) [11], but data are more limited in people with less severe CKD. In this report, we focused on the prognostic value of three specific uremic solutes – asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and trimethylamine-N-oxide (TMAO), in people with diabetes and CKD.

SDMA and ADMA arise from the metabolism of proteins with methylated arginine residues [12]. ADMA is metabolized in the kidney; both ADMA and SDMA are cleared by the kidneys and accumulate with decreasing glomerular filtration rate (GFR) [13–15]. Associations of ADMA and SDMA with CVD have been inconsistent in the general population, populations with CKD with our without diabetes, and in those with KFRT [16–23].

TMAO is derived from the metabolism of compounds with a quaternary ammonia structure, such as choline or carnitine, by intestinal flora [24–26]. The resulting trimethylamine is absorbed and oxidized in the liver to TMAO, which is cleared primarily by filtration and secretion in the kidney [27,28]. Extremely high TMAO levels have been detected in patients with KFRT [27,29], though less is known about concentrations in CKD patients [30], especially in high risk populations with diabetes. In prior studies, plasma TMAO levels were associated with CVD events in the general population, even after adjusting for traditional risk factors, including eGFR [24–26].

Despite some precedent to implicate ADMA, SDMA and TMAO as biomarkers for CVD in the context of DKD, there has been insufficient validation for clinical utilization. In the current study, we assayed plasma and urine for ADMA, SDMA and TMAO in a random subcohort of participants with diabetes and baseline eGFR <60 ml/min/1.73 m^2 from the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort study. These measures permitted study of associations with plasma and urine concentrations, as well as urine:plasma (U/P) solute ratios with the primary outcome, CV mortality, and secondary outcomes, all-cause mortality and incident KFRT.

Methods

Study population.

The REGARDS study is a prospective cohort study designed to investigate regional differences in stroke incidence among Black and white adults ≥45 years of age. The study design has been reported previously [31]. Briefly, participants were recruited from the 48 contiguous U.S. and Washington DC. The study oversampled for Black people,

and those residing in the stroke belt region of the southeastern US. Trained interviewers collected socio-demographic, CVD risk factors, and use of anti-hypertensive, anti-glycemic, and cholesterol-lowering medication information by phone interviews. Trained personnel then conducted an in-home study visit that included an electrocardiogram, height, weight and blood pressure measurements, medication reconciliation, and blood and urine sample collection. Between January 2003 and October 2007, 30,239 individuals (42% Black, 55% women) were enrolled. Participants or their proxies were then contacted by telephone every six months to assess outcomes. Among this sample, 1,145 had diabetes and eGFR <60 ml/min/1.73m^2 at baseline. From this group, we randomly selected a subcohort of 600 REGARDS participants, of whom 555 had plasma and urine samples available at baseline (Figure 1). Baseline characteristics for included and excluded participants are shown in Table S1. Urine albumin and UACR were not significantly different between the included and excluded groups ($p = 0.575$ and $p = 0.539$ respectively, by non-parametric median tests). The REGARDS study protocol was approved by the Institutional Review Boards from each participating institution and all participants provided informed consent.

Materials.

Deuterated trimethylamine N-oxide (TMAO-d9) and deuterated asymmetric dimethylarginine (ADMA-d7) were purchased from Cambridge Isotope Labs. Deuterated symmetric dimethylarginine (SDMA-d6) was purchased from Toronto Research Chemicals (Toronto, ON). HPLC grade solvents were purchased from Thermo Fisher Scientific (Waltham, MA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Thermo Fisher.

Sample preparation.

Urine and blood were collected at study entry, centrifuged, and aliquots were barcoded and stored at −80° C at the REGARDS biorepository at the University of Vermont. Samples were shipped on dry ice to Case Western Reserve University, where they were maintained at −80° C until biomarker assays were performed. Freeze/thaw experiments demonstrated a high degree of correlation across a broad concentration range (Table S2). To 20 µl of plasma or 20 µl diluted urine samples (1:20 in HPLC grade water), 80 µl of internal standard mixture (containing 10 µM TMAO-d9, 1 µM of ADMA-d7 and 1 µM of SDMA-d6) in methanol was added, according to published methods [27]. Protein in the samples was precipitated by vortexing for 2 min and then the supernatant was recovered following centrifugation (20,000 × g, 4° C, 10 min).

Liquid chromatography-mass spectrometry (LC-MS/MS) analysis.

Five µl supernatant was injected into a Luna Silica column (2 × 150 mm, 5 µm silica, 00F-4274-B0, Phenomenex, Torrance, CA). LC-MS/MS analysis employed a Shimadzu Prominence LC system coupled to an API 4000 Q-TRAP mass spectrometer (AB Sciex, Framingham, MA). Binary flow was generated to resolve the analytes by using mobile phases A (0.1 % propionic acid in H_2O) and B (0.1 % acetic acid in methanol) at 0.2 ml/min flow rate. The analytes, TMAO, TMAO-d9, SDMA, SDMA-d6, ADMA and ADMA-d7 were monitored using electrospray ionization in positive-ion mode with multiple reaction monitoring of precursor and characteristic product ion transitions of m/z 76.0 → 59.0 amu,

85.0 → 66.0 amu, 203.1 → 172.0 amu, 209.2 → 70.0 amu, 203.1 → 70.0 amu and 210.1 → 77.2 respectively. The parameters for the ion monitoring were as follows: Ionization voltage, 5.5 kV; ion source temperature 650 °C; curtain gas, 40; GS1, 40; GS2, 55; CAD gas, 4; DP, 50; CE, 25.0 volts; CXP, 11; EP, 10. Nitrogen (99.95% purity) was used as the only gas.

Calibration curves were generated using TMAO, SDMA and ADMA standards at six different concentrations, to which internal standards (TMAO-d9, SDMA-d6 and ADMA-d7) were added. Curves were analyzed by linear regression and 1/x weighting, using Analyst software (version 1.6, Framingham, MA) for TMAO and SDMA. Because of an overlapping peak with ADMA, MultiQuant SignalFinder software (version 3.0, Framingham, MA) was used to achieve superior peak modeling and ADMA quantification. Quality control samples of three different concentrations were run with each assay batch. The coefficients of variation (CVs) for the inter-assay quality control samples, and intra-assay calibration curves, were consistently <15%. The mean CVs for blind duplicate plasma TMAO, SDMA and ADMA values were 8.85%, 11.62% and 6.71%, respectively. Prior studies in subjects with similar baseline eGFR values yielded similar plasma ADMA, SDMA and TMAO levels [14,20,30,32]. For urine assays, the mean blind duplicate CVs were 5.84%, 6.89% and 7.38% for TMAO, SDMA and ADMA.

Outcomes of interest.

