Renal tubular secretion in chronic kidney disease: description, determinants, and outcomes

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ABSTRACT

Renal tubular secretion in chronic kidney disease:
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Background The presence and severity of chronic kidney disease are currently assessed by glomerular filtration rate and urinary albumin excretion. Other kidney functions, such as proximal tubular secretion, are not typically quantified. Tubular secretion is capable of clearing metabolites from the blood more efficiently than filtration, suggesting important clinical consequences of secretion dysfunction. Measuring tubular secretion as an independent marker of kidney function may provide insight into kidney disease etiology and prediction of adverse outcomes.

Methods We developed and validated mass spectrometry assays to measure hippurate and cinnamoylglycine, which are primarily cleared by renal tubular secretion. We estimated secretion function in a prospective cohort study of 298 CKD patients using timed urinary clearance of these molecules. We examined associations between renal secretion and estimated filtration (average of creatinine and urea clearance), related clinical characteristics, and mortality.

Results Tubular secretion correlated with glomerular filtration, but considerable residual variability remained. Tubular secretion was significantly greater among men. For a given level of filtration function, diminished hippurate clearance was associated with increased risk of death (hazard ratio comparing low to high secretion groups: 2.3, 95% confidence interval: 1.1-4.7). Diminished cinnamoylglycine clearance, for a given level of filtration, was associated with increased risk of starting dialysis (hazard ratio comparing low to high secretion groups: 4.5, 95% confidence interval: 1.4-14.4).

Conclusion Proximal tubular secretion function comprises a measurement of kidney function that modestly correlates with filtration function and may be associated with mortality and starting dialysis.

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Introduction

Glomerular filtration and urinary protein excretion are the measures of kidney function most widely used to evaluate chronic kidney disease (CKD) in clinic and research.[1-4] The glomerular filtration rate (GFR) defines CKD stage, informs optimal medication dosing, and predicts the risks of progression to end stage renal disease (ESRD), cardiovascular events, and death.[5-11] Urinary albumin excretion typically measured by the albumin to creatinine ratio (ACR) is a complementary measure of kidney disease severity that is additionally informative for prediction of ESRD and cardiovascular events.[5,6] Enhanced precision, accuracy, and predictive power afforded by use of multiple solute measures to estimate glomerular function further underscore the clinical importance of developing comprehensive measurements of kidney function.[8,9]

In additional to filtration and reclamation of filtered albumin, vital kidney functions include secretion of retained solutes and synthesis of the homeostatic hormones erythropoietin and calcitriol. In addition to GFR and ACR, a measure of proximal tubular secretion may serve as an independent marker of kidney function because secretion is an active solute elimination process that has high specificity for both endogenous and exogenous metabolites, including many drug products.[4,12-15] Despite the biological importance of proximal tubule secretion and its potential to represent a unique aspect of kidney function, routine measurement of secretion has not been utilized in clinical care or research because assessment is generally cumbersome and expensive.

We developed a tandem mass spectrometry assay to quantify the concentrations of hippurate and cinnamoylglycine, which are primarily cleared by proximal tubule secretion.[4,13,15,16] We estimated renal tubular secretion function as the clearance of these molecules using timed urine and serum samples from approximately 300 participants in a prospective cohort study of CKD patients. We compared estimated tubular secretion with glomerular filtration and urinary albumin excretion; evaluated associations with individual-level characteristics; and evaluated associations with CKD progression and death during follow-up.

Methods

Study Population

The Seattle Kidney Study (SKS) is a clinic-based, prospective cohort study of CKD in nearly 700 patients recruited from nephrology clinics at the Veteran's Administration Puget Sound Health Care System (VA), Harborview Medical Center (HMC), and University of Washington Medical Center (UWMC), in Seattle, WA. Patients were eligible if they were at least 18 years old and not receiving any form of chronic renal replacement therapy, including kidney transplant or dialysis. Exclusion criteria for SKS included dementia, residency in an institution, inability to provide informed consent, being non-English speaking, or expecting to initiate dialysis within 6 months. Inclusion criteria for the current study include the following: availability of serum and timed urine samples—many SKS subjects provided only spot urine samples; urine collection time spanning 8-36 hours total; per-day urine volume 0.25-10.0 liters; per-day creatinine excretion >8 mg/kg lean body mass (LBM) for women or >10 mg/kg LBM for men; albumin-creatinine ratio (ACR) <3000. For subjects with multiple eligible clinic visits, we assayed serum and urine samples from the first available date as that subject's baseline. The current study population consisted of 298 subjects.

Measurement of exposures

We quantified hippurate (HA) and cinnamoylglycine (CMG) using timed urine and serum samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We used isotope dilution with stable isotope-labeled internal standards and external calibration materials to allow maximal precision and accuracy in quantification of solute concentrations. Ion ratios were validated and monitored for specificity. Matrix effects were minimal at low concentrations of analytes for both urine and serum samples. We identified minimal detectible fractions for each analyte for sensitivity purposes, as serum concentrations would be expected to decrease and urine concentrations to increase in a setting of reduced kidney function.

The process of tandem mass spectrometry (MS/MS) involves several steps. First, the sample is eluted on a solid-phase chromatography column, to allow for a crude separation of various solutes ions, predominantly using molecule size and polarity to produce eluent over a given time; the selection of an appropriate elution column and the time period within which each solute is generally eluted was optimized during assay development. Second, the molecules are sprayed into a quadrupole mass spectrometer, where they are ionized during desolvation based on their chemical structure. Then, ion molecules undergo a specific energy-based collision with a gaseous carrier. Fragmentation follows, in highly specific manner, determined in part by the energy and that is optimized during the assay development step. These ion fragments are selected based on mass/charge ratio and sent through a second quadrupole, where they are further fragmented, allowing for final detection of a diagnostic daughter ion and crude quantification. Precise specification of molecule quantity is standardized to internal standard reagents, which are selected during the assay development stage.

We defined the clearance of solutes (HA, CMG) as the volume of blood fully cleared of solute X per unit time: $Clearance_X = XCl = (U_X * V) / P_X$, where U_X is the urinary concentration of solute X, P_X the plasma concentration of solute X, and V the urine volume over a given period of time.[17]

Measurement of outcomes

Deaths were identified via proxies during surveillance calls, during scheduling calls for annual visits, and by consulting the national Social Security death index. Study coordinators screened for hospitalizations and procedures semi-annually, at clinical exams and during interim telephone interviews. Major longitudinal analysis end points (death, ESRD, dialysis) were adjudicated in independent review by 2 nephrologists; assembled adjudication packets included hospital discharge summaries, imaging documents, consultation requests, laboratory results, and procedure reports.

Measurement of other study data

Enrolled SKS participants completed annual health questionnaires and clinical examinations. Participants self-reported age, gender, race (categorized in current study as white, black, or other), current smoking status, and current alcohol use. Study coordinators averaged three seated blood pressure readings at annual exams and also measured weight and height. Prevalent type 2 diabetes mellitus (T2DM) was assessed based on self report, use of insulin or hypoglycemic medications, fasting glucose levels ≥7.0 mmol/L, or random glucose levels ≥11.1 mmol/L. Urine albumin excretion rate (AER, mg/day) was measured using timed urine samples, and therefore used in analyses where possible; otherwise, the roughly equivalent measure of urine albumin creatinine ratio (mg/g) was used.[18] Combined cardiovascular disease (CVD) was identified using hospitalization and procedures reporting as for the major longitudinal outcomes, and included acute myocardial infarction, cardiac arrest, coronary artery bypass graft, percutaneous coronary intervention, stroke, transient ischemic attack, carotid endarterectomy, or heart failure. Use of medications was ascertained from the computerized pharmacy database for participants from VA and by direct transcription of medication bottle labels for participants from HMC. Participants submitted blood and timed urine samples at annual study visits, which were either assayed for standard clinical lab measures or frozen at -80^{o}C for future use.

Statistical analyses

All analyses were performed using Stata (v.13). When appropriate, the problem of multiple comparisons was addressed by comparing estimated P-values with a Bonferroni-corrected P-value.

