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Results of the HEMO Study suggest that p-cresol sulfate and indoxyl sulfate are not associated with cardiovascular outcomes

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Abstract

Cardiovascular disease, the leading cause of mortality in hemodialysis patients, is not fully explained by traditional risk factors. To help define non-traditional risk factors we determined the

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Disclosure

Conflict of Interest: None

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association of predialysis total p-cresol sulfate, indoxyl sulfate, phenylacetylglutamine and hippurate with cardiac death, sudden cardiac death, and first cardiovascular event in the 1,273 participants of the HEMO Study. The results were adjusted for potential demographic, clinical, and laboratory confounders. The mean age of the patients was 58 years, 63% were Black and 42% were male. Overall, there was no association between the solutes and outcomes. However, in subgroup analyses, among patients with lower serum albumin (under 3.6 g/dL), a two-fold higher p-cresol sulfate was significantly associated with a 12% higher risk of cardiac death (hazard ratio 1.12; 95% confidence interval, 0.98–1.27) and 22% higher risk of sudden cardiac death (1.22, 1.06–1.41). Similar trends were also noted with indoxyl sulfate. Trial interventions did not modify the association between these solutes and outcomes. Routine clinical and lab data explained less than 22% of the variability in solute levels. Thus, in prevalent hemodialysis patients participating in a large U.S. hemodialysis trial, uremic solutes p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine were not associated with cardiovascular outcomes. However, there were trends of toxicity among patients with lower serum albumin.

Keywords

Cardiovascular Mortality; Dialysis Outcomes; P-cresol sulfate; Indoxyl Sulfate; Hippurate; Phenylacetylglutamine

INTRODUCTION

Cardiovascular disease morbidity and mortality in dialysis patients remains high and unexplained by traditional risk factors.[1] Despite the majority of US patients meeting target Kt/Vurea goal, the median survival after starting dialysis is approximately 3.5 years and over half of all deaths are due to cardiovascular causes.[2] Uremic toxins, substances that are cleared by the kidney and retained in kidney failure are possible contributors to accelerated cardiovascular disease in dialysis patients. Identification of these toxins is essential to develop therapies, both dialytic and non-dialytic, that can lower solute concentrations and hopefully improve survival of dialysis patients.[3]

P-cresol sulfate and indoxyl sulfate are among the most commonly studied uremic solutes. Substantial evidence has been accumulated that both these may cause vascular injury and have other toxic effects.[4] They share the property of generation by colon microbes followed by colonic absorption, conjugation, and clearance from the circulation by tubular secretion. Phenylacetylglutamine is also generated exclusively and hippurate partially through the action of colon microbes. Prior studies, including our work, also suggest that phenylacetylglutamine retention may also contribute to cardiovascular events in patients on hemodialysis[5] and also in patients with earlier stages of chronic kidney disease.[6] All these solutes share the property that they are cleared largely by secretion in the normal kidney. Because dialysis does not replicate secretory processes, their concentrations rise much higher relative to normal than concentrations of urea and creatinine in patients maintained on dialysis.[7]

We measured predialysis levels of p-cresol sulfate, indoxyl sulfate, hippurate and phenylacetylglutamine in specimens of the Hemodialysis (HEMO) Study, a U.S. multicenter

trial of hemodialysis dose and flux.[8] The goal of our study was to analyze the longitudinal association between these solutes and physician-adjudicated cardiovascular outcomes in the HEMO Study. The large sample size of the HEMO Study, its national multicenter design and inclusion of patients without significant residual kidney function provided us with a unique opportunity to examine the associations between these solutes and cardiovascular outcomes in hemodialysis patients.

RESULTS

Participant Characteristics

Baseline characteristics of the 1273 participants included in this study are presented in Table 1. Mean age of the participants was 57 years, 63% were Black and 57% were female. The participants included in this study were generally similar to the 1846 participants of the HEMO Study (Table S1), except for lower baseline cardiac disease (79% versus 83%), less years of prior dialysis (3.5 versus 4.4 years), higher residual urea clearance (0.3 versus 0.2 ml/min/35L), and lower serum β2-microglobulin (36 versus 38 mg/L).

Outcomes during Follow-Up

There were 221 cardiac deaths during 3,282 person-years of follow-up (median, 2.3 years) with a crude cardiac death rate of 67 per 1000 person-years. The adjudicated causes of cardiac death included ischemic heart disease (62.3%), congestive heart failure (11.4%), arrhythmias and other conduction disorders (15.0%) and other heart diseases (11.4%). During follow-up, there were 127 sudden cardiac deaths (crude mortality rate, 39 per 1000 person years), 641 cardiovascular events or any-cause deaths (crude event rate, 273 per 1000 person-years) and 563 any-cause deaths (crude mortality rate, 172 per 1000 person-years).