Definitions of the primary outcome (CV death) and the secondary outcome (all-cause mortality) were previously published [33]. Briefly, deaths were identified by report from next-of-kin and online sources (Social Security Death Index, National Death Index). Information from medical records, death certificates and interviews with surviving family members was compiled and reviewed by physician adjudicators to determine cause of death, which was classified by ICD-9 or −10 codes. CV death was defined as death due to circulatory disease (I00-I19, I26-I59, I70-I99) or coronary heart disease (CHD) (I20-I25) or stroke (I60-I69).

Incident KFRT data reported here have been supplied by the United States Renal Data System (USRDS) through 2014. The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy or interpretation of the U.S. government.

Covariates of interest.

Age, sex, race, smoking history, and education level were determined by self-report. Body mass index (BMI) was determined using height and weight obtained at the baseline home visit. Blood pressure was defined as the average of two measures while seated and after a 5-minute rest. Medications for hypertension were obtained by self-report. History of CHD was defined as evidence of myocardial infarction on the baseline electrocardiograph, self-report of prior myocardial infarction, coronary artery bypass surgery or emergent percutaneous coronary intervention. History of stroke was determined by self-report. Estimated GFR (eGFR) was determined from isotope dilution mass spectrometry-traceable serum creatinine concentration measurements and the CKD Epidemiology Collaboration equation [34]. Urine albumin concentration was measured on a BNII ProSpec nephelometer (Siemens AG,

Munich, Germany) and urine creatinine concentration was measured by the Jaffé method (Roche/Hitachi, Basel, Switzerland). The urine albumin-to-creatinine ratio (UACR) was expressed as mg/g.

Statistical analyses.

Descriptive statistics were used to characterize the study cohort across quartiles of plasma and urine:plasma uremic solute ratios. U/P ratios were used rather than fractional excretions because the latter requires incorporation of serum creatinine data, which may induce collinearity with eGFR in the same models. Instead, we adjusted for urine creatinine (to account for differences in urine tonicity at the time of urine specimen sampling) [35] and eGFR as separate covariates in multivariable models. Age- and sex- adjusted Pearson partial correlations were used to examine associations of plasma and urine biomarkers with each other and with eGFR and UACR [36]. After confirming the assumption of proportionality of hazards, Cox regression models were used to estimate the hazard ratios (HR) for incident CV death, all-cause mortality and incident KFRT as a function of baseline biomarkers. The relationship between the plasma biomarkers and the primary outcome, CV mortality, was linear (Figure S1). Model 1 adjusted for age, sex, race, education, and urine creatinine. Model 2 was further adjusted for BMI, systolic blood pressure, use of hypertension medications, smoking status, history of CHD, history of stroke, LDL, HDL, lipid-lowering medications, baseline urine albumin and eGFR. Missing data were taken into account using multiple imputation with chained equations in all regression analyses [37]. Resulting estimates were combined using Rubin's rules to account for the variability in the imputation procedure [38]. In both models 1 and 2, plasma biomarkers and U/P biomarker ratios were analyzed in quartiles (median values represented by the cut-off between quartiles 2 and 3), with the lowest plasma and highest U/P ratio quartiles serving as the reference groups. U/P biomarker data were also analyzed on a continuous scale after log base 2 transformation (i.e., per two-fold lower concentration of each U/P biomarker). Because the plasma biomarker inter-quartile ranges were generally less than half the median, regression analyses were conducted using continuous function (based on standard deviation). A two-tailed P value was determined with IBM SPSS version 26 software (Armonk, NY: IBM Corp), and $P<0.05$ was considered statistically significant.

Results

Characteristics of the study population.

Among the 555 participants with diabetes and eGFR <60 ml/min/1.73 m^2 in the cohort, the mean age was 70 years, 53% were Black, and 53% were women. The prevalence of hypertension, CHD and stroke was 88%, 42% and 16%, respectively. The mean eGFR (SD) at baseline was 44 (12) ml/min/1.73 m^2, and median (interquartile range) urine albumin to creatinine ratio (UACR) was 32 (11, 203) mg/g. Participant characteristics across baseline quartiles of plasma ADMA, SDMA and TMAO are shown in Tables S2 through S4, respectively. Individuals of female gender, those with less education, and white individuals demonstrated higher levels of ADMA (Table S2); male gender and Black race were associated with higher SDMA levels (Table S3); male gender and white race were associated with higher TMAO levels (Table S4).

Biomarker correlations.

Pairwise age- and sex-adjusted Spearman correlations between plasma and U/P ratio biomarker concentrations, eGFR, and UACR are shown in Table 1. All three plasma uremic solutes inversely and moderately correlated with eGFR, with the strongest correlation with SDMA. The plasma concentrations were also directly correlated with UACR, but these correlations were weaker than with eGFR. For the U/P ratios of the uremic solutes, very strong pairwise correlations were observed with eGFR and urine creatinine, and moderately strong correlations were observed with UACR (Table 1).

Association of plasma solutes with CV mortality.

During a mean follow-up period of 6.2 (3.5) years, there were 120 CV deaths (mean rate of 3.31%/yr). Composite event numbers are shown in Table S6. The CV mortality rate increased across ascending quartiles of each of the three plasma solutes (Table 2). HRs were attenuated after adjustment for demographic and traditional CV risk factors including eGFR and UACR. Results did not differ when HRs were adjusted using a race-independent GFR estimating equation (Table S7) [39]. A statistically significant association was observed for higher plasma ADMA with CV mortality in the multivariable-adjusted model, wherein each SD higher ADMA concentration was associated with a 20% higher risk of CV mortality. The association also increased monotonically across quartiles of plasma ADMA (Table 2).

Table 3 demonstrates the associations of U/P ratios of the uremic solutes with CV death. In this analysis, lower U/P ratios of all three solutes were independently associated with CV mortality. The strengths of association ranged from 38% higher risk per two-fold lower TMAO U/P ratio to 69% higher risk for SDMA U/P ratio. Neither the urinary concentrations nor fractional excretion of ADMA, SDMA and TMAO were significantly associated with CV death.

Association of uremic solutes with all-cause mortality.

A total of 285 all-cause deaths occurred over the course of the study (7.67% per year). Higher plasma concentrations of all three solutes were significantly associated with all-cause mortality, and associations were particularly strong for ADMA (Table 4). Lower U/P ratios were also consistently associated with all-cause mortality, and again these relationships appeared monotonic across respective U/P ratio quartiles (Table 5).

Association of uremic solutes with KFRT.

There were 89 participants who developed incident KFRT. Ascending quartiles of plasma SDMA concentration were strongly and significantly associated with incident KFRT risk in the multivariable-adjusted models; however, the association did not reach significance in the continuous model (Table 6). Higher plasma ADMA was also associated with incident KFRT in the multivariable-adjusted model by inter-quartile comparisons only. There were no statistically significant associations among plasma TMAO (Table 6), or any of the U/P solute ratios (Table S8) with KFRT.