Glomerular filtration was described as the average of urea and creatinine (Ur-Cr) clearance, to examine differences among different levels of tubular secretion function, standardized to a functional range-independent estimate of filtration-specific solute clearance.[19,20] Secretion function was

described as the clearance of secretion-specific solutes, hippuric acid (HA) and cinnamoylglycine (CMG). Secretion-beyond-filtration was evaluated based on a three-part categorization of the population, using quantile regressions of secretory solute (HA, CMG) clearance over filtered solute (Ur-Cr) clearance, at the 33rd and 67th percentiles, separating subjects into high, medium, and low secretion function per their on-average level of filtration function. Categorization was done separately for HA clearance and CMG clearance, relative to Ur-Cr clearance. Categorical regressions distinguish high (Category 3), medium (Category 2), and low (Category 1) secretion-beyond-filtration.

Crude measures of secretion (HA, CMG clearance) were directly compared with conventional markers of kidney function, specifically filtration function and injury, using Pearson's correlation coefficients. Filtration-standardized secretion measures (Category 1, Category 2, Category 3 categories of secretion-beyond-filtration) were evaluated in association with conventional demographic, behavioral, and clinical determinants of CKD as well as laboratory measures of conventional clinical consequences of CKD using category-stratified means and standard deviations, unadjusted linear tests for trend across categories, and logistic regressions with robust standard errors estimation, comparing the lowest category (Category 1) with the highest category (Category 3) of secretion, adjusted for age, sex, race, estimated GFR (2009 CKD EPI equation), and standardized daily creatinine excretion (24-hour creatinine, per kilogram of lean body mass). Supplemental linear regressions with similar adjustment schemes were used to maximize power. Adjustment for standardized daily creatinine excretion was done to account for the possibility of incomplete urine collection; since creatinine is excreted by muscle at a relatively consistent inter-individual rate, any observed variability in this measure is likely to be due to participant errors in recorded urine collection times. Continuous exposures (determinants) were modeled per standard deviation, to allow direct comparison of odds ratios across different factors.

Crude measures of secretion (on the $1/\log_2$ scale to allow interpretation of risk per halving of the exposure) and filtration-standardized secretion categories (comparing Category 1 & Category 2 with the referent Category 3) were evaluated in association with risk of long-term outcomes using Cox proportional hazards regressions with robust standard errors estimation. Outcomes included time to death from any cause, loss of >30% eGFR since baseline, or initiation of dialysis; sensitivity analyses also evaluated time to first of either loss of 30% GFR or initiation of dialysis. Censoring events included loss to follow-up, death, or end of study period (January 1, 2012). Additional sensitivity analyses evaluated initiation of dialysis with death included as a competing risk, using the subdistribution hazard method.[21] Adjustment for confounders included age, sex, race, body-size-standardized daily creatinine excretion, and filtration function; additional sensitivity analyses included adjustment for components of the Tangri model, which include age, sex, eGFR, macroalbuminuria, and serum calcium, phosphorus, bicarbonate, and albumin.[22] Final Cox PH regressions for death and dialysis outcomes directly compared two secretion measures (HA, CMG clearance) with filtration (Ur-Cr clearance) used nested models without adjustment (Model 1), with adjustment for the Tangri model components (Model 2), and with mutual adjustment (Model 3; adjustment variables modeled on the continuous, arithmetic scale). The proportional hazards assumption was confirmed using visual inspection of log-log plots.

Results

Participants in this study population were middle-aged (mean 59.6 years) and predominantly male (**Table 1**). The majority of participants has stage III-IV CKD; 53.0% had type 2 diabetes. Non-white races comprised over one-third of the cohort (black 26.2%, other non-white 9.1%). Estimated GFR (eGFR) based on both the CKD-EPI 2009 (creatinine only) and 2012 (creatinine & cystatin C) equations comprised similar ranges, with the majority of subjects between 28 and 63 mL/min; urine

albumin excretion rate (AER) was consistent with predominant albuminuria, with a median of 180.9 mg/day (microalbuminuria).

Table 1: Selected subject characteristics

	N = 298
Age (years), mean (SD)	59.6 (13.8)
Male gender, n (%)	236 (79.2%)
Black race, n (%)	78 (26.2%)
Other non-white race, n (%)	27 (9.1%)
Prevalent diabetes, n (%) †	158 (53.0)
Conventional Measures of Kidney Function:	
Average UR-CR clearance (mL/min), median (IQR)	47.5 (29.4-72.4)
Creatinine clearance, ml/min, median (IQR)	69.1 (44.7-107.0)
eGFR (CKDEPI 2009, mL/min/1.73m3), median (IQR)	41.1 (28.1-63.1)
eGFR (CKDEPI 2012, mL/min/1.73m3), median (IQR) ‡	41.8 (28.5-59.9)
Albumin excretion rate (AER, mg/day), median (IQR)	180.9 (20.9-918.0)

† Prevalent diabetes based on self report, use of T2DM medications, or fasting glucose
‡ CKD EPI 2009 based on serum creatinine, age, race, sex; CKD EPI 2012 added serum cystatin C

Hippurate (HA) and cinnamoylglycine (CMG) estimated much larger ranges of function, compared with the average of urea and creatinine (Ur-Cr): HA clearance IQR 180-618 mL/min (max 5400 mL/min), CMG clearance IQR 61-207 mL/min (max: 3100 mL/min); Ur-Cr clearance IQR 29-72 mL/min (max 204 mL/min).

Substantial inter-individual variation in estimated secretion was observable across the measured range of estimated filtration (**Figure**), although variation was similar for hippurate (HA) and cinnamoylglycine (CMG) clearance, with increased variance at highest levels of urea-creatinine (Ur-Cr) clearance. On the logarithmic scale, the cohort was divided into three equal-sized, roughly-homoscedastic groups based on secretion function relative to filtration function (Fig 1-C, 1-D).