Association between Solutes and Outcomes

The association between the solutes and outcomes visualized using plots of age, sex and race adjusted mortality rates appeared linear and did not show a higher death rate with higher solute concentrations (Figure S1). In unadjusted and sequentially adjusted Cox models (Table 2), there were no associations between p-cresol sulfate, indoxyl sulfate, hippurate or phenylacetylglutamine and any of the outcomes. Table 3 presents the minimum hazard ratio that could be detected in this study with 90% power and alpha of 0.05, given the observed number of events and the correlation between solutes and other covariates. The study had at least 90% power to detect a hazard ratio for cardiac death of 1.19 for p-cresol sulfate, 1.29 for indoxyl sulfate, 1.19 for hippurate and 1.26 for phenylacetylglutamine. Prespecified sub-group analyses are presented in Table S2-S5. The results should be interpreted with caution due to multiple comparisons and a p-value of 0.05/11=0.004 is suggested as a significant interaction between the groups. Using this threshold, among those with serum albumin below median (<3.6 g/dL), p-cresol sulfate was associated with higher risk of cardiac death (HR per 2-fold increase, 1.12; 95% CI, 0.98–1.27; p-interaction <0.001) and sudden cardiac death (HR per 2-fold increase, 1.22; 95% CI, 1.06–1.41; p-interaction <0.001). Similar trend was noted for indoxyl sulfate in patients with serum albumin <3.6 g/dL (HR for cardiac death per 2-fold increase, 1.09; 95% CI, 0.91–1.31; p-interaction 0.003). There were also

trends towards higher risk of death with p-cresol sulfate and indoxyl sulfate in patients without diabetes.

Other Analyses

Analysis of solutes modeled as quintiles yielded results similar to the primary analyses (Table S6). Further adjustment of the outcomes model for trimethylamine-N-oxide (TMAO), asymmetric and symmetric dimethylarginines (ADMA and SDMA) did not change the associations (Table 2). Table 4 presents the results of univariate and multivariate cross-sectional associations of the solutes. In multivariate model, using forward selection (p entry ≤ 0.05), there was no consistent pattern of variables associated with all solutes. Importantly, Kt/Vurea and treatment time did not predict solute concentrations.

DISCUSSION

In this study with measurement of four uremic solutes in samples from 1,273 prevalent hemodialysis patients participating in a national, multicenter trial in the US, we found no association between predialysis p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine and adjudicated cardiovascular outcomes or all-cause mortality. In subgroup analyses, among patients with low serum albumin, p-cresol sulfate was associated with a 12% higher risk of cardiac death and 22% higher risk of sudden cardiac death, whereas indoxyl sulfate was associated with a 9% higher risk of cardiac death and 8% risk of sudden cardiac death.

P-cresol sulfate and indoxyl sulfate are among the most well studied uremic toxins. P-cresol sulfate has been reported to induce vasculotoxicity by a number of mechanisms, including endothelial dysfunction and leukocyte activation.[4] Indoxyl sulfate has been reported to have toxic effects on renal tubular cells as well as on the vasculature. [4] Indoxyl sulfate could contribute to morbid events in dialysis patients by causing loss of residual kidney function (RKF), which is strongly associated with mortality.[9, 10] Since both solutes are predominantly removed by tubular secretion, RKF is a major confounder in the observational studies of these solutes and outcomes. As the HEMO Study included only prevalent patients and excluded patients with significant RKF (residual urea clearance >1.5 ml/min per 35L total body water) it may have avoided this confounding.

We noted that patients with diabetes had higher p-cresol sulfate levels compared to patients without diabetes, in both univariate and multivariable models (Table 4). The association of diabetes with higher p-cresol concentrations mirrors previous findings.[11–13] Similar to a prior study, although p-cresol concentrations were higher in patients with diabetes, the risk of cardiovascular outcomes with p-cresol sulfate was higher in patients without diabetes compared to patients with diabetes (p-interaction 0.04 for cardiac death and 0.06 for sudden cardiac death). Due to multiple comparisons, we consider these findings as hypothesis generating. The mechanism of higher p-cresol sulfate in patients with diabetes in not known. As p-cresol is generated from the gut microbiome, alteration of the microbiome in patients with diabetes, either due to diabetes itself[14] or the effect of antidiabetic medications[15], could play a role.

Our study is the largest measurement of p-cresol sulfate, indoxyl sulfate, hippurate and phenylacetylglutamine in a hemodialysis cohort. Our findings of the overall lack of association between these solutes and outcomes despite adequate statistical power (Table 3) contradict some prior epidemiological studies of chronic kidney disease and dialysis patients,[13, 16] including some of our own prior work.[5] We previously reported that among incident hemodialysis participants of the Choices for Healthy Outcomes in Caring for ESRD (CHOICE) Study, free (but not total) p-cresol sulfate and free phenylacetylglutamine were associated with cardiovascular mortality, whereas there was no association with outcomes noted with total or free indoxyl sulfate.[5, 17] Although in our HEMO analyses, the overall associations of p-cresol sulfate and indoxyl sulfate with mortality were negative, we noticed statistically significant interactions in our sub-group analyses. In particular, total p-cresol sulfate (Table S2) and indoxyl sulfate (Table S3) were associated with cardiac death and sudden cardiac death in patients with lower serum albumin (<3.6 g/dL), even though lower serum albumin was associated with lower levels of these solutes (Table 4).