Discussion

We examined the associations between plasma and urine ADMA, SDMA and TMAO concentrations, collectively termed uremic solutes, and CV death, in community-living individuals with both diabetes and eGFR <60 ml/min/kg/1.73 m^2. We found that higher plasma ADMA and lower U/P ratios for all three uremic solutes were significantly associated with CV death, even after adjusting for demographic and traditional CV risk factors, eGFR, and urine ACR. Plasma and U/P ratios for all three solutes were also significantly associated with the secondary outcome, all-cause mortality. SDMA was the only plasma uremic solute to be associated with incident KFRT and, none of the U/P ratios was associated with KFRT. Our observations that U/P ratios of all solutes have stronger associations with CV mortality, compared with the associations of plasma levels of these solutes suggest that renal handling and clearance of uremic solutes may influence CVD pathogenesis.

The associations between concentrations of plasma uremic solutes and CV death in this REGARDS DKD population confirmed prior published findings. TMAO, and to a lesser extent, ADMA and SDMA, have been linked to CVD in the general population [16–18,24–26], and plasma concentrations of all three solutes are increased in the context of CKD [13–15,27,29]. We observed associations between increased plasma ADMA and CV death, and all three plasma solutes with all-cause mortality. However, significant associations were more consistently observed for low U/P ratios of the uremic solutes. U/P ratios of urea and creatinine have typically been employed as measures of urine concentrating capacity, and an index of tubular integrity. Applications to renal pathophysiology have included distinction between pre-renal vs. tubular injury as the etiology of acute kidney injury [40], as well as prediction of polycystic kidney disease progression [41]. All three solutes are small, uncharged, and not protein-bound, implying that they should undergo glomerular filtration. But because U/P ratios of all three solutes remained significantly associated with CV mortality, after adjusting for eGFR, we propose that additional mechanisms of impaired renal clearance are plausible. TMAO may undergo proximal tubule secretion via organic anion transporters [42,43], though reports are contradictory regarding whether TMAO achieves net secretion (clearance exceeds GFR) [27,28,42,44]. Furthermore, evidence for impaired secretion contributing to elevated plasma levels of TMAO in CKD is lacking [45]. The renal tubular contributions to ADMA and SDMA excretions are even less clear, since fractional excretion of both molecules is less than 100% in rats [13] and ADMA undergoes significant extrarenal clearance [15]. An alternative explanation for the significant association of U/P ratios with CV outcomes, after eGFR adjustment, is that the U/P solute ratios could represent markers of filtration, which are not fully captured by the creatinine-based eGFR equation. Establishing renal handling with more precision, and whether uremic solute U/P ratios represent mechanistic biomarkers, will require further investigation.

Increased plasma ADMA concentration was significantly associated with both CV and all-cause mortality. These data are consistent with most prior studies evaluating these markers in the general, pre-KFRT CKD and KFRT populations [16,19,20,22], though few studies have been conducted in high risk, diabetic CKD populations [21,23]. Potential pathophysiologic mechanisms of ADMA-induced CVD include inhibition of nitric oxide synthase, thereby

contributing to vasoconstriction, hypertension and ischemia, as well as inflammation, oxidative stress and altered macrophage lipid metabolism [12,46].

Of these uremic solutes, only plasma levels of SDMA were associated with incident KFRT. Two small studies support a relationship between elevated plasma SDMA in CKD [47] and progression to KFRT [48]. Compared to ADMA, SDMA is a relatively weak inhibitor of nitric oxide synthase [49], but may exert other noxious effects on the vasculature, including activation of inflammatory and pro-oxidant pathways [12,50]. Additional studies will be required to both corroborate the association of SDMA with KFRT, and to determine pathobiological mechanisms of SDMA-mediated DKD progression.

Strengths of this study include the large, well-curated REGARDS diabetic CKD subcohort, with robust representation by Black persons, and assays of three specific solutes with high quality control standards. The study also has important limitations. First, despite adjusting for multiple covariates, residual confounding by GFR remains a possibility, since the correlations of uremic solutes with eGFR were strong, and eGFR has known imprecision relative to measured GFR, and can vary over time. Second, participants with relatively advanced CKD were evaluated, and may predispose to index event bias, which may not be fully addressed with adjustment for the baseline eGFR. Third, only samples from a REGARDS diabetic subcohort were analyzed; confirmation in other cohorts is therefore warranted. Fourth, our findings could be impacted by the stability of the three solutes, which may not be reflected by the one-week freeze-thaw experiments (Table S2). However, measurement bias generally influences findings toward the null hypothesis, so our observed findings should be robust. Fifth, dietary intake data, specifically for L-carnitine and choline precursors to TMAO, were not obtained in REGARDS. Finally, uremic solutes were measured at one point in time. Future studies evaluating trajectories of changes of uremic solutes with cardiovascular outcomes are required.

In conclusion, increased plasma ADMA and decreased U/P concentrations of ADMA, SDMA and TMAO are independently associated with the primary outcome, CV death, among community-living persons with both diabetes and CKD. Because the strongest associations were observed for lower U/P ratios of the uremic solutes, which may reflect diminished GFR as well as tubular dysfunction, we suggest that U/P ADMA, SDMA and TMAO represent unique links between DKD and CV death. Each uremic solute has been implicated in CVD pathophysiology, implying that in instances where plasma levels were elevated, the biomarkers might be mechanistic, and contribute to the risk of DKD for CVD by enhancing atherogenesis and thrombosis, although this requires future study. Nevertheless, these three biomarkers have not yet achieved clinical utility. If validated in other cohorts, diminished U/P ratios of ADMA, SDMA and TMAO could be used to identify the subset of patients with diabetes and CKD who are at particularly high risk for CV and all-cause mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors thank the staff and the participants of the REGARDS Study for their valuable contributions.

Peer Review:

Received September 17, 2021. Evaluated by 3 external peer reviewers and a statistician, with editorial input from an Acting Editor-in-Chief (Editorial Board Member Ifeoma Ulasi, FWACP). Accepted in revised form February 6, 2022. The involvement of an Acting Editor-in-Chief to handle the peer-review and decision-making processes was to comply with AJKD's procedures for potential conflicts of interest for editors, described in the Information for Authors & Journal Policies.

Support:

HS, HB, THH and JRS were supported by the CKD BioCon Consortium through NIDDK grant U01 DK106965. This research project is also supported by cooperative agreement U01 NS041588 co-funded by the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute on Aging (NIA), National Institutes of Health, Department of Health and Human Services. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NINDS or NIA. NIH officials (PLK) participated in the study design, but the findings do not necessarily reflect the opinion of the National Institute of Diabetes, Digestive and Kidney Diseases, the National Institutes of Health, the Department of Health and Human Services or the government of the United States.