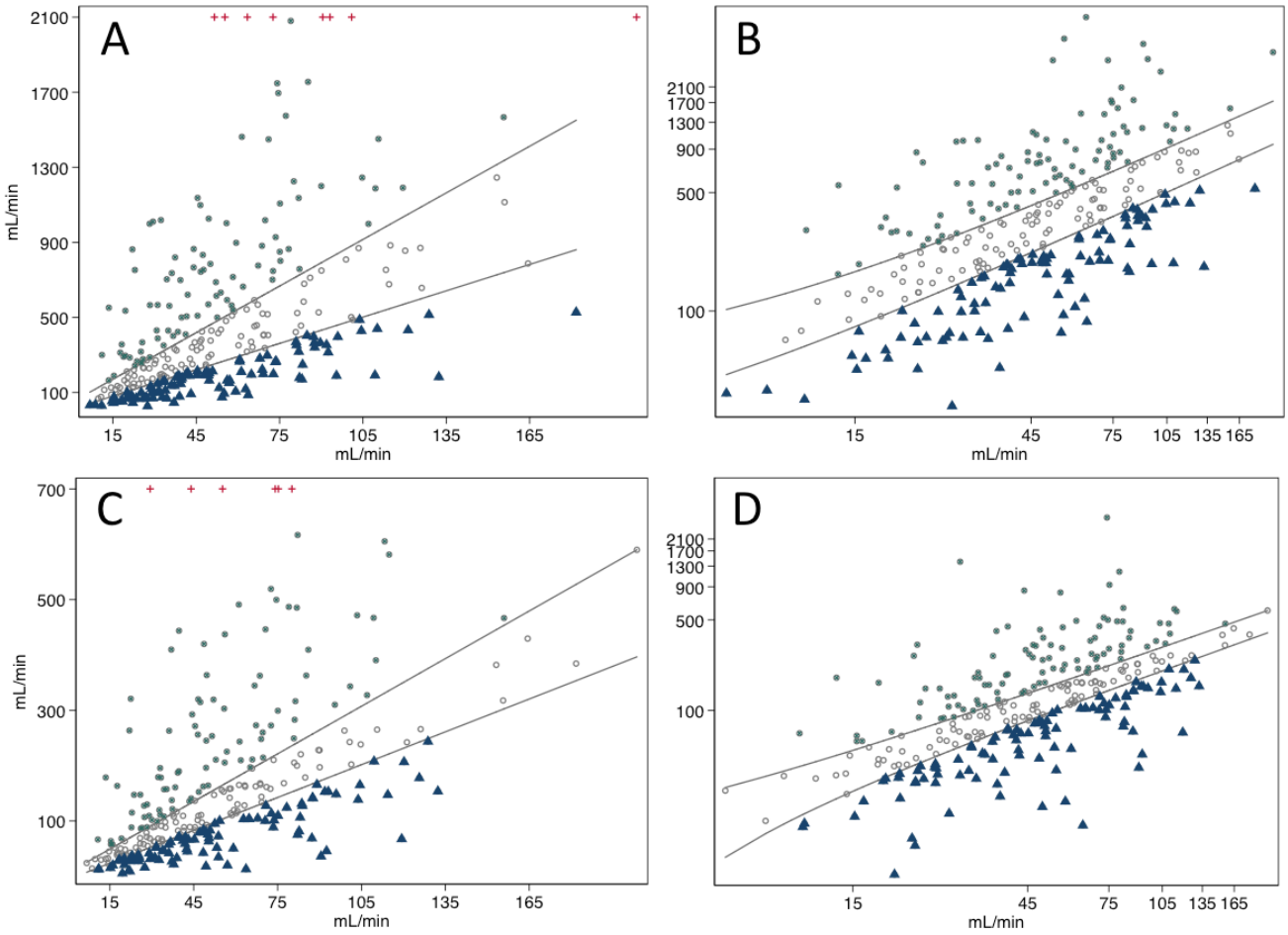

Figure: **Scatter plots of hippurate (HA; left) and cinnamoylglycine (CMG; right) clearance, over average of urea and creatinine (Ur-Cr) clearance**. *Continuous scale (top) and natural logarithmic scale (bottom) shown, for visual examination of the range, variability, and interindividual differences between two measures of secretion (HA, CMG clearance) and filtration (Ur-Cr clearance). Units given as volume of blood completely cleared of the given solute, per unit time (mL/min). Red crosses at the top (1-A, 1-C) indicate subjects with extremely high secretion clearances, who are displayed at an arbitrary maximal range value for graphical examination purposes; these subjects are portrayed in the natural log-scaled plots (1-B, 1-D) and included in all statistical analyses using the true data value. Population separated into three categories using percentile regressions; includes subjects with high (green crosses;* Category *3), medium (grey circles;* Category *2), and low (blue triangles;* Category *1) secretion per average level of filtration function (Ur-Cr clearance). Regression lines at 33rd and 67th percentiles of secretion-beyond-filtration are shown.*

Secretory solute clearances were modestly correlated with each other, and with filtration solute clearance; correlations with measures of albuminuria were low (**Table 2**). Pearson's linear, product-moment correlation coefficient (ρ) between HA clearance and CMG clearance was 0.38; between these solutes (respectively) and Ur-Cr clearance the coefficient (ρ) was 0.42 and 0.35. Evaluations of filtration function using creatinine (Cr) clearance or estimated GFR based on the 2009 CKD EPI equation provided similar results. AER—the gold standard for albuminuria, for which ACR serves as a proxy—correlated with HA and CMG clearance at estimated coefficients (ρ), respectively, 0.026 and 0.156. Log-transformed HA and CMG clearance correlation coefficients with arithmetic Ur-Cr clearance were 0.566 and 0.588 (data not shown).

Table 2: Correlations among measures of tubular secretion and glomerular filtration

	Hippurate (HA) clearance, mL/min		Cinnamoylglycine (CMG) clearance, mL/min	
	(ρ) †	*P-value*	(ρ) †	*P-value*
Urea-Creatinine clearance, mL/min	0.421	*<0.0001*	0.345	*<0.0001*
Creatinine clearance, mL/min	0.416	*<0.0001*	0.341	*<0.0001*
Estimated GFR, mL/min/1.73m^3	0.365	*<0.0001*	0.319	*<0.0001*
Albumin excretion rate (AER), mg/day	0.026	*0.655*	0.156	*0.007*
Cinnamoylglycine clearance, mL/min	0.379	*<0.0001*	-	-

† Pearson (pairwise) product-moment correlation coefficient (ρ); analyses on arithmetic scale

Secretion function, relative to filtration function (secretion-beyond-filtration), tended to differ in a linear test across categories by gender, by tobacco and alcohol use, by use of certain medications, by level of HbA1c, and by fractional excretion of potassium (HA: **Table 3-A**, CMG: **Table 3-B**); however, none of these distinctions excluded the possibility of chance after accounting for the problem of multiple testing. Men tended to have greater secretion relative to filtration; (HA) Category 3 was 84% male, vs. Category 1 at 70%; (CMG) Category 3 was 85% male vs. Category 1 at 69%. Current use of tobacco, alcohol, aspirin, or statins were also associated with greater secretion relative to filtration: proportion

differences comparing Category 3 with Category 1 were (HA) 8% / ~ / 17% / 14%, respectively; (CMG) 8% / 15% / 10% / 13%. Similar comparisons for loop diuretics indicated 7% lower secretion (CMG) for current users. Highest hemoglobin-A1c levels (HA: mean 6.8; CMG: mean 6.7) and highest fractional excretion of potassium (HA: mean 15.9; CMG: 17.3) were observed in the lowest (Category 1) secretion category relative to filtration.

Table 3-A: Secretion-beyond-filtration & CKD correlates – Comparing low (Category 1), medium (Category 2), and high (Category 3) hippurate clearance, for same Urea-Creatinine clearance

Characteristics:	Category 1 (Low) N=98	Category 2 (Medium) N=99	Category 3 (High) N=99	*P for trend*
Age, years mean(SD)	59.8 (13.8)	59.4 (14.0)	59.4 (13.5)	*0.96*
Male gender, n(%)	69 (70%)	82 (83%)	83 (84%)	*0.04*
Black race, n(%)	31 (32%)	24 (24%)	23 (23%)	*0.34*
Other non-white race, n(%)	8 (8%)	9 (9%)	10 (10%)	*0.89*
Urea-creatinine clearance, mL/min m(SD)	54.2 (31.7)	54.3 (34.1)	54.1 (31.6)	*1.00*
Daily creatinine excretion / lean body mass, mg/kg m(SD) †	29.4 (11.1)	30.7 (12.4)	32.2 (12.9)	*0.27*
Glomerular filtration rate, m(SD) †	50.0 (27.1)	46.2 (26.3)	46.2 (25.1)	*0.50*
Body mass index, kg/m^2 m(SD)	31.9 (8.1)	32.1 (8.0)	30.9 (6.3)	*0.50*
Systolic blood pressure, mmHg m(SD)	132.0 (21.7)	131.3 (21.4)	129.9 (20.1)	*0.77*
Current smoking, n(%)	15 (15%)	19 (19%)	23 (23%)	*0.39*
Current alcohol use, n(%)	30 (31%)	34 (34%)	31 (31%)	*0.84*
Medications use:				
Aspirin, n(%)	39 (40%)	32 (32%)	47 (47%)	*0.09*
Statin, n(%)	48 (49%)	57 (58%)	62 (63%)	*0.15*
H2 blocker, n(%)	7 (7%)	8 (8%)	11 (11%)	*0.59*
Loop diuretic, n(%)	38 (39%)	42 (42%)	39 (39%)	*0.86*
Thiazide, n(%)	12 (12%)	16 (16%)	15 (15%)	*0.72*
ACE inhibitor, n(%)	52 (53%)	56 (57%)	55 (56%)	*0.88*
Angiotensin-R blocker, n(%)	34 (35%)	35 (35%)	43 (43%)	*0.37*
Colchicine, n(%)	5 (5%)	5 (5%)	7 (7%)	*0.78*
EPO, n(%)	6 (6%)	1 (1%)	6 (6%)	*0.13*
Insulin, n(%)	26 (27%)	24 (24%)	30 (30%)	*0.62*
Oral diabetes medication, n(%) †	21 (21%)	21 (21%)	17 (17%)	*0.70*
Prevalent diabetes, n(%) †	53 (54%)	53 (54%)	51 (52%)	*0.93*
Prevalent cardiovascular disease, n(%) †	8 (8%)	9 (9%)	7 (7%)	*0.87*
Laboratory measures:				
Hemoglobin A1c, % m(SD)	6.8 (1.9)	6.4 (1.2)	6.4 (1.3)	*0.09*
Cholesterol, mg/dL m(SD)	175.7 (63.9)	168.6 (50.7)	169.8 (52.0)	*0.63*
C-Reactive Protein, mg/L m(SD)	6.2 (10.0)	5.6 (8.5)	4.9 (6.9)	*0.58*
CO2 (bicarbonate), mmol/L m(SD)	24.7 (3.6)	24.73 (3.4)	24.9 (3.6)	*0.93*
Fractional excretion Phosphorus, % m(SD)	29.4 (16.1)	29.40 (13.2)	29.0 (19.0)	*0.98*
Fractional excretion Uric Acid, % m(SD)	7.0 (3.9)	7.06 (4.0)	6.9 (6.9)	*0.96*
Fractional excretion Calcium, % m(SD)	1.3 (1.2)	1.37 (2.5)	1.2 (1.4)	*0.78*
Fractional excretion Sodium, % m(SD)	1.6 (1.5)	1.57 (1.2)	1.6 (1.5)	*0.94*
Fractional excretion Potassium, % m(SD)	15.9 (15.1)	14.82 (10.1)	13.8 (9.4)	*0.45*
Albumin excretion rate, mg/dL m(SD)	539.6 (704.5)	790.7 (1125.8)	719.3 (1254.9)	*0.23*

