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Assessment of Proximal Tubular Function by Tubular Maximum Phosphate Reabsorption Capacity in Heart Failure

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Significance statement

Proximal tubular function is of great relevance to patients with heart failure, since the proximal tubule plays a vital role in sodium handling of the kidney, sodium retention, and diuretic response. The tubular maximum phosphate reabsorption capacity (TmP/GFR) indicates the maximum capacity of the kidney to reabsorb phosphate in the proximal tubule. As such, TmP/GFR can be considered a parameter of proximal tubular function, and has never been investigated in heart failure. This study shows that TmP/GFR is frequently reduced in heart failure, and associated with markers of tubular damage and worse clinical outcomes.

Abstract

Introduction

The glomerular filtration rate (eGFR) is a crucial parameter in heart failure. Much less is known about the importance of tubular function. We addressed the impact of tubular maximum phosphate reabsorption capacity (TmP/GFR), a parameter of proximal tubular function, in patients with heart failure.

Methods

We established TmP/GFR (Bijvoet-formula) in 2,085 patients with heart failure and studied its association with deterioration of kidney function (>25% eGFR decrease), plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) doubling (baseline–9 months), and clinical outcomes. Additionally, we evaluated the effect of sodium-glucose transport protein 2-inhibition by empagliflozin on TmP/GFR in 78 patients with acute heart failure.

Results

Low TmP/GFR (<0.80 mmol/L) was observed in 1,392(67%) and 21(27%) patients. Patients with lower TmP/GFR had more advanced heart failure, lower eGFR, and increased levels of tubular damage markers. The main determinant of lower TmP/GFR was higher fractional excretion of urea ($P<0.001$). Lower TmP/GFR was independently associated with higher risk of plasma NGAL doubling (OR 2.20(1.05–4.66), $P=0.038$), but not with deterioration of kidney function. Lower TmP/GFR was associated with higher risk of all-cause mortality (HR 2.80(1.37–5.73), $P=0.005$), heart failure hospitalization (HR 2.29(1.08–4.88), $P=0.031$), and their combination (HR 1.89(1.07–3.36), $P=0.030$) after multivariable adjustment. Empagliflozin significantly increased TmP/GFR compared with placebo after one day ($P=0.004$), but not after adjustment for eGFR change.

Conclusions

TmP/GFR, a measure of proximal tubular function, is frequently reduced in heart failure, especially in patients with more advanced heart failure. Lower TmP/GFR is furthermore associated with future risk of plasma NGAL doubling and worse clinical outcomes, independent of glomerular function.

Abbreviations

BIOSTAT-CHF	A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure
CKD	Chronic Kidney Disease
CI	Confidence Interval
eGFR	estimated Glomerular Filtration Rate
EMPA-RESPONSE-AHF	Randomized, double-blind, placebo-controlled, multicentre pilot study on the effects of empagliflozin on clinical outcomes in patients with acute decompensated heart failure
FE _{Na}	Fractional Excretion of sodium
FE _{Urea}	Fractional Excretion of urea
FGF23	Fibroblast Growth Factor 23
HR	Hazard Ratio
KIM-1	Kidney Injury Molecule-1
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NT-proBNP	N-Terminal pro Brain Natriuretic Peptide
OR	Odds Ratio
SGLT2	Sodium-glucose cotransporter 2
TmP/GFR	Tubular maximum phosphate reabsorption capacity
UACR	Urine Albumin-to-Creatinine Ratio

Introduction

Impaired kidney function is common and associated with greater risk of poor outcomes in patients with heart failure.(1,2) Many studies have addressed the cardiorenal syndrome, but almost exclusively focused on glomerular filtration rate (GFR). Much less is known about prevalence, predictors, and clinical outcome of tubular function in patients with heart failure.(3,4) Since proximal tubular function is of vital importance for sodium handling of the kidney, sodium retention, and diuretic response, it is of great relevance to patients with heart failure.(4,5) Recent studies have drawn attention to the proximal tubule by showing that sodium-glucose cotransporter 2(SGLT2)-inhibitors improve outcomes in patients with heart failure with reduced ejection fraction.(6,7)

The tubular maximum phosphate reabsorption capacity (TmP/GFR) indicates the maximum capacity of the kidney to reabsorb phosphate in the proximal tubule independent of GFR and net inflow of phosphate.(8,9) As such, TmP/GFR can be considered a parameter of proximal tubular function. In experimental studies, expression of sodium phosphate cotransporters decreased in response to kidney injury, ranging from injury due to ischemia-reperfusion to hereditary kidney diseases, thereby decreasing tubular phosphate reabsorption.(10–15) Due to its high rates of oxygen consumption and relatively sparse endogenous antioxidant defenses, the proximal tubule as a whole is particularly vulnerable to injury.(16) TmP/GFR of living kidney donors predicts recipient measured GFR independent of donor measured GFR, confirming TmP/GFR as a functional tubular parameter not primarily driven by GFR.(17) As such, TmP/GFR might be useful to detect damage and dysfunction of the proximal tubule and investigate its consequences. We therefore aimed to study the clinical value of TmP/GFR and investigate effects of SGLT2-inhibition on TmP/GFR in heart failure.