Financial Disclosure:

Dr. Gutierrez reports receiving grant funding and honoraria from Akebia and GSK; honoraria from AstraZeneca, Ardelyx, and Reata; grant funding from GSK; and serving on the Data Monitoring Committee for a clinical trial from QED. The remaining authors declare that they have no relevant financial interests.

References

1. Weiner DE, Tighiouart H, Amin MG, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol. 2004;15(5):1307–1315. doi:10.1097/01.asn.0000123691.46138.e2 [PubMed: 15100371]
2. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med. 2004;351(13):1296–1305. doi:10.1056/NEJMoa041031 [PubMed: 15385656]
3. Palsson R, Patel UD. Cardiovascular complications of diabetic kidney disease. Adv Chronic Kidney Dis. May 2014;21(3):273–80. doi:10.1053/j.ackd.2014.03.003 [PubMed: 24780455]
4. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97(18):1837–1847. doi:10.1161/01.cir.97.18.1837 [PubMed: 9603539]
5. Weiner DE, Tighiouart H, Elsayed EF, et al. The Framingham predictive instrument in chronic kidney disease. J Am Coll Cardiol. 2007;50(3):217–224. doi:10.1016/j.jacc.2007.03.037. [PubMed: 17631213]
6. Tangri N, Kitsios GD, Inker LA, et al. Risk prediction models for patients with chronic kidney disease: a systematic review. Ann Intern Med. 2013;158(8):596–603. doi:10.7326/0003-4819-158-8-201304160-00004 [PubMed: 23588748]
7. Meyer TW, Hostetter TH. Uremia. N Engl J Med. 9/27/2007 2007;357(13):1316–1325. 10.1056/NEJMc073016 [PubMed: 17898101]
8. Shah VO, Townsend RR, Feldman HI, Pappan KL, Kensicki E, Vander Jagt DL. Plasma metabolomic profiles in different stages of CKD. Clin J Am Soc Nephrol. 2013;8(3):363–70. doi:10.2215/cjn.05540512 [PubMed: 23220422]
9. Rhee EP, Souza A, Farrell L, et al. Metabolite profiling identifies markers of uremia. J Am Soc Nephrol. 2010;21(6):1041–1051. doi:10.1681/asn.2009111132 [PubMed: 20378825]
10. Vanholder R, Meert N, Schepers E, et al. Review on uraemic solutes II-variability in reported concentrations: causes and consequences. Nephrol Dial Transplant. 2007;22(11):3115–21. doi:10.1093/ndt/gfm151 [PubMed: 17550927]

11. Moradi H, Sica DA, Kalantar-Zadeh K. Cardiovascular burden associated with uremic toxins in patients with chronic kidney disease. Am J Nephrol. 2013;38(2):136–48. doi:10.1159/000351758 [PubMed: 23941724]
12. Schepers E, Speer T, Bode-Boger SM, Fliser D, Kielstein JT. Dimethylarginines ADMA and SDMA: the real water-soluble small toxins? Semin Nephrol. 2014;34(2):97–105. doi:10.1016/j.semnephrol.2014.02.003 [PubMed: 24780466]
13. Nijveldt RJ, Teerlink T, van Guldener C, et al. . Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia. Nephrol Dial Transplant. 2003;18(12):2542–50. doi:10.1093/ndt/gfg452 [PubMed: 14605276]
14. Fliser D, Kronenberg F, Kielstein JT, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. J Am Soc Nephrol. 2005;16(8):2456–61. doi:10.1681/asn.2005020179 [PubMed: 15930091]
15. Oliva-Damaso E, Oliva-Damaso N, Rodriguez-Esparragon F, et al. Asymmetric (ADMA) and symmetric (SDMA) dimethylarginines in chronic kidney disease: A clinical approach. Int J Mol Sci. 2019;20(15):3668. doi:10.3390/ijms20153668
16. Boger RH, Sullivan LM, Schwedhelm E, et al. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. Circulation. 2009;119(12):1592–600. doi:10.1161/circulationaha.108.838268 [PubMed: 19289633]
17. Gore MO, Luneburg N, Schwedhelm E, et al. Symmetrical dimethylarginine predicts mortality in the general population: observations from the Dallas heart study. Arterioscler Thromb Vasc Biol. 2013;33(11):2682–8. doi:10.1161/atvbaha.113.301219 [PubMed: 24008162]
18. Schwedhelm E, Wallaschofski H, Atzler D, et al. Incidence of all-cause and cardiovascular mortality predicted by symmetric dimethylarginine in the population-based study of health in pomerania. PLoS One. 2014;9(5):e96875. doi:10.1371/journal.pone.0096875 [PubMed: 24819070]
19. Young JM, Terrin N, Wang X, et al. Asymmetric dimethylarginine and mortality in stages 3 to 4 chronic kidney disease. Clin J Am Soc Nephrol. 2009;4(6):1115–20. doi:10.2215/cjn.06671208 [PubMed: 19389824]
20. Emrich IE, Zawada AM, Martens-Lobenhoffer J, et al. Symmetric dimethylarginine (SDMA) outperforms asymmetric dimethylarginine (ADMA) and other methylarginines as predictor of renal and cardiovascular outcome in non-dialysis chronic kidney disease. Clin Res Cardiol. 2018;107(3):201–213. doi:10.1007/s00392-017-1172-4 [PubMed: 29101459]
21. Lajer M, Tarnow L, Jorsal A, Teerlink T, Parving HH, Rossing P. Plasma concentration of asymmetric dimethylarginine (ADMA) predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care. 2008;31(4):747–52. doi:10.2337/dc07-1762 [PubMed: 18162497]
22. Schlesinger S, Sonntag SR, Lieb W, Maas R. Asymmetric and symmetric dimethylarginine as risk markers for total Mortality and cardiovascular outcomes: A systematic review and meta-analysis of prospective studies. PLoS One. 2016;11(11):e0165811. doi:10.1371/journal.pone.0165811
23. Zobel EH, von Scholten BJ, Reinhard H, et al. Symmetric and asymmetric dimethylarginine as risk markers of cardiovascular disease, all-cause mortality and deterioration in kidney function in persons with type 2 diabetes and microalbuminuria. Cardiovasc Diabetol. 2017;16(1):88. doi:10.1186/s12933-017-0569-8 [PubMed: 28697799]
24. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57–63. doi: 10.1038/nature09922 [PubMed: 21475195]
25. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013;19(5):576–85. doi:10.1038/nm.3145 [PubMed: 23563705]
26. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013;368(17):1575–84. doi:10.1056/NEJMoa1109400 [PubMed: 23614584]

