Proximal Tubular Secretory Clearance
A Neglected Partner of Kidney Function

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Abstract
The secretion of small molecules by the proximal tubules of the kidneys represents a vital homeostatic function for rapidly clearing endogenous solutes and medications from the circulation. After filtration at the glomerulus, renal blood flow is directed through a network of peritubular capillaries, where transporters of the proximal tubules actively secrete putative uremic toxins and hundreds of commonly prescribed drugs into the urine, including protein-bound substances that cannot readily cross the glomerular basement membrane. Despite its central physiologic importance, tubular secretory clearance is rarely measured or even estimated in clinical or research settings. Major barriers to estimating tubular solute clearance include uncertainty regarding optimal endogenous secretory markers and a lack of standardized laboratory assays. The creation of new methods to measure tubular secretion could catalyze advances in kidney disease research and clinical care. Differences in secretory clearance relative to the GFR could help distinguish among the causes of CKD, particularly for disorders that primarily affect the tubulointerstitium. As the primary mechanism by which the kidneys excrete medications, tubular secretory clearance offers promise for improving kidney medication dosing, which is currently exclusively based on the GFR. The differing metabolic profiles of retained solutes eliminated by secretion versus glomerular filtration suggest that secretory clearance could uniquely inform uremic toxicity, refine existing measures of residual kidney function, and improve prediction of cardiovascular and kidney disease outcomes. Interdisciplinary research across clinical, translational, and laboratory medicine is needed to bring this often neglected kidney function into the limelight.


Introduction
Our seawater ancestors were in osmotic equilibrium with their environment, excreting waste through primitive agglomerular kidneys into coelomic sacs connected to the exterior. Recognition that this excreted waste consisted primarily of nitrogenous breakdown products formed the earliest recognition of tubular secretion as an essential mechanism for eliminating toxic substances (1). Glomerular function evolved later when some species of fish moved to freshwater and required the recovery of filtered water. As freshwater fish used glomerular filtration as the new way to maintain homeostasis and osmotic integrity, tubular secretion remained an important mechanism for the removal of retained solutes. Some species of freshwater fish returned to seawater, and their readaptation to a saline environment was accompanied by a decrease in the number and size of evolved glomeruli (2).

The concept of kidney tubular secretion in humans was first appreciated during the mid-19th century by William Bowman, who speculated that nephrons might secrete “specific constituents of urine” just like other secretory organs, such as the salivary glands and pancreas. In 1874, Bowman and Heidenhain proposed that the kidneys are primarily secretory glands, with the tubules responsible for secretion (3). E.K. Marshall provided the first experimental evidence of tubular secretion by showing avid uptake of phenol red, a protein-bound dye, by the proximal tubules after experimental blockade of glomerular filtration. Marshall and Vickers (4) further observed that the single-pass kidney elimination of phenol red was far greater than that predicted for a filtered substance. Smith (2) subsequently measured tubular secretory clearance in humans by administering p-aminohippurate (PAH), a partially protein-bound organic acid that resembles many uremic toxins. This was the first study to show efficient elimination of endogenous organic acids by the kidney via tubular secretion, and it established PAH as the prototype compound for assessing secretory function and renal plasma flow (2,5). Beyenbach (6) further proposed a potential role of tubular secretion in salt and water balance by showing active secretion of hypertonic fluid, including sodium, by the proximal tubules.

Molecular Mechanisms of Proximal Tubular Secretion
Advances in molecular cell biology forged a more detailed understanding of the processes regulating the secretion of substances across the proximal tubules. The primary transporters initiating uptake on the basolateral surface are the organic anion transporters (OATs) and organic cation transporters (OCTs) of the solute carrier 22 family (Figure 1) (7). All have a
similar general predicted structure that includes 12 α-helical transmembrane domains, a large glycosylated extracellular loop, and at least one intracellular motif (8). The basolateral uptake of anions and cations does not rely directly on ATP hydrolysis. The inward transmembrane sodium gradient generated by the basolateral sodium-potassium-ATPase drives intracellular movement of the endogenous dicarboxylic acid α-ketoglutarate, which is subsequently exchanged for organic anions via OAT1 and OAT3. The driving ion for OAT2 is unknown (9–11). Cation uptake occurs via facilitated diffusion down an electrochemical gradient. Basolateral transporters have broad specificity for a wide variety of substances, including signaling molecules, putative uremic toxins, and medications (Table 1). On the apical surface of the proximal tubules, anion efflux into the urine is an energy-dependent process mediated by members of the ATP binding cassette transporter family, including multidrug-resistant proteins 2 and 4 (12). Cation efflux is mediated by several transporters, including multidrug and toxin extrusion protein 1, human kidney-specific multidrug and toxin extrusion, and organic cation/carnitine transporter 1 via antiport proton exchange (13).

