Kidney360

Urinary Metabolite Profile Predicting the Progression of CKD

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Key Points

- As a biomarker, urinary metabolites could bridge the gap between genetic abnormalities and phenotypes of diseases.
- We found that levels of betaine, choline, fumarate, citrate, and glucose were significantly correlated with kidney function and could predict kidney outcomes, providing prognostic biomarkers in CKD.

Abstract

Background Because CKD is caused by genetic and environmental factors, biomarker development through metabolomic analysis, which reflects gene-derived downstream effects and host adaptation to the environment, is warranted.

Methods We measured the metabolites in urine samples collected from 789 patients at the time of kidney biopsy and from urine samples from 147 healthy participants using nuclear magnetic resonance. The composite outcome was defined as a 30% decline in eGFR, doubling of serum creatinine levels, or end-stage kidney disease.

Results Among the 28 candidate metabolites, we identified seven metabolites showing (*1*) good discrimination between healthy controls and patients with stage 1 CKD and (*2*) a consistent change in pattern from controls to patients with advanced-stage CKD. Among the seven metabolites, betaine, choline, glucose, fumarate, and citrate showed significant associations with the composite outcome after adjustment for age, sex, eGFR, the urine protein–creatinine ratio, and diabetes. Furthermore, adding choline, glucose, or fumarate to traditional biomarkers, including eGFR and proteinuria, significantly improved the ability of the net reclassification improvement ($P < 0.05$) and integrated discrimination improvement ($P < 0.05$) to predict the composite outcome.

Conclusion Urinary metabolites, including betaine, choline, fumarate, citrate, and glucose, were found to be significant predictors of the progression of CKD. As a signature of kidney injury–related metabolites, it would be warranted to monitor to predict the renal outcome.

KIDNEY360 4: 1048–1057, 2023. doi: https://doi.org/10.34067/KID.0000000000000158

Introduction

CKD is among the leading causes of disease burden worldwide in both disease outcome and health care costs. Because there are few disease-specific treatment measures in the advanced stage, risk stratification for early intervention has a critical role in managing CKD.

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Although estimation of the GFR or quantification of urinary protein may indicate the function of the kidneys and disease activity, these biomarkers have limited utility in predicting long-term clinical outcomes, especially in patients with early-stage CKD. Consequently, invasive diagnostic procedures such as kidney biopsy have been implemented in current medicine.

The human metabolome reflects genetic variability, environmental challenges, intrinsic biochemical processes, and the complex interactions of all these factors.[1] Thus, metabolites are involved in the pathophysiology of complex diseases such as CKD and could serve as a indicative biomarker that bridges the gap between genetic abnormalities and phenotypes of diseases.[2] In addition, changes in urine metabolites may be not only a consequence of kidney injury but also a host response to kidney injury. Therefore, urinary metabolomic analysis could offer comprehensive information on alteration in metabolism that could be involved in the actual disease processes occurring in kidney disease. Taken together, these results indicate that some urinary metabolites could serve as noninvasive biomarkers and/or treatment targets for kidney diseases.

Previous studies showed changes in the urinary metabolites of patients with CKD regarding kidney function and disease activity.[3,4] In addition, several reports presented specific metabolites such as trimethylamine-N-oxide (TMAO), C-glycosyltryptophan, and phosphatidylcholine related to the prognosis of kidney diseases.[5,6] However, the results might not be consistent because of the different characteristics of the participants and the timing of biospecimen acquisition. Therefore, we aimed to determine the urinary metabolomic signature indicative of kidney injury and progression of CKD using urine samples at the time of kidney biopsy from a large number of participants. To this end, we assessed metabolites that were differentially expressed based on kidney function and could predict the progression of CKD.

Methods

Study Participants and Specimens

We included participants with an eGFR ≥30 ml/min per 1.73 m^2 at the time of kidney biopsy. Participants who provided informed consent to donate kidney tissue were enrolled in this study, and only native kidney biopsies were included for analysis. Serum and urine samples at the time of kidney biopsy were collected between December 2010 and June 2017. Biospecimens were stored at −80°C according to the standard operating procedure.[7] In addition, for the control samples, we used biospecimens from healthy volunteers with no underlying diseases, including hypertension, diabetes, and kidney diseases. The baseline clinical characteristics and biospecimens, including morning random urine samples, were collected. The eGFR was calculated with the CKD Epidemiology Collaboration equation.[8] All the participants who were followed up over 3 months were included, and the participants were followed until August 2021. We identified the development of the composite outcome, defined as the initiation of renal replacement therapy, doubling of serum creatinine, or a 30% decline in eGFR from baseline. For the participants who did not develop the composite outcome until August 2021, the latest visit date before August 2021 was regarded as the last visit for follow-up.

^{1}H NMR-Based Metabolic Profiling

We performed nuclear magnetic resonance (NMR)–based urine metabolite analysis following a previously reported method.[9,10] In brief, all urine samples were centrifuged at 12,700 rpm and 4°C for 15 minutes to remove proteins in those samples using centrifugal filters (Amicon Ultra, 3K, Merck Millipore). Next, 330 μl of 0.2 M sodium phosphate buffer (pH 7.0) and 70 μl of 5 mM 3-(trimethylsilyl) propionic 2,2,3,3-d4 acid sodium salt (TSP, 98 atom %) were added to 300 μl of filtered urine. After mixing, 600 μl of the sample was transferred into a 5-mm NMR tube. Prepared urine samples were analyzed by using one-dimensional (1D) ^{1}H NMR spectra with 64 transients at 298 K using a Bruker Avance III HD 800-MHz NMR spectrometer (Bruker BioSpin, Germany) equipped with a Bruker 5-mm CPTCI Z-GRD probe using a NOESYPRESAT pulse sequence. TopSpin 3.1 and AMIX (Bruker BioSpin) were used for phase and baseline correction of all acquired ^{1}H NMR spectra, respectively. The processed NMR spectra were imported into Chenomx (version 7.1, Edmonton, AB, Canada) to identify and quantify the urine metabolites. The urine metabolites were identified using the 800 MHz library of Chenomx, 2D NMR spectra, and spiking experiments. The levels of urinary metabolites were quantified by integrating the peak areas of metabolites compared with the areas of the 5 mM 3-(trimethylsilyl) propionic 2,2,3,3-d4 acid sodium salt (TSP, 98 atom %) using Chenomx software. Finally, the concentration of each metabolite was adjusted with urine creatinine levels.