† Oral diabetes medications metformin or sulfonylurea; prevalent diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD)

Table 3-B: Secretion-beyond-filtration & CKD correlates – Comparing low (Category 1), medium (Category 2), and high (Category 3) cinnamoylglycine clearance, for same Urea-Creatinine clearance

Filtration-defined CKD determinants:	Category 1 (Low) N=98	Category 2 (Medium) N=99	Category 3 (High) N=99	*P for trend*
Age, years mean(SD)	60.5 (14.5)	59.5 (14.5)	59.0 (12.0)	*0.75*
Male gender, n(%)	67 (69.1%)	82 (82.8%)	85 (85.0%)	*0.01*
Black race, n(%)	21 (21.6%)	29 (29.3%)	28 (28.0%)	*0.43*
Other non-white race, n(%)	13 (13.4%)	8 (8.1%)	6 (6.0%)	*0.18*
Urea-creatinine clearance, mL/min m(SD)	54.6 (30.6)	55.1 (38.2)	53.2 (27.7)	*0.92*
Daily creatinine excretion / lean body mass, mg/kg m(SD) †	48.3 (28.1)	46.4 (26.7)	47.9 (23.6)	*0.87*
Glomerular filtration rate, m(SD) †	31.0 (12.8)	30.8 (12.8)	30.5 (11.0)	*0.96*
Body mass index, kg/m^2 m(SD)	31.5 (7.7)	31.5 (7.2)	32.0 (7.7)	*0.87*
Systolic blood pressure, mmHg m(SD)	132.6 (22.2)	130.9 (20.7)	129.4 (20.1)	*0.55*
Current smoking, n(%)	16 (16%)	17 (17%)	24 (24%)	*0.29*
Current alcohol use, n(%)	23 (23.7%)	32 (32.3%)	39 (39.0%)	*0.07*
Medications use:				
Aspirin, n(%)	34 (35.1%)	38 (38.4%)	45 (45.0%)	*0.35*
Statin, n(%)	49 (50.5%)	54 (54.5%)	63 (63.0%)	*0.20*
H2 blocker, n(%)	8 (8.2%)	10 (10.1%)	9 (9.0%)	*0.90*
Loop diuretic, n(%)	42 (43.3%)	40 (40.4%)	36 (36.0%)	*0.57*
Thiazide, n(%)	12 (12.4%)	15 (15.2%)	16 (16.0%)	*0.75*
ACE inhibitor, n(%)	55 (56.7%)	50 (50.5%)	59 (59.0%)	*0.46*
Angiotensin-R blocker, n(%)	36 (37.1%)	40 (40.4%)	35 (35.0%)	*0.73*
Colchicine, n(%)	7 (7.2%)	4 (4.0%)	6 (6.0%)	*0.63*
EPO, n(%)	3 (3.1%)	6 (6.1%)	4 (4.0%)	*0.58*
Insulin, n(%)	25 (25.8%)	26 (26.3%)	28 (28.0%)	*0.93*
Oral diabetes medication, n(%) †	21 (21.6%)	18 (18.2%)	21 (21.0%)	*0.81*
Prevalent diabetes, n(%) †	50 (51.5%)	52 (52.5%)	55 (55.0%)	*0.88*
Prevalent cardiovascular disease, n(%) †	8 (8.2%)	12 (12.1%)	5 (5.0%)	*0.19*
Laboratory measures:				
Hemoglobin A1c, % m(SD)	6.7 (1.9)	6.4 (1.4)	6.5 (1.2)	*0.26*
Cholesterol, mg/dL m(SD)	174.4 (61.5)	176.6 (59.1)	162.8 (44.2)	*0.17*
C-Reactive Protein, mg/L m(SD)	5.9 (10.7)	5.1 (6.6)	5.8 (8.0)	*0.77*
CO2 (bicarbonate), mmol/L m(SD)	24.5 (3.6)	24.8 (3.7)	25.0 (3.3)	*0.60*
Fractional excretion Phosphorus, % m(SD)	29.4 (13.0)	31.4 (16.3)	27.3 (19.0)	*0.21*
Fractional excretion Uric Acid, % m(SD)	6.9 (3.9)	7.6 (4.2)	6.5 (6.7)	*0.30*
Fractional excretion Calcium, % m(SD)	1.3 (1.3)	1.5 (2.5)	1.1 (1.2)	*0.40*
Fractional excretion Sodium, % m(SD)	1.6 (1.4)	1.7 (1.4)	1.5 (1.5)	*0.38*
Fractional excretion Potassium, % m(SD)	17.3 (16.5)	14.9 (9.4)	12.5 (7.5)	*0.02*
Albumin excretion rate, mg/dL m(SD)	598.9 (851.8)	524.6 (728.0)	839.3 (1283.8)	*0.06*

† Oral diabetes medications metformin or sulfonylurea; prevalent diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD)

With adjustment for age, sex, race, estimated GFR, and daily creatinine excretion standardized to lean body mass, associations between categories of secretion relative to filtration and clinical characteristics were similar to crude associations (HA: **Table 4-A**, CMG: **Table 4-B**). Male gender was associated with better secretion function; men had 50% to 70% lower odds of being "low secretors" (Category 1), compared with women, independent of age, race, estimated GFR, and accounting for measurement error in timed urines (HA OR: 0.5, CMG OR: 0.3). This association was not significant for HA, but was for CMG (HA 95% CI: 0.2-1.1; CMG 95% CI: 0.1-0.6, P<0.001); the Bonferroni threshold to account for multiple testing in these analyses was P=0.001. Older age, higher fractional excretion of potassium, and higher HbA1c levels were associated with worse secretion function, although these associations were not statistically significant. Non-white race may be associated with lower secretion, but these analyses provided inconsistent direction of the relationship for subjects with African heritage ("Black") and other non-whites ("Other"—predominantly includes Asian, Native American, Native Alaskan, and non-white Hispanic). Current smoking was associated with better secretion, with odds of being a "low secretor" 40% (CMG) to 50% (HA) lower comparing current smokers with non-smokers; however, the association for smoking was not robust to additional adjustment for levels of parathyroid hormone (results not shown). Use of medications, especially aspirin and statins, were associated with better secretion, although these associations were not adjusted for indications for medication use.

