Impaired Tubular Secretion of Organic Solutes in Acute Kidney Injury

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Abstract
Background Impairment of kidney function is routinely assessed by measuring the accumulation of creatinine, an organic solute cleared largely by glomerular filtration. We tested whether the clearance of solutes that undergo tubular secretion is reduced in proportion to the clearance of creatinine in humans with AKI.

Methods Four endogenously produced organic solutes (phenylacetylglutamine [PAG], hippurate [HIPP], indoxyl sulfate [IS], and p-cresol sulfate [PCS]) were measured in spot urine and plasma samples from ten patients with AKI and 17 controls. Fractional clearance relative to creatinine was calculated to assess tubular secretion. Fractional clearance values were calculated in terms of the free, unbound levels of HIPP, IS, and PCS that bind to plasma proteins.

Results Fractional clearance values for PAG, HIPP, IS, and PCS were 1.0 in patients with AKI as well as controls, indicating that these solutes were still secreted by the tubules of the injured kidneys. Fractional clearance values were, however, significantly lower in patients with AKI than controls, indicating that kidney injury reduced tubular secretion more than glomerular filtration (AKI versus control: PAG, 2.1±0.7 versus 4.6±1.4, P<0.001; HIPP, 10±5 versus 15±7, P=0.02; IS, 10±6 versus 28±7, P<0.001; PCS, 3.3±1.8 versus 10±3, P<0.001). Free plasma levels rose out of proportion to total plasma levels for each of the bound solutes in AKI, so that calculating their fractional clearance in terms of their total plasma levels failed to reveal their impaired secretion.

Conclusions Tubular secretion of organic solutes can be reduced out of proportion to glomerular filtration in AKI. Impaired secretion of protein-bound solutes may be more reliably detected when clearances are expressed in terms of their free, unbound levels in the plasma.

Introduction
The severity of kidney injury is most often assessed by measuring the plasma creatinine level, which serves as a surrogate for the GFR. Many waste solutes, however, are removed from the body through proximal tubular secretion (1–4). This study tested whether renal clearances of secreted solutes decline in proportion to the GFR in AKI. The number of secreted solutes is large and we face the problem of assessing secretory function on the basis of the behavior of a set of them. Our study assessed the fractional clearances of four endogenously produced organic solutes that are normally cleared by tubular secretion and are bound to plasma proteins to varying degrees (5). As originally described by Marshall (6,7) the combination of reversible protein binding and tubular secretion allows the kidney to achieve clearances in excess of the renal plasma flow and thereby keep the free solute levels that body cells are exposed to very low. Subsequent studies have identified both endogenous organic solutes and pharmaceuticals that are efficiently cleared by the combination of protein binding and tubular secretion (5,8). A particular aim of this study was to assess the effect of AKI on such highly efficient secretory clearances. We therefore calculated the fractional clearance of the protein-bound solutes hippurate, indoxyl sulfate, and p-cresol sulfate expressed in terms of their free, unbound solute levels as well as their total solute levels in the plasma. We further assessed the effect of impaired secretion on the plasma accumulation of normally secreted solutes in patients with AKI and compared them with solute levels in patients with anuria on thrice weekly hemodialysis.

Materials and Methods
Patient Enrollment
Ten patients with AKI, 17 controls, and 15 patients with anuria on thrice weekly hemodialysis were included in the study. Patients with AKI were recruited from May 2016 to August 2016. Controls and patients...
on hemodialysis were from prior studies (9–11). Written informed consent was obtained from all participants (including written consent from a legally authorized representative for three patients with AKI). The study was approved by the Stanford Institutional Review Board and was conducted in accordance with the Declaration of Helsinki.

Patients with AKI were included if they met at least one of the Kidney Disease Improving Global Outcomes (KDIGO) criteria of (1) increase in plasma creatinine level ≥0.3 mg/dl within 48 hours or (2) increase in plasma creatinine level ≥1.5 times baseline within the prior 7 days (12,13). KDIGO criteria were employed to select patients according to a widely recognized categorization; however, individual patients falling within this categorization may vary widely. Patients were excluded if they were anuric or if they were receiving heparin because heparin causes error in the measurement of the free, unbound levels of protein-bound solutes (14). The presumed causes of AKI were infection in five patients and cardiovascular decompensation in five patients. Nine patients were on antibiotics. Eight patients were on diuretics (two on a loop diuretic only, five on a thiazide and a loop diuretic, and one on a mineralocorticoid blocking agent). Controls were from a prior study and included if they were >18 years old and had no known kidney disease (9). Plasma solute levels in patients with AKI and controls were compared with those in patients on hemodialysis from previous studies who were anuric and maintained on thrice weekly treatment, providing adequate treatment by current standards, with single pool Kt/V<sub>urea</sub> >1.4 (10,11).