Liquid Chromatography–Mass Spectrometry Analysis

Because several peaks in the NMR spectrum slightly overlapped, we performed targeted analysis using liquid chromatography–mass spectrometry to validate the metabolites, including betaine, choline, and TMAO. To extract those metabolites, 30 μl of urine was mixed with 90 μl of cold methanol, vigorously vortexed for 1 minute, and kept at −20°C for 30 minutes. After centrifuging at 12,500 rpm and 4°C for 20 minutes, the supernatant was transferred into a new 1.5 ml tube and dried. The extract was resolved with 300 μl of water/acetonitrile (8:2 [v/v]) and diluted with the same solvent to 10× or 100×. After 180 μl of each sample was mixed with 20 μl of internal standard (betaine-d11, 500 ng/ml), 1 μl was injected into an Agilent 1290 Infinity LC and 6495 Triple Quadrupole MS system equipped with an Agilent Jet Stream ESI source (Agilent Technologies). An acquity UPLC BEH Amide column (2.1×100 mm, 1.7 μm; Waters Corp) was used to separate urinary metabolites for 5.10 minutes at 25°C. The mobile phase for gradient elution consisted of 0.3% formic acid in water (A) and 0.3% formic acid in acetonitrile (B). The linear gradients were as follows: 85% B for 1.0 minute, 85%–40% B for 1.5 minute, 40% B for 0.5 minute, 40%–85% B for 0.1 minute, and 85% B for 2.0 minutes. Multiple reaction monitoring experiments were conducted in positive ion mode with the following parameters: capillary voltage, 3.5 kV; nebulizer gas, nitrogen at 40 psi; drying gas temperature, 120°C; drying gas flow rate, 11 L/min; sheath gas temperature, 350°C; and sheath gas

flow rate, 12 L/min. Representative multiple reaction monitoring chromatograms of betaine, choline, and TMAO in urine are shown in Supplemental Figure 1. Finally, metabolite concentrations were adjusted with urine creatinine levels, which were assessed according to the manufacturer's instructions (Abcam, Cambridge, UK, Cat No. ab65340). As an internal validation, we compared the concentration of metabolites between NMR and liquid chromatography–mass spectrometry methods using a linear regression model. In this validation method, we included metabolites of the choline pathway (Supplemental Figure 2).

Statistical Analysis

The baseline characteristics of the study participants were analyzed using the chi-squared test, one-way ANOVA, and independent *t* test. Continuous and categorical variables are presented as the mean±SD and numbers with percentages, respectively. We described *P* for trend for variables showing a normal distribution. The concentration of each metabolite was compared according to the stage of CKD using the Jonckheere–Terpstra test. The value of each metabolite is represented as the median and interquartile range.

We evaluated the association between the metabolite and the prognosis of CKD using the categorical values of metabolites divided into tertiles. Among all participants, we included only those with an eGFR ≥30 ml/min per 1.73 m^2 and follow-up over 3 months in the outcome analysis. We used Kaplan–Meier survival analysis and the Cox proportional hazards model. In addition, the continuous net reclassification improvement (cNRI) and integrated discrimination improvement (IDI) were calculated to determine the extent to which adding metabolites improved the predictive ability for composite outcomes. Conventional biomarkers such as eGFR and urine protein–creatinine ratio (uPCR) have a significant statistical power to reveal the kidney function; we used reclassification calibration to provide a statistic for comparing the overall reclassification of a new model compared with a reference model which has a strong statistical power.[11,12] We performed the statistical analysis using the programs SPSS 20.0 (IBM Statistics) and SAS 9.4 (SAS Institute) with a two-sided *P*-value of < 0.05 as the criterion for statistical significance.

Ethical Considerations

This study was conducted following the Declaration of Helsinki. The study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea (H-1707-176-875). All biospecimens and clinical parameters were prospectively collected with informed consent.

Results

Study Populations

The baseline clinical characteristics are shown in Table 1. A total of 936 samples were obtained, comprising 147 (15.7%) healthy controls and 340 (36.3%), 230 (24.5%), and 219 patients with (23.3%) stage 1, 2, and 3 CKD, respectively. Regarding the advanced stage of CKD, age was higher, and levels of serum phosphate, glucose, blood urea nitrogen, and uric acid were increased; however, serum hemoglobin levels, platelet count, total CO_2, and eGFR were decreased. When comparing healthy controls and participants with stage 1 CKD, healthy controls were older, more likely to be male, and had higher serum hemoglobin, serum calcium, serum protein, and serum albumin levels and lower serum glucose and cholesterol levels. By contrast, the eGFR was higher in participants with CKD stage 1 than in healthy controls, suggesting hyperfiltration (Table 1).

The prevalence of hypertension and diabetes was 16.7% and 17.7%, respectively (Supplemental Table 1). Most patients were diagnosed with glomerular disease, and the most common pathologic finding was IgA nephropathy (45.8%) (Supplemental Table 1).

Differentially Expressed Metabolites between the Healthy Controls and Patients in Different Stages of CKD

A total of 28 urinary metabolites were identified and quantified. The resonance assignment of urinary metabolites is listed in Supplemental Table 2. To identify indicative biomarkers, we explored metabolites under three conditions: (*1*) differentially expressed between patients and controls with CKD stage 1, (*2*) differential metabolites according to the stage of CKD, and (*3*) consistent pattern according to the stage of CKD with a significant Jonckheere–Terpstra test result. The concentrations of 28 metabolites in 147 healthy controls and 789 patients are shown in Table 2. Among the 28 metabolites, we identified eight metabolites satisfying the aforementioned three conditions. There were six metabolites (betaine, choline, dimethylamine, fumarate, glucose, and TMAO) representing consistently increasing patterns according to the degree of kidney dysfunction. On the other hand, two metabolites, citrate and methylnicotinamide, showed consistently decreasing patterns according to the degree of kidney dysfunction (Figure 1).

We also evaluated the metabolite concentration according to the grade of proteinuria <0.3, 0.3–1.0, 1.0–3.0, and ≥3.0 g/gCr. We found that 14 metabolites showed significantly different concentrations according to the grade of proteinuria. Except for urine creatinine, the concentration of 13 metabolites incrementally increased with a higher grade of proteinuria (Supplemental Table 3).

Urinary Metabolites Associated with the Progression of CKD

Among the eight differentially expressed metabolites, we tried to determine which metabolites could predict complex results. Over 59.3±33.2 (62.0, interquartile range 36.0–83.0) months, 309 (39.2%) patients showed the composite outcome. The risk for the composite outcome was incrementally increased among patients in the third tertile of expression of the metabolites, including betaine ($P < 0.001$), choline ($P < 0.001$), dimethylamine ($P = 0.005$), fumarate ($P < 0.001$), glucose ($P < 0.001$), and TMAO ($P = 0.038$). The lowest tertile of citrate ($P < 0.001$) was significantly associated with better outcomes, but methylnicotinamide did not significantly affect the outcome (Figure 2).

