RESEARCH LETTER

Tubular Secretion Markers, Glomerular Filtration Rate, Effective Renal Plasma Flow, and Filtration Fraction in Healthy Adolescents

To the Editor:

The diagnosis and prognosis of kidney disease in clinical practice remain limited to estimation of glomerular filtration rate (GFR) by serum creatinine and/or cystatin C measurements and glomerular integrity assessment with urinary albumin measurement. A broader assessment of global kidney health and function would substantially improve clinical practice and research methods, especially if such measurements could be made less invasively.

One critical aspect of kidney function is delivery of renal blood and plasma flow to the kidneys. Effective renal plasma flow (ERPF) can be measured by infusion of *p*-aminohippurate (PAH). PAH is secreted with high fidelity by the proximal tubule cells, with most cleared by a single pass through the kidney when infused at a low concentration, thus approximating ERPF.[1] Studies evaluating ERPF are arduous and rarely performed due to the required intravenous infusion for several hours to achieve steady state and accurate timed collections of blood and/or urine. In recent years, ERPF studies are scarce because injectable PAH is not commercially available and requires US Food and Drug Administration Investigational New Drug application.

Recently, Sirich et al[2] used mass spectrometry to study endogenous compounds secreted by the kidney. Evaluating healthy controls who provided blood and timed urine collections, the investigators identified 13 endogenous compounds secreted by kidney tubules. Other groups demonstrated that lower tubule secretion of these compounds was associated with higher mortality risk and suggested a higher risk for chronic kidney disease progression; however, the latter finding did not reach statistical significance.[3] These secreted molecules included compounds structurally similar to PAH. It is unknown whether measurement of these compounds in paired plasma and urine could provide a simple way to estimate ERPF. In this pilot study, we hypothesized that measurement of these endogenous secretion markers may provide a less invasive and pragmatic way to assess ERPF.

Protocol information and analytical methods are provided in Item S1. Briefly, 16 healthy adolescents provided paired plasma and spot urine specimens at baseline and subsequently underwent iohexol GFR and PAH plasma clearance studies over 250 minutes. The endogenous markers were measured in paired plasma and spot urine specimens at baseline and in the 250-minute urine collection. We evaluated baseline spot urine to plasma ratios and also used baseline plasma concentrations combined with urine flow rate and secretion marker concentrations during the 250-minute urine collection to measure secretion marker clearances. Thus, the clearance of endogenous secretion markers was assessed contemporaneously with the PAH and iohexol infusion.

Participant demographics, GFR, and ERPF measurements are depicted in Table 1. Mean GFR was 139 ± 20 mL/min and ERPF by PAH clearance was 615 ± 62 mL/min. Mean fractional excretion measurements at baseline using spot specimens and 250-minute clearance of secretion markers are also depicted in Table 1. About half the spot secretion markers were strongly correlated with the 250-minute timed secretion measure (M-hydroxyhippuric acid, tiglylglycine, cinnamoylglycine, and trimethyluric acid), while the rest were not.

Table 2 illustrates Spearman correlations of each marker with ERPF. In spot urine specimens, the strongest correlations with ERPF were in the inverse direction rather than direct, as hypothesized (indoxyl sulfate). The strongest direct correlation was between fractional excretion of tiglylglycine and ERPF, but this was of only moderate strength ($r = 0.37$). When evaluating 250-minute measured clearance of secretion markers, all except one (M-hydroxy hippurate) were inversely correlated with ERPF. All 24 correlations evaluated were statistically unrelated to ERPF. Results were similar if normalized for body surface area, evaluating PAH clearance instead of ERPF, and when associations were individually or mutually adjusted for GFR, age, sex, and body weight.

The modest direct correlations observed between several of the spot fractional excretion measurements with ERPF were almost universally rendered inverse when evaluated using 250-minute measured clearances. One possible explanation for this finding is potential competition for secretion of the endogenous secretion markers with PAH. Timed urine measurements were made concurrent with PAH infusion. Because PAH is highly secreted, the renal transporters may become saturated with infusion of PAH. Such a phenomenon would potentially confound clearance rates of the secreted markers during concurrent PAH infusion. Nevertheless, baseline paired plasma and spot urine measurements collected before the PAH infusion also failed to correlate with ERPF.

Strengths of this pilot study include the well-characterized healthy adolescents with availability of ERPF and GFR measurements concurrent with endogenous secretion markers in paired blood and urine collections. Limitations include its small sample size and heterogeneity in sex distribution and body size, which may have confounded our data. Additionally, evaluation of healthy adolescents precludes generalizability to other age groups or persons with comorbid diseases, most notably those with decreased GFR.

Table 1. Characteristics of 16 Healthy Adolescent Volunteers

Measurement	Mean ± SD, No. (%), or Median [IQR]
Age, y	16.6 ± 2.8
Tanner staging (1-5)	5 [4-5]
Male sex	4 (25%)
Body weight, kg	65.3 ± 14.4
Iohexol GFR, mL/min	139 ± 20
ERPF, mL/min	615 ± 62

Endogenous Secretion Markers	No. With Available Data	Spot Fractional Excretion, %	250-Min Clearance. mL/min	Spearman Correlation (ρ)
M-Hydroxyhippuric acid	16	535.9 ± 287.5	1,317.2 ± 746.5	0.69[a]
2-Furoylglycine	5	823.1 ± 774.6	1,473.2 ± 1,869.1	0.90[a]
Tiglylglycine	16	376.3 ± 137.7	863.1 ± 528.1	0.71[a]
Phenylacetylglutamine	16	292.9 ± 65.1	813.8 ± 367.4	0.29
Hippuric acid	15	375.3 ± 109.3	860.6 ± 486.0	0.49
Cinnamoylglycine	16	115.9 ± 67.6	345.6 ± 244.3	0.65[a]
Indoxyl sulfate	16	41.6 ± 12.2	116.3 ± 54.8	0.38
Trimethyluric acid	4	74.4 ± 31.4	167.2 ± 83.2	0.90[a]
Dimethyluric acid	7	278.1 ± 81.9	583.1 ± 264.0	0.26
Adipic acid	12	76.1 ± 28.9	242.0 ± 50.6	0.62
p-Cresol sulfate	16	16.7 ± 4.0	50.3 ± 22.9	0.35

Note: Data shown are mean ± standard deviation unless otherwise indicated.
Abbreviations: ERPF, effective renal plasma flow; GFR, glomerular filtration rate; IQR, interquartile range; SD, standard deviation.
[a]$P < 0.05$.

In conclusion, this pilot study does not support the hypothesis that renal clearance of the endogenous secretion markers evaluated here serves as noninvasive surrogates for ERPF.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Item S1: Supplementary Methods

Table 2. Correlations of Endogenous Secretion Markers With Effective Renal Plasma Flow

	No. With Available Data	Correlations (ρ)	
		Spot FE	250-Min Clearance
M-OH-hippurate	16	0.28	0.09
2-Furoylglycine	5	−0.62	−0.54
Tiglylglycine	16	0.37	−0.02
Phenylacetylglutamine	16	0.19	−0.14
Hippurate	15	0.06	−0.10
Cinnamoylglycine	16	−0.02	−0.11
Indoxyl sulfate	16	−0.69	−0.11
Trimethylurate	4	−0.13	−0.27
Dimethylurate	7	−0.70	−0.64
Adipic acid	12	0.07	−0.13
p-Cresol sulfate	16	0.12	−0.11

Abbreviation: FE, fractional excretion.

ARTICLE INFORMATION

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