**Sample Processing**

Simultaneous spot urine and plasma samples were obtained in patients with AKI and controls. Among the patients with AKI, urine was obtained from a Foley catheter in nine and spontaneously voided in one. Plasma samples in patients on hemodialysis were obtained pretreatment at a midweek session. Free solute levels were measured in plasma ultrafiltrate obtained using Nanosep 30K Omega separators (Pall, Ann Arbor, MI). Total solute levels were measured in plasma samples deproteinized 1:4 vol:vol with methanol, dried, and reconstituted in water. Urine samples were diluted with water to a concentration approximating a urine flow of 10 ml/min for patients with AKI and 40 ml/min for controls. Phenylacetylglutamine, hippurate, indoxyl sulfate, and p-cresol sulfate were measured using stable isotope dilution liquid chromatography with tandem mass spectrometry as previously described (5). Urea was measured using an enzymatic assay and creatinine was measured using HPLC.

**Calculations**

The fractional clearance of each solute was calculated as the urine-to-plasma concentration ratio of the solute divided by the urine-to-plasma concentration ratio of creatinine. For the protein-bound solutes hippurate, indoxyl sulfate, and p-cresol sulfate, fractional clearance was calculated in two ways: first, in terms of the free, unbound solute concentration measured in the plasma ultrafiltrate, and then also in terms of the total solute concentration in the plasma. The free fraction of the bound solutes was calculated as the plasma ultrafiltrate concentration divided by the total plasma concentration.

**Statistical Analyses**

Values for the fractional clearance and free fraction of each solute in patients with AKI and controls were compared using the Mann–Whitney U<sub>1</sub> test. Solute levels among the hemodialysis, AKI, and control groups were log-transformed and then compared by ANOVA, with significance of pairwise comparisons assessed using the Tukey method. Statistical analysis was performed using SPSS version 24.

**Results**

The characteristics of the patients with AKI are summarized in Table 1. The patients had been hospitalized for an average of 17±18 days before the study, with a range of 4–49 days. Their BP averaged 131±19/60±9 mm Hg and heart rate 92±16 beats per minute. The plasma creatinine level averaged 3.3±1.3 mg/dl and plasma urea nitrogen level averaged 89±25 mg/dl. The rate of rise was 0.32±0.23 mg/dl per day for the plasma creatinine level and 6.2±3.3 mg/dl per day for plasma urea nitrogen level over a preceding period of up to 7 days, for which values are available as illustrated in Supplemental Figure 1 (average 6.2±1.1 days, range 4–7 days). Urine output averaged over a 16-hour period around the study sample collection averaged 1.7±0.8 L (range 0.55–2.9 L). The fractional excretion of sodium was <1% in three patients and >1% in seven patients, with an average of 4.7%±4.9%. Of the ten patients, five who had plasma creatinine level of 3.9±1.6 mg/dl at the time of sample collection initiated RRT 5±6 days later, as illustrated in Supplemental Figure 2. Four of these five patients died during hospitalization at 49±44 days after sample collection without recovery of kidney function, and one remained alive on chronic RRT. Of the remaining five patients who had plasma creatinine 3.0±1.2 mg/dl at the time of sample collection, four were discharged 11±9 days later with creatinine 2.0±0.4 mg/dl and one died. The characteristics of the controls and patients on hemodialysis are summarized in Supplemental Table 1.