**Homeostatic Functions of Proximal Tubular Secretion**

Proximal tubular secretion is capable of rapidly removing retained solutes and medications from the circulation, including protein-bound molecules, which are minimally filtered due to size and charge specificity of the glomerular basement membrane. Approximately 20% of renal plasma flow is filtered at the glomerulus and enters Bowman’s capsule. The remaining 80% flows through the peritubular capillaries, where active transporters on the basolateral surface of the proximal tubules interact with solutes and drugs, enabling near-complete clearance in a single pass through the kidneys. The robust clearance properties of tubular secretion suggest an important role in eliminating the most toxic substances. Many candidate uremic solutes identified by the European Uremic Toxin Work Group are known substrates of OATs and OCTs, such as hippuric acid, indoleacetic acid, and indoxyl sulfate (14,15). These metabolites are synthesized in the gut microbiome, are processed by hepatic enzymes, and circulate bound to plasma proteins, minimizing effective removal by filtration or current dialysis modalities (16–18). Among patients with ESKD, substances that are cleared primarily by tubular secretion accumulate in substantially higher concentrations compared with those eliminated by filtration (19).
Proximal tubular secretion represents the primary kidney mechanism for eliminating hundreds of commonly prescribed medications, including cephalosporins, quinolone antibiotics, diuretics, antidepressants, diabetics medications, antiviral agents, and chemotherapies (20). For example, furosemide is a substrate of the basolateral OAT1 and OAT3, whereas metformin and atenolol are substrates of OCT2 (21,22).

Unlike glomerular filtration, secretory clearance is a competitive process subject to interactions among different medications and among medications and circulating solutes.

Tubular solute clearance may inform the degree of tubular injury, which is an important component of many CKDs, including those traditionally classified as “glomerular.” For example, diabetic kidney disease is often considered a glomerular disease, but diabetic kidney disease often includes tubular injury, and the degree of tubulointerstitial scarring on biopsy is among the strongest prognostic determinants of kidney disease progression in diabetes and other glomerular disorders (23,24). Sodium-glucose cotransporter 2 inhibitors, which block proximal tubule glucose uptake, show kidney-protective effects in CKD presumably by reducing glomerular hyperfiltration (25).

To examine the potential prognostic significance of proximal tubular clearance, we measured the clearances of four candidate secretory markers from timed urine and serum samples in 299 patients with CKD due to heterogeneous causes (26). We found the clearance of secretory markers to be modestly correlated with creatinine clearance measured from the same timed urine samples; however, substantial interindividual variation was present, suggesting differences in secretion relative to filtration among individual patients with CKD. We further observed that the net clearance of one of the secretion markers, cinnamoylglycine, was associated with a greater risk of progression to ESKD. These findings, obtained from a relatively small study, require replication.

Challenges to Developing Reliable Tests of Proximal Tubular Secretion

Despite its physiologic importance, tubular secretory clearance is rarely measured or even estimated in clinical or research settings. The development of accurate and potentially clinically useful tests of tubular secretion faces several challenges. One obstacle is the absence of gold standard measures of secretory clearance. Some secreted molecules, such as PAH, have supraphysiologic avidity for proximal tubular transporters, such that the measured clearance approaches the entirety of plasma flow to functioning tubules (27). Other secreted markers, such as indoxyl sulfate, have much lower rates of tubular clearance (26), presumably due to extensive protein binding, but they may be more representative of the “natural” secretory clearance of suspected uremic toxins and medications. There is no definitive method to prove that a substance is cleared exclusively by tubular secretion. Characteristics that suggest secretion as a major modality of elimination include in vitro evidence of uptake and efflux through known proximal tubular transporters, demonstrated kidney clearances that exceed the GFR, a high degree of protein binding suggestive of minimal filtration, and negligible nonkidney routes of elimination. It is probable that more than one marker may be needed to comprehensively characterize tubular secretory clearance.

Potential tests of secretory function could be performed by administering exogenous substances or measuring endogenous secretion markers in the serum and urine. Exogenous tests would be limited by feasibility and cost; nonetheless, technologic advances in point of care testing may enable practical application of such methods in the future. A simplified exogenous test of proximal tubular clearance is the pharmacodynamic response to furosemide, which is highly protein bound, is minimally filtered, and eliminated almost entirely via secretion (28). A diminished response to a single dose of furosemide is strongly associated with worsening kidney function among patients with AKI (29). A more specific test of proximal tubular secretion would be the kidney pharmacokinetic response to a fixed dose of furosemide, which should not be affected by sodium delivery, distal reabsorption, or the actions of the drug in the loop of Henle.