Secondary analyses with adjustment for Ur-Cr clearance instead of eGFR, or with no adjustment for filtration function, were also done and were not substantively different from those reported (data not shown). Sensitivity analyses comprised of similarly-adjusted linear regressions of continuous hippurate clearance (**Supplemental Table A**) and cinnamoylglycine clearance (**Supplemental Table B**) as a function of the same CKD correlates offered similar associations; positive associations with secretion measures included eGFR, systolic blood pressure and smoking, and negative associations included male sex, non-white race, HbA1c, and fractional excretion of solutes.

Table 4-A: Secretion beyond filtration and CKD correlates – Odds of having Low versus High (referent) hippurate clearance, for same Urea-Creatinine clearance		
	OR (95%CI) †	*P-value*
Age, years	1.2 (0.8, 1.7)	*0.33*
Male gender	0.5 (0.2, 1.1)	*0.07*
Black race	1.5 (0.7, 2.9)	*0.27*
Other non-white race	0.9 (0.3, 2.6)	*0.81*
Glomerular filtration rate, mL/min/1.7m^3 ‡	1.2 (0.9, 1.7)	*0.20*
Standard daily creatinine excretion, mg/kg ‡	0.8 (0.6, 1.1)	*0.23*
Body mass index, kg/m^2	1.1 (0.8, 1.5)	*0.42*
Systolic blood pressure, mmHg	1.2 (0.9, 1.6)	*0.27*
Current smoking	0.5 (0.2, 0.9)	*0.04*
Current alcohol use	1.0 (0.6, 1.9)	*0.97*
Aspirin use	0.7 (0.4, 1.2)	*0.20*
Statins use	0.6 (0.3, 1.0)	*0.07*
H2 blocker use	0.6 (0.2, 1.7)	*0.30*
Loop diuretic use	1.0 (0.5, 1.8)	*0.90*
Thiazide use	0.7 (0.3, 1.6)	*0.35*
ACE inhibitor use	0.8 (0.5, 1.5)	*0.50*
Angiotensin-R blocker use	0.7 (0.4, 1.2)	*0.21*
Colchicine use	0.6 (0.1, 2.7)	*0.51*
EPO use	1.5 (0.4, 5.1)	*0.52*
Insulin use	0.8 (0.4, 1.6)	*0.57*
Oral diabetes medication use ‡	1.1 (0.5, 2.3)	*0.88*
Diabetes ‡	1.0 (0.5, 1.8)	*0.96*
Cardiovascular disease ‡	1.3 (0.4, 4.0)	*0.67*
Hemoglobin A1c, %	1.2 (0.9, 1.5)	*0.24*
Cholesterol, mg/dL	1.1 (0.8, 1.4)	*0.67*
C-Reactive Protein, mg/L	1.1 (0.8, 1.5)	*0.44*
CO2 (bicarbonate), mmol/L	0.9 (0.7, 1.2)	*0.46*
Fractional excretion Phosphorus, %	1.3 (0.7, 2.4)	*0.45*
Fractional excretion Uric Acid, %	1.1 (0.7, 1.7)	*0.60*
Fractional excretion Calcium, %	1.2 (0.7, 1.9)	*0.58*
Fractional excretion Sodium, %	1.1 (0.8, 1.7)	*0.52*
Fractional excretion Potassium, %	1.5 (1.0, 2.2)	*0.07*
Albumin excretion rate (timed), mg/dL	0.9 (0.7, 1.2)	*0.36*

† Logistic regression modeled per yes/no exposure (dichotomous) or per SD exposure (continuous), comparing Category 1 to Category 3 (referent) outcome; adjusted for age, sex, black race, other non-white race, estimated GFR per 2009 CKD EPI equation, 24-hr Creatinine excretion per kg lean body mass. Analyses do not include Category 2 (middle; N=99)

‡ Oral diabetes medications metformin or sulfonylurea; Type 2 diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD); Glomerular filtration rate (GFR) based on 2009 CKD EPI equation, units mL/min/1.73m^3; daily creatinine excretion is for calculated 24-hour period & given per kg lean body mass (LBM).

Table 4-B: Secretion beyond filtration and CKD correlates – Odds of having Low versus High (referent) cinnamoylglycine clearance, for same Urea-Creatinine clearance		
	OR (95%CI) †	*P-value*
Age, years	1.5 (1.0, 2.1)	*0.05*
Male gender	0.3 (0.1, 0.6)	*0.001*
Black race	0.7 (0.3, 1.4)	*0.30*
Other non-white race	2.7 (0.9, 7.5)	*0.06*
Glomerular filtration rate, mL/min/1.7m^3 ‡	1.1(0.8, 1.6)	*0.49*
Standard daily creatinine excretion, mg/kg ‡	1.2 (0.9, 1.7)	*0.17*
Body mass index, kg/m^2	1.3 (0.9, 1.7)	*0.16*
Systolic blood pressure, mmHg	0.6 (0.3, 1.3)	*0.16*
Current smoking	0.6 (0.3, 1.0)	*0.06*
Current alcohol use	0.6 (0.3, 1.1)	*0.11*
Aspirin use	0.6 (0.3, 1.0)	*0.07*
Statins use	0.8 (0.3, 2.3)	*0.66*
H2 blocker use	1.4 (0.7, 2.6)	*0.37*
Loop diuretic use	0.8 (0.4, 1.7)	*0.53*
Thiazide use	0.9 (0.5, 1.6)	*0.65*
ACE inhibitor use	1.1 (0.6, 2.0)	*0.78*
Angiotensin-R blocker use	0.9 (0.3, 3.1)	*0.92*
Colchicine use	0.8 (0.2, 3.7)	*0.78*
EPO use	1.0 (0.5, 2.0)	*0.99*
Insulin use	0.7 (0.3, 1.6)	*0.41*
Oral diabetes medication use ‡	0.8 (0.5, 1.5)	*0.51*
Diabetes ‡	1.7 (0.5, 5.6)	*0.39*
Cardiovascular disease ‡	1.2 (0.9, 1.6)	*0.22*
Hemoglobin A1c, %	1.3 (0.9, 1.8)	*0.16*
Cholesterol, mg/dL	1.1 (0.8, 1.4)	*0.71*
C-Reactive Protein, mg/L	0.9 (0.6, 1.1)	*0.27*
CO2 (bicarbonate), mmol/L	1.5 (0.5, 4.1)	*0.47*
Fractional excretion Phosphorus, %	1.2 (0.6, 2.4)	*0.55*
Fractional excretion Uric Acid, %	1.3 (0.8, 2.2)	*0.30*
Fractional excretion Calcium, %	1.4 (0.8, 2.4)	*0.30*
Fractional excretion Sodium, %	2.1 (1.2, 3.7)	*0.01*
Fractional excretion Potassium, %	0.9 (0.7, 1.1)	*0.31*
Albumin excretion rate (timed), mg/dL	1.5 (1.0, 2.1)	*0.05*

† Logistic regression modeled per yes/no exposure (dichotomous) or per SD exposure (continuous), comparing Category 1 to Category 3 (referent) outcome; adjusted for age, sex, black race, other non-white race, estimated GFR per 2009 CKDEPI equation, 24-hr Creatinine excretion per kg lean body mass. Analyses do not include Category 2 (middle; N=99)

‡ Oral diabetes medications metformin or sulfonylurea; Type 2 diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD); Glomerular filtration rate (GFR) based on 2009 CKD EPI equation, units mL/min/1.73m^3; daily creatinine excretion is for calculated 24-hour period & given per kg lean body mass (LBM).

During a median of 3.1 years of follow-up (IQR 1.9, 4.0) there were 43 deaths, or 5.1 deaths per 100 person-years. Lower HA clearance secretion function, relative to filtration, was independently associated with increased risk of death (**Table 5-A**). After adjustment for age, sex, race, and filtration-based kidney disease (based on eGFR), the lowest HA clearance category of secretion-beyond-filtration (Category 1) was associated with an estimated 2.3-fold greater risk of death (95% CI: 1.1-4.7, *P=0.025*), compared with the highest category (Category 3). A similar, but not as extreme, association was observed comparing the middle category (Category 2) with the highest category (Category 3). Evaluation of the proportionality of hazards assumption using log-log plots indicated a minor departure from the assumption in the earliest time points (data not shown). Secondary analyses for death or dialysis outcome, with adjustment for components of the Tangri model for progression of CKD (age, sex, eGFR, microalbuminuria, and serum albumin, calcium, phosphorus, and bicarbonate), provided similar results, although none was statistically significant (data not shown). Sensitivity analyses for the dialysis outcome with death modeled as a competing risk were not markedly different, although power was limited (data not shown).