Methods

Patient populations

We used the index cohort of A systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF), an investigator- driven multicenter clinical study consisting of 2,516 patients with the aim to identify patients with a poor outcome despite currently recommended treatment with angiotensin-converting enzyme-inhibitors/angiotensin receptor blockers and beta blockers.(18)

Additionally, we evaluated TmP/GFR in the randomized, double-blind, placebo-controlled, multicenter pilot study on the effects of empagliflozin on clinical outcomes in patients with acute decompensated heart failure (EMPA-RESPONSE-AHF) cohort. The main outcomes have been published.(19) In brief, patients hospitalized for acute heart failure treated with (iv) loop diuretics were included. Patients were randomly assigned to empagliflozin 10 mg/day or placebo within 24 hours of presentation and treated for 30 days.

Both studies were conducted in accordance with the declaration of Helsinki, national ethics and legal requirements, and relevant EU legislation. All participants provided written informed consent for study participation.

Calculations and biomarker measurements

TmP/GFR could be calculated in 2,085 patients in BIOSTAT-CHF and 78 patients in EMPA-RESPONSE-AHF using the formula described by Payne(8) and originally devised by Bijvoet.(20) The study subset did not differ substantially from patients without available TmP/GFR (Supplementary Table 1). First, fractional tubular reabsorption of phosphate (TRP) was calculated:

$$TRP = 1 - \frac{\text{serum creatinine} \times \text{urine phosphate}}{\text{urine creatinine} \times \text{serum phosphate}}$$

When TRP was ≤ 0.86 , indicating maximal phosphate reabsorption and a linear relationship between serum phosphate concentration and excretion, TmP/GFR was calculated by:

$$TmP/GFR = TRP \times serum\ phosphate$$

When TRP was > 0.86 , indicating a curvilinear relationship between serum phosphate concentration and excretion, TmP/GFR was calculated by:

$$TmP/GFR = \frac{0.3 \times TRP}{1 - (0.8 \times TRP)} \times serum\ phosphate$$

Urinary phosphate and other urinary measurements were measured in non-fasting spot urine using standardized methods in both cohorts. The normal reference range of 0.80–1.35 mmol/L for TmP/GFR was derived from data in healthy individuals 65–75 years old.(8)

The association between TmP/GFR and doubling of plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) levels and deterioration of kidney function ($>25\%$ eGFR decrease) was analyzed between baseline–9 months.(21,22) Sensitivity analyses were conducted for $>30\%$ and $>40\%$ eGFR decrease. eGFR was calculated using the CKD-EPI creatinine formula.(23) Presence of CKD was defined as eGFR <60 mL/min/1.73m². After blood was drawn by venipuncture, samples were stored at -80°C . If possible, analyses were performed directly locally with standardized international methods, otherwise they were performed in a central laboratory. Urinary Kidney Injury Molecule (KIM)-1 and NGAL were measured using an in-house developed and validated multiplex immunoassay (xMAP; Luminex, Austin, USA).(24) Measurement of additional biomarkers was performed as previously described.(25–27) Endogenous fractional excretion of lithium was measured prior to empagliflozin administration using inductively coupled plasma mass spectrometry in EMPA-RESPONSE-AHF (Supplementary Methods 1).

Study endpoints

The relation of TmP/GFR with all-cause mortality, heart failure hospitalization, and those combined was evaluated. Endpoints were adjusted for BIOSTAT risk models created for each specific outcome.(28)

Statistical analysis

Data are presented as means $\pm$ SD when normally distributed, as medians (Q1-Q3) when skewed, and as frequencies (percentage) when categorical, and statistically tested using analysis of variance, Kruskal-Wallis H test, and Chi-Squared tests respectively. Trends were tested with Cochran-Armitage trend test, Jonckheere-Terpstra test, or linear regression for categorical, skewed variables, and normally distributed variables, respectively.

Determinants of TmP/GFR were analyzed using linear regression. All variables with $P < 0.1$ univariably were included in multivariable analysis and subjected to backward elimination. Variables with $P < 0.05$ were retained in the final multivariable regression model. Prior to linear regression, normal distribution of residuals was checked, as well as presence of outliers. If necessary, variables were transformed using natural logarithm, including TmP/GFR. The association between log TmP/GFR and risk of plasma NGAL doubling and deterioration of kidney function was evaluated using logistic regression. Results were adjusted for log plasma and urinary NGAL, eGFR, log urine albumin-to-creatinine ratio (UACR), log KIM-1, log fractional excretion of sodium (FE_{Na}), and log fractional excretion of urea (FE_{Urea}). Results are expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Cox proportional hazard models were constructed to evaluate prognostic value of log TmP/GFR and adjusted for BIOSTAT risk models, phosphate, eGFR, and New York Heart Association class. Results are expressed as hazard ratios (HRs) with 95% CIs. Kaplan-Meier plots were constructed using the survminer package. The dendrogram was constructed using

the Hmisc package. Analysis of covariance was used to study effects of empagliflozin versus placebo on TmP/GFR, controlling for baseline TmP/GFR. A two-tailed p-value <0.05 was considered statistically significant. Statistical analyses were performed with R (version 1.3.1073).

Results

Baseline characteristics

In BIOSTAT-CHF, median TmP/GFR was 0.67 (0.46–0.88) mmol/L. Low TmP/GFR (<0.80 mmol/L) was observed in 1,392 (67%) patients. Patients with lower TmP/GFR were older, had lower levels of serum phosphate and higher fractional excretion of phosphate ($FE_{\text{Phosphate}}$), lower eGFR, higher doses of loop diuretics, and higher levels of NT-proBNP, FE_{Na} , FE_{Urea} , plasma and urinary NGAL, and UACR (Table 1). Baseline characteristics by median TmP/GFR and presence/absence of CKD are shown in Supplementary Table 2, and according to low, normal, and high TmP/GFR in Supplementary Table 3, the latter yielding similar trends to Table 1.