There were seven metabolites that satisfied both conditions, showing significant discrimination according to the stage of kidney dysfunction and a meaningful association with the composite outcome. These metabolites were differentiated by the metabolism pathway: (*1*) choline metabolism (*e.g.*, betaine, choline, dimethylamine, TMAO), (*2*) carbohydrate metabolism (*e.g.*, glucose), and (*3*) tricarboxylic acid (TCA) cycle (*e.g.*, fumarate and citrate). In the

Table 1. The clinical characteristics of the study participants

Variables	Control (n=147)	CKD Stage 1 (n=340)	CKD Stage 2 (n=230)	CKD Stage 3 (n=219)	P for Trend	Jonckheere–Terpstra P Value	P Value[a]
Age, yr	49.7 (17.6)	38.5 (15.1)	48.4 (15.0)	51.6 (14.3)	<0.001	<0.001	<0.001
Male, n (%)	89 (60.5)	171 (50.3)	137 (59.6)	124 (56.6)	0.075[b]	NA	0.02
BMI, kg/m^2	23.6 (3.2)	23.7 (3.6)	24.9 (4.0)	24.4 (4.1)	0.002	0.046	0.885
WBC, $10^3/\mu$l	6.0 (1.6)	7.9 (2.4)	8.0 (2.6)	7.9 (2.7)	<0.001	<0.001	<0.001
Hemoglobin, g/dl	14.5 (1.3)	12.6 (1.7)	12.2 (2.1)	11.1 (2.0)	<0.001	<0.001	<0.001
Platelet, $10^3/\mu$l	242.3 (53.2)	235.8 (64.4)	233.7 (76.8)	226.0 (81.6)	<0.001	0.166	0.367
Calcium, mg/dl	9.4 (0.4)	8.8 (0.7)	8.8 (0.7)	8.8 (0.7)	<0.001	<0.001	<0.001
Phosphate, mg/dl	3.5 (0.4)	3.6 (0.6)	3.5 (0.6)	3.6 (0.7)	<0.001	0.168	0.061
Glucose, mg/dl	97.5 (11.0)	103.3 (36.2)	109.5 (33.7)	112.5 (52.7)	<0.001	0.001	0.011
BUN, mg/dl	13.5 (3.9)	12.9 (3.6)	18.2 (7.7)	25.5 (9.5)	<0.001	<0.001	0.238
Creatinine, mg/dl	0.8 (0.2)	0.7 (0.2)	1.0 (0.2)	1.6 (0.3)	<0.001	<0.001	<0.001
Uric acid, mg/dl	5.6 (1.3)	5.6 (1.5)	6.6 (1.8)	7.1 (2.1)	<0.001	<0.001	0.485
Cholesterol, mg/dl	197.5 (37.6)	223.7 (92.4)	215.6 (75.6)	189.6 (66.0)	0.002	<0.001	<0.001
Protein, g/dl	7.5 (0.4)	6.2 (1.1)	6.2 (1.1)	6.5 (1.1)	<0.001	<0.001	<0.001
Albumin, g/dl	4.4 (0.3)	3.5 (0.8)	3.4 (0.8)	3.5 (0.7)	<0.001	<0.001	<0.001
Sodium, mEq/L	141.6 (1.9)	140.0 (2.2)	139.9 (2.6)	139.7 (3.0)	<0.001	<0.001	<0.001
Potassium, mEq/L	4.3 (0.3)	4.1 (0.3)	4.3 (0.4)	4.4 (0.6)	<0.001	<0.001	<0.001
Chloride, mEq/L	104.1 (2.1)	104.9 (2.5)	104.6 (2.8)	105.8 (3.9)	<0.001	<0.001	0.001
Total CO_2, mmol/L	28.7 (2.8)	27.4 (2.6)	27.1 (3.1)	25.2 (3.1)	<0.001	<0.001	<0.001
uPCR, g/g	NA	2.9 (4.2)	3.8 (9.0)	3.4 (4.4)	0.099	0.194	NA
eGFR, ml/min per 1.73 m^2	96.8 (15.4)	111.1 (14.3)	76.0 (8.5)	45.1 (8.5)	<0.001	<0.001	<0.001

NA, not applicable; BMI, body mass index; WBC, white blood cell; uPCR, urine protein to creatinine ratio.
[a]P-value: control versus CKD stage 1.
[b]P-value for Pearson chi-squared analysis.

unadjusted Cox proportional analysis, seven metabolites were associated with a significantly increased risk for the composite outcome according to concentration. After adjusting for age, sex, eGFR, the uPCR, diabetes, hypertension, and body mass index, this relationship was maintained in betaine, choline, glucose, fumarate, and citrate (Table 3).

Additive Effect of Metabolites to Predict Kidney Outcome

Among the seven metabolites that were selected in the above processes, six metabolites, excluding TMAO, showed statistically significant improvement in cNRI after adjusting for age and sex. In addition, fumarate (P = 0.013) and glucose (P = 0.033) showed significant improvement in cNRI after adjusting for age, sex, eGFR, and the uPCR ($P < 0.05$). The IDI was also significantly improved across all metabolites except dimethylamine and TMAO after adjusting for age and sex. The addition of eGFR and the uPCR to choline (P = 0.020), glucose (P = 0.027), or fumarate (P = 0.013) also improved model performance (Table 4).

Combining two or more metabolites significantly increased the predictive power in the choline metabolism pathway. Among the various conditions, the choline and betaine combination showed the best result in the cNRI and IDI indexes. However, the result was not improved after adding dimethylamine and/or TMAO to the betaine and choline combination. In the TCA cycle, the fumarate and citrate combination attenuated the predictive significance compared with the effect of fumarate alone on cNRI. However, the combination of metabolites increased the significance of the predictive power of IDI (Table 4).

Discussion

In this study, we identified a signature of kidney injury–related metabolites that could be used to predict the prognosis of kidney disease. These metabolites showed significant differences in their levels between the healthy controls and patients with stage 1 CKD, where the GFR remains normal. In addition, these metabolites showed changes in their levels that were proportional to the CKD stage, reflecting kidney injury. Moreover, we found that adding these metabolites to traditional risk factors, such as eGFR and proteinuria, could significantly improve the prediction of the renal outcome.