During a similar follow-up time period (mean 2.8 years), 20 subjects initiated dialysis, or 2.5 per 100 person-years. Although risk of starting dialysis may be increased among those with low secretion function, the confidence intervals were wide and included the null hypothesis of no association. Following subjects until loss of 30% or more filtration function (eGFR) since baseline visit or a combined outcome of either 30% loss or initiation of renal replacement therapy (RRT; including dialysis or transplant) was not associated with HA clearance-based secretion function categories (data not shown).

In contrast, during the same follow-up period, lower CMG clearance secretion function, relative to filtration, was not associated independently with risk of death (**Table 5-B**), but was associated with initiation of dialysis. After adjustment for age, sex, race, and filtration-based kidney disease (based on eGFR), the lowest CMG clearance category of secretion-beyond-filtration (Category 1) was

associated with an estimated 4.5-fold greater risk of starting dialysis (95% CI: 1.4-14.4, *P=0.012*), compared with the highest category (Category 3). A similar, but not as extreme, association was observed comparing the middle category (Category 2) with the highest category (Category 3). The 30% loss and the combined outcomes were similarly not associated with CMG clearance-based secretion function categories. Secondary analyses for death or dialysis outcomes with adjustment for components of the Tangri model for progression of CKD provided similar results (data not shown), with risk of dialysis comparing low secretors with high secretors approximately 4.8-fold (95% CI: 1.6-13.8). Sensitivity analyses for the dialysis outcome with death modeled as a competing risk were not markedly different, although power was limited (data not shown).

Table 5-A: Secretion-beyond-filtration and longitudinal outcomes – Comparing categories of hippurate clearance, for same Urea-Creatinine clearance

	Incidence / 100 person years (N cases)	HR (95% CI)
Outcome: Death †		
HA: Low (vs. High)	6.8 (19)	2.3 (1.1, 4.7)
HA: Medium (vs High)	4.4 (12)	1.3 (0.6, 2.9)
HA: High *(Referent)*	3.8 (11)	*Ref*
Outcome: Dialysis †		
HA: Low (vs. High)	2.7 (7)	1.6 (0.4, 5.5)
HA: Medium (vs High)	2.6 (7)	1.1 (0.4, 3.4)
HA: High *(Referent)*	2.1 (6)	*Ref*
Outcome: loss 30% GFR ‡		
HA: Low (vs. High)	10.7 (18)	0.9 (0.5, 1.7)
HA: Medium (vs High)	10.1 (17)	0.8 (0.4, 1.5)
HA: High *(Referent)*	12.8 (22)	*Ref*

† Cox proportional hazards models, comparing categories of secretion-beyond-filtration; adjusted for age, sex, black race, other non-white race, and estimated GFR (2009 CKD EPI)
‡ Cox proportional hazards models, comparing categories of secretion-beyond-filtration; adjusted for age, sex, black race, other non-white race; outcome of loss of 30% GFR or combined outcome of 30% loss and/or initiation of dialysis modeled per (approximately annual) clinic visit; incidence rates given in person-visits

Table 5-B: Secretion-beyond-filtration and longitudinal outcomes – Comparing categories of cinnamoylglycine clearance, for same Urea-Creatinine clearance

	Incidence / 100 person years (N cases)	HR (95% CI)
Outcome: Death †		
CMG: Low (vs High)	4.4 (12)	0.9 (0.4, 1.8)
CMG: Medium (vs High)	5.8 (16)	1.1 (0.6, 2.2)
CMG: High *(Referent)*	5.1 (15)	*Ref*
Outcome: Dialysis †		
CMG: Low (vs High)	3.5 (9)	4.5 (1.4, 14.4)
CMG: Medium (vs High)	2.3 (6)	1.3 (0.4, 5.1)
CMG: High *(Referent)*	1.4 (4)	*Ref*
Outcome: loss 30% GFR ‡		
CMG: Low (vs High)	10.4 (17)	1.0 (0.5, 1.8)
CMG: Medium (vs High)	11.0 (18)	1.0 (0.6, 1.8)
CMG: High *(Referent)*	11.9 (22)	*Ref*

† Cox proportional hazards models, comparing categories of secretion-beyond-filtration; adjusted for age, sex, black race, other non-white race, and estimated GFR (2009 CKD EPI)
‡ Cox proportional hazards models, comparing categories of secretion-beyond-filtration; adjusted for age, sex, black race, other non-white race; outcome of loss of 30% GFR or combined outcome of 30% loss and/or initiation of dialysis modeled per (approximately annual) clinic visit; incidence rates given in person-visits

Modeled per halving in clearance, lower secretion function was associated with approximately two-fold increased risk of death and dialysis in unadjusted analyses (**Table 6-A**), remained consistent after adjusting for components of the Tangri model for progression of CKD (age, sex, eGFR, serum calcium, phosphorus, bicarbonate and albumin, and macroalbuminuria), and was minimally attenuated with additional adjustment for filtration function (Ur-Cr clearance). Risk of death per halving in HA clearance, independent of all other factors, was 1.4-fold (95% CI: 1.0-1.9). Similar models per halving in Ur-Cr clearance produced similar results; overall risk was approximately two-fold, and remained consistent with adjustment for factors related to progression of kidney disease, and for hippurate clearance. Models examining risk of death per halving in CMG clearance (**Table 6-B**) also provided similar associations, although full adjustment attenuated the effect estimates for both death and dialysis to null effect.

Table 6-A : Secretion versus Filtration -- comparing hippurate clearance and Ur-Cr clearance, with respect to risk of death or dialysis		
	Secretion (HA) HR (95% CI)	Filtration (Ur-Cr) HR (95% CI)
Outcome: Death †		
M1: per halving in exposure	1.7 (1.3, 2.1)	2.2 (1.6, 3.0)
M2: + adjust Tangri model	1.7 (1.2, 2.3)	2.9 (1.7, 5.1)
M3: + mutually adjusted	1.4 (1.0, 1.9)	2.5 (1.4, 4.4)
Outcome: Dialysis †		
M1: per halving in exposure	1.7 (1.2, 2.5)	3.9 (2.4, 6.3)
M2: + adjust Tangri model	1.3 (0.9, 1.9)	2.5 (1.2, 4.9)
M3: + mutually adjusted	1.1 (0.7, 1.6)	2.8 (1.3, 5.8)

† First model analyses unadjusted (M1); second model (M2) adjusted for adjusted for age, sex, eGFR, albumin, calcium, phosphorus, bicarbonate, and macroalbuminuria (ACR>300); third model also mutually adjusted for (continuous, arithmetic) Ur-Cr clearance or HA clearance (M3)

Table 6-B : Secretion versus Filtration -- comparing cinnamoylglycine clearance and Ur-Cr clearance, with respect to risk of death or dialysis		
	Secretion (CMG)	Filtration (Ur-Cr)
Outcome: Death †	HR (95% CI)	HR (95% CI)
M1: per halving in exposure	1.3 (1.1, 1.6)	2.2 (1.6, 3.0)
M2: + adjust Tangri model	1.2 (0.9, 1.7)	2.9 (1.7, 5.1)
M3: + mutually adjusted	0.9 (0.7, 1.3)	3.3 (1.8, 6.0)
Outcome: Dialysis †		
M1: per halving in exposure	1.8 (1.3, 2.5)	3.9 (2.4, 6.3)
M2: + adjust Tangri model	1.5 (0.9, 2.3)	2.5 (1.2, 4.9)
M3: + mutually adjusted	1.2 (0.8, 1.9)	2.6 (1.3, 5.2)

† First model analyses unadjusted (M1); second model (M2) adjusted for adjusted for age, sex, eGFR, albumin, calcium, phosphorus, bicarbonate, and macroalbuminuria (ACR>300); third model also mutually adjusted for (continuous, arithmetic) Ur-Cr clearance or CMG clearance (M3)

Discussion

We measured proximal tubular secretion function in a cohort study of 298 CKD patients using assays with internal standards and external calibrations to allow accurate quantification of solutes. Tubular secretion correlated modestly with glomerular filtration function, although there was considerable interindividual variability. Lower secretion function was associated with female sex, older age, greater fractional excretion of potassium, and elevated serum levels of HbA1c. Although associations were not consistent for the two solutes examined, this study provided evidence that impairment of secretion may be associated with increased likelihood of need for initiation of dialysis and of death.