Association between TmP/GFR and clinical variables

Multivariable linear regression for log TmP/GFR including serum phosphate is shown in Supplementary Table 4 (adjusted $R^2=0.832$). Upon exclusion of phosphate, the strongest predictors of log TmP/GFR were log FE_{Urea} , log urea, hemoglobin, log urinary creatinine, and log loop diuretic dose (Table 2; adjusted $R^2=0.242$).

Correlation plots and hierarchical cluster analysis of TmP/GFR

Correlation plots showing the association between TmP/GFR and eGFR, plasma NGAL, urea, and FE_{Urea} are displayed in Figure 1. In hierarchical cluster analysis (Figure 2), TmP/GFR clustered closely with urinary creatinine, FE_{Na} , age, and hemoglobin. TmP/GFR did not directly cluster with serum phosphate or eGFR.

TmP/GFR predicts doubling of plasma NGAL over time

Deterioration of kidney function occurred in 221 (19%) of 1,168 patients with available measurements. Log decrease of TmP/GFR was neither a significant predictor for deterioration of kidney function overall (OR 0.53, 95% CI:0.27–1.04, P=0.064) nor in a subgroup of patients without CKD (n=569; OR 0.65, 95% CI:0.24–1.78, P=0.403). Sensitivity analyses for >30% and >40% decrease in eGFR yielded consistent results. Doubling of plasma NGAL between baseline–9 months occurred in 413 (35%) of 1,178 patients with available measurements. Log TmP/GFR decrease was a significant predictor for doubling of plasma NGAL after adjustment for baseline plasma and urinary NGAL, eGFR, urinary KIM-1, UACR, FE_{Na}, and FE_{Urea} (Table 3; OR 2.20, 95% CI:1.05–4.66, P=0.038).

Lower TmP/GFR predicts poor clinical outcomes

Overall, 559 (27%) patients died, 535 (26%) were hospitalized for heart failure, and in 878 (42%) one or both occurred during a median follow-up of 21 (15–27) months. In unadjusted Cox regression, TmP/GFR was significantly associated with all three outcomes, and remained so after adjustment for the previously described risk models, serum phosphate, eGFR, and New York Heart Association class (mortality HR per log decrease=2.80, 95% CI:1.37–5.73, P=0.005, heart failure hospitalization HR per log decrease=2.29, 95% CI:1.08–4.88, P=0.031, and combined endpoint HR per log decrease=1.89, 95% CI:1.07–3.36, P=0.030; Table 4). Serum phosphate was not associated with any of these outcomes (Supplementary Table 5). Kaplan Meier curves for the combined endpoint per quartile of TmP/GFR illustrate increasing risk with lower quartiles of TmP/GFR (Supplementary Figure 1; Log-rank P<0.001).

The combination of low TmP/GFR and CKD was significantly associated with higher risk of poor outcomes after adjustment (Supplementary Tables 6 and 7). Kaplan Meier curves also revealed worst survival for this combination (Supplementary Figure 2).

Effect of empagliflozin on TmP/GFR

Median TmP/GFR in EMPA-RESPONSE-AHF was 0.97 (0.78–1.13) mmol/L. Low TmP/GFR was observed in 21 (27%) patients. Patients with TmP/GFR below the median had higher levels of NT-proBNP, FE_{Urea} , and $FE_{Phosphate}$, and lower levels of hemoglobin, urinary creatinine, and serum phosphate (Supplementary Table 8; all $P < 0.05$).

Only 1 day after administration, patients randomized to empagliflozin had higher TmP/GFR compared with placebo (1.17 (1.00–1.33) mmol/L versus 0.92 (0.70–1.10) mmol/L, F -value=8.983, $P=0.004$, adjusted for baseline TmP/GFR) as shown in Figure 3. However, after additional adjustment for eGFR change between baseline–day 1, this difference was no longer statistically significant. Urinary phosphate and urinary glucose were not correlated at day 1 in patients allocated to empagliflozin.

Baseline TmP/GFR correlated with fractional excretions of lithium ($\rho=-0.513$, $P < 0.001$), glucose ($\rho=-0.325$, $P=0.005$), uric acid ($\rho=-0.354$, $P=0.002$), urea ($\rho=-0.552$, $P < 0.001$), and sodium ($\rho=-0.329$, $P=0.003$), but not with serum bicarbonate. For all fractional excretions, it should be kept in mind that all incorporated serum/urinary creatinine.

Discussion

In this study, we showed that TmP/GFR is frequently reduced in patients with heart failure, especially in patients with more advanced heart failure, worse kidney function, and increased signs of tubular damage. TmP/GFR predicted plasma NGAL doubling over time after adjustment for several kidney parameters, but not deterioration of kidney function. TmP/GFR was independently associated with both all-cause mortality and heart failure hospitalization. Finally, empagliflozin increased TmP/GFR compared with placebo one day after start of administration, but not after adjustment for eGFR change.