As an essential nutrient, choline is involved in three main metabolic pathways for the synthesis of (*1*) acetylcholine, (*2*) betaine, (*3*) phospholipids, and (*4*) trimethylamine (TMA). Following the metabolic pathways, choline could be detected in urine with betaine and TMA. An increased level of plasma choline has been suggested to be a sign of tubular dysfunction and atherogenesis and is also associated with kidney dysfunction in CKD.[5,13,14] However, there have been no previous reports regarding the relationship between urinary choline levels and kidney dysfunction. Because most choline is metabolized into acetylcholine, phosphatidylcholine, and betaine aldehyde, only a small amount of choline can be excreted into the urine.[15] In this study, we found that an increased urinary choline level was significantly related to kidney dysfunction and poor prognosis. Although we could not determine the causal relationship, the relationship between urinary choline and kidney dysfunction could be linked to the production of TMAO and betaine, considering the complicated metabolic pathway of choline.

Table 2. Concentrations of metabolites in healthy controls and participants with CKD

Variable	Control (n=147)	CKD Stage 1 (n=340)	CKD Stage 2 (n=230)	CKD Stage 3 (n=219)	Jonckheere–Terpstra Statistic	P Value (1)	P Value (2)	P Value (3)	P Value (4)
Acetate	4.667 (3.165–7.301)	5.243 (3.444–8.798)	5.675 (3.788–9.500)	5.609 (3.236–8.990)	1.950	0.051	0.103	0.026	0.484
Acetone	2.194 (1.553–4.023)	1.812 (1.124–3.125)	1.815 (1.153–3.158)	2.748 (1.686–4.886)	2.915	0.004	0.725	0.629	0.039
Alanine	26.338 (17.095–36.036)	39.402 (30.095–59.174)	37.561 (26.179–53.068)	34.753 (22.971–57.849)	3.507	<0.001	<0.001	0.313	0.639
Betaine	11.031 (7.303–16.060)	15.698 (9.905–28.800)	19.789 (11.599–37.861)	29.999 (16.074–59.074)	10.722	<0.001	<0.001	0.071	<0.001
Choline	2.353 (1.653–3.743)	4.453 (2.840–7.129)	6.057 (3.375–11.359)	10.317 (4.980–25.218)	15.246	<0.001	<0.001	0.008	<0.001
Citrate	345.159 (178.178–595.663)	317.298 (177.718–528.377)	250.976 (132.666–406.263)	168.583 (89.775–273.460)	−9.088	<0.001	0.091	0.003	<0.001
Creatine	11.878 (6.221–31.115)	16.479 (10.040–75.642)	12.778 (8.120–30.244)	13.745 (9.568–27.024)	−0.262	0.794	0.002	0.026	0.650
Dimethylamine	38.732 (31.733–51.443)	46.887 (38.938–59.140)	50.290 (39.519–67.108)	56.361 (45.398–74.596)	8.843	<0.001	0.001	0.213	0.033
Dimethylglycine	4.608 (2.907–6.784)	6.303 (4.157–9.596)	6.091 (3.812–9.226)	6.716 (3.797–10.010)	3.685	<0.001	0.507	0.402	0.214
Formate	17.859 (9.496–28.338)	23.311 (15.471–34.072)	17.638 (9.753–29.340)	11.704 (6.328–21.213)	−6.234	<0.001	0.001	0.273	0.257
Fumarate	0.270 (0.167–0.477)	0.338 (0.000–0.670)	0.435 (0.110–0.977)	0.768 (0.170–1.633)	7.123	<0.001	0.022	0.007	0.005
Glucose	51.648 (39.709–72.577)	52.651 (40.486–74.541)	61.008 (39.631–130.392)	70.372 (39.425–197.467)	3.966	<0.001	0.008	0.965	0.505
Glycerol	690.886 (465.887–1009.953)	50.393 (31.780–90.542)	56.488 (31.014–95.777)	70.067 (40.801–121.037)	−9.300	<0.001	<0.001	0.879	0.033
Glycine	77.047 (49.174–115.894)	114.684 (76.066–167.389)	86.248 (54.209–137.342)	72.595 (41.638–137.428)	−3.318	0.001	<0.001	0.957	0.256
Hydroxyisobutyrate	7.211 (5.590–9.441)	7.537 (5.874–9.427)	6.979 (5.177–8.665)	6.387 (4.801–8.038)	−5.037	<0.001	0.942	0.018	0.011
Indoxylsulfate	18.807 (11.286–27.956)	17.304 (8.041–30.286)	17.331 (9.134–29.430)	22.023 (13.323–36.911)	2.033	0.042	0.870	0.632	0.971
Isoleucine	1.421 (1.109–1.726)	2.088 (1.615–2.773)	1.837 (1.327–2.666)	1.838 (1.288–2.915)	3.538	<0.001	0.873	0.999	0.089
Lactate	9.949 (6.886–13.560)	15.017 (9.836–23.819)	13.885 (8.696–23.399)	17.684 (10.234–29.786)	6.523	<0.001	<0.001	0.227	0.995
Leucine	3.136 (2.470–4.050)	4.986 (3.816–6.309)	4.668 (3.249–6.827)	4.777 (3.072–7.156)	5.801	<0.001	<0.001	0.690	0.019
Methylhistidine	20.664 (17.383–26.109)	5.822 (4.670–7.412)	5.260 (3.914–7.539)	4.892 (3.363–7.467)	2.600	<0.001	<0.001	0.824	0.095
Methylnicotinamide	6.228 (3.872–10.715)	4.418 (2.691–7.469)	4.333 (2.566–6.660)	3.941 (2.256–6.011)	−5.273	<0.001	<0.001	0.938	0.510
Phenylalanine	30.785 (13.261–62.279)	34.693 (17.582–65.706)	37.297 (19.185–71.489)	51.026 (26.508–91.948)	5.423	<0.001	0.588	0.125	0.007
Pyruvate	2.034 (1.247–2.861)	3.748 (2.550–5.222)	3.316 (2.051–5.159)	3.384 (2.295–5.405)	5.476	<0.001	<0.001	0.462	0.262
Taurine	162.589 (113.690–238.590)	172.042 (114.344–252.347)	174.114 (105.643–253.353)	160.440 (94.312–231.019)	−1.118	0.264	0.218	0.315	0.629
Threonine	13.075 (9.404–19.088)	19.742 (14.967–27.493)	16.691 (11.752–24.083)	14.986 (8.702–27.808)	−0.016	0.987	<0.001	0.696	0.371
TMAO	52.447 (26.521–115.009)	85.924 (42.980–195.251)	107.220 (54.524–249.696)	128.553 (68.933–291.752)	7.852	<0.001	0.004	0.084	0.191
Tryptophan	7.116 (5.106–9.113)	8.242 (5.975–11.075)	7.409 (5.395–10.204)	6.860 (4.396–9.357)	−1.869	0.062	<0.001	0.212	0.351
Valine	3.957 (3.223–4.912)	5.822 (4.670–7.412)	5.260 (3.914–7.539)	4.892 (3.363–7.467)	2.600	0.009	<0.001	0.824	0.095

P-value (1): Jonckheere–Terpstra test; P-value (2): control versus CKD stage 1; P-value (3): CKD stage 1 versus stage 2; P-value (4): CKD stage 2 versus stage 3. All the concentration of each metabolite was adjusted by urine creatinine. TMAO, trimethylamine-N-oxide.