Solute fractional clearances in patients with AKI and controls are summarized in Table 2 and depicted in Figure 1. As expected, the fractional clearance of urea in both groups was <1.0, indicating tubular reabsorption, with a lower average value in patients with AKI than in controls (0.40±0.11 versus 0.57±0.14; <i>P</i>=0.004) indicating greater reabsorption of urea in the setting of acute injury. In contrast, the fractional clearance of phenylacetylglutamine in both groups was >1.0, indicating tubular secretion. The fractional clearance of phenylacetylglutamine in controls was close to five, consistent with prior reports that tubular secretion provides a clearance of this organic solute which is close to the renal plasma flow (5). The fractional clearance of phenylacetylglutamine was, however, greatly reduced in patients with AKI as compared with controls (2.1±0.7 versus 4.6±1.4; <i>P</i><0.001), indicating that injury reduced secretion out of proportion to the GFR.

For the protein-bound solutes hippurate, indoxyl sulfate, and p-cresol sulfate, fractional clearance values were first calculated in terms of the free, unbound plasma concentrations to which body cells are exposed. In accord with prior
Table 1. Characteristics of patients with AKI

| Characteristics         | Results          |
|-------------------------|------------------|
| Age, yr                 | 66±16            |
| Male/female             | 9/1              |
| BSA, m²                 | 1.8±0.2          |
| Weight, kg              | 79±16            |
| Plasma creatinine, mg/dl| 3.3±1.3          |
| Plasma urea nitrogen, mg/dl| 89±25         |
| Hemoglobin, g/dl        | 9.1±1.0          |
| Albumin, g/dl           | 2.7±1.3          |
| Fractional excretion sodium, % | 4.7±4.9 |

Results are shown as mean±SD. Three patients met both AKI criteria and seven patients met one criteria (two met criteria 1, five met criteria 2, as defined in the Materials and Methods). Albumin levels for five patients were not available on the day of sample collection. The fractional excretion of sodium was <1% in three patients and >1% in seven patients. BSA, body surface area.

Table 2. Solute fractional clearances

| Solute     | Fractional Clearance | % Free |
|------------|----------------------|--------|
|            | Control | AKI     | AKI/Control | P Value | Control | AKI | P Value |
| UreaN      | 0.57±0.14 | 0.40±0.11 | 0.7 | 0.004 | — | — | — |
| PAG        | 4.6±1.4  | 2.1±0.7  | 0.5 | <0.001 | — | — | — |
| HIPP       | 15±7     | 10±5     | 0.7 | 0.02  | 32±3 | 51±14 | 0.002 |
| Total      | 4.8±2.9  | 4.7±2.0  | 1.0 | 0.93  | — | — | — |
| IS         | 28±7     | 10±6     | 0.4 | <0.001 | 1.8±0.4 | 9.7±7.0 | <0.001 |
| Total      | 0.48±0.07 | 0.63±0.23 | 1.3 | 0.04  | 3.3±1.8 | 0.3 | <0.001 |
| PCS        | 0.18±0.03 | 0.29±0.28 | 1.7 | 0.26  | 1.9±0.4 | 7.9±5.6 | <0.001 |

Fractional clearance for each solute was calculated as the urine-to-plasma concentration ratio of the solute divided by the urine-to-plasma concentration ratio of creatinine. The fractional clearance for the bound secreted solutes was expressed in two ways: in terms of the plasma free, unbound level and in terms of the plasma total level. Fractional clearance could not be calculated for HIPP in one control, for IS in one patient with AKI and two controls, and for PCS in one patient with AKI, because of free levels that were below the assay ranges. Total fractional clearance could not be calculated for PAG in one patient with AKI and two controls, for HIPP in one patient with AKI and one control, and for IS in one patient with AKI and one control, because of plasma levels that were below the assay ranges. % Free represents the proportion of solute that is not bound to protein and was calculated as the free level divided by the total level for each solute. % Free could not be calculated for HIPP in one patient with AKI and two controls, for IS in one patient with AKI and two controls, and for PCS in one patient with AKI and one control, because of plasma free and plasma total levels that were below the assay range. Results are mean±SD. UreaN, urea nitrogen; —, not applicable; PAG, phenylacetylglutamine; HIPP, hippurate; IS, indoxyl sulfate; PCS, p-cresol sulfate.
urea nitrogen. Of note, although the creatinine level in patients with AKI was much lower than the creatinine level in patients on hemodialysis (AKI versus hemodialysis: 3.5 \pm 1.4 versus 9.7 \pm 2.7 mg/dl), the urea nitrogen level in patients with AKI exceeded that in patients on hemodialysis (AKI versus hemodialysis: 89 \pm 29 versus 56 \pm 22 mg/dl). Plasma levels of the normally secreted solutes exhibited a different pattern. In accord with previous reports, plasma levels of these solutes were much lower and also more variable among controls than were levels of creatinine and urea (16). With the increase in their free fractions, the free levels of the bound solutes rose to proportionally higher degrees than their total levels in patients with AKI. However, the increases in plasma levels in AKI as compared with controls were statistically significant only for phenylacetylglutamine and the free levels of indoxyl sulfate and p-cresol sulfate. It was notable that the levels of the normally secreted solutes were much lower in patients with AKI than in patients with anuria maintained on conventional hemodialysis.