Importance of proximal tubular function in heart failure

Symptoms of heart failure are primarily driven by congestion, for which loop diuretics are the cornerstone of treatment.(29) However, a large number of patients with heart failure do not appropriately respond to loop diuretics.(29) Since approximately 65% of sodium handling occurs at the proximal tubule, this kidney segment is of particular relevance to patients with heart failure.(4) However, the proximal tubule is one of the most vulnerable segments; for this reason, proximal tubule cells are early and central sensors, effectors, and injury recipients,(16,30) leading to multiple features of CKD.(16,31,32) Proximal tubule cells are as such suggested a primary player in the progression of acute kidney injury and CKD.(16) Following damage, proximal tubules also become evidently less functional due to pathological changes impairing reabsorption and secretion.(33) In heart failure, hypoperfusion of the kidney and congestion(1) might cause proximal tubular dysfunction and damage. This, vice versa, might in theory (counterbalancing natriuretic peptide release) cause congestion through increased sodium and water reabsorption downstream, similar to mechanisms involved in diuretic resistance(29) and lead to progression of heart failure. This has for example been shown in a study by Rao et al., which addressed mechanisms of diuretic

resistance and showed that compensatory distal tubular sodium reabsorption makes the largest relative contribution (79%) to diuretic induced increase in FENa.(34) Additionally, sympathetic nervous system activation in heart failure with formation of reactive oxygen species may also have detrimental effects on proximal tubular cells.(35)

For all these reasons, a relevant metric of proximal tubular function that allows heart failure specialists to look beyond eGFR will be highly relevant. Several heart failure studies have focused on tubular damage markers, such as NGAL or KIM-1,(36) yet these do not assess tubular function. As such, TmP/GFR might be a clinically relevant metric of proximal tubular function in heart failure.

Suitability and clinical relevance of TmP/GFR as a parameter of proximal tubular function in heart failure

Originally, TmP/GFR has been invented to help distinguish hypercalcemia due to hyperparathyroidism from other causes.(20) We now know that expression of (sodium) phosphate (co)transporters (i.e. NaPi-IIa, NaPi-IIc, and PiT-2, that determine TmP/GFR) decreases in response to injury.(10–15,33) In studies conducted in rats, expression of phosphate (co)transporters also decreased in the setting of acute hypertension.(37) TmP/GFR is thought to be independent of glomerular function but instead represents functioning of phosphate reabsorption in the proximal tubule.(8,17) TmP/GFR thus shows promise as a functional proximal tubular parameter. TmP/GFR has already been suggested useful for monitoring kidney tubular response to therapy in tubular damage over two decades ago,(8) and has since been reported as a marker of tubular dysfunction.(14,15) Additionally, TmP/GFR of living kidney donors independently predicted recipient measured GFR independent of donor measured GFR, but not NGAL or KIM-1.(17)

In the current study, we observed that TmP/GFR is frequently reduced in patients with heart failure, more often so in BIOSTAT-CHF (67%) than in EMPA-RESPONSE-AHF (27%).

This difference might be explained by EMPA-RESPONSE-AHF including a higher percentage of de novo heart failure compared to BIOSTAT-CHF (47% versus 28%) and less frequently of an ischemic etiology (29% versus 61%). Longer existence of heart failure and ischemic injury might result in more proximal tubular dysfunction. Indeed, in heart failure patients with proven renovascular disease, an ischemic etiology was more often present compared with patients without renovascular disease.(38)

Second, lower TmP/GFR was indeed associated with higher doses of loop diuretics, higher levels of bioactive adrenomedullin (a congestion marker(39)) and NT-proBNP, and higher fractional excretion of lithium, providing hints towards patients with lower TmP/GFR having more congestion and/or diuretic resistance.

Lower TmP/GFR was also associated with higher levels of tubular damage markers, such as NGAL, but less strongly than with higher FE_{Na} and FE_{Urea} , which may also be considered functional parameters.(33,40) This seems logical, precisely because NGAL levels do not necessarily indicate tubular function, and furthermore originate from multiple tubular segments. The association between TmP/GFR and FE_{Urea} might well be explained by the fact that a large proportion of urea is already absorbed in the proximal tubule and is rather a reflection of proximal peritubular forces than distal forces.(40) In addition, TmP/GFR was independently associated with future doubling of plasma NGAL and poor clinical outcomes, further underlining its clinical relevance, independent of glomerular function. Of course, its association with clinical outcomes might partly be influenced by other forces driving phosphate metabolism, but our findings that 1) TmP/GFR did not cluster with fibroblast growth factor (FGF)23, neither was FGF23 a significant determinant of TmP/GFR, and 2) serum phosphate, by far the largest determinant of TmP/GFR, was not associated with any

outcome in this study, make it less likely that these effects were of a large magnitude. Lastly, one might postulate that TmP/GFR decline could also be a physiological compensatory mechanism in preventing hyperphosphatemia, but values below lower reference limit of serum phosphate (lowest two quartiles of TmP/GFR) imply an “overshoot” of TmP/GFR decline that is likely attributable to proximal tubular dysfunction.

Finally, SGLT2-inhibition significantly increased TmP/GFR one day after administration after adjustment for baseline TmP/GFR, but not after additional adjustment for eGFR change.(41) This was contrary to our expectation, first because of the anatomical proximity of SGLT2 to NaPi-IIa (believed to be responsible for 70% of phosphate reabsorption), both primarily located in the S1 segment.(42,43) Second, SGLT2-inhibitors have an effect on phosphate homeostasis by increasing serum phosphate, both short- and long term.(44,45) Third, prevention of sodium reabsorption by SGLT2-inhibition causes the sodium gradient to be preserved for, among others, sodium-dependent phosphate transporters.(44) Alternatively, changes in urine osmolality induced by empagliflozin (although no correlation between urinary phosphate and urinary glucose was observed)(41) or neurohormonal influences might also influence phosphate transport.