27. Hai X, Landeras V, Dobre MA, DeOreo P, Meyer TW, Hostetter TH. Mechanism of prominent trimethylamine oxide (TMAO) accumulation in hemodialysis patients. PLoS One. 2015;10(12):e0143731. doi:10.1371/journal.pone.0143731
28. Pelletier CC, Croyal M, Ene L, et al. Elevation of trimethylamine-N-oxide in chronic kidney disease: Contribution of decreased glomerular filtration rate. Toxins. 2019;11(11):635. doi:10.3390/toxins11110635
29. Bain MA, Faull R, Fornasini G, Milne RW, Evans AM. Accumulation of trimethylamine and trimethylamine-N-oxide in end-stage renal disease patients undergoing haemodialysis. Nephrol Dial Transplant. 2006;21(5):1300–4. doi:10.1093/ndt/gfk056 [PubMed: 16401621]
30. Tang WH, Wang Z, Kennedy DJ, et al. . Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. Circ Res. 2015;116(3):448–55.doi:10.1161/circresaha.116.305360 [PubMed: 25599331]
31. Howard VJ, Cushman M, Pulley L, et al. The reasons for geographic and racial differences in stroke study: objectives and design. Neuroepidemiology. 2005;25(3):135–43. doi:10.1159/000086678 [PubMed: 15990444]
32. Hov GG, Aasarød KI, Sagen E, Åsberg A. Arginine, dimethylated arginine and homoarginine in relation to cardiovascular risk in patients with moderate chronic kidney disease. Clin Biochem. 2015;48(10–11):646–51. doi:10.1016/j.clinbiochem.2015.03.012 [PubMed: 25828044]
33. Malek AM, Vladutiu CJ, Meyer ML, et al. The association of age at menopause and all-cause and cause-specific mortality by race, postmenopausal hormone use, and smoking status. Prev Med Rep. 2019;15:100955. doi:10.1016/j.pmedr.2019.100955 [PubMed: 31367516]
34. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–12. [PubMed: 19414839]
35. Wettersten N, Katz R, Shlipak MG, et al. Urinary bomarkers and kidney outcomes: Impact of indexing versus adjusting for urinary creatinine. Kidney Med. 2021;3(4):546–554.e1. doi:10.1016/j.xkme.2021.02.013 [PubMed: 34401722]
36. Snedecor GW, Cochran WG. Statistical Methods. 7th ed. Iowa State University Press; 1980.
37. Royston P. Multiple imputation of missing values STATA J. 2004;4:227–241.
38. Rubin DB. Multiple Imputation for Nonresponse in Surveys. Wiley; 1987.
39. Inker LA, Eneanya ND, Coresh J, et al. New creatinine- and cystatin C-based equations to estimate GFR without race. N Engl J Med. 2021;385(19):1737–1749. doi:10.1056/NEJMoa2102953 [PubMed: 34554658]
40. Miller TR, Anderson RJ, Linas SL, et al. Urinary diagnostic indices in acute renal failure: a prospective study. Ann Intern Med. 1978;89(1):47–50. doi:10.7326/0003-4819-89-1-47 [PubMed: 666184]
41. Heida JE, Gansevoort RT, Messchendorp AL, et al. Use of the urine-to-plasma urea ratio to predict ADPKD progression. Clin J Am Soc Nephrol. 2021;16(2):204–212. doi:10.2215/cjn.10470620 [PubMed: 33504546]
42. Miyake T, Mizuno T, Mochizuki T, et al. Involvement of organic cation transporters in the kinetics of trimethylamine N-oxide. J Pharm Sci. 2017;106(9):2542–2550. doi:10.1016/j.xphs.2017.04.067 [PubMed: 28479364]
43. Wu W, Bush KT, Nigam SK. Key role for the organic anion transporters, OAT1 and OAT3, in the in vivo handling of uremic toxins and solutes. Sci Rep. 2017;7(1):4939. doi:10.1038/s41598-017-04949-2 [PubMed: 28694431]
44. Stubbs JR, House JA, Ocque AJ, et al. Serum trimethylamine-N-oxide is elevated in CKD and correlates with coronary atherosclerosis burden. J Am Soc Nephrol. 2016;27(1):305–13. doi:10.1681/asn.2014111063 [PubMed: 26229137]
45. Mair RD, Sirich TL, Meyer TW. Uremic toxin clearance and cardiovascular toxicities. Toxins. 2018;10(6):226. doi:10.3390/toxins10060226
46. Lim YJ, Sidor NA, Tonial NC, Che A, Urquhart BL. Uremic toxins in the progression of chronic kidney disease and cardiovascular disease: Mechanisms and therapeutic targets. Toxins. 2021;13(2)doi:10.3390/toxins13020142

47. Fleck C, Schweitzer F, Karge E, Busch M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases. Clin Chim Acta. 2003;336(1–2):1–12. doi:10.1016/s0009-8981(03)00338-3 [PubMed: 14500028]

48. Busch M, Fleck C, Wolf G, Stein G. Asymmetrical (ADMA) and symmetrical dimethylarginine (SDMA) as potential risk factors for cardiovascular and renal outcome in chronic kidney disease - possible candidates for paradoxical epidemiology? Amino Acids. 2006;30(3):225–32. doi:10.1007/s00726-005-0268-8 [PubMed: 16680555]

49. Bode-Böger SM, Scalera F, Kielstein JT, et al. Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. J Am Soc Nephrol. 2006;17(4):1128–34. doi:10.1681/asn.2005101119 [PubMed: 16481412]

50. Speer T, Rohrer L, Blyszczuk P, et al. Abnormal high-density lipoprotein induces endothelial dysfunction via activation of Toll-like receptor-2. Immunity. 2013;38(4):754–68. doi:10.1016/j.immuni.2013.02.009 [PubMed: 23477738]