**Discussion**

Kidney function is currently assessed largely by estimating the GFR, with a rise in the plasma creatinine used to detect reduced glomerular filtration. Urea, the only other organic waste solute routinely measured, is passively reabsorbed by the kidney tubules. Thus, in relying on creatinine and urea, we may fail to detect impairment of the tubular secretory mechanisms by which the kidney removes a wide variety of waste solutes (1–4).

This study showed that the secretion of four endogenous organic waste solutes was depressed out of proportion to glomerular filtration in humans with AKI. A notable finding was that the impairment in tubular secretion of the three protein-bound solutes became apparent only when the fractional clearances were calculated in terms of their free, unbound levels in the plasma. The total plasma levels of the bound solutes rose less than their free levels, and calculations of fractional clearance in terms of the total levels did not reveal the extent to which their tubular secretion was impaired. Protein-bound solutes are of particular interest.
because the combination of reversible protein binding and active tubular secretion allows their clearances to exceed the renal plasma flow (5–8). Such high secretory clearances serve to keep the free solute levels to which body cells are exposed very low. Although the toxicity of individual waste solutes secreted by the kidney remains largely unknown, we presume that high clearance rates evolved to minimize the plasma levels of toxins.

A further notable finding was that the free fraction of the bound solutes was increased in AKI to nearly the same extent as in patients with end-stage renal failure maintained on hemodialysis (10,17). Some increase in the free fraction could be accounted for by competition for binding sites by either medications or endogenous solutes that accumulate in the plasma when kidney function declines. However, the free fraction of the bound solutes in patients with AKI increased to the degree previously observed in patients on hemodialysis, whereas the absolute levels of these solutes were much lower in patients with AKI than in patients on hemodialysis, as shown in Supplemental Table 3. This suggests modification of plasma albumin may contribute more importantly to reduction in protein binding than competition by accumulating solutes, as has recently been demonstrated for solutes, including p-cresol sulfate and indoxyl sulfate, in patients with end-stage renal failure (18). The reduction in albumin concentration in patients with AKI would not by itself cause a notable reduction in solute binding unless the aggregate levels of bound solutes were causing competition for binding sites. Post-translational modifications of albumin and other changes in plasma composition, including pH, may contribute (19–21).

Previous studies of proximal tubular secretion in AKI have most often assessed the clearance of para-aminohippurate (PAH). Myers et al. (22) found that the normal ratio of PAH clearance to GFR of approximately 5 was well preserved in a group of nonoliguric patients with AKI with average GFR reduced to 15 ml/min after cardiac surgery. Westenfelder et al. (23) likewise reported that the ratio of PAH clearance to GFR remained normal when the GFR reduced to about one tenth of normal in rats with glycerol-induced AKI. Other studies, however, have reported that PAH clearance relative to GFR is reduced in humans with oliguric AKI and in rats with AKI induced by cisplatin (24,25). When considering these findings, we would note that PAH was adopted for measurement of renal plasma flow by Homer Smith because it is not significantly protein bound in humans and because the tubular capacity for PAH secretion is extraordinarily high (26). Presumably, the secretory clearances of individual solutes may be reduced to a variable extent as the kidney is injured. This is in contrast to the GFR, which is a passive process and provides uniform clearance for filtered solutes.

