AJKD

Correspondence

RESEARCH LETTER

Utility of Spot Urine Specimens to Assess Tubular Secretion

To the Editor:

We have previously shown that markers of tubule cell injury,[1] fibrosis,[2] abnormal acid-base homeostasis,[3] and diminished proximal tubule reabsorptive capacity[4] are associated with more rapid chronic kidney disease (CKD) progression, independent of eGFR and albuminuria. Another key function of kidney tubules is secreting many toxins and medications. Whether secretory capacity gives insights into kidney tubule health is uncertain.

Recently, several endogenous metabolites that are actively secreted and measurable by liquid chromatography–tandem mass spectroscopy (LC-MS/MS) were identified in healthy individuals using 24-hour urine collections.[5] Further, lower secretory function, measured using 2 of these markers, is associated with mortality independent of eGFR and albuminuria in persons with advanced CKD.[6] Thus, endogenous markers of tubular secretion may provide insights to tubular health. However, few large-scale epidemiologic studies have 24-hour urine collection data. Whether spot urine specimens are a suitable alternative to assess tubular secretion is uncertain, so we investigated their utility.

In phase 1 of our study, we identified 21 men from a large blood pressure intervention trial[7] who were selected a priori to span a wide eGFR range (23-103 mL/min/1.73 m^2). Participants provided fasting morning serum and spot urine specimens at the baseline trial visit. We used an LC-MS/MS platform designed to assess metabolism deficits in pediatric patients (Item S1)[8] that measures 16 metabolites, 6 of which had been identified as endogenous secretion markers in healthy controls.[5]

We measured serum and spot urine concentrations of the metabolites and creatinine and calculated each secretion marker's FE. Because FE gives the instantaneous clearance relative to creatinine and creatinine clearance (CL_{cr}) approximates GFR, we defined FE $>$ 100% as net tubular secretion. By generating a 2×2 table of markers expected to be secreted (based on prior data[5]) vs not and with measured FE $>$ 100% vs not on spot specimens, we evaluated statistical significance using the Fisher exact test. We also compared FE on spot specimens to that reported using 24-hour urine samples in prior studies.[5]

The 21 trial participants had a mean age of 74 ± 8 years and eGFR of 60 ± 26 mL/min/1.73 m^2 (Item S1). Of the 6 metabolites hypothesized to be secreted, 5 had FE $>$ 100% (Table 1). In contrast, none of the 10 with unknown renal handling had FE $>$ 100% ($P = 0.001$). Among the 5 secreted markers, mean FEs (172%-713%) appeared consistently lower than those reported in 24-hour urine collections in healthy volunteers.[5]

In phase 2, we determined the stability of the 6 endogenous secretion markers over 24 hours. Three healthy male volunteers provided serum samples at 4 PM immediately before initiating a 24-hour urine collection and again at 4 PM immediately post-collection. During the intervening period, volunteers provided spot urine aliquots at 4-hour intervals. In each urine and serum sample, the 6 endogenous secretion markers and creatinine were measured by LC-MS/MS. We calculated FE at each time point, followed by the mean, SD, and maximum-minimum ratio (max/min) over 24 hours. Max/min served as our primary indicator of stability. We also compared the FE of metabolites at the start of the urine collection to the mean 24-hour urine FE to compare one spot assessment to 24-hour clearance assessment. A *t* test with a 2-sided $P < 0.05$ was considered statistically significant. All participants provided written informed consent and the study was approved by the San Diego VA Medical Center IRB (#H160110).

Table 1. FE of Selected Metabolites in Spot Specimens Among Trial Participants

Metabolite	Spot FE ± SD From This Study, %	Expected FE ± SD From Prior Study,[a] %
Hippuric acid[b]	713.59 ± 681.27	1,200 ± 600
Isovalerylglycine[b]	330.38 ± 236.05	1,100 ± 200
Phenylacetylglutamine[b]	310.01 ± 210.72	320 ± 282
Tiglylglycine[b]	109.61 ± 57.82	800 ± 200
Cinnamoylglycine[b]	172.38 ± 173.44	5,500 ± 500
Dimethyl L arginine	50.60 ± 24.29	
Suberic acid[b]	40.51 ± 34.33	800 ± 100
1-Pyrroline-5-carboxylic acid	19.41 ± 11.75	
Guanadinoacetic acid	18.11 ± 14.18	
Pyroglutamic acid	13.31 ± 10.14	
Uric acid	8.95 ± 5.35	
Sebacic acid	3.85 ± 2.78	
Creatine	1.52 ± 2.62	
Arginine	0.52 ± 0.31	
Hydroxyproline	0.26 ± 0.32	
Proline	0.03 ± 0.03	

Abbreviations: eGFR, estimated glomerular filtration rate; FE, fractional excretion; SD, standard deviation.

[a]Study in 5 healthy controls with mean eGFR of 142 ± 22 mL/min/1.73 m^2.[5]

[b]Hypothesized to be secreted based on prior data.[5]

Volunteers had a mean age of 38 ± 4 years and CL_{cr} of 138 ± 28 mL/min (Item S1). Stability is shown in Fig 1, and the FE of markers at every time point, their mean, SD, and max/min are given in Item S1. All 6 had FE $>$ 100% at each time point. Phenylacetylglutamine showed the greatest stability (max/min, 1.9), whereas cinnamoylglycine showed the greatest variability (max/min, 3.2). No clear diurnal pattern was evident. FE values obtained using one spot serum and urine measurement were not statistically different from those obtained over 24 hours (Item S1).

Post hoc, we noted that among the 16 metabolites evaluated, all 4 that were glycine conjugates (hippuric acid, isovalerylglycine, tiglylglycine, and cinnamoylglycine) were secreted. Of the 10 metabolites that were not secreted, none were glycine conjugates. Glycination is a component of phase 2 liver detoxification, thought previously to increase serum solubility and enhance renal filtration. We show that the 4 glycinated compounds are not only filtered but also secreted by the kidney. Thus, we hypothesize that liver glycination may serve as a biochemical flag to enhance renal tubular secretion. Future studies are required to evaluate this hypothesis.

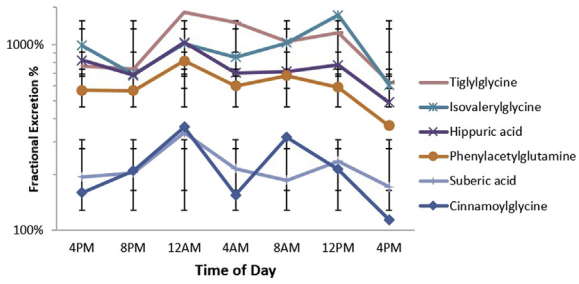

Figure 1. FE of secretion markers on spot urine specimens over 24 hours in healthy volunteers. Each data point shows the mean FE of the candidate secretion marker averaged across the 3 study participants. Solid lines represent the 24-hour trend of each marker, and the bars at each point represent the SD.

In conclusion, we found that FE using spot serum and urine samples can give tubular secretion data similar to that using 24-hour urine samples. Overall, FE markers tubular secretion markers appear relatively stable, without clear diurnal variation. These findings support using spot urine specimens to assess tubular secretion. Future studies are required to determine whether secretion can identify persons at higher risk for CKD progression.

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Peer Review: Evaluated by an external peer reviewer, a Statistical Editor, a Co-Editor, and Editor-in-Chief Levey.

Supplementary Material

Item S1: Detailed methods, baseline characteristics, stability of FE markers, spot vs 24-h comparison.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2016.12.016) is available at www.ajkd.org

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