The protein-binding of p-cresol sulfate and indoxyl sulfate is an important consideration for the interpretation of our results. Both p-cresol sulfate and indoxyl sulfate are largely protein-bound. Free p-cresol sulfate and indoxyl sulfate levels may be a better indicator for potential toxicity of uremic solutes as tissues are exposed to the free solutes. However, the free levels are also more likely to be influenced by other biological factors such as the presence of other unmeasured protein-bound solutes that displace p-cresol sulfate and indoxyl sulfate from their binding sites, leading to higher free p-cresol sulfate and indoxyl sulfate levels. These unmeasured solutes could be either directly toxic or exert toxicity via increasing free levels of p-cresol sulfate and indoxyl sulfate. For example, Dou et al. reported that in 120 patients with chronic kidney disease (73 treated with hemodialysis), higher indole-3 acetic acid was associated with cardiovascular events, whereas, p-cresol sulfate and indoxyl sulfate were not associated with outcomes.[18] Indole-3 acetic acid is a protein-bound solute produced by gut microbiome from tryptophan (similar to indoxyl sulfate) and demonstrates in vitro endothelial toxicity. The in vivo toxicity of protein-bound solutes such as indole-3 acetic acid could be either from direct toxicity, or indirect effects via increasing free levels of other protein-bound solutes, or both. The net effect of these factors would be to induce uncontrolled confounding in an epidemiological study. Although we measured both total and free concentrations of these solutes, our measurements of free fractions in some HEMO samples were in excess compared with samples obtained locally, as we have previously described.[19] We presume but cannot prove that this effect could have been due to sample collection in HEMO after heparin administration. Heparin administration prior to sample collection can increase free solute levels in samples tubes above in vivo levels.[20] This artifactual increase is due to release of lipolytic enzymes from capillary endothelium into plasma, increasing free fatty acid release which displaces solutes (and drugs) from their protein binding sites. Therefore, we have related outcomes to total solute concentrations. Results for free solutes are included in supplemental materials (Table S7 to S12) but their interpretation is limited.

There are other limitations to our study. Solutes were measured at single time point and their concentration may change over time. Although the HEMO Study was a large national trial with 15 clinical centers and 72 dialysis clinics, the eligibility criteria excluded patients with

serum albumin <2.6 g/dL and inability to achieve high dose (spKt/V=1.7) over a 4.5-hour treatment; the latter criterion resulted in exclusion of very heavy patients and 97% of the patients weighed <100 kg. Due to inclusion of mostly urban clinical centers, the study population was 63% Black, a higher proportion than the general population of hemodialysis patients. Although patients with diabetes and cardiac disease were well-represented and the death rate was similar to the general population of hemodialysis patients, the eligibility criteria could have introduced selection bias reducing the generalizability of our findings. These limitations are balanced by major strengths of the study that includes a large, national, prospective design, exclusion of patients with significant RKF, careful collection of samples, long duration of follow-up and carefully adjudicated cardiovascular outcomes.

In conclusion, p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine were not associated with CV outcomes or death in 1,273 prevalent hemodialysis patients participating in the HEMO Study. However, subgroup analyses suggested a higher risk of cardiac death and sudden cardiac death with higher p-cresol sulfate and indoxyl sulfate levels in patients with low serum albumin levels. The concentrations of these solutes are dramatically elevated in dialysis patients and the 30% higher Kt/Vurea achieved in HEMO did not markedly reduce their concentrations so that a threshold effect may be responsible for the lack of observed associations. As there is strong scientific rationale for toxicity of p-cresol sulfate and indoxyl sulfate, only a randomized controlled trial that targets lowering of these solutes can resolve the question of uremic toxicity from these solutes.

METHODS

Study Design

The HEMO Study was a clinical trial that randomized 1846 prevalent hemodialysis patients to standard or high dialyzer urea clearance (assessed by Kt/V_{UREA}, an index of urea clearance by dialysis) and to low-flux or high-flux dialysis membranes (assessed by β2-microglobulin clearance).[8, 21] The patients were enrolled from May 1995 to February 2001 from 15 clinical centers in the US comprising 72 dialysis units and followed for outcomes until death, kidney transplantation or end of study in December 2001. Major exclusion criteria included residual urea clearance >1.5 mL/min/35 L urea volume of distribution, unstable angina, active systemic infection, New York Heart Association class IV congestive heart failure and severe hypoalbuminemia (<2.6 g/dL). Our study sample included 1,273 HEMO study participants that had available predialysis serum samples collected between 3 to 6 months post-randomization. We selected this time-point as it allowed adequate separation of uremic solutes between the trial intervention arms. The serum samples used in the study were collected at the time of the monthly kinetic modeling session. The samples were stored in the central repository at -80°C until they were shipped to Stan ford University for analyses. The participating institutions' institution review boards reviewed and approved the study. The Johns Hopkins Medicine Institutional Review Board reviewed and approved this study.