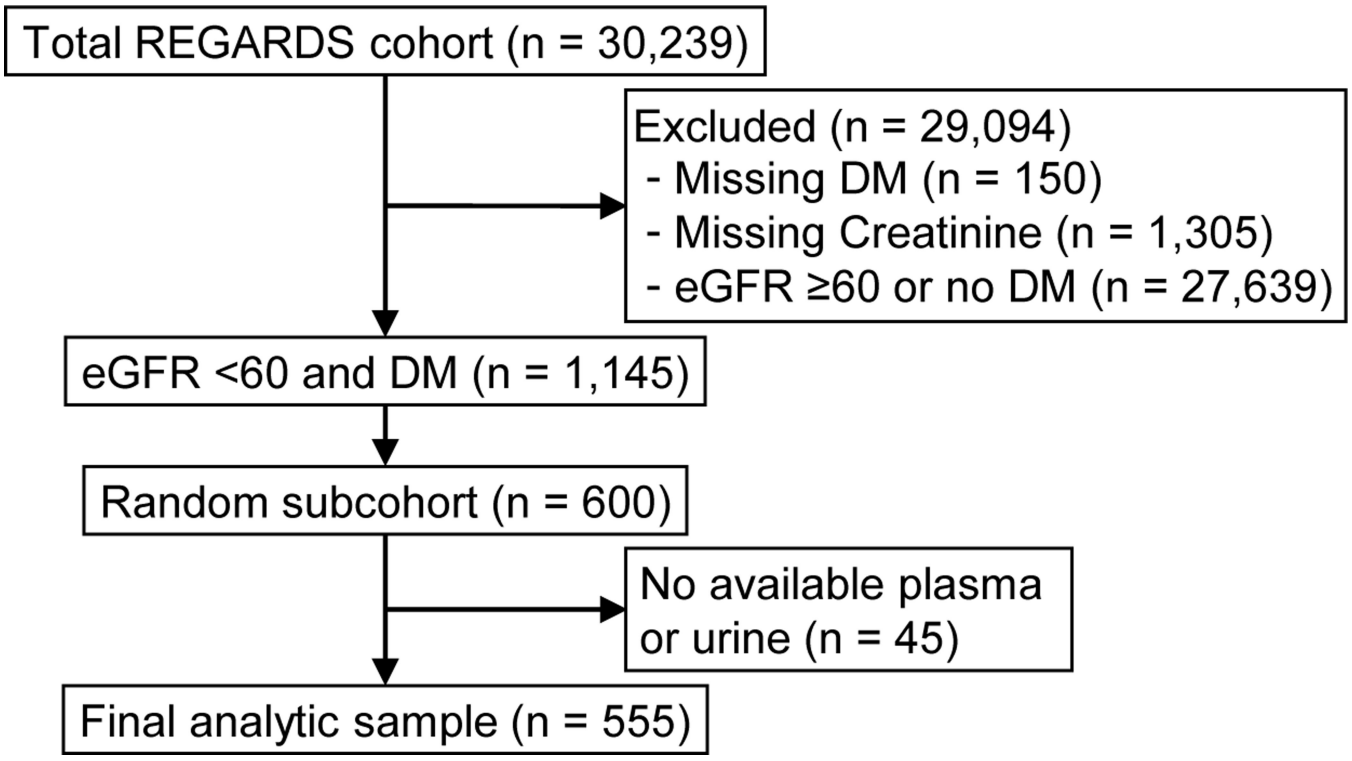


Figure 1.

Sampling of REGARDS population to achieve study subcohort. Among 30,239 REGARDS participants, a total of 1,145 had eGFR <60 mL/min/1.73m^2 and diabetes at study entry, and a subcohort of 600 individuals was randomly selected from those participants.

Table 1.

Spearman correlations

		Plasma						
		ADMA	**SDMA**	**TMAO**	**Ualb**	**UCr**	**UACR**	**eGFR**
Plasma	**ADMA**	1.0 00	0.351*	0.221*	0.117*	−0.098	0.135*	−0.204
	SDMA		1.0 00	0.410*	0.290*	−0.194	0.334*	−0.603
	TMAO			1.000	0.135*	−0.308	0.213*	−0.396

		U/P						
		ADMA	**SDMA**	**TMAO**	**Ualb**	**UCr**	**UACR**	**eGFR**
U/P	**ADMA**	1.000	0.929*	0.875*	−0.074	0.816*	−0.294*	0.512*
	SDMA		1.000	0.893*	−0.075	0.810*	−0.296*	0.519*
	TMAO			1.000	−0.060	0.789*	−0.277*	0.473*

*Correlation is significant at the P <0.01 level (2-tailed).

Table 2.

Association of baseline plasma ADMA, SDMA and TMAO with CV mortality

		Quartiles (range)			
Plasma ADMA	**Continuous (per SD = 0.15)**	**Q1 (<0.601 μM)**	**Q2 (0.601 – 0.670)**	**Q3 (0.671 – 0.760)**	**Q4 (>0.760)**
N (# of events)		151 (21)	136 (30)	132 (32)	136 (37)
Mortality rate (%/yr)		1.91	3.18	3.87	4.88
Unadjusted HR (95% CI)	1.35 (1.17, 1.57)	1.00 (ref)	1.68 (0.96, 2.94)	2.08 (1.20, 3.61)	2.73 (1.59, 4.66)
Model 1 HR (95% CI)	1.33 (1.13, 1.56)	1.00 (ref)	1.57 (1.15, 3.52)	2.01 (1.15, 3.52)	2.63 (1.51, 4.56)
Model 2 HR (95% CI)	1.20 (1.01, 1.43)	1.00 (ref)	1.40 (0.79, 2.49)	1.72 (0.97, 3.03)	1.93 (1.08, 3.45)
Plasma SDMA	**Continuous (per SD = 0.50)**	**Q1 (<0.854 μM)**	**Q2 (0.854 – 1.045)**	**Q3 (1.046 – 1.330)**	**Q4 (>1.330)**
N (# of events)		140 (26)	136 (25)	145 (32)	134 (37)
Mortality rate (%/yr)		2.56	2.80	3.44	4.71
Unadjusted HR (95% CI)	1.33 (1.14, 1.55)	1.00 (ref)	1.12 (0.65, 1.93)	1.39 (0.83, 2.33)	1.94 (1.17, 3.21)
Model 1 HR (95% CI)	1.33 (1.15, 1.54)	1.00 (ref)	1.07 (0.61, 1.87)	1.21 (0.72, 2.05)	1.97 (1.17, 3.34)
Model 2 HR (95% CI)	1.28 (1.02, 1.60)	1.00 (ref)	1.05 (0.60, 1.85)	1.03 (0.59, 1.81)	1.51 (0.79, 2.87)
Plasma TMAO	**Continuous (per SD = 14.06)**	**Q1 (<5.900 μM)**	**Q2 (5.901 – 9.155)**	**Q3 (9.156 – 15.300)**	**Q4 (>15.300)**
N (# of events)		142 (22)	136 (29)	142 (34)	135 (35)
Mortality rate (%/yr)		2.25	3.37	3.61	4.15
Unadjusted HR (95% CI)	1.06 (0.91.1.24)	1.00 (ref)	1.54 (0.88, 2.67)	1.64 (0.96, 2.80)	1.93 (1.13, 3.30)
Model 1 HR (95% CI)	1.07 (0.91. 1.27)	1.00 (ref)	1.53 (0.88, 2.67)	1.56 (0.91, 2.70)	1.96 (1.14, 3.39)
Model 2 HR (95% CI)	0.97 (0.78, 1.19)	1.00 (ref)	1.41 (0.80, 2.50)	1.39 (0.77, 2.50)	1.34 (0.73, 2.48)

Model 1 = age, sex, race, education

Model 2 = Model 1 + BMI, SBP, hypertension meds, smoking, CHD, stroke, LDL, HDL, lipid lowering meds, UACR and eGFR

Table 3.