Our study did not identify mechanisms responsible for the impaired secretion of organic solutes in AKI. It is known, however, that secretion is an active process requiring different families of transporters on the basolateral and apical surfaces of the proximal tubule cell, as well as cellular production of energy and countertransport ions (1). Impairment of secretory function in renal insufficiency could therefore result from reduced expression of the transporters, loss of cell polarity, or impairment of cellular metabolism (27–29). Modifications of albumin, and reduced concentrations of albumin and solute in the capillaries around actively secreting tubule segments, could also contribute (19). Studies in rat models of acute kidney failure have demonstrated reduced kidney expression of organic anion transporter 1 and organic anion transporter 3 (27,30,31). Animal studies have not currently established that expression of the transporters is reduced out of proportion to the GFR. However, studies using knockout mice have provided valuable insight into the specificity of transporters for solutes on the basis of their chemical properties and/or their potential role in regulating pathways outside of the kidneys (32–34). These and other studies suggest that transport for solutes with lesser affinity for individual transporters may become impaired to a greater degree than those with higher affinity. Moreover, transporter affinity may be altered by the effect of other accumulated solutes or by the inflammatory environment in AKI (35). Secretion in AKI may also be impaired by competition among accumulating solutes, including medications (36,37). Indeed, seven of the patients with AKI were on diuretics, which may use the same transport mechanisms

| Solute        | Control, mg/dl | AKI, mg/dl | Hemodialysis, mg/dl | AKI/Control | Hemodialysis/Control |
|---------------|----------------|------------|---------------------|-------------|----------------------|
| Creatinine    | 0.95±0.20      | 3.5±1.4*   | 9.7±2.7*            | 3.6         | 10                   |
| UreaN         | 14±2           | 89±29a     | 56±22a              | 6.5         | 4.1                  |
| PAG           | 0.029±0.018    | 0.34±0.39* | 5.3±2.8*            | 12          | 187                  |
| HIPP Free     | 0.047±0.039    | 0.26±0.47  | 2.7±1.5*            | 5.6         | 57                   |
| HIPP Total    | 0.16±0.12      | 0.49±0.76  | 5.0±2.6*            | 3.1         | 31                   |
| IS Free       | 0.002±0.001    | 0.032±0.073* | 0.24±0.16*        | 19          | 139                  |
| IS Total      | 0.09±0.04      | 0.23±0.31  | 2.7±1.2*            | 2.5         | 29                   |
| PCS Free      | 0.005±0.002    | 0.048±0.048* | 0.28±0.17*        | 8.8         | 51                   |
| PCS Total     | 0.32±0.15      | 0.64±0.60  | 4.1±2.0*            | 2.0         | 13                   |

Results are mean±SD. Plasma free levels were below the assay range for HIPP in one control, for IS in one patient with AKI and two controls, and for PCS in one patient with AKI and one control. Plasma total levels were below the assay range for PAG in one patient with AKI and two controls, for HIPP in one patient with AKI and one control, and for IS in one patient with AKI. UreaN, urea nitrogen; PAG, phenylacetyleglutamine; HIPP, hippurate; IS, indoxyl sulfate; PCS, p-cresol sulfate.

*P<0.05, levels different from control.

**P<0.05, levels different from AKI. Solute levels among the control, AKI, and hemodialysis groups were compared by ANOVA, with significance of pairwise comparisons made using the Tukey method.
of the proximal tubule as waste solutes. Our study was too small, however, to test whether diuretics interfered with the secretion of organic solutes. Enhancement of secretion has also been shown in animal studies via increased expression of transporters and remodeling of kidney tissue induced by higher solute levels (38,39). Additional studies are needed to test these hypotheses.

Reduced fractional clearances of the secreted solutes phenylacetylglutamine and of free hippurate, indoxyl sulfate, and p-cresol sulfate were accompanied by increases in the plasma levels in our patients with AKI. However, plasma levels of these solutes were much lower in patients with AKI than in anuric outpatients receiving adequate hemodialysis according to current guidelines. One factor that may have limited the rise in their levels is reduced production. A recent study found that plasma levels of indoxyl sulfate and p-cresol sulfate declined over 4 days, whereas the plasma creatinine level remained nearly stable in patients with AKI (16). It was noted that these solutes are produced by colon microbes and that their production in patients with AKI was likely reduced by antibiotic administration and decreased oral feeding. We presume that the production of the secreted solutes measured in our patients may have been similarly reduced, as phenylacetylglutamine is also produced entirely, and hippurate partially, by colon microbes (9).