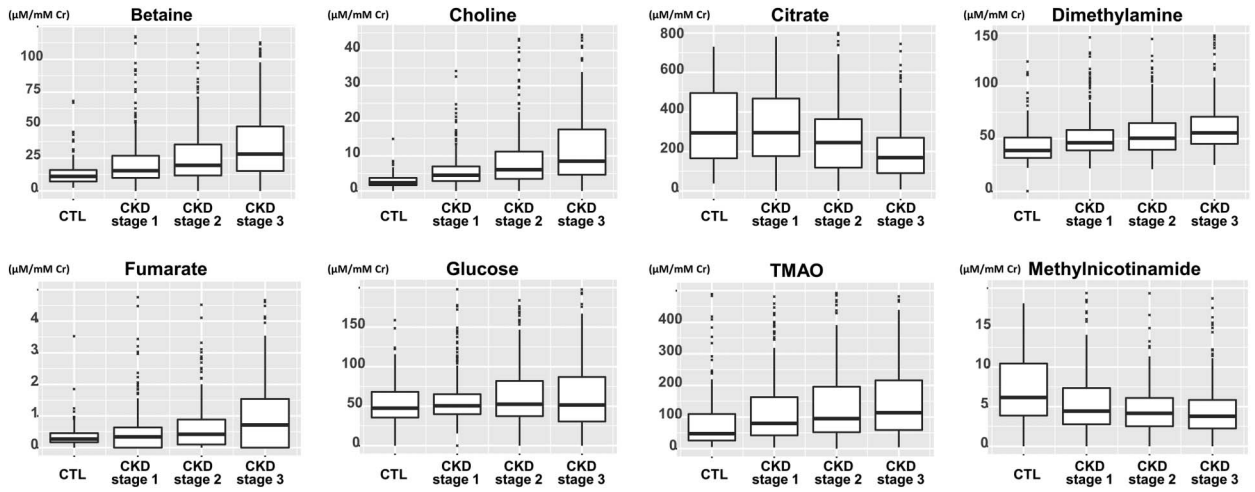

Figure 1. The quantitative concentrations of eight selected metabolites. Box plots showing the creatinine-adjusted quantitative urinary metabolite concentration. The *y* axis indicates the creatinine-adjusted urine concentration (μM/mM Cr). The box plot shows the interquartile ranges, with the horizontal lines indicating the median values. Each dot represents an outlier over the 95th percentile range. CTL, control.

TMAO is closely linked to the choline metabolism pathway and is metabolized from TMA by the gut microbiota.[16] Increased serum TMAO levels were associated with increased risks for major cardiovascular events and all-cause mortality.[17] In addition, the concentration was increased with kidney dysfunction, and a higher level of TMAO has a harmful cardiovascular effect in patients with CKD.[5] In this study, urinary TMAO levels were well discriminated according to CKD stage. However, this metabolite was not significantly correlated with the composite outcome. Considering the metabolic pathway, the production of TMAO is closely linked to the gut microbiome; thus, outcome predictability could not be derived using urine samples.

Betaine has an essential role in the generation and maintenance of methionine and contributes 50% of the homocysteine methylation capacity of the liver.[15] Although it is minimally excreted in the urine, it also has a role in essential osmolytes in the kidney. The concentration of betaine in the kidney is controlled by tonicity through the regulation of betaine homocysteine methyltransferase. Finally, it protects kidney resident cells from high concentrations of

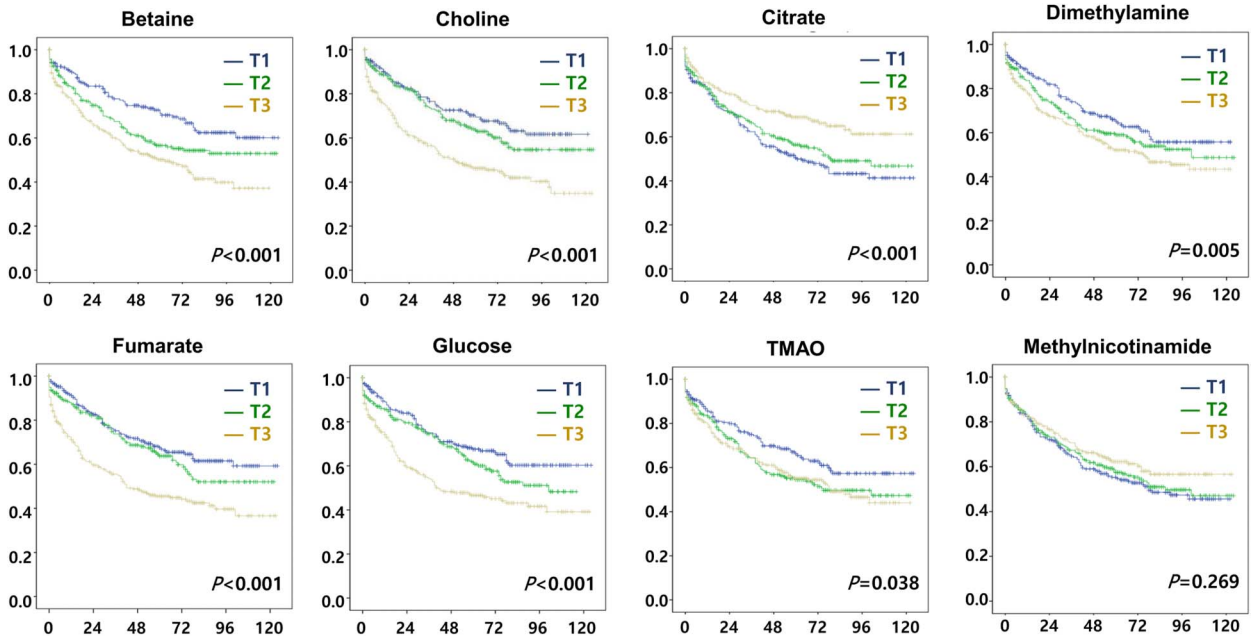

Figure 2. Kaplan–Meier curves for the composite outcome according to each metabolite. The risk for the composite outcome is presented according to the tertiles of each metabolite. Blue, green, and yellow lines indicate the first, second, and third tertiles, respectively. The *y* axis indicates the risk proportion, and the *x* axis shows the follow-up period in months. TMAO, trimethylamine-N-oxide.

