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Plasma Potassium Negatively Correlates With Sodium Chloride Cotransporter Abundance and Phosphorylation in Urinary Extracellular Vesicles From Patients With Chronic Kidney Disease

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ABSTRACT

Aim: Using urinary extracellular vesicles (uEVs), we have demonstrated the functional 'renal-K switch' mechanism (the WNK-SPAK-NCC pathway) in both healthy subjects and those with primary aldosteronism. The close relationship between blood pressure and CKD has led to the hypothesis that high potassium intake may be reno-protective through the same mechanism. This study used uEVs to evaluate whether plasma potassium negatively correlates with NCC and its phosphorylation (pNCC) in patients with CKD.

Methods: Morning blood and second morning urine were collected on a single occasion between 8 and 11 AM from patients with various CKD stages. Plasma potassium levels were assessed by a local pathology laboratory. uEVs were obtained by progressive ultracentrifugation, and NCC and pNCC were analysed by western blotting.

Results: Correlation analyses among 23 patients with CKD revealed the abundance of NCC ($R^2 = 0.46$, $p = 0.0003$) and pNCC ($R^2 = 0.30$, $p = 0.0067$) strongly and negatively correlate with plasma potassium. The negative correlations persist among 18 patients who did not receive SGLT2 inhibitors or K-binders (NCC: $R^2 = 0.5$, $p = 0.002$; pNCC: $R^2 = 0.30$, $p = 0.03$) and the negative trends remain among 5 patients who received either SGLT2 inhibitors or K-binders (NCC: $R^2 = 0.64$, $p = 0.11$; pNCC: $R^2 = 0.42$, $p = 0.24$).

Conclusion: In patients with CKD, there are negative correlations between NCC and pNCC in uEVs and plasma potassium, which appear independent of eGFR. This suggests that the mechanism at play is distinct from the overall kidney function, and potassium supplement within a safe level may assist in natriuresis and improve cardiovascular outcomes.

1 | Introduction

Hypertension is a leading cause of chronic kidney disease (CKD) due to the deleterious effect of elevated blood pressure on the kidney vasculature. In turn, CKD is a major risk factor for hypertension and cardiovascular disease. Potassium (K^+) often has negative implications in the setting of kidney dysfunction as patients with CKD are prone to develop hyperkalaemia. This drives recommendations for dietary K^+ restriction, diuretics and/or K^+ binders to lower plasma K^+ in patients with moderate and severe CKD [1]. However, low dietary K^+ intake (below 1.5 g per day) has been associated with an elevation in blood pressure and an increased risk of stroke in populations previously experiencing cardiovascular events [2–4].

Recent evidence has highlighted that dietary potassium exerts a critical influence on sodium reabsorption and blood pressure regulation [5]. Specifically, low dietary potassium intake has been associated with increased phosphorylation and abundance of the thiazide-sensitive cotransporter (NCC) via the chloride-sensitive with no lysine kinase (WNK)-Ste20-related proline-alanine-rich kinase (SPAK) network in the distal convoluted tubule [6, 7], resulting in increased sodium retention, extracellular fluid expansion, and elevated blood pressure. In contrast, high dietary potassium intake suppresses NCC activity and under certain conditions, promotes natriuresis and a reduction in blood pressure. This 'renal-K switch' mechanism not only provides a novel explanation for the inverse relationship between dietary potassium and blood pressure but also underpins the potential reno-protective benefits of optimising K^+ consumption in CKD patients.

Epidemiological studies and clinical trials have demonstrated that high dietary potassium levels are linked with a lower incidence of cardiovascular events, for example, stroke [8–11], suggesting that adequate potassium intake may mitigate the cardiovascular risk in CKD. Emerging data in early-stage CKD indicate that higher urinary K^+ excretion, as a proxy for higher dietary K^+ intake, is associated with better kidney outcomes [12–18]. A recent clinical trial demonstrated the feasibility of moderate K^+ supplementation to blunt CKD progression [19]. However, a thorough exploration of these mechanisms in CKD remains limited.

Urinary extracellular vesicles (uEVs) are a non-invasive source and a reliable tool to identify potential biomarkers to mirror molecular processes and physiological and pathological conditions in the kidney and other urinary tract tissues [20]. Human uEVs have recently been used as a readout to assess the inhibitory effect of potassium on NCC in health and in patients with primary aldosteronism [21, 22]. To assess the relevance of the switch mechanism in patients with CKD, we conducted a cross-sectional observational study using uEVs to determine the association between plasma K^+ levels and NCC abundance and phosphorylation.

2 | Materials and Methods

This study was conducted at the University of Queensland Endocrine Hypertension Research Centre (EHRC) and The Princess Alexandra Hospital (PAH) Department of Kidney Services from May 2022 to November 2023 in Brisbane, Australia. Ethical approval was granted by the Metro South Human Research Ethics Committee (HREC/18/QPAH/103).