Data Collection

Laboratory Measurements—We measured total p-cresol sulfate, indoxyl sulfate, hippurate, and phenylacetylglutamine by stable isotope dilution LC/MC/MS as previously described.[19] The coefficient of variation for quality control samples run with each assay for total solute concentration was 3% for p-cresol sulfate, 9% for indoxyl sulfate, 6% for hippurate, and 6% for phenylacetylglutamine. For other laboratory tests including urea, albumin and β2-microglobulin, we used data collected as part of the HEMO Study. Details of trimethylamine-N-oxide (TMAO), asymmetric and symmetric dimethylarginines (ADMA and SDMA) measurements have been previously described.[19, 22]

Outcomes—The primary outcomes for our analyses were cardiac death, sudden cardiac death and first cardiovascular event (composite of first cardiovascular hospitalization or death from any cause), as defined in the HEMO Study. Secondary outcome was all-cause mortality. Cardiac death included deaths due to coronary events, heart failure, arrhythmias and other heart diseases and conditions. Sudden cardiac death was defined as a witnessed death with preceding duration of symptoms less than 24 hours or unwitnessed unexpected death with symptom duration less than the interval since the last dialysis session.[23] Cardiovascular hospitalizations were defined as hospitalizations for ischemic heart disease, heart failure, arrhythmias, other cardiac conditions, hypertension and peripheral vascular disease. Causes for death and hospitalizations in HEMO Study were adjudicated by an outcomes committee that was unaware of treatment-group assignments.[24]

Other Covariates—Demographics and clinical information was available for all participants at baseline. For comorbidity assessment, we used the Index of Coexisting Disease (ICED) score which was assessed by chart abstraction by trained nurses at baseline and then annually. The final ICED score ranges from 0 to 3 with higher numbers indicating greater comorbidity. We assessed dietary information which was collected at baseline and then annually using 2-day assisted recall. We assessed residual kidney function at baseline from a timed urine collection with measurement of urinary urea clearance. We used data for systolic blood pressure, weight and volume removed on dialysis collected as per the dialysis unit routine and recorded on the monthly HEMO kinetic modelling day, the same date as the blood sample collection. We calculated relative volume removed as predialysis weight minus post dialysis weight divided by predialysis weight and body mass index as target weight in kg divided by height in m^2. We used data for Kt/Vurea and normalized protein catabolic rate (an index of protein intake) provided in the HEMO database. Variables measured at baseline included: age, sex, race, ICED score, cause of end-stage renal disease, residual kidney function, and nutritional parameters (adjusted protein intake, fat percent, and carbohydrate percent). Variables measured at the same time point as solutes included: body mass index, systolic blood pressure, relative volume removed on dialysis, serum albumin, and nPCR.

Statistical Analysis

We analyzed the baseline characteristics of the participants overall and compared difference in included and excluded participants using chi-squared test for categorical variables and linear regression for continuous variables. Covariates with missing values included race (0.1%) cause of end-stage renal disease (2.3%), systolic blood pressure (0.1%), albumin

(0.5%), and residual kidney function (0.1%). To avoid listwise deletion,[25] we imputed missing data with 10 data replicates. We censored participants at kidney transplantation or end of the study for mortality analyses and also for transfer to non-participating clinical centers for hospitalization analyses, as the hospitalization information was not collected after transfer. For survival analyses, we set the time origin as the date of dialysis initiation with at-risk time starting at the date of sample collection (left censoring; accounts for duration of dialysis prior to enrollment). We visualized the functional form of minimally adjusted association between the solutes and outcomes by calculating age, sex and race adjusted incidence rates using a Poisson regression model with the solutes modeled as restricted cubic spline (5 knots). We used Cox proportional hazards models to analyze the association between the solutes and outcomes modeling the solutes as a natural log. We checked proportionality assumptions by Schoenfeld residual plots. We adjusted the Cox models for the following prespecified factors: age, sex, race, ICED score, cause of end-stage renal disease, body mass index (categorized as <18, 18 to 25 and >25 kg/m^2), systolic blood pressure categorized as <130, 130–160 and >160 mm Hg, relative volume removed, serum albumin and residual kidney function (urinary standard Kt/V_{UREA} calculated from urinary urea clearance). To assess the power for observed associations, we calculated the minimum detectable hazard ratio with 90% power and alpha of 0.05 for these analyses. We prespecified the following subgroup analyses: age (above or below median), sex, race (Blacks versus non-Blacks), diabetes, cardiac disease, gastrointestinal disease, BMI (<18 or 18 to 25 or >25), albumin (above or below median), residual kidney function (any versus none) and trial interventions. In additional analyses, we analyzed solutes as quintiles, explored further adjustment for TMAO, ADMA, and SDMA, and determined predictors of the solutes concentrations using univariate and multivariate linear regression. We considered two sided $p<0.05$ as statistically significant. We conducted all analyses using SAS 9.4 (SAS Institute Inc., Cary, NC) and STATA 13.0.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline Characteristics of the 1273 HEMO Participants

Characteristics	Results
Total P-Cresol Sulfate, mg/dL	
Mean ± SD	3.3 ± 1.7
Median [25th to 75th percentiles]	3.3 [2.2–4.4]
Total Indoxyl Sulfate, mg/dL	
Mean ± SD	2.5 ± 1.2
Median [25th to 75th percentiles]	2.4 [1.7–3.3]
Total Hippurate, mg/dL	
Mean ± SD	5.4 ± 4.3
Median [25th to 75th percentiles]	4.5 [2.3–7.4]
Total Phenylacetylglutamine, mg/dL	
Mean ± SD	4.5 ± 2.8
Median [25th to 75th percentiles]	4.0 [2.5–6.0]
Demographics	
Age, years	57.5 ± 14.0
Sex, Female %	723 (56.8)
Race, Black %	799 (62.8)
Clinical Characteristics	
Diabetes, %	574 (45.1)
Cardiac Disease, %	1003 (78.8)
ICED Score, %	2.0 ± 0.8
Gastrointestinal Disease, %	477 (37.5)
Residual Kidney Urea Clearance, ml/min/35L TBW	0.3 ± 0.5
Body Mass Index, Kg/m^2 †	25.7 ± 5.4
Body Surface Area, m^2 †	1.8 ± 0.2
Dialysis Characteristics	
Years of prior dialysis	3.5 ± 4.1