Table 3. Hazard ratios of metabolites for the composite outcome

Metabolites	Unadjusted		Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Choline metabolism										
Betaine T1 (ref)										
Betaine T2	1.31 (0.98 to 1.77)	0.070	1.20 (0.89 to 1.63)	0.228	1.18 (0.87 to 1.60)	0.293	1.15 (0.84 to 1.56)	0.381	1.18 (0.87 to 1.61)	0.288
Betaine T3	1.96 (1.47 to 2.61)	<0.001	1.71 (1.27 to 2.31)	<0.001	1.60 (1.18 to 2.17)	0.002	1.42 (1.03 to 1.95)	0.030	1.48 (1.07 to 2.03)	0.017
Choline T1 (ref)										
Choline T2	1.43 (1.06 to 1.94)	0.020	1.43 (1.05 to 1.95)	0.024	1.44 (1.06 to 1.97)	0.021	1.38 (1.01 to 1.89)	0.041	1.45 (1.06 to 1.99)	0.021
Choline T3	2.27 (1.71 to 3.01)	<0.001	2.14 (1.59 to 2.87)	<0.001	1.99 (1.47 to 2.71)	<0.001	1.84 (1.35 to 2.51)	<0.001	1.90 (1.39 to 2.60)	<0.001
Dimethylamine T1 (ref)										
Dimethylamine T2	1.22 (0.91 to 1.62)	0.186	1.19 (0.88 to 1.60)	0.268	1.15 (0.85 to 1.56)	0.360	1.10 (0.81 to 1.49)	0.554	1.10 (0.81 to 1.50)	0.532
Dimethylamine T3	1.58 (1.20 to 2.08)	0.001	1.45 (1.08 to 1.95)	0.015	1.38 (1.02 to 1.86)	0.036	1.27 (0.94 to 1.72)	0.128	1.27 (0.94 to 1.72)	0.123
TMAO T1 (ref)										
TMAO T2	1.25 (0.94 to 1.65)	0.131	1.16 (0.87 to 1.55)	0.314	1.12 (0.84 to 1.50)	0.440	1.08 (0.81 to 1.45)	0.588	1.08 (0.81 to 1.45)	0.595
TMAO T3	1.34 (1.01 to 1.77)	0.041	1.19 (0.89 to 1.59)	0.235	1.13 (0.84 to 1.51)	0.414	1.01 (0.75 to 1.36)	0.935	1.02 (0.76 to 1.37)	0.913
Carbohydrate metabolism										
Glucose T1 (ref)										
Glucose T2	1.31 (0.97 to 1.76)	0.081	1.22 (0.90 to 1.66)	0.206	1.25 (0.92 to 1.71)	0.154	1.25 (0.91 to 1.70)	0.170	1.25 (0.91 to 1.70)	0.170
Glucose T3	2.21 (1.67 to 2.92)	<0.001	1.96 (0.47 to 2.62)	<0.001	1.90 (1.42 to 2.54)	<0.001	1.68 (1.23 to 2.30)	0.001	1.76 (1.28 to 2.41)	0.001
TCA cycle										
Fumarate T1 (ref)										
Fumarate T2	1.23 (0.90 to 1.66)	0.190	1.21 (0.89 to 1.64)	0.220	1.23 (0.90 to 1.67)	0.196	1.22 (0.90 to 1.66)	0.202	1.22 (0.90 to 1.66)	0.209
Fumarate T3	2.07 (1.57 to 2.73)	<0.001	2.03 (1.52 to 2.71)	<0.001	1.92 (1.43 to 2.59)	<0.001	1.73 (1.27 to 2.35)	0.001	1.78 (1.30 to 2.42)	<0.001
Citrate T1 (ref)										
Citrate T2	0.88 (0.63 to 1.15)	0.351	0.87 (0.67 to 1.12)	0.282	0.93 (0.71 to 1.21)	0.585	0.92 (0.70 to 1.20)	0.522	0.94 (0.72 to 1.23)	0.637
Citrate T3	0.69 (0.52 to 0.92)	0.012	0.67 (0.50 to 0.89)	0.007	0.77 (0.56 to 1.06)	0.105	0.69 (0.51 to 0.96)	0.025	0.72 (0.52 to 1.00)	0.047

Model 1: adjusted with age, sex. Model 2: adjusted with age, sex, eGFR, urinary protein/creatinine ratio. Model 3: adjusted with age, sex, eGFR, urinary protein/creatinine ratio, diabetes. Model 4: adjusted with age, sex, eGFR, urinary protein/creatinine ratio, diabetes, hypertension, body mass index. HR, hazard ratio; CI, confidence interval; TMAO, trimethylamine-N-oxide; TCA, tricarboxylic acid.

Table 4. Prediction of kidney outcomes according to each metabolite and combination of metabolites