Our study has several limitations. First, our study was small and was not designed to relate impaired secretion to either the patients’ current clinical conditions or their longer-term outcomes. Analyses of a much larger group would be required to determine if impaired secretory clearance can be used as a guide to therapy or even serve as a predictor of the course of renal injury. Larger studies could also obtain a more accurate picture of secretory function in AKI by refined characterization of patients’ hemodynamic status and longitudinal measurements. Second, we calculated the fractional clearances of phenylacetylglutamine, hippurate, indoxyl sulfate, and p-cresol sulfate relative to creatinine. Creatinine undergoes variable tubular secretion and so our calculations could understate the clearances of the other solutes relative to the GFR. Our finding that secretory clearance of these solutes relative to GFR is reduced remains firm unless the secretion of creatinine is greatly increased in AKI. Of note, Myers et al. (22) found that creatinine clearance provided an accurate measure of GFR in nonoliguric patients with AKI. Third, we measured solute clearances at a single time point and did not assess potential differences in the volumes of distribution or rates of production for individual solutes that may occur in AKI. The rates of solute production could be altered in the nonsteady-state environment of AKI. Finally, hippurate, indoxyl sulfate, and p-cresol are secreted as organic anions, whereas the mechanism for phenylacetylglutamine secretion is unknown. Our measurements of these four solutes may therefore provide only a limited view of the extent to which tubular secretion is impaired in AKI. AKI may have different effects on solutes secreted by different mechanisms, and these mechanisms are complex. Secretion of creatinine, for instance, may involve the participation of the organic anion transporter 2 as well as the organic cation transporter 2 on the proximal tubule basolateral membrane, and the multidrug and toxin extrusion proteins 1 and 2 on the luminal membrane (1).

In conclusion, the tubular secretion of some organic waste solutes is impaired out of proportion to GFR to a variable degree in AKI. For protein-bound solutes, impaired secretion is reliably detected only when clearances are expressed in terms of their free, unbound levels in the plasma. The mechanism and clinical significance of this impairment in secretory function in AKI remain to be established.

Disclosures
T. Meyer has a patent “Sorbent processing of dialysate to increase solute removal during hemodialysis” pending. All remaining authors have nothing to disclose.

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Author Contributions
F. O’Brien, R. Mair, T. Meyer, S. Sutherland, and T. Sirich, conceptualized the study, were responsible for study supervision, formal analysis, study investigation, methodology, project administration, resources, validation, and visualization, wrote the original draft, and reviewed and edited the manuscript; F. O’Brien, R. Mair, T. Meyer, and T. Sirich were responsible for funding acquisition; and all authors were responsible for data curation and approved the final version of the manuscript.

Supplemental Material
This article contains supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0001632020/-/DCSupplemental.

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Supplemental Table 1 –

Characteristics of Control Subjects and Hemodialysis Patients

|                        | Control          | Hemodialysis     |
|------------------------|------------------|------------------|
| Age (years)            | 50 ± 14          | 63 ± 13          |
| M / F                  | 11 / 6           | 12 / 3           |
| BSA (m²)               | 1.8 ± 0.2        | 1.9 ± 0.3        |
| Weight (kg)            | 70 ± 12          | 76 ± 18          |
| Dialysis vintage (years)| -            | 3.2 ± 1.9        |
| spKt/V<sub>urea</sub>  | -                | 1.6 ± 0.4        |

Results are mean ± standard deviation.

The height was missing for three hemodialysis patients so BSA could not be calculated. All hemodialysis patients were maintained on thrice weekly treatment.
Supplemental Table 2 –