Characteristics	Results
Predialysis Systolic Blood Pressure, mm Hg †	152.4 ± 25.8
Post Dialysis Weight, Kg †	70.0 ± 15.3
Relative Volume Removed, % †	4.1 ± 1.7
Dose Intervention	633 (49.7)
Flux Intervention	632 (49.6)
Treatment Time, min †	206.8 ± 28.3
Blood Flow Rate, ml/min †	343.0 ± 60.7
Dialysate Flow Rate, ml/min †	673.3 ± 129.8
Predialysis Laboratory Tests	
Blood Urea Nitrogen, mg/dL †	59.7 ± 18.8
Single-pool Kt/V_{UREA} †	1.5 ± 0.3
Serum albumin, g/dL †	3.6 ± 0.4
Serum β2-microglobulin, mg/L †	36.7 ± 14.2
Nutritional Parameters	
Equilibrated nPCR, g/kg/day †	1.0 ± 0.3
Adjusted protein intake, g/kg/day (ABW) *	0.9 ± 0.3
Fat, %	35.5 ± 7.6
Carbohydrate, %	48.4 ± 9.3

Abbreviations: SE, Standard Error; ICED, Index of Coexistent Disease; Equilibrated nPCR, equilibrated normalized protein catabolic rate; TBW, total body water; SD, Standard Deviation; IQR, Interquartile Range [25th to 75th percentiles]; ABW, adjusted body weight

Data are presented as Mean ± SD or Median [IQR] for continuous variables and N (%) for categorical variables.

† Measured at the same time as solutes. Remaining variables are measured at baseline.

* If body weight was <90% or >120% of median standard body weight (SBW) as determined from the National Health and Nutrition Examination Survey II (NHANES II) data then protein intakes were normalized to an adjusted body weight (ABW) to standardize nutrient intake using the formula: ABW = ([Actual weight - SBW)] × 0.25) + SBW.[26]

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Table 2

Association of Uremic Solutes with Outcomes in the Hemodialysis Study

	Model 1		Model 2		Model 3		Model 4 (Final)		Model 5 (Additional Analyses)	
	Unadjusted		Adjusted: Age, Sex, Race		Adjusted: Model 2 + Comorbidity + Clinical + Labs + Residual Kidney Function		Adjusted: Model 3 + Nutritional Parameters		Adjusted: Model 4 + TMAO + ADMA + SDMA	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Cardiac Death (Events=221; IR=67.5)										
PCS	0.97 (0.88–1.08)	0.59	0.97 (0.89–1.07)	0.55	0.98 (0.90–1.07)	0.7	0.98 (0.89–1.07)	0.62	0.99 (0.91–1.07)	0.82
IS	0.88 (0.76–1.03)	0.11	0.91 (0.77–1.07)	0.24	0.96 (0.81–1.14)	0.64	0.95 (0.81–1.11)	0.52	0.94 (0.80–1.11)	0.47
HIPP	1.05 (0.93–1.18)	0.42	1.06 (0.96–1.18)	0.25	1.09 (0.98–1.22)	0.1	1.10 (0.99–1.22)	0.09	1.09 (0.98–1.22)	0.11
PAG	**1.18 (1.02–1.36)**	**0.03**	**1.17 (1.01–1.36)**	**0.03**	1.09 (0.94–1.26)	0.24	1.08 (0.93–1.26)	0.32	1.09 (0.94–1.26)	0.24
Sudden Cardiac Death (Events=127; IR=38.8)										
PCS	0.96 (0.84–1.11)	0.62	0.97 (0.86–1.10)	0.66	0.99 (0.88–1.10)	0.8	0.99 (0.88–1.12)	0.91	1.00 (0.90–1.11)	0.97
IS	0.86 (0.70–1.04)	0.12	0.88 (0.71–1.08)	0.22	0.94 (0.76–1.18)	0.61	0.95 (0.77–1.17)	0.63	0.93 (0.75–1.14)	0.46
HIPP	0.99 (0.87–1.13)	0.91	1.00 (0.90–1.12)	0.95	1.03 (0.91–1.17)	0.61	1.05 (0.93–1.18)	0.45	1.03 (0.92–1.17)	0.58
PAG	1.17 (0.95–1.43)	0.15	1.16 (0.95–1.43)	0.15	1.08 (0.90–1.30)	0.41	1.10 (0.89–1.35)	0.36	1.07 (0.88–1.32)	0.49
First CV Event (Events=641; IR=273.3)										
PCS	**0.95 (0.91–0.99)**	**0.03**	**0.96 (0.92–0.99)**	**0.01**	0.98 (0.94–1.01)	0.14	0.98 (0.94–1.02)	0.24	0.97 (0.93–1.01)	0.16
IS	**0.91 (0.86–0.97)**	**0.005**	0.95 (0.88–1.01)	0.12	0.99 (0.92–1.07)	0.8	0.99 (0.92–1.07)	0.83	0.99 (0.91–1.07)	0.74
HIPP	0.96 (0.91–1.01)	0.11	0.97 (0.93–1.02)	0.21	0.98 (0.94–1.03)	0.48	0.99 (0.95–1.04)	0.73	0.98 (0.94–1.03)	0.42
PAG	1.04 (0.97–1.11)	0.31	1.03 (0.96–1.10)	0.46	0.99 (0.92–1.05)	0.65	0.99 (0.92–1.06)	0.71	0.98 (0.91–1.05)	0.5
Any-Cause Death (Events=563; IR=171.8)										
PCS	**0.94 (0.89–0.99)**	**0.02**	**0.93 (0.90–0.97)**	**0.002**	**0.96 (0.92–1.00)**	**0.05**	0.96 (0.92–1.01)	0.1	0.96 (0.92–1.01)	0.13
IS	**0.88 (0.82–0.95)**	**<0.001**	**0.90 (0.83–0.97)**	**0.006**	0.97 (0.90–1.05)	0.43	0.98 (0.91–1.06)	0.59	0.97 (0.90–1.05)	0.5
HIPP	0.96 (0.89–1.04)	0.36	0.98 (0.92–1.05)	0.58	1.03 (0.97–1.10)	0.33	1.04 (0.98–1.11)	0.17	1.04 (0.98–1.10)	0.24
PAG	**1.09 (1.00–1.19)**	**0.04**	**1.09 (1.00–1.18)**	**0.04**	1.05 (0.97–1.13)	0.21	1.06 (0.98–1.15)	0.12	1.06 (0.97–1.16)	0.17