Association of baseline urine/plasma (U/P) ADMA, SDMA and TMAO with CV mortality

		Quartiles (range)			
U/P ADMA	**Continuous (log2, per halving)**	**Q1 (<17.49)**	**Q2 (17.49 – 28.78)**	**Q3 (28.79 – 46.35)**	**Q4 (>46.35)**
N (# of events)		137 (39)	140 (37)	138(24)	140 (20)
Mortality rate (%/yr)		4.90	4.20	2.57	1.97
Unadjusted HR (95% CI)	1.45 (1.24, 1.71)	2.67 (.155, 4.58)	2.24 (1.30, 3.86)	1.36 (0.75, 2.46)	1.00 (ref)
Model 1 HR (95% CI)	1.52 (1.29, 1.79)	3.16 (1.83, 5.45)	2.44 (1.41, 4.24)	1.48 (0.81, 2.69)	1.00 (ref)
Model 2 HR (95% CI)	1.53 (1.23, 1.90)	2.57 (1.33, 4.96)	2.37 (1.33, 4.24)	1.37 (0.75, 2.52)	1.00 (ref)
U/P SDMA	**Continuous (log2, per halving)**	**Q1 (<17.34)**	**Q2 (17.34 – 31.19)**	**Q3 (31.20 – 49.65)**	**Q4 (>49.65)**
N (# of events)		137 (42)	139 (36)	140 (25)	139(17)
Mortality rate (%/yr)		5.53	4.01	2.63	1.67
Unadjusted HR (95% CI)	1.52 (1.29, 1.78)	3.56 (2.03, 6.27)	2.49 (1.40, 4.44)	1.60 (0.87, 2.97)	1.00 (ref)
Model 1 HR (95% CI)	1.61 (1.37, 1.91)	4.04 (2.28, 7.15)	2.42 (1.36, 4.32)	1.57 (0.84, 2.91)	1.00 (ref)
Model 2 HR (95% CI)	1.69 (1.37, 2.09)	3.94 (2.03, 7.62)	2.56 (1.40, 4.67)	1.41 (0.75, 2.64)	1.00 (ref)
U/P TMAO	**Continuous (log2, per halving)**	**Q1 (<22.62)**	**Q2 (22.62 – 42.13)**	**Q3 (42.14 – 71.16)**	**Q4 (>71.16)**
N (# of events)		139 (43)	139 (28)	149 (30)	138(19)
Mortality rate (%/yr)		5.47	3.19	3.08	1.92
Unadjusted HR (95% CI)	1.34 (1.20, 1.49)	3.09 (1.80, 5.31)	1.74 (0.97, 3.12)	1.64 (0.92, 2.92)	1.00 (ref)
Model 1 HR (95% CI)	1.36 (1.22, 1.52)	3.26 (1.89, 5.63)	1.71 (0.95, 3.06)	1.65 (0.93, 2.93)	1.00 (ref)
Model 2 HR (95% CI)	1.38 (1.21, 1.57)	3.31 (1.76, 6.23)	1.86 (1.02, 3.42)	1.77 (0.98, 3.18)	1.00 (ref)

Model 1 = age, sex, race, education, urine creatinine

Model 2 = Model 1 + BMI, SBP, hypertension meds, smoking, CHD, stroke, LDL, HDL, lipid lowering meds, urine albumin and eGFR

Table 4.

Association of baseline plasma ADMA, SDMA and TMAO with all-cause mortality

		Quartiles (range)			
Plasma ADMA	**Continuous (per SD = 0.15)**	**Q1 (<0.601 μM)**	**Q2 (0.601 – 0.670)**	**Q3 (0.671 – 0.760)**	**Q4 (>0.760)**
N (# of events)		151(57)	136(64)	132 (74)	136 (90)
Mortality rate (%/yr)		5.05	6.63	8.70	11.63
Unadjusted HR (95% CI)	1.37 (1.24, 1.50)	1.00 (ref)	1.34 (0.94, 1.91)	1.78 (1.26, 2.52)	2.50 (1.79, 3.49)
Model 1 HR (95% CI)	1.38 (1.24, 1.53)	1.00 (ref)	1.31 (0.91, 1.87)	1.77 (1.25, 2.52)	2.52 (1.25, 2.52)
Model 2 HR (95% CI)	1.31 (1.17, 1.47)	1.00 (ref)	1.17 (0.81, 1.69)	1.60 (1.12, 2.29)	2.00 (1.39, 2.86)
Plasma SDMA	**Continuous (per SD = 0.50)**	**Q1 (<0.854 μM)**	**Q2 (0.854 – 1.045)**	**Q3 (1.046 – 1.330)**	**Q4 (>1.330)**
N (# of events)		140 (47)	136 (65)	145 (83)	134 (90)
Mortality rate (%/yr)		4.48	7.00	8.77	11.33
Unadjusted HR (95% CI)	1.44 (1.33, 1.56)	1.00 (ref)	1.61 (1.11, 2.35)	2.02 (1.41, 2.89)	2.68 (1.88, 3.81)
Model 1 HR (95% CI)	1.45 (1.33, 1.57)	1.00 (ref)	1.31 (0.91, 1.87)	1.77 (1.25, 2.52)	2.68 (1.88, 3.81)
Model 2 HR (95% CI)	1.42 (1.26, 1.60)	1.00 (ref)	1.17 (0.81, 1.69)	1.60 (1.12, 2.29)	2.05 (1.31, 2.20)
Plasma TMAO	**Continuous (per SD = 14.06)**	**Q1 (<5.900 μM)**	**Q2 (5.901 – 9.155)**	**Q3 (9.156 – 15.300)**	**Q4 (>15.300)**
N (# of events)		142 (51)	136 (65)	142 (83)	135 (86)
Mortality rate (%/yr)		4.99	7.46	8.60	10.00
Unadjusted HR (95% CI)	1.15 (1.07, 1.24)	1.00 (ref)	1.55 (1.07, 2.23)	1.76 (1.24, 2.50)	2.10 (1.48, 2.96)
Model 1 HR (95% CI)	1.18 (1.08, 1.29)	1.00 (ref)	1.59 (1.10, 2.30)	1.76 (1.23, 2.51)	2.13 (1.50, 3.04)
Model 2 HR (95% CI)	1.13 (1.02, 1.24)	1.00 (ref)	1.46 (1.00, 2.12)	1.47 (1.01, 2.14)	1.62 (1.09, 2.40)

Model 1 = age, sex, race, education

Model 2 = Model 1 + BMI, SBP, hypertension meds, smoking, CHD, stroke, LDL, HDL, lipid lowering meds, UACR and

Table 5.