Potential tests of secretory function on the basis of endogenous markers would require identification of internally produced substances that are cleared primarily by secretion. Ideal endogenous secretion markers would be excreted unchanged into the urine and accurately quantified by standardized laboratory assays. Given the identification of such markers, tubular solute clearance could theoretically be estimated from paired plasma and timed urine samples of a candidate marker:

$$\text{Clearance} = \frac{(U_X \times V)}{(P_X)},$$  \hspace{1cm} (1)

where $U_X$ and $P_X$ represent urine and plasma concentrations of the candidate marker, respectively, and $V$ represents urine volume over time. The key assumption of the clearance equation is that $P_X$ represents the steady-state plasma concentration of a marker over the timed urine collection period. However, candidate secretory markers, many of which are synthesized by the gut microbiome, may exhibit diurnal, dietary, or other biologic variation in circulating concentrations, violating the steady-state assumption (30). Fluctuations in $P_X$ could be addressed in research settings by obtaining multiple plasma measurements over a timed urine collection period. However, clinically applicable tests
of secretory clearance will require further identification of endogenous markers that are produced at a relatively constant rate or those for which production can be accurately predicted using easily obtainable characteristics, such as age, race, sex, and weight. Liquid chromatography-mass spectrometry is a promising method to develop potential assays for markers of proximal tubule secretion.

Despite these challenges, the successful development of equations to estimate GFR should offer hope that a similar solution could be applied to tubular secretory clearance. External standards are available for many endogenously produced secretory solutes, enabling future standardization of laboratory assays. Among a wide range of potential secretory solutes, some are expected to have low diurnal variation, permitting valid assessment of clearance from paired timed urine and blood samples. Clinical application of secretory clearance measures would be facilitated by the development of simplified equations to predict the timed clearance of secretory solutes from spot blood and/or urine samples.

Potential Applications of Measuring Proximal Tubular Secretion

The potential applications of proximal tubular solute clearance beyond existing measures of GFR and albuminuria will require empirical testing in future research studies. Tubular solute clearance is a not a biomarker but an intrinsic kidney function that differs across a range of kidney diseases, with potential implications for clinical diagnosis and prognosis.

Early Identification of Specific Kidney Diseases

Differences in the natural history of many kidney diseases, particularly those affecting the proximal tubules, could manifest as measurable contrasts in tubular solute clearance (Table 2). For example, cyst formation in autosomal dominant polycystic kidney disease occurs via proliferation of tubular epithelial cells, including in the proximal tubule and loop of Henle (31). Abnormalities in proximal tubular architecture are observed early in the course of the autosomal dominant polycystic kidney disease, whereas a reduction in GFR typically occurs later (32). It is possible that reduced secretory clearance relative to GFR could be detected during early stages of cyst formation and growth when filtration remains normal. Similarly, acquired causes of proximal tubulopathy, such as exposure to tenofovir, cisplatin, or heavy metals, or conditions, such as multiple myeloma or immunoglobulin G4-related disease, could be identified by a relative reduction in tubular secretory capacity relative to GFR. Conversely, peritubular capillary blood flow increases during early stages of some forms of experimental GN (33,34). It is possible that higher proximal tubular clearance relative to GFR might suggest the presence of acute GN. Future studies examining net differences in tubular clearance relative to GFR among major causes of CKD will be helpful to determine the diagnostic effect of tubular secretion measures beyond established clinical kidney function measurements.

Optimization of Kidney Medication Dosing

The ideal kidney medication dosing strategy would be on the basis of the actual kidney mechanism of drug elimination. For most administered medications that are cleared by the kidneys, this mechanism is (predominantly) proximal tubular secretion. Nonetheless, kidney medication dosing strategies remain exclusively on the basis of measures of filtration, such as GFR or creatinine clearance, because they can be readily estimated in clinical settings with reasonable accuracy. The concept that GFR can be used as a proxy measure of tubular clearance is on the basis of the intact nephron hypothesis, which surmises that a loss of kidney function, regardless of cause, promotes a compensatory increase in the excretory functions of remaining nephrons (35). Although intact nephrons of diseased kidneys can substantially augment the excretion of retained solutes, such as sodium and phosphate, a purely idealized model of coupled glomerular and tubular loss is unlikely for several reasons. First, proximal tubular secretion is an active process, requiring synchronized transport and energy, whereas glomerular filtration is passive. Second, postglomerular blood flow from one glomerulus may be directed to other proximal tubules (36). Third, the severity of tubulointerstitial disease is often dissociated from the extent of glomerular involvement across many kidney diseases (37). The concept that GFR may be an imperfect measure of kidney medication clearance is supported by a recent review of published pharmacokinetic data, which found differences between GFR-predicted and actual kidney elimination in 48% of the studied drugs (38). The hypothesis that direct measurements of tubular secretion will more strongly predict kidney pharmacokinetics compared with GFR requires empirical testing. Optimizing kidney medication dosing strategies is relevant for not only patients with CKD but also, patients with AKI, who are often hospitalized and receive multiple medications with clinically important side effects.