The modest correlation between secretion and filtration and the large range of variability in secretion over filtration (Figure 1) suggests that the prevailing framework for examining kidney function is incomplete. In addition, secreted clearances were estimated at values exceeding the rate of renal blood flow, suggesting that rapid ion-protein binding makes these solutes' free fractions much lower than total serum concentrations, emphasizing the usual role of active elimination processes.[4] Further, the absence of strong associations for tubular secretion with many conventional determinants of glomerular filtration-defined kidney disease suggests that glomerular dysfunction and tubular dysfunction comprise different disease processes. Measurement of secretory solutes as biomarkers for proximal tubule function presents an opportunity to examine facets of kidney disease that differ from those of filtration dysfunction.

The findings also suggest that different secretory solutes (HA, CMG) may represent distinct facets of tubular secretion. Despite being members of related metabolic pathways,[23] hippurate and cinnamoylglycine were not consistently associated with the same characteristics, risk factors, and outcomes. It is possible that different basolateral membrane transporters, such as OAT1/3 and OAT2, have differing influence on the transference and accumulation of secretory solutes, as well as on solute representativeness as biomarkers for proximal tubular secretion function.[24-27]

There are a number of limitations in this study. Using calculated solute clearances to represent renal function—an established field standard—may be statistically prone to misleading inference in some circumstances.[28] The biomechanistic processes of renal filtration and renal secretion are known to overlap; for example, creatinine is cleared an estimated 20% by secretion.[19] Categorizing subjects for secretion-specific solute clearance per level of filtration-non-specific creatinine clearance may remove a proportion of interindividual variance from the estimates of tubular secretion function, which has potential to bias detectable associations towards the null. In addition, since the average of Cr-Ur clearance has been demonstrated as comprising a consistent, unbiased estimate of filtration function over the range of GFR, this effect is non-differential in these analyses.[20] Therefore, any detected associations for secretion are likely to be truly independent of filtration.

The observed associations between higher tubular secretion function and current smoking, current alcohol use, or use of medications may be confounded by unmeasured dietary or behavioral choices, comorbidities or health factors, or other metabolic or hormone states, such as increased levels of parathyroid hormone (PTH)—which removed the observed effect with smoking when included as an adjustment factor.

Timed urine collections may be prone to interindividual data collection errors, as participants may not correctly record the time during which all voids over a 24-hour period are produced, often missing the accumulation time for the first void. However, both secretion and filtration measures were similarly subject to these errors, and adjustment for body mass-standardized daily creatinine excretion in primary regressions helped account for the effect of such misclassification on the results.

Our data included 1 “outlier” with unreasonably high values for estimated clearance (>50 L/min) but which were consistent in repeat assays; this subject was excluded on that basis. Our data also included 1 “outlier” with exceedingly low values for estimated solute clearance (0.6-2 mL/min), and which was several standard deviations lower than the rest of the population; this subject was

excluded from primary analyses on this “extreme value” basis, but was included in sensitivity analyses. Inclusion of this subject did not substantively change any of the results (data not shown).

These data are generated from a cohort of several hundred adults with chronic kidney disease in the Pacific Northwest with baseline estimated GFR between 15 and 120 mL/min/m^2; findings may not be generalizable to other groups. Finally, the size of the study population limited our ability to address some questions.

In summary, this study provides the first characterization of proximal tubular secretion as an independent marker of kidney function in a longitudinal cohort. These data suggest that tubular secretion is an independent marker of kidney dysfunction, and loss of renal tubular secretion function may independently contribute to risk of adverse outcomes.

Supplemental Table A: Hippurate clearance and CKD correlates; adjusted linear regressions		
	Beta (95%CI) †	*P-value*
Age, years	-14.7 (-87.7, 58.2)	*0.691*
Male gender	-193.6 (-359.0, -28.2)	*0.022*
Black race	1.6 (-190.4, 193.6)	*0.987*
Other non-white race	-134.8 (-249.8, -19.8)	*0.022*
Glomerular filtration rate, mL/min/1.7m^3 ‡	8.7 (4.0, 13.4)	*<0.001*
Standard daily creatinine excretion, mg/kg ‡	132.6 (47.3, 217.8)	*0.002*
Body mass index, kg/m^2	37.0 (-14.7, 88.7)	*0.160*
Systolic blood pressure, mmHg	-52.5 (-98.9, -6.1)	*0.027*
Current smoking	219.2 (8.5, 429.9)	*0.042*
Current alcohol use	-6.6 (-147.8, 134.6)	*0.927*
Aspirin use	21.8 (-103.1, 146.7)	*0.732*
Statins use	62.1 (-54.8, 179.1)	*0.297*
H2 blocker use	94.2 (-173.3, 361.7)	*0.489*
Loop diuretic use	29.8 (-88.8, 148.4)	*0.621*
Thiazide use	32.3 (-164.0, 228.6)	*0.746*
ACE inhibitor use	9.5 (-119.6, 138.7)	*0.884*
Angiotensin-R blocker use	93.9 (-66.8, 254.6)	*0.251*
Colchicine use	98.6 (-131.3, 328.6)	*0.399*
EPO use	-29.7 (0198.9, 139.5)	*0.730*
Insulin use	-10.6 (0144.5, 123.4)	*0.876*
Oral diabetes medication use ‡	-111.3 (-251.9, 29.3)	*0.120*
Type 2 diabetes ‡	-117.0 (-246.7,12.7)	*0.077*
Cardiovascular disease ‡	-64.8 (0180.7, 51.1)	*0.272*
Hemoglobin A1c, %	-67.4 (-122.7, -12.1)	*0.017*
Cholesterol, mg/dL	-37.9 (-99.0, 23.1)	*0.222*
C-Reactive Protein, mg/L	38.4 (-48.8, 125.7)	*0.386*
CO2 (bicarbonate), mmol/L	-10.1 (-65.9, 45.7)	*0.721*
Fractional excretion Phosphorus, %	8.3 9-89.6, 106.1)	*0.868*
Fractional excretion Uric Acid, %	-87.7 (-15.3, -22.1)	*0.009*
Fractional excretion Calcium, %	-17.4 (-58.4, 23.6)	*0.405*
Fractional excretion Sodium, %	-7.1 (-64.9, 50.7)	*0.809*
Fractional excretion Potassium, %	-32.9 (-80.4, 14.5)	*0.173*
Albumin excretion rate, mg/dL	20.6 (-51.4, 92.7)	*0.573*

† Linear regression per yes/no exposure (dichotomous) or per SD exposure (continuous) of continuous arithmetic secretion measure (outcome); adjusted for age, sex, black race, other non-white race, estimated GFR, 24-hr Creatinine excretion per kg lean body mass

‡ Oral diabetes medications metformin or sulfonylurea; Type 2 diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD); Glomerular filtration rate (GFR) based on 2009 CKD EPI equation, units mL/min/1.73m^3; daily creatinine excretion calculated for 24-hour period per kg lean body mass