Urine Concentration, Plasma Concentration, and Urine to Plasma Concentration Ratio in Control Subjects and Acute Kidney Injury Patients

| Solute      | Control | AKI          |
|-------------|---------|--------------|
|             | Urine (mg/dl) | Plasma (mg/dl) | Urine/Plasma | Urine (mg/dl) | Plasma (mg/dl) | Urine/Plasma |
| Creatinine  | 103 ± 74 | 0.95 ± 0.20  | 111 ± 78 | 50 ± 23 | 3.5 ± 1.4 | 17 ± 11 |
| Urea        | 760 ± 422 | 14 ± 2 | 55 ± 28 | 526 ± 269 | 89 ± 29 | 6.6 ± 4.5 |
| PAG         | 12 ± 9 | 0.029 ± 0.018 | 463 ± 297 | 6.6 ± 5.2 | 0.34 ± 0.39 | 36 ± 25 |
| HIPP Free   | 60 ± 75 | 0.047 ± 0.039 | 1759 ± 1586 | 15 ± 13 | 0.26 ± 0.47 | 157 ± 115 |
| HIPP Total  | 4.6 ± 2.8 | 0.16 ± 0.12 | 502 ± 492 | 0.49 ± 0.76 | 64 ± 35 |
| IS Free     | 0.002 ± 0.001 | 3375 ± 2647 | 1.5 ± 1.5 | 0.032 ± 0.073 | 199 ± 174 |
| IS Total    | 0.094 ± 0.042 | 55 ± 43 | 0.23 ± 0.31 | 11 ± 8 |
| PCS Free    | 5.6 ± 4.2 | 0.32 ± 0.15 | 1121 ± 726 | 2.3 ± 2.4 | 0.048 ± 0.048 | 64 ± 50 |
| PCS Total   | 0.005 ± 0.002 | 19 ± 14 | 0.64 ± 0.60 | 3.9 ± 2.4 |

Results are mean ± standard deviation. AKI, acute kidney injury; PAG, phenylacetylglutamine; HIPP, hippurate; IS, indoxyl sulfate; PCS, p-cresol sulfate.

Free refers to the unbound solute concentration in the plasma and Total refers to the total solute concentration in the plasma for the protein-bound solutes.
Supplemental Table 3 –

Solute Free Concentration and % Free in Acute Kidney Injury and Hemodialysis Patients

| Solute | AKI         | HD        |
|--------|-------------|-----------|
|        | Free concentration (mg/dl) | 0.26 ± 0.47 | 2.7 ± 1.5 |
| HIPP   | % Free      | 51 ± 14   | 54 ± 8   |
|        | Free concentration (mg/dl) | 0.032 ± 0.073 | 0.24 ± 0.16 |
| IS     | % Free      | 9.7 ± 7.0 | 8.0 ± 3.0 |
|        | Free concentration (mg/dl) | 0.048 ± 0.048 | 0.28 ± 0.17 |
| PCS    | % Free      | 7.9 ± 5.6 | 6.8 ± 2.5 |

Results are mean ± standard deviation. AKI, acute kidney injury; HD, hemodialysis; HIPP, hippurate; IS, indoxyl sulfate; PCS, p-cresol sulfate.
Supplemental Figure 1 –

Evolution of Plasma Creatinine Level in Acute Kidney Injury Patients

The black circles and solid lines represent the 7 AKI patients with FENa >1%. The open circles and dashed lines represent the 3 AKI patients with FENa <1%. Data for plasma creatinine levels are shown for up to the four days preceding the study and for one day after the study. The dotted vertical line represents the day of sample collection for solute fractional clearance measurements for the study.
10 AKI patients (plasma Cr 3.3±1.3 mg/dl)

5 AKI patients started RRT (plasma Cr 3.9±1.6 mg/dl)
  • 4 AKI patients died
  • 1 AKI patient remained on RRT

5 AKI patients did not start RRT (plasma Cr 3.0±1.2 mg/dl)
  • 1 AKI patient died
  • 4 AKI patients discharged (plasma Cr 2.0±0.4 mg/dl)

AKI, acute kidney injury; RRT, renal replacement therapy.
Supplemental Figure 3 –

Urine to Plasma Concentration Ratio of Solute Relative to Creatinine

Urea

Phenylacetylglutamine

Hippurate

Indoxyl Sulfate

p-Cresol Sulfate
Supplemental Figure 3 Legend

The urine to plasma concentration ratio (U/P) of each solute is plotted versus the urine to plasma concentration ratio of creatinine. For the protein-bound solutes hippurate, indoxyl sulfate, and p-cresol sulfate, the urine to plasma concentration ratios are expressed in terms of the free, unbound plasma solute level (U/P_{Free}). The circles represent the 10 AKI patients and the triangles represent the 17 control subjects.