Certain associations of TmP/GFR deserve additional discussion. First, low TmP/GFR was associated with higher albuminuria. This might be explained by the fact that decreased albumin reabsorption in tubules due to tubular damage can contribute to albuminuria.(1) Second, FGF23 not clustering with nor being a determinant of TmP/GFR was unexpected, since FGF23 regulates urinary phosphate excretion.(46) Likely FGF23 is stronger affiliated with different pathways in heart failure, like development of ventricular hypertrophy,(47) sodium homeostasis,(48) volume overload, and more pronounced RAAS activation.(25,46) Third, low TmP/GFR was not associated with eGFR decline. This might be explained by

eGFR changes not necessarily reflecting changes in intrinsic kidney (tubular) damage.(3) We showed that low TmP/GFR and low eGFR often occur together and confer a very poor survival and cardiorenal profile if they do, but they might not necessarily determine one another, consistent with regression and cluster analysis.

Clinical relevance and future perspectives

To our knowledge, this is the first study to pose TmP/GFR as a potentially clinically relevant parameter of proximal tubular function in heart failure (Concept Figure). Looking beyond glomerular function with a relevant metric of proximal tubular function will provide valuable cardiorenal insights because it might ultimately contribute to therapies that modulate proximal tubular function and improve sodium homeostasis in heart failure. Further research is warranted to study dynamics of TmP/GFR and its value in prospectively predicting (long-term) future CKD onset. Additionally, since we could only include derivatives of sodium homeostasis and diuretic response and EMPA-RESPONSE-AHF had a small sample size, extended exploration of the role of TmP/GFR in this sense will be important. Finally, exploration of other parameters reflecting tubular (sodium) transport will also be highly valuable to further increase our knowledge of proximal tubular function in heart failure.

Strengths and limitations

We investigated TmP/GFR in relation to a wide panel of variables in a large, heterogeneous, multinational heart failure population, a strength of our study. Furthermore, this study included a good balance of patients with and without CKD, allowing reliable subgroup analyses. Additional insights were provided by studying effects of empagliflozin on TmP/GFR in a small, but very well-defined cohort in acute heart failure patients. However, we are mainly able to describe associations, not causality. We also realize that this study does

not provide definitive proof of the relationship of TmP/GFR with actual proximal tubular damage or overall reduced sodium reabsorption of the proximal tubule, as previous experimental studies in rats have detected upregulation of the NHE3 transporter in response to heart failure.(49–51) Necessity for collecting urinary samples is a disadvantage. Also, the formula is quite complex, but this can easily be solved by implementation in electronic patient systems. Furthermore, urinary NGAL would have been preferred over plasma NGAL, but unfortunately no measurements were available at 9 months. We were only able to show dynamic changes of TmP/GFR in EMPA-RESPONSE-AHF, since no repeat measurements were performed in BIOSSTAT-CHF. Finally, no true GFR or parathyroid hormone levels were measured, and patients were not fasted nor had consistent sample collection times, which would have provided more consistency in TmP/GFR readouts.

Conclusion

TmP/GFR, a metric of proximal tubular function, is frequently reduced in patients with heart failure, especially in patients with more advanced heart failure. Lower TmP/GFR is associated with plasma NGAL doubling over time and poor outcomes, independent of glomerular function. SGLT2-inhibition increased TmP/GFR, but not after adjustment for eGFR change.

Author contributions

JEE, MHdB, and JMtM designed the study; EMB, KDamman, DJvV, KDickstein, CCL, NJS, PP, LLN, SDA, GF, MM, and AAV acquired data; JEE analyzed the data and made tables/figures; JEE, MHdB, EMB, KDamman, GN, AAV, and JMtM drafted and revised the paper (supported by feedback from all authors); all authors approved the final version of the manuscript.

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JEE, EMB, GN, DJvV, KDickstein, CCL, NJS, PP, LLN, and JMtM reported no conflicts of interest.

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Table 1. Baseline characteristics of BIOSTAT-CHF index cohort stratified by quartiles of TmP/GFR