Abbreviation: IR, Incidence Rate per 1,000 person-years; HR, Hazard Ratio; CI, Confidence Interval; PCS, P-Cresol Sulfate; IS, Indoxyl Sulfate; HIPP, Hippurate; PAG, Phenylacetylglutamine; TMAO, Trimethylamine-N-Oxide; ADMA, Asymmetric Dimethylarginine; SDMA, Symmetric Dimethylarginine

HR represents increase in risk per 2-fold increase in solute concentrations. Modeled as natural log transformed variable/natural log of 2.

Model 1 was unadjusted.
Model 2 adjusted for age, sex and race.

Model 3 adjusted for variables in Model 2 + Index of Coexisting Disease (ICED) severity score, cause of end-stage renal disease, body mass index (categorized as <18, 18 to 25 and >25 kg/m^2), systolic blood pressure (categorized as <130, 130–160 and >160 mm Hg), albumin, relative volume removed on dialysis, and residual kidney function (urinary stdKt/V_{UREA} calculated from urinary urea clearance).
Model 4 adjusted for variables in Model 3 + nPCR, adjusted protein intake, g/kg/day (ABW), fat percent, CHO percent.
Model 5 adjusted for variables in Model 4 + TMAO, ADMA and SDMA.

Note: Variables measured at baseline: age, sex, race, Index of Coexisting Disease (ICED) severity score, cause of end-stage renal disease, residual kidney function (urinary stdKt/V_{UREA} calculated from urinary urea clearance), adjusted protein intake, g/kg/day (adjusted body weight), fat percent, and carbohydrate percent. Variables measured at the same time point as solutes: body mass index (categorized as <18, 18 to 25 and >25 kg/m^2), systolic blood pressure (categorized as <130, 130–160 and >160 mm Hg), albumin, relative volume removed on dialysis, and nPCR.

Table 3

Minimum Detectable Hazard Ratio of the Association between Solutes and Outcomes

		Cardiac Death	Sudden Cardiac Death	First CV Event	Any-Cause Death
Number of Events		221	127	641	563
Event Probability		17.4%	10.0%	54.2%	44.2%
Solutes	Standard Deviation	HR	HR	HR	HR
PCS	1.351	1.19	1.25	1.14	1.11
IS	0.930	1.29	1.40	1.22	1.17
HIPP	1.369	1.19	1.26	1.15	1.12
PAG	1.031	1.26	1.35	1.20	1.15

Abbreviation: HR, Hazard Ratio; PCS, P-Cresol Sulfate; IS, Indoxyl Sulfate; HIPP, Hippurate; PAG, Phenylacetylglutamine

Note: The minimum detectable hazard ratio per 2-fold increase in solute was calculated assuming 90% power and alpha=0.05. Observed standard deviation of the solutes and R^2 from the linear regression of solutes on predictors in the fully adjusted model (Model 4) were calculated from the data.

Note: Variables measured at baseline: age, sex, race, Index of Coexisting Disease (ICED) severity score, cause of end-stage renal disease, residual kidney function (urinary stdKt/V_{UREA} calculated from urinary urea clearance), adjusted protein intake, g/kg/day (ABW), fat percent, and CHO percent. Variables measured at the same timepoint as solutes: body mass index (categorized as <18, 18 to 25 and >25 kg/m^2), systolic blood pressure (categorized as <130, 130–160 and >160 mm Hg), albumin, relative volume removed on dialysis, and nPCR.