Variables Included in Model	cNRI Model 1		cNRI Model 2		IDI Model 1		IDI Model 2	
	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value	Estimate (95% CI)	*P* Value
Choline metabolism								
Betaine (B)	0.051 (0.011 to 0.196)	0.013	0.045 (−0.044 to 0.209)	0.193	0.010 (0.000 to 0.038)	0.027	0.001 (−0.001 to 0.016)	0.252
Choline (C)	0.189 (0.103 to 0.280)	<0.001	0.113 (−0.006 to 0.230)	0.053	0.038 (0.015 to 0.071)	<0.001	0.010 (0.001 to 0.029)	0.020
Dimethylamine (D)	0.080 (0.005 to 0.176)	0.040	0.034 (−0.047 to 0.150)	0.233	0.005 (0.000 to 0.021)	0.073	0.001 (−0.001 to 0.011)	0.179
TMAO (T)	0.102 (−0.018 to 0.164)	0.093	0.097 (−0.057 to 0.172)	0.259	0.004 (0.000 to 0.019)	0.047	0.001 (−0.001 to 0.013)	0.206
C+B	0.189 (0.118 to 0.297)	<0.001	0.141 (0.004 to 0.247)	0.047	0.039 (0.017 to 0.077)	<0.001	0.011 (0.002 to 0.036)	0.027
C+D	0.214 (0.105 to 0.295)	<0.001	0.100 (0.013 to 0.225)	0.020	0.038 (0.015 to 0.074)	<0.001	0.010 (0.002 to 0.034)	0.007
C+T	0.187 (0.117 to 0.301)	<0.001	0.124 (0.001 to 0.221)	0.047	0.039 (0.019 to 0.077)	<0.001	0.010 (0.002 to 0.033)	<0.001
C+B+D	0.189 (0.115 to 0.303)	<0.001	0.141 (0.023 to 0.229)	0.007	0.039 (0.020 to 0.078)	<0.001	0.011 (0.004 to 0.040)	<0.001
C+B+T	0.213 (0.111 to 0.288)	<0.001	0.128 (0.020 to 0.217)	0.020	0.040 (0.018 to 0.076)	<0.001	0.011 (0.004 to 0.038)	0.013
C+B+D+T	0.215 (0.103 to 0.300)	<0.001	0.128 (0.024 to 0.231)	0.020	0.040 (0.022 to 0.088)	<0.001	0.011 (0.006 to 0.040)	<0.001
Carbohydrate metabolism								
Glucose	0.230 (0.119 to 0.293)	<0.001	0.203 (0.034 to 0.275)	0.033	0.031 (0.013 to 0.055)	<0.001	0.011 (0.001 to 0.030)	0.027
TCA cycle								
Fumarate	0.227 (0.136 to 0.310)	<0.001	0.198 (0.038 to 0.265)	0.013	0.043 (0.020 to 0.080)	<0.001	0.015 (0.004 to 0.041)	0.013
Citrate	0.109 (0.034 to 0.182)	0.007	0.075 (−0.038 to 0.153)	0.186	0.014 (0.003 to 0.036)	<0.001	0.001 (−0.001 to 0.015)	0.252
Fumarate+citrate	0.242 (0.131 to 0.329)	<0.001	0.088 (0.026 to 0.251)	0.007	0.064 (0.034 to 0.113)	<0.001	0.02 (0.005 to 0.054)	<0.001

Model 1: adjusted with age, sex. Model 2: adjusted with age, sex, eGFR, urinary protein/creatinine ratio. When the 95% CI was greater than zero, the model has better prognostic predictability after adding the variables. When the 95% CI crosses zero, the difference in prognostic predictability between the models is nonsignificant. cNRI, continuous net reclassification improvement; IDI, integrated discrimination improvement; CI, confidence interval; TMAO, trimethylamine-N-oxide; TCA, tricarboxylic acid.

electrolytes and urea.[15,18] Thus, the increased excretion of betaine in urine represented imbalances in renal osmolyte regulation and could be related to renal cell damage and the progression of CKD.[19] In this study, we also revealed that urinary betaine has the strength to indicate kidney dysfunction and predict kidney outcomes.

Citrate and fumarate are metabolites involved in the TCA cycle, which is an essential process for harvesting the energy needed by living cells to grow and divide. Hypocitraturia is a well-known risk factor for developing kidney stones.[20] In addition, urinary citrate normalized to creatinine is a good marker of acid-base status, representing acid retention with reduced GFR in CKD.[21,22] Although we did not evaluate the acid-base status, urinary citrate was an excellent parameter for identifying decreased kidney function with a significant predictive ability for the outcome. In this study, the urinary citrate level was well discriminated between the healthy population and patients with early-stage CKD with preserved kidney function. Thus, checking urinary citrate is helpful for identifying kidney dysfunction or predicting kidney outcomes even in patients with preserved kidney function.

Previous studies have shown that fumarate has a role in various kidney diseases.[9,23,24] Fumarate is crucial in mediating the effects of NADPH oxidase isoform 4 in diabetic kidney disease.[23] Podocyte-specific induction of NADPH oxidase isoform 4 might induce glomerular injury in diabetic kidney disease.[24] In addition, significant changes in the levels of urinary fumarate were observed in diabetic patients with kidney dysfunction.[25] In addition, we previously reported that fumarate could play a role in podocyte injury and be a potential target for the treatment of phospholipase A2 receptor–associated membranous nephropathy.[9] We found that high urinary fumarate levels could predict the composite outcome of phospholipase A2 receptor–associated membranous nephropathy. Urinary fumarate could be a good biomarker for predicting kidney outcomes, but it has a limitation to applicate in the clinical field because of its very low concentration levels in urine.

Increased urinary glucose levels have various clinical implications, such as diabetes and tubular disorders. Glycosuria is a well-known marker representing proximal renal tubular dysfunction, especially in participants without diabetes. It is usually regarded as benign without serious consequences.[26] Nevertheless, glycosuria in patients with glomerular disease was significantly related to pronounced tubular atrophy and interstitial fibrosis with a poorer prognosis.[27,28] Using the samples obtained from patients with kidney disease, urinary glucose was also found to have a significant role in representing deteriorated kidney function even after adjusting for diabetes status.

This study identified metabolites that represent current kidney dysfunction with an excellent predictive ability for kidney outcomes. One of the strengths of this study was an assessment of the metabolites in a relatively large number of participants with biopsy-proven kidney disease with an unbiased method. Nevertheless, there are several limitations to be discussed. First, although we successfully identified meaningful metabolites, we could not evaluate the causal relationship between each metabolite and kidney outcomes. A future study is warranted to determine causality. Second, we could not validate the significance of the urinary metabolites in an independent cohort or a repetitive measurement. Third, there were structural limitations because of the retrospective study design. Finally, we did not focus on specific glomerular diseases; further research is necessary to apply these metabolites in the clinical field.

In conclusion, urinary choline, betaine, fumarate, citrate, and glucose significantly correlate with kidney dysfunction and represent the prognosis of kidney dysfunction. These metabolites could be candidates to improve the predictability of kidney outcomes in addition to eGFR and proteinuria.

Disclosures

S.S. Han reports the following—research funding: Daewoong Pharmaceutical. C.S. Lim reports the following—advisory or leadership role: President, Korean Society of Nephrology. All remaining authors have nothing to disclose.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant (NRF-2021R1A2C2094586, NRF-2020R1G1A1008959) and the Korea Basic Science Institute (Fund No. C270200).

Acknowledgments

The biospecimens and data used in this study were provided by the Biobank of Seoul National University Hospital, a member of the Korea Biobank Network (KBN4_A03).

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Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/KN9/A369.

Supplemental Figure 1. Representative multiple reaction monitoring chromatograms of betaine, choline, and TMAO in quality control samples obtained from CKD patients.

Supplemental Figure 2. Internal validation results comparing the urine metabolites of choline pathway measured by NMR method and LC-MS method.

Supplemental Table 1. ^{1}H NMR peak assignments for identified metabolites in urine sample.

Supplemental Table 2. ^{1}H NMR peak assignments for identified metabolites in urine sample.