	TmP/GFR (mmol/L)			
Variable	Q1, <i>n</i> = 522 0.32 (0.19 – 0.40) [min: -0.38, max: 0.46]	Q2, <i>n</i> = 521 0.57 (0.52 – 0.62) [min: 0.46, max: 0.67]	Q3, <i>n</i> = 521 0.77 (0.72 – 0.82) [min: 0.67, max: 0.88]	Q4, <i>n</i> = 521 1.06 (0.95 – 1.25) [min: 0.88, max: 6.81]
<i>Clinical characteristics</i>				
Age (years)	72 ± 11	70 ± 11	68 ± 12	66 ± 13
Sex (male), <i>n</i> (%)	385 (74)	389 (75)	383 (74)	369 (71)
BMI (kg/m ²)	27 (24 – 30)	27 (24 – 31)	27 (24 – 30)	27 (24 – 33)
New York Heart Association classification (III/IV), <i>n</i> (%)	323 (64)	320 (63)	305 (60)	282 (56)
Systolic blood pressure (mmHg)	124 ± 21	127 ± 22	124 ± 22	124 ± 23
Diastolic blood pressure (mmHg)	73 ± 24	75 ± 13	75 ± 13	76 ± 15
Heart rate (bpm)	75 (65 – 85)	76 (67 – 90)	76 (67 – 88)	80 (70 – 92)
Presence of atrial fibrillation/flutter, <i>n</i> (%)	164 (31)	196 (38)	169 (32)	164 (31)
Left ventricular ejection fraction (%)	30 (25 – 38)	30 (25 – 37)	30 (25 – 36)	30 (25 – 35)
Diabetes mellitus, <i>n</i> (%)	170 (33)	184 (35)	159 (31)	162 (31)
Smoking (past or current), <i>n</i> (%)	340 (65)	320 (61)	311 (60)	340 (65)
<i>Primary heart failure etiology, n (%)</i>				
Ischemic heart disease	273 (52)	238 (46)	210 (40)	207 (40)

Hypertension	33 (6)	78 (15)	54 (10)	60 (12)
Cardiomyopathy	115 (22)	108 (21)	154 (30)	142 (27)
Medication, n (%)				
ACE inhibitor/angiotensin receptor blocker	350 (67)	378 (73)	385 (74)	385 (74)
Beta-blocker	437 (84)	423 (82)	439 (84)	425 (82)
Loop diuretics	521 (99)	519 (99)	516 (99)	519 (99)
Loop diuretic dose (mg furosemide equivalent)	60 (40 – 125)	40 (40 – 80)	40 (40 – 80)	40 (40 – 80)
Aldosterone antagonist	258 (49)	271 (52)	279 (54)	288 (55)
Laboratory values				
Hemoglobin (g/dL)	12.5 ± 1.9	13.2 ± 1.7	13.5 ± 1.9	13.7 ± 1.9
Sodium (mEq/L)	140 (137 – 142)	140 (137 – 142)	140 (137 – 142)	140 (137 – 141)
Potassium (mEq/L)	4.2 (3.9 – 4.6)	4.2 (3.9 – 4.6)	4.2 (3.9 – 4.6)	4.3 (4.0 – 4.6)
Phosphate (mg/dL)	1.80 (1.39 – 2.14)	2.38 (2.14 – 2.63)	2.88 (2.63 – 3.16)	3.59 (3.19 – 4.00)
NT-proBNP (ng/L)	3281 (1361 – 7709)	2840 (1269 – 5547)	2382 (1115 – 5144)	2568 (1147 – 5604)
ASAT (U/L)	24 (18 – 34)	26 (20 – 35)	25 (20 – 35)	26 (20 – 36)
ALAT (U/L)	21 (15 – 32)	25 (16 – 40)	25 (18 – 38)	28 (19 – 42)
Kidney function				
Creatinine (mg/dL)	1.39 (1.10 – 1.82)	1.15 (0.95 – 1.48)	1.10 (0.92 – 1.37)	1.05 (0.90 – 1.27)
eGFR (mL/min/1.73m ²)	50 ± 22	60 ± 21	64 ± 22	68 ± 22
Plasma NGAL (ng/mL)	71 (44 – 115)	59 (36 – 95)	55 (35 – 92)	57 (37 – 87)
Urea (mg/dL)	82.3 (52.3 – 126.6)	63.1 (43.8 – 100.2)	64.3 (44.2 – 102.3)	63.1 (42.6 – 96.5)

Urinary creatinine (mg/dL)	49.8 (27.1 – 84.3)	62.2 (32.8 – 105.2)	64.5 (30.5 – 116.5)	63.3 (30.5 – 112.0)
Urinary KIM-1 (ng/gCr)	2085 (1051 – 4001)	1868 (878 – 3600)	1787 (851 – 3325)	1802 (845 – 3605)
Urinary NGAL (µg/gCr)	42 (18 – 106)	30 (14 – 83)	30 (15 – 68)	30 (15 – 65)
UACR (mg/gCr)	38 (9 – 153)	25 (8 – 108)	19 (7 – 74)	22 (7 – 74)
FE _{Na} (%)	1.35 (0.64 – 3.24)	0.91 (0.44 – 1.93)	0.90 (0.39 – 2.01)	0.86 (0.37 – 1.90)
FE _{Urea} (%)	34 (22 – 45)	30 (21 – 41)	27 (18 – 39)	25 (16 – 37)
FE _{Phosphate} (%)	46 (35 – 65)	25 (18 – 32)	17 (13 – 23)	10 (6 – 16)
Neurohormonal activation				
Aldosterone (pg/mL)	76 (37 – 170)	98 (45 – 204)	89 (44 – 188)	109 (51 – 220)
Renin (µUI/mL)	96 (35 – 277)	77 (25 – 235)	86 (29 – 224)	100 (29 – 292)
Aldosterone-renin ratio (ng/dL/ng/mL)	147 (37 – 429)	199 (54 – 551)	175 (54 – 542)	202 (61 – 542)
Biomarkers				
FGF23 (RU/mL)	359 (148 – 973)	193 (111 – 512)	199 (114 – 450)	199 (113 – 437)
Bio-ADM (pg/mL)	40 (26 – 66)	33 (23 – 49)	30 (21 – 48)	30 (21 – 50)
PENK (pmol/L)	102 (75 – 149)	85 (62 – 117)	82 (61 – 111)	79 (61 – 106)
Pro-ADM (ng/mL)	0.67 (0.39 – 1.04)	0.50 (0.32 – 0.76)	0.47 (0.30 – 0.74)	0.45 (0.28 – 0.73)
Galectin-3 (ng/mL)	24 (17 – 33)	21 (15 – 29)	20 (15 – 28)	21 (15 – 28)
GDF-15 (ng/L)	3186 (1924 – 5659)	2668 (1735 – 4536)	2655 (1572 – 4350)	2573 (1644 – 4223)
IL-6 (pg/mL)	6.1 (3.1 – 13.1)	5.1 (2.8 – 10.0)	4.9 (2.7 – 9.1)	5.2 (2.8 – 10.2)