Table 4

Predictors of Total Solutes in 1273 Patients of the HEMO Study

		Total PCS (mg/dL)				Total IS (mg/dL)				Total HIPP (mg/dL)				Total PAG (mg/dL)			
		Mean (SD) = 3.3 (1.7)				Mean (SD) = 2.5 (1.2)				Mean (SD) = 5.4 (4.3)				Mean (SD) = 4.5 (2.8)			
		Median [IQR] = 3.3 [2.2–4.4]				Median [IQR] = 2.4 [1.7–3.3]				Median [IQR] = 4.5 [2.3–7.4]				Median [IQR] = 4.0 [2.5–6.0]			
		Univariate		Multivariable		Univariate		Multivariable		Univariate		Multivariable		Univariate		Multivariable	
Characteristics	**Modeling**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Demographic																	
Age, years	Per 10 year higher	0.066 (0.034)	0.06	—	—	−0.094 (0.024)	**<.001**	—	—	−0.247 (0.085)	**0.004**	—	—	0.051 (0.057)	0.4	0.165 (0.054)	**0.002**
Sex, Female %	Female vs. Male	0.051 (0.097)	0.6	—	—	−0.319 (0.068)	**<.001**	−0.180 (0.064)	**0.005**	−0.345 (0.241)	0.2	—	—	0.215 (0.161)	0.2	0.350 (0.147)	**0.02**
Race, Black %	Black vs. Non-Black	0.211 (0.099)	**0.03**	0.296 (0.095)	**0.002**	0.079 (0.070)	0.3	—	—	−0.840 (0.246)	**<.001**	−0.879 (0.239)	**<.001**	−0.064 (0.165)	0.7	—	—
Clinical Characteristics																	
Diabetes, %	Yes vs. No	0.604 (0.095)	**<.001**	0.695 (0.096)	**<.001**	−0.342 (0.068)	**<.001**	—	—	−0.667 (0.240)	**0.005**	—	—	0.591 (0.159)	**<.001**	0.833 (0.155)	**<.001**
Cardiac Disease, %	Yes vs. No	0.012 (0.117)	0.9	—	—	−0.168 (0.083)	**0.04**	—	—	−0.730 (0.292)	**0.01**	—	—	0.137 (0.195)	0.5	—	—
ICED Score, %	<3 vs. 3	−0.016 (0.102)	0.9	—	—	−0.255 (0.072)	**<.001**	—	—	−0.370 (0.255)	0.1	—	—	0.379 (0.170)	**0.03**	—	—
Gastrointestinal Disease, %	Yes vs. No	−0.000 (0.099)	0.9	—	—	−0.212 (0.070)	**0.002**	—	—	−0.061 (0.247)	0.8	—	—	0.378 (0.164)	**0.02**	0.436 (0.151)	**0.004**
Residual Kidney Urea Clearance, ml/min/35L TBW	Per 0.5 mL/min/35 L TBW higher	0.074 (0.049)	0.1	—	—	−0.197 (0.034)	**<.001**	−0.130 (0.033)	**<.001**	−0.852 (0.119)	**<.001**	−0.599 (0.121)	**<.001**	−0.473 (0.080)	**<.001**	−0.246 (0.076)	**0.001**
Body Mass Index, Kg/m^2 [3]	≥25 Kg/m^2 vs. <25 Kg/m^2	0.066 (0.096)	0.5	—	—	−0.088 (0.068)	0.2	—	—	−0.230 (0.239)	0.3	—	—	−0.175 (0.160)	0.3	−0.319 (0.150)	**0.03**
Body Surface Area, m^2 [3]	Per 0.5 m^2 higher	0.218 (0.121)	0.07	—	—	0.169 (0.086)	**0.05**	—	—	−0.185 (0.303)	0.5	—	—	−0.118 (0.201)	0.6	—	—
Dialysis Characteristics																	
Years of prior dialysis	Per 1 year higher	−0.054 (0.011)	**<.001**	−0.038 (0.011)	**0.001**	0.037 (0.008)	**<.001**	—	—	0.141 (0.029)	**<.001**	—	—	0.039 (0.019)	**0.04**	—	—
Predialysis Systolic Blood Pressure, mm Hg [3]	Per 10 mm Hg higher	0.034 (0.019)	0.07	—	—	−0.000 (0.013)	0.9	—	—	−0.010 (0.046)	0.8	—	—	0.070 (0.031)	**0.02**	—	—
Post Dialysis Weight, Kg [†]	Per 10 kg higher	0.048 (0.031)	0.1	—	—	0.024 (0.022)	0.3	—	—	−0.049 (0.078)	0.5	—	—	−0.050 (0.052)	0.3	—	—
Relative Volume Removed, % [†]	Per 1% higher	−0.016 (0.028)	0.6	−0.098 (0.028)	**<.001**	0.023 (0.020)	0.2	—	—	0.412 (0.070)	**<.001**	0.222 (0.070)	**0.002**	0.139 (0.047)	**0.003**	—	—
Dose Intervention	High Dose vs. Standard Dose	0.061 (0.096)	0.5	0.358 (0.107)	**<.001**	−0.303 (0.068)	**<.001**	—	—	−0.217 (0.239)	0.4	—	—	−0.318 (0.159)	**0.05**	—	—
Flux Intervention	High Flux vs. Low Flux	−0.030 (0.096)	0.8	—	—	−0.075 (0.068)	0.3	—	—	−0.287 (0.239)	0.2	—	—	−0.339 (0.159)	**0.03**	—	—