Supplemental Table B: Cinnamoylglycine clearance and CKD correlates; adjusted linear regressions		
	Beta (95%CI) †	*P-value*
Age, years	-12.2 (-36.4, 12.0)	*0.322*
Male gender	-83.6 (-137.6, -29.6)	*0.003*
Black race	-6.5 (-57.1, 44.0)	*0.799*
Other non-white race	-64.1 (-107.5, -20.8)	*0.004*
Glomerular filtration rate, mL/min/1.7m^3 ‡	2.8 (1.0, 4.6)	*0.003*
Standard daily creatinine excretion, mg/kg ‡	32.5 (12.4, 52.6)	*0.002*
Body mass index, kg/m^2	-0.49(-22.9, 21.9)	*0.966*
Systolic blood pressure, mmHg	-13.4 (-37.4, 10.5)	*0.270*
Current smoking	64.4 (-45.4, 174.3)	*0.249*
Current alcohol use	27.7 (-19.0, 74.5)	*0.244*
Aspirin use	56.6 (-10.5, 123.6)	*0.098*
Statins use	8.0 (-48.8, 64.8)	*0.782*
H2 blocker use	19.2 (-42.0, 80.4)	*0.537*
Loop diuretic use	-23.2 (-59.6, 13.3)	*0.212*
Thiazide use	-22.6 (-69.8, 24.5)	*0.345*
ACE inhibitor use	16.1 (-30.6, 62.9)	*0.497*
Angiotensin-R blocker use	-7.4 (-51.9, 37.1)	*0.743*
Colchicine use	28.3 (-36.9, 93.5)	*0.394*
EPO use	-22.0 (-67.5, 23.4)	*0.341*
Insulin use	-24.6 (-65.9, 16.7)	*0.243*
Oral diabetes medication use ‡	-18.7 (-78.2, 40.9)	*0.538*
Type 2 diabetes ‡	-34.2 (-87.8, 19.3)	*0.209*
Cardiovascular disease ‡	-32.4 (-70.2, 5.5)	*0.094*
Hemoglobin A1c, %	-24.4 (-48.5, -0.2)	*0.048*
Cholesterol, mg/dL	3.6 (-40.6, 47.9)	*0.872*
C-Reactive Protein, mg/L	6.7 (-23.6, 36.9)	*0.665*
CO2 (bicarbonate), mmol/L	14.6 (-12.9, 42.0)	*0.297*
Fractional excretion Phosphorus, %	-42.4 (-73.6, -11.1)	*0.008*
Fractional excretion Uric Acid, %	-18.3 (-42.0, 5.4)	*0.129*
Fractional excretion Calcium, %	-8.1 (-19.4, 3.2)	*0.158*
Fractional excretion Sodium, %	-21.5 (-45.5, 2.4)	*0.078*
Fractional excretion Potassium, %	-21.9 (-40.4, -3.5)	*0.020*
Albumin excretion rate, mg/dL	39.4 (-22.1, 100.9)	*0.208*

† Linear regression per yes/no exposure (dichotomous) or per SD exposure (continuous) of continuous arithmetic secretion measure (outcome); adjusted for age, sex, black race, other non-white race, estimated GFR, 24-hr Creatinine excretion per kg lean body mass

‡ Oral diabetes medications metformin or sulfonylurea; Type 2 diabetes based on self-report, physician diagnosis, use of DM medications, or fasting glucose; cardiovascular disease (CVD) defined as coronary artery disease (CAD), heart failure (HF), peripheral vascular disease (PVD), or cerebrovascular disease (CBVD); Glomerular filtration rate (GFR), units mL/min/1.73m^3; daily creatinine excretion calculated for 24-hour period per kg lean body mass (LBM).

References

1. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney international.* Jul 2011;80(1):17-28.

2. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney international.* Jun 2005;67(6):2089-2100.

3. Eknoyan G, Hostetter T, Bakris GL, et al. Proteinuria and other markers of chronic kidney disease: a position statement of the national kidney foundation (NKF) and the national institute of diabetes and digestive and kidney diseases (NIDDK). *American journal of kidney diseases : the official journal of the National Kidney Foundation.* Oct 2003;42(4):617-622.

4. Sirich TL, Aronov PA, Plummer NS, Hostetter TH, Meyer TW. Numerous protein-bound solutes are cleared by the kidney with high efficiency. *Kidney international.* May 1 2013.

5. Astor BC, Matsushita K, Gansevoort RT, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney international.* Jun 2011;79(12):1331-1340.

6. Gansevoort RT, Matsushita K, van der Velde M, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. *Kidney international.* Jul 2011;80(1):93-104.

7. van der Velde M, Matsushita K, Coresh J, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney international.* Jun 2011;79(12):1341-1352.

8. Peralta CA, Shlipak MG, Judd S, et al. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA : the journal of the American Medical Association.* Apr 20 2011;305(15):1545-1552.

9. Abboud H, Henrich WL. Clinical practice. Stage IV chronic kidney disease. *The New England journal of medicine.* Jan 7 2010;362(1):56-65.

10. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *Journal of the American Geriatrics Society.* Apr 1985;33(4):278-285.

11. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *The New England journal of medicine.* Sep 9 1982;307(11):652-659.

12. Meyer TW, Walther JL, Pagtalunan ME, et al. The clearance of protein-bound solutes by hemofiltration and hemodiafiltration. *Kidney international.* Aug 2005;68(2):867-877.

13. Amorim LC, Alvarez-Leite EM. Determination of o-cresol by gas chromatography and comparison with hippuric acid levels in urine samples of individuals exposed to toluene. *Journal of toxicology and environmental health.* Mar 1997;50(4):401-407.

14. Meyer TW, Hostetter TH. Uremia. *The New England journal of medicine.* Sep 27 2007;357(13):1316-1325.

15. Rhee EP, Souza A, Farrell L, et al. Metabolite profiling identifies markers of uremia. *Journal of the American Society of Nephrology : JASN.* Jun 2010;21(6):1041-1051.

16. Mulder TP, Rietveld AG, van Amelsvoort JM. Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. *The American journal of clinical nutrition.* Jan 2005;81(1 Suppl):256S-260S.

17. Doolan PD, Alpen EL, Theil GB. A clinical appraisal of the plasma concentration and endogenous clearance of creatinine. *The American journal of medicine.* Jan 1962;32:65-79.

18. National Kidney Disease Education Program IWGoSoAiU. Urine Albumin-to-Creatinine Ratio (UACR) in Evaluating Patients with Diabetes for Kidney Disease. *NIH Publication No. 08-6286.* 2008.

19. Levey AS. Measurement of renal function in chronic renal disease. *Kidney international.* Jul 1990;38(1):167-184.

20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of internal medicine.* Mar 16 1999;130(6):461-470.

21. Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc.* 1999;94(446):496-509.

22. Tangri N, Stevens LA, Griffith J, et al. A predictive model for progression of chronic kidney disease to kidney failure. *JAMA : the journal of the American Medical Association.* Apr 20 2011;305(15):1553-1559.

23. Hoskins JA, Holliday SB, Greenway AM. The metabolism of cinnamic acid by healthy and phenylketonuric adults: a kinetic study. *Biomedical mass spectrometry.* Jun 1984;11(6):296-300.

24. Anzai N, Kanai Y, Endou H. Organic anion transporter family: current knowledge. *Journal of pharmacological sciences.* 2006;100(5):411-426.

25. Sekine T, Cha SH, Endou H. The multispecific organic anion transporter (OAT) family. *Pflugers Archiv : European journal of physiology.* Jul 2000;440(3):337-350.

26. Tsutsumi Y, Deguchi T, Takano M, Takadate A, Lindup WE, Otagiri M. Renal disposition of a furan dicarboxylic acid and other uremic toxins in the rat. *The Journal of pharmacology and experimental therapeutics.* Nov 2002;303(2):880-887.

27. Deguchi T, Takemoto M, Uehara N, Lindup WE, Suenaga A, Otagiri M. Renal clearance of endogenous hippurate correlates with expression levels of renal organic anion transporters in uremic rats. *The Journal of pharmacology and experimental therapeutics.* Aug 2005;314(2):932-938.

28. Kronmal RA. Spurious Correlation and the Fallacy of the Ratio Standard Revisited. *Journal of the Royal Statistical Society.* 1993;156(3):379-392.