Abbreviations: ACE, angiotensin-converting enzyme; ALAT, alanine transaminase; ASAT, aspartate aminotransferase; Bio-ADM, bioactive adrenomedullin; BMI, body mass index; eGFR, estimated glomerular filtration rate; FE, fractional excretion; FGF23, fibroblast growth factor 23; gCR, gram of urinary creatinine; GDF-15, growth differentiation factor 15;

IL-6, interleukin 6; KIM-1, kidney injury molecule-1; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; pro-ADM, proadrenomedullin; UACR, urine albumin-to-creatinine ratio

Table 2. Multivariable regression analysis for log TmP/GFR* in BIOSTAT-CHF

Variable	Standardized Beta	<i>T</i>	P-value
Log FE _{Urea}	-0.455	-12.286	<0.001
Log urea	-0.460	-10.742	<0.001
Hemoglobin	0.196	7.265	<0.001
Log urinary creatinine	-0.300	-5.854	<0.001
Log loop diuretic dose**	-0.093	-3.688	<0.001
Log FE _{Na}	-0.123	-2.910	0.004
Female sex	0.073	2.802	0.005
Log plasma NGAL	0.076	2.761	0.006
Log aldosterone	0.069	2.755	0.006
Log ALAT	0.066	2.647	0.008
Current smoker	0.063	2.590	0.010
Log urinary osteopontin	-0.089	-2.390	0.017
Log urinary NGAL	-0.056	-2.061	0.040

*After exclusion of serum phosphate. Complete case analysis N = 1,318, R² = 0.242

** In furosemide equivalent

Abbreviations: FE, fractional excretion; NGAL, Neutrophil Gelatinase-Associated Lipocalin;

TmP/GFR, tubular maximum phosphate reabsorption capacity

Table 3. Logistic regression analysis for incidence of doubling of plasma NGAL*

Variable	Odds ratio	95% CI	P-value
Per log baseline TmP/GFR decrease†	2.81	1.65 – 4.84	<0.001
Per log baseline plasma NGAL decrease †	4.45	3.61 – 5.54	<0.001
TmP/GFR adjusted for log baseline plasma NGAL	5.90	3.17 – 11.13	<0.001
TmP/GFR adjusted for above + baseline eGFR	2.48	1.27 – 4.87	0.008
TmP/GFR adjusted for above + log baseline UACR	2.36	1.20 – 4.66	0.013
TmP/GFR adjusted for above + log baseline KIM-1	2.41	1.23 – 4.77	0.011
TmP/GFR adjusted for above + log baseline urinary NGAL	2.36	1.20 – 4.67	0.014
TmP/GFR adjusted for above + log baseline FE_{Na}	2.22	1.06 – 4.69	0.035
TmP/GFR adjusted for above + log baseline FE_{Urea}	2.20	1.05 – 4.66	0.038

*Between baseline and 9 months, complete case analysis N = 1,178

† At baseline, unadjusted

Abbreviations: eGFR, estimated glomerular filtration rate; FE, fractional excretion; KIM-1, kidney injury molecule-1; NGAL, Neutrophil Gelatinase-Associated Lipocalin; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

Table 4. Cox proportional hazards analysis per log TmP/GFR decrease predicting all-cause mortality, hospitalization, or the composite endpoint

	Event rates, n (%)	Unadjusted		Adjusted for BIOSTAT risk score*, serum phosphate, eGFR, and New York Heart Association class	
Outcomes		HR (95% CI)	P-value	HR (95% CI)	P-value
All-cause mortality	559 (27)	3.94 (2.69 – 5.77)	<0.001	2.80 (1.37 – 5.73)	0.005
Heart failure hospitalization	535 (26)	3.20 (2.16 – 4.74)	<0.001	2.29 (1.08 – 4.88)	0.031
All-cause mortality or heart failure hospitalization	878 (42)	3.14 (2.31 – 4.26)	<0.001	1.89 (1.07 – 3.36)	0.030

* Variables in BIOSTAT risk score:

All-cause mortality: age, log blood urea nitrogen, log NT-proBNP, hemoglobin, and beta-blocker use at baseline

Heart failure hospitalization: age, heart failure hospitalization in previous year, peripheral edema, systolic blood pressure, and eGFR

Combined endpoint: age, heart failure hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline

Complete case analysis in adjusted analyses N=2,021 for all three endpoints.