A related application of measuring tubular solute clearance is the potential to detect early kidney toxicity of specific medications. Many drugs can cause direct injury to the proximal tubules, such as tenofovir, aminoglycosides, and cisplatin (39,40). These effects may be detected more readily by changes in tubular secretory clearance than a decline in GFR, which may occur days to weeks after drug exposure.

<table>
<thead>
<tr>
<th>Medication-induced injury</th>
<th>Nucleotide reverse transcription inhibitors (tenofovir)</th>
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<tr>
<td>Anticancer drugs (cisplatin, ifosfamide)</td>
<td>Antibiotics (aminoglycosides)</td>
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<td>Ig disorders</td>
<td>Multiple myeloma</td>
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<td>Light-chain tubulopathy</td>
<td>Amyloidosis</td>
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<td>IGG4 disease</td>
<td>Heavy metal exposure (lead, cadmium, mercury)</td>
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<td>Other</td>
<td>Aristoloichic acid nephropathy</td>
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<td>Sjogren syndrome</td>
<td>Inherited genetic disorders (polycystic kidney disease, cystinosis, glycogen storage disease)</td>
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<td>ICG4, immunoglobulin G4-related disease.</td>
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Estimating Residual Kidney Function in ESKD

Current methods of providing chronic dialysis are inadequate for removing uremic solutes. Increasing the intensity or frequency of maintenance hemodialysis does not improve the clearance of many protein-bound toxins (17,18). Consequently, residual kidney function remains an important mechanism for solute removal in patients on hemodialysis and patients on peritoneal dialysis (19,41). Residual kidney function is currently estimated by measures of filtration, such as creatinine clearance or the average of creatinine plus urea clearance. However, such measures may downplay the contribution of residual tubular secretion, which is critical for eliminating protein-bound solutes that are poorly cleared by current dialysis modalities. Moreover, a decline in kidney function before dialysis initiation is accompanied by an increase in net secretory clearance, possibly reflecting an adaptive response (42,43). The inclusion of tubular secretory measures into current estimates of residual kidney function could help inform decision making surrounding dialysis initiation and dialysis dosing in the setting of ESKD.

Predicting Metabolic Complications of CKD

Measurements of tubular secretory clearance could provide useful informing regarding other essential proximal tubular functions. The proximal tubules are the primary site for reclaiming bicarbonate and synthesizing ammonia, a principal buffer for accepting hydrogen ions excreted by the distal tubules. Lower urinary ammonium excretion is associated with kidney disease progression and mortality in patients with CKD, but it is only modestly correlated with GFR (44,45). The proximal tubules also play important roles in the pathogenesis of CKD-mineral and bone disorder by augmenting phosphate excretion in response to parathyroid hormone and fibroblast growth factor-23 and synthesizing calcitriol from precursor 25-hydroxyvitamin D.

Estimates of proximal tubular secretory function could potentially add novel information to existing measures of kidney function for associations with cardiovascular and kidney disease outcomes. Lower eGFR is independently associated with greater risks of ischemic cardiovascular events, heart failure, and progression to dialysis dependence (46,47). The combination of serum creatinine, serum cystatin C, and albuminuria yields greater predictive accuracy for dialysis and mortality outcomes than any individual marker, suggesting that aggregate measures of kidney function can provide a more complete assessment of kidney disease complications (48). The contrasting metabolic profiles of secreted and filtered solutes suggest that the toxicities of impaired secretory function may be distinct from those observed with diminished GFR. For instance, indoxyl sulfate and p-cresyl sulfate can accelerate myocardial hypertrophy and fibrosis in animal models (49,50), and they are associated with cardiovascular death among patients with CKD and patients on chronic dialysis (51,52). Both solutes also promote glomerular and tubular injury through activation of cytokine and reactive oxygen species (53–55). Therefore, measurements of tubular secretory clearance could suggest new markers and pathways of cardiovascular disease development and kidney disease progression.

Conclusion

The development of standardized equations to estimate GFR advanced the field of nephrology by promoting the early detection of CKD and increasing recognition of the burden of kidney disease in populations. Complementary methods to estimate proximal tubular solute clearance could catalyze new advances, including improved detection and diagnosis of CKD, optimized kidney medication dosing strategies, and refined estimation of residual kidney function in dialysis. Collaborative research efforts will be required to overcome the significant obstacles impeding the creation of accurate and practical methods to measure tubular solute clearance. If successful, broadening measurements of the multidimensional functions of the kidneys could pave the way for new insights into the causes of kidney disease and promote novel diagnostic and therapeutic approaches.

Acknowledgments

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Disclosures

None.

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