Association of baseline urine/plasma (U/P) ADMA, SDMA and TMAO with all-cause mortality

		Quartiles (range)			
U/P ADMA	**Continuous (log2, per halving)**	**Q1 (<17.49)**	**Q2 (17.49 – 28.78)**	**Q3 (28.79 – 46.35)**	**Q4 (>46.35)**
N (# of events)		137 (90)	140 (75)	138 (65)	140 (55)
Mortality rate (%/yr)		11.08	8.32	6.78	5.27
Unadjusted HR (95% CI)	1.37 (1.23, 1.52)	2.23 (1.59, 3.12)	1.65 (1.16, 2.33)	1.32 (0.92, 1.89)	1.00 (ref)
Model 1 HR (95% CI)	1.43 (1.29, 1.59)	2.67 (1.90, 3.75)	1.87 (1.32, 2.67)	1.44 (1.01, 2.07)	1.00 (ref)
Model 2 HR (95% CI)	1.34 (1.17, 1.55)	1.96 (1.29, 2.97)	1.63 (1.12, 2.36)	1.31 (0.90, 1.89)	1.00 (ref)
U/P SDMA	**Continuous (log2, per halving)**	**Q1 (<17.34)**	**Q2 (17.34 – 31.19)**	**Q3 (31.20 – 49.65)**	**Q4 (>49.65)**
N (# of events)		137 (95)	139(73)	140 (65)	139(52)
Mortality rate (%/yr)		12.27	7.94	6.70	4.93
Unadjusted HR (95% CI)	1.39 (1.26, 1.55)	2.66 (1.90, 3.73)	1.66 (1.17, 2.37)	1.37 (0.95, 1.98)	1.00 (ref)
Model 1 HR (95% CI)	1.46 (1.31, 1.62)	2.88 (2.04, 4.06)	1.64 (1.15, 2.35)	1.31 (0.91, 1.90)	1.00 (ref)
Model 2 HR (95% CI)	1.37 (1.19, 1.58)	2.25 (1.50, 3.39)	1.53 (1.05, 2.24)	1.19 (0.82, 1.72)	1.00 (ref)
U/P TMAO	**Continuous (log2, per halving)**	**Q1 (<22.62)**	**Q2 (22.62 – 42.13)**	**Q3 (42.14 – 71.16)**	**Q4 (>71.16)**
N (# of events)		139 (93)	139(74)	149 (63)	138 (55)
Mortality rate (%/yr)		11.62	8.11	6.39	5.40
Unadjusted HR (95% CI)	1.27 (1.18, 1.37)	2.30 (164, 3.21)	1.54 (1.09, 2.19)	1.20 (0.84, 1.72)	1.00 (ref)
Model 1 HR (95% CI)	1.31 (1.21, 1.42)	2.41 (1.72, 3.37)	1.52 (1.07, 2.16)	1.18 (0.82, 1.69)	1.00 (ref)
Model 2 HR (95% CI)	1.26 (1.15, 1.39)	2.01 (1.35, 3.00)	1.47 (1.02, 2.13)	1.23 (0.85, 1.78)	1.00 (ref)

Model 1 = age, sex, race, education, urine creatinine

Model 2 = Model 1 + BMI, SBP, hypertension meds, smoking, CHD, stroke, LDL, HDL, lipid lowering meds, urine albumin and eGFR

Table 6.

Association of baseline plasma ADMA, SDMA and TMAO with incident KFRT

		Quartiles (range)			
Plasma ADMA	**Continuous (per SD = 0.15)**	**Q1 (>0.601 μM)**	**Q2 (0.601 – 0.670)**	**Q3 (0.671 – 0.760)**	**Q4 (>0.760)**
N (# of events)		151 (15)	136 (23)	132 (19)	136(32)
KFRT incidence (%/yr)		1.40	2.55	2.35	4.60
Unadjusted HR (95% CI)	1.29 (1.08, 1.55)	1.00 (ref)	1.80 (0.94, 3.46)	1.69 (0.86, 3.34)	3.29 (1.78, 6.08)
Model 1 HR (95% CI)	1.41 (1.16, 1.70)	1.00 (ref)	1.70 (0.88, 3.28)	1.95 (0.98, 3.87)	3.93 (2.10, 7.35)
Model 2 HR (95% CI)	1.22 (0.97, 1.52)	1.00 (ref)	1.62 (0.80, 3.28)	2.03 (0.95, 4.34)	2.75 (1.38, 5.48)
Plasma SDMA	**Continuous (per SD = 0.50)**	**Q1 (<0.854 μM)**	**Q2 (0.854 – 1.045)**	**Q3 (1.046 – 1.330)**	**Q4 (>1.330)**
N (# of events)		140 (<10)	136 (11)	145 (24)	134 (48)
KFRT incidence (%/yr)		0.58	1.20	2.73	7.47
Unadjusted HR (95% CI)	1.65 (1.50, 1.82)	1.00 (ref)	2.10 (0.78, 5.68)	4.75 (1.94, 11.63)	13.32 (5.69, 31.20)
Model 1 HR (95% CI)	1.66 (1.48, 1.86)	1.00 (ref)	2.23 (0.82, 6.07)	5.06 (2.06, 12.45)	10.89 (4.58, 25.88)
Model 2 HR (95% CI)	1.20 (0.99, 1.46)	1.00 (ref)	1.67 (0.60, 4.65)	2.28 (0.89, 5.79)	2.96 (1.10, 7.97)
Plasma TMAO	**Continuous (per SD = 14.06)**	**Q1 (<5.900 μM)**	**Q2 (5.901 – 9.155)**	**Q3 (9.156 – 15.300)**	**Q4 (>15.300)**
N (# of events)		142 (13)	136 (14)	142 (27)	135 (35)
KFRT incidence (%/yr)		1.31	1.65	3.02	4.69
Unadjusted HR (95% CI)	1.31 (1.15.1.49)	1.00 (ref)	1.26 (0.59, 2.69)	2.28 (1.18, 4.42)	3.52 (1.86, 6.67)
Model 1 HR (95% CI)	1.32 (1.16. 1.51)	1.00 (ref)	1.31 (0.62, 2.80)	2.73 (1.40, 5.32)	3.84 (2.01, 7.32)
Model 2 HR (95% CI)	1.06 (0.88, 1.28)	1.00 (ref)	1.03 (0.48, 2.23)	0.97 (0.47, 2.02)	0.98 (0.45, 2.16)

Model 1 = age, sex, race, education

Model 2 = Model 1 + BMI, SBP, hypertension meds, smoking, CHD, stroke, LDL, HDL, lipid lowering meds, UACR and eGFR