Abbreviations: BIOSTAT, A systems BIOlogy Study to Tailored Treatment in Chronic

Heart Failure; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard

ratio; NT-proBNP, N terminal pro brain natriuretic peptide; TmP/GFR, tubular maximum phosphate reabsorption capacity

Concept Figure

The nephron, with the proximal tubule highlighted, in A) the normal situation, and B) in heart failure. A) Proximal tubule cells are vulnerable to injury and are therefore central sensors and effectors of injury. The sodium phosphate cotransporters NaPi-IIa, NaPi-IIc, and PiT-2 determine the maximum capacity of the kidney to reabsorb phosphate in the proximal tubule, which is reflected by TmP/GFR. TmP/GFR is calculated by first determining the fractional tubular reabsorption of phosphate (TRP) using formula (1). When TRP is ≤ 0.86 , indicating maximum phosphate reabsorption, there is a linear relationship between serum phosphate concentration and excretion, and TmP/GFR is subsequently calculated by formula (2). When TRP is > 0.86 , TmP/GFR is calculated using a different second formula, which was not included in this figure for purposes of comprehensibility. This resulted in TmP/GFR values, a parameter of proximal tubular function. B) In heart failure, hypoxia, congestion, and

formation of reactive oxygen species can lead to the development of tubular damage, notably of the proximal tubule. Damage to proximal tubular cells triggers multiple features of CKD, such as fibrosis, inflammation, and capillary rarefaction, and might lead to impaired sodium handling of the kidney. In response to injury, expression of the sodium phosphate cotransporters declines, resulting in decreased tubular phosphate reabsorption and thus a decreased TmP/GFR. Decreased TmP/GFR is associated with increased fractional excretion of sodium and urea, increased levels of KIM-1, NGAL, and NT-proBNP, higher doses of loop diuretics, and with a poor prognosis.

Abbreviations: CKD, chronic kidney disease; FE_{Na} , fractional excretion of sodium; FE_{Urea} , fractional excretion of urea; KIM-1, Kidney Injury Molecule-1; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; SCr, serum creatinine; SPhos, serum phosphate; UCr, urinary creatinine; UPhos, urinary phosphate; SGLT2, sodium-glucose cotransporter 2; TmP/GFR, tubular maximum phosphate reabsorption capacity; TRP, tubular reabsorption of phosphate

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Figure 1. Correlation plots of TmP/GFR with glomerular and tubular parameters

Correlation coefficients: eGFR, 0.287 (Pearson); FE_{Urea} , -0.173 (Spearman); serum urea, -0.121 (Spearman); plasma NGAL, -0.106 (Spearman)

Complete case analysis N=2,042.

Abbreviations: eGFR, estimated glomerular filtration rate; FE_{Urea} , fractional excretion of urea; NGAL, Neutrophil Gelatinase-Associated Lipocalin; TmP/GFR, tubular maximum phosphate reabsorption capacity

Figure 2. Position of TmP/GFR depicted in hierarchical cluster analysis

Hierarchical cluster analysis of TmP/GFR based on the Spearman's rho correlation coefficient. The further down the scale a variable is positioned, the higher the correlation coefficient between a cluster of variables.

Abbreviations: BioADM, bioactive adrenomedullin; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FE, fractional excretion; FGF23, fibroblast growth factor 23; HR, heart rate; KIM-1, kidney injury molecule-1; LVEF, left ventricular ejection fraction; NGAL, Neutrophil Gelatinase-Associated Lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; PENK, proenkephalin; SBP, systolic blood pressure; TmP/GFR, tubular maximum phosphate reabsorption capacity; UACR, urine albumin-to-creatinine ratio

Figure 3. TmP/GFR over time stratified by empagliflozin versus placebo

TmP/GFR over time after administration of either empagliflozin or placebo in EMPA-RESPONSE-AHF. Error bars represent the interquartile range (Q1-Q3). P-values are controlled for baseline TmP/GFR and change in eGFR between the respective timepoints.

Complete case analysis N=88.

Abbreviations: TmP/GFR, tubular maximum phosphate reabsorption capacity

Figure Legends

Concept Figure

The nephron, with the proximal tubule highlighted, in A) the normal situation, and B) in heart failure. A) Proximal tubule cells are vulnerable to injury and are therefore central sensors and effectors of injury. The sodium phosphate cotransporters NaPi-IIa, NaPi-IIc, and PiT-2 determine the maximum capacity of the kidney to reabsorb phosphate in the proximal tubule, which is reflected by TmP/GFR. TmP/GFR is calculated by first determining the fractional tubular reabsorption of phosphate (TRP) using formula (1). When TRP is ≤ 0.86 , indicating maximum phosphate reabsorption, there is a linear relationship between serum phosphate concentration and excretion, and TmP/GFR is subsequently calculated by formula (2). When TRP is > 0.86 , TmP/GFR is calculated using a different second formula, which was not included in this figure for purposes of comprehensibility. This resulted in TmP/GFR values, a parameter of proximal tubular function. B) In heart failure, hypoxia, congestion, and formation of reactive oxygen species can lead to the development of tubular damage, notably of the proximal tubule. Damage to proximal tubular cells triggers multiple features of CKD, such as fibrosis, inflammation, and capillary rarefaction, and might lead to impaired sodium handling of the kidney. In response to injury, expression of the sodium phosphate cotransporters declines, resulting in decreased tubular phosphate reabsorption and thus a decreased TmP/GFR. Decreased TmP/GFR is associated with increased fractional excretion of sodium and urea, increased levels of KIM-1, NGAL, and NT-proBNP, higher doses of loop diuretics, and with a poor prognosis.

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