		Total PCS (mg/dL)				Total IS (mg/dL)				Total HIPP (mg/dL)				Total PAG (mg/dL)			
		Mean (SD) = 3.3 (1.7)				Mean (SD) = 2.5 (1.2)				Mean (SD) = 5.4 (4.3)				Mean (SD) = 4.5 (2.8)			
		Median [IQR] = 3.3 [2.2–4.4]				Median [IQR] = 2.4 [1.7–3.3]				Median [IQR] = 4.5 [2.3–7.4]				Median [IQR] = 4.0 [2.5–6.0]			
		Univariate		Multivariable		Univariate		Multivariable		Univariate		Multivariable		Univariate		Multivariable	
Characteristics	**Modeling**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Treatment Time, min †	Per 30 minute higher	−0.010 (0.051)	0.9	−0.114 (0.056)	**0.04**	−0.060 (0.036)	0.1	—	—	−0.106 (0.127)	0.4	—	—	−0.131 (0.085)	0.1	—	—
Blood Flow Rate, ml/min †	Per 50 ml/min higher	0.064 (0.039)	0.1	—	—	−0.029 (0.028)	0.3	—	—	−0.119 (0.098)	0.2	—	—	−0.191 (0.066)	**0.004**	—	—
Dialysate Flow Rate, ml/min †	Per 100 ml/min higher	−0.012 (0.037)	0.7	—	—	−0.047 (0.026)	0.08	—	—	−0.028 (0.092)	0.8	—	—	−0.079 (0.062)	0.2	—	—
Predialysis Laboratory Tests																	
Blood Urea Nitrogen, mg/dL †	Per 10 mg/dL higher	0.224 (0.025)	**<.001**	0.235 (0.026)	**<.001**	0.211 (0.017)	**<.001**	0.176 (0.017)	**<.001**	0.489 (0.062)	**<.001**	0.358 (0.063)	**<.001**	0.460 (0.041)	**<.001**	0.471 (0.038)	**<.001**
Single-pool Kt/V_{UREA} †	Per 0.2 higher	0.016 (0.035)	0.6	—	—	−0.082 (0.024)	**<.001**	—	—	0.005 (0.087)	0.9	—	—	−0.031 (0.057)	0.6	—	—
Serum albumin, g/dL †	Per 0.5 g/dL higher	0.344 (0.061)	**<.001**	0.362 (0.061)	**<.001**	0.427 (0.043)	**<.001**	0.331 (0.041)	**<.001**	0.962 (0.153)	**<.001**	0.721 (0.150)	**<.001**	0.070 (0.104)	0.5	—	—
Serum β2-microglobulin, mg/L †	Per 10 mg/L higher	−0.080 (0.034)	**0.02**	—	—	0.208 (0.024)	**<.001**	0.163 (0.023)	**<.001**	0.695 (0.084)	**<.001**	0.553 (0.084)	**<.001**	0.465 (0.056)	**<.001**	0.486 (0.054)	**<.001**
Nutritional Data																	
Equilibrated nPCR, g/kg/day †	Per 0.2 g/kg/day higher	0.299 (0.036)	**<.001**	—	—	0.241 (0.025)	**<.001**	—	—	0.665 (0.090)	**<.001**	—	—	0.597 (0.058)	**<.001**	—	—
Adjusted protein intake, g/kg/day (ABW) *	Per 10 g/kg/day higher	−1.208 (1.378)	0.38	—	—	1.379 (0.980)	0.16	—	—	2.351 (3.442)	0.49	—	—	1.115 (2.297)	0.63	—	—
Fat, %	Per 10% higher	0.047 (0.063)	0.46	—	—	−0.009 (0.045)	0.85	—	—	−0.434 (0.156)	0.005	—	—	0.081 (0.104)	0.44	—	—
Carbohydrate, %	Per 10% higher	−0.102 (0.051)	**0.05**	—	—	0.012 (0.037)	0.75	—	—	0.528 (0.128)	**<.001**	0.472 (0.124)	**<.001**	−0.092 (0.086)	0.28	—	—
Multivariable Model Characteristics																	
Root mean square error (RMSE)	—	—	—	2.48	—	—	—	1.15	—	—	—	15.52	—	—	—	6.15	—
R^2	—	—	—	14.9%	—	—	—	22.2%	—	—	—	15.9%	—	—	—	20.3%	—

Abbreviations: PCS, P-cresol Sulfate; IS, Indoxyl Sulfate, HIPP, Hippurate; PAG, Phenylacetylglutamine; SE, Standard Error; ICED, Index of Coexistent Disease; Equilibrated nPCR, equilibrated normalized protein catabolic rate; TBW, total body water; ABW, adjusted body weight

Note: βcoefficients are from linear regression of PCS, IS, HIPP or PAG (natural scale) on predictors in separate models (univariate associations) and in a forward selection model with model entry specified as p≤0.05 (multivariable model). Interpretation of β coefficients: change in value of solute per modeled change in predictor. Positive values mean higher solute concentration and negative values mean lower solute concentration.

† Measured at the same time as solutes. Remaining variables are measured at baseline.

* If body weight was <90% or >120% of median standard body weight (SBW) as determined from the National Health and Nutrition Examination Survey II (NHANES II) data then protein intakes were normalized to an adjusted body weight (ABW) to standardize nutrient intake using the formula: ABW = ([Actual weight - SBW)] × 0.25) + SBW.[26]