Supplemental Table 3. Different concentration of each metabolite according to the grade of proteinuria.

References

1. Nicholson JK, Lindon JC. Systems biology: metabonomics. *Nature.* 2008;455(7216):1054–1056. doi:10.1038/4551054a
2. Guma M, Tiziani S, Firestein GS. Metabolomics in rheumatic diseases: desperately seeking biomarkers. *Nat Rev Rheumatol.* 2016;12(5):269–281. doi:10.1038/nrrheum.2016.1
3. Posada-Ayala M, Zubiri I, Martin-Lorenzo M, et al. Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. *Kidney Int.* 2014;85(1):103–111. doi:10.1038/ki.2013.328
4. Gao X, Chen W, Li R, et al. Systematic variations associated with renal disease uncovered by parallel metabolomics of urine and serum. *BMC Syst Biol.* 2012;6(S1):S14. doi:10.1186/1752-0509-6-s1-s14
5. Missailidis C, Hallqvist J, Qureshi AR, et al. Serum trimethylamine-N-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. *PloS One.* 2016; 11(1):e0141738. doi:10.1371/journal.pone.0141738
6. Steinbrenner I, Schultheiss UT, Kotsis F, et al. Urine metabolite levels, adverse kidney outcomes, and mortality in CKD patients: a metabolome-wide association study. *Am J Kidney Dis.* 2021; 78(5):669–677.e1. doi:10.1053/j.ajkd.2021.01.018
7. Kang E, Kim Y, Kim YC, et al. Biobanking for glomerular diseases: a study design and protocol for KOrea Renal biobank NEtwoRk System TOward NExt-generation analysis (KORNERSTONE). *BMC Nephrol.* 2020;21(1):367. doi:10.1186/s12882-020-02016-z
8. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9): 604–612. doi:10.7326/0003-4819-150-9-200905050-00006
9. Jo HA, Hyeon JS, Yang SH, et al. Fumarate modulates phospholipase A2 receptor autoimmunity-induced podocyte injury in membranous nephropathy. *Kidney Int.* 2021;99(2):443–455. doi:10.1016/j.kint.2020.06.031
10. Park S, Lee J, Yang SH, et al. Comprehensive metabolomic profiling in early IgA nephropathy patients reveals urine glycine as a prognostic biomarker. *J Cell Mol Med.* 2021;25(11): 5177–5190. doi:10.1111/jcmm.16520
11. Cook NR, Paynter NP. Performance of reclassification statistics in comparing risk prediction models. *Biom J.* 2011;53(2): 237–258. doi:10.1002/bimj.201000078
12. Paynter NP, Cook NR. A bias-corrected net reclassification improvement for clinical subgroups. *Med Decis Making.* 2013; 33(2):154–162. doi:10.1177/0272989x12461856
13. Rhee EP, Clish CB, Ghorbani A, et al. A combined epidemiologic and metabolomic approach improves CKD prediction. *J Am Soc Nephrol.* 2013;24(8):1330–1338. doi:10.1681/ASN.2012101006
14. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature.* 2011;472(7341):57–63. doi:10.1038/nature09922
15. Craig SA. Betaine in human nutrition. *Am J Clin Nutr.* 2004; 80(3):539–549. doi:10.1093/ajcn/80.3.539
16. Arias N, Arboleya S, Allison J, et al. The relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. *Nutrients.* 2020; 12(8):2340. doi:10.3390/nu12082340
17. Stubbs JR, House JA, Ocque AJ, et al. Serum trimethylamine-N-oxide is elevated in CKD and correlates with coronary atherosclerosis burden. *J Am Soc Nephrol.* 2016;27(1):305–313. doi:10.1681/ASN.2014111063
18. Kwon ED, Zablocki K, Peters EM, Jung KY, Garcia-Perez A, Burg MB. Betaine and inositol reduce MDCK cell glycerophosphocholine by stimulating its degradation. *Am J Physiol.* 1996;270(1):C200–C207. doi:10.1152/ajpcell.1996.270.1.C200
19. Gil RB, Ortiz A, Sanchez-Nino MD, et al. Increased urinary osmolyte excretion indicates chronic kidney disease severity and progression rate. *Nephrol Dial Transplant.* 2018;33(12): 2156–2164. doi:10.1093/ndt/gfy020
20. Curhan GC, Taylor EN. 24-h Uric acid excretion and the risk of kidney stones. *Kidney Int.* 2008;73(4):489–496. doi:10.1038/sj.ki.5002708
21. Gianella FG, Prado VE, Poindexter JR, et al. Spot urinary citrate-to-creatinine ratio is a marker for acid-base status in chronic kidney disease. *Kidney Int.* 2021;99(1):208–217. doi:10.1016/j.kint.2020.07.006
22. Goraya N, Simoni J, Sager LN, Madias NE, Wesson DE. Urine citrate excretion as a marker of acid retention in patients with chronic kidney disease without overt metabolic acidosis. *Kidney Int.* 2019;95(5):1190–1196. doi:10.1016/j.kint.2018.11.033
23. You Y-H, Quach T, Saito R, Pham J, Sharma K. Metabolomics reveals a key role for fumarate in mediating the effects of NADPH oxidase 4 in diabetic kidney disease. *J Am Soc Nephrol.* 2016;27(2):466–481. doi:10.1681/ASN.2015030302
24. Gorin Y, Block K, Hernandez J, et al. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. *J Biol Chem.* 2005;280(47):39616–39626. doi:10.1074/jbc.M502412200
25. Liu JJ, Liu S, Gurung RL, et al. Urine tricarboxylic acid cycle metabolites predict progressive chronic kidney disease in type 2 diabetes. *J Clin Endocrinol Metab.* 2018;103(12):4357–4364. doi:10.1210/jc.2018-00947
26. Hung CC, Lin HY, Lee JJ, et al. Glycosuria and renal outcomes in patients with nondiabetic advanced chronic kidney disease. *Sci Rep.* 2016;6(1):39372. doi:10.1038/srep39372
27. Woronik V, Freitas IF, Saldanha LB, Sabbaga E, Marcondes M. Glycosuria in glomerular diseases: histopathology and clinical correlations. *Braz J Med Biol Res.* 1998;31(5):633–637. doi:10.1590/s0100-879x1998000500005
28. Ormonde C, Laranjinha I, Gil C, Gonçalves M, a Gaspar A. Glycosuria in primary glomerulopathies: prevalence and prognostic significance. *J Bras Nefrol.* 2022;44(1):26–31. doi:10.1590/2175-8239-jbn-2021-0115

Received: January 10, 2023 **Accepted:** May 17, 2023
Published Online Ahead of Print: June 9, 2023

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