2.1 | Patient Selection and CKD Staging

Patients with CKD at all stages and who had two kidneys were invited to participate. Patients were included if they had a confirmed diagnosis of CKD as defined by the KDIGO guidelines, exhibited stable renal function for at least 3 months prior to enrolment, and provided informed consent. Exclusion criteria were established to minimise confounding factors, including the presence of acute kidney injury, active infections, recent hospitalisation, prior kidney transplantation or concurrent participation in other intervention studies. These criteria were set to ensure that the cohort was representative of a stable CKD population. CKD stages were classified using estimated glomerular filtration rate (eGFR, measured by the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation).

2.2 | Sample Collection and Measurements

Morning blood was collected from 8 to 11 AM for measuring plasma aldosterone, plasma direct renin, urea and electrolyte concentrations. Second morning urine was collected immediately after the blood draw for uEV isolation and measurements of spot urine creatinine, total protein and neutrophil gelatinase-associated lipocalin (NGAL). Measurements on blood were performed by the Pathology Queensland Laboratory immediately after collection was completed. Plasma aldosterone was determined by LC–MS/MS [23], and direct renin concentration was measured using a chemiluminescent immunoassay [24]. Spot urine creatinine (EIACUN, Invitrogen, USA), total protein (QuantiChrom, BioAssay Systems, USA) and NGAL (DuoSet ELISA, R&D Systems, USA) were measured in the EHRC and Centre for Kidney Disease Research laboratories using assay kits.

2.3 | uEV Isolation and Characterisation

Second morning urine samples were immediately treated with protease inhibitors (cOmplete EDTA-free, Roche, Switzerland) and phosphatase inhibitors (Sigma Aldrich, USA) before aliquoting and freezing at −80°C. uEVs were isolated using progressive ultracentrifugation techniques with dithiothreitol treatment as previously described [25]. uEV pellets were resuspended in 1× phosphate buffered saline (PBS) containing 0.1% v/v sodium dodecyl sulfate (SDS), followed by on-ice sonicating on a Bioruptor with approximately 5–10 cycles at 50% power (10 s on/off) and 10 min centrifugation at 17000 × *g* to pellet insoluble residues. Total protein concentration of the obtained supernatant was measured by NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, USA). uEVs were characterised by Zetasizer size distribution using two uEV pool samples (each pool sample containing 1 uL of uEV resuspension from each of

26 participants' uEV samples) and by the presence of EV marker proteins (ALIX [ALG-2-Interacting Protein X] and TSG101 [Tumour Susceptibility Gene 101 Protein]) by western blotting for all participants.

2.4 | Western Blotting

Precast SDS-PAGE TGX midi gels (4%–15%, Bio-Rad, USA) were used. Each well of the gel was loaded with a uEV suspension containing 10 μg of total protein, sample buffer and reducing agent. Blot transfer was performed using the Bio-Rad Turbo transfer system, followed by blocking with 5% bovine serum albumin (A3858, Sigma-Aldrich, USA) before overnight 4°C incubation with the following primary antibodies: rabbit anti-NCC (1/1000; AB3553, Merck Millipore, Germany), rabbit anti-T60pNCC (1/2500) [26], rabbit anti-ALIX (1/2000; ABC40, Merck Millipore, Germany) or rabbit anti-TSG101 (1/2000; MASBC649, Merck Millipore, Germany). HRP-conjugated goat anti-rabbit IgG antibody (12–348, Merck Millipore, Germany) was used as the secondary antibody at a 1/20000 dilution for luminol-based enhanced chemiluminescence (1 705 061, Bio-Rad, USA) before exposure to configure signal accumulation mode using a Bio-Rad ChemiDoc XRS+ Imager on configure signal accumulation mode with Image Lab software. Images that did not exceed saturation were exported for analysis.

2.5 | Statistical Analyses

Calculations were processed with R (version 4.4.2) [27]. Protein abundance was analysed by ImageJ; relative protein abundance was determined by dividing the protein band intensity by the sum abundance of 2 EV markers (ALIX+TSG101). Unpaired Wilcoxon tests were performed to compare the sex differences of biochemical features and the log10-transformed relative protein abundance. One-way Analysis of Variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) was used to compare NCC and pNCC at difference stages (G1–G5) of CKD. Pearson's correlation was assessed between log10-transformed relative protein abundance and biochemical parameters (including plasma levels of K^+, Na^+, Cl^-, bicarbonate, aldosterone, renin, aldosterone-to-renin ratio). A $p < 0.05$ was considered significant. Data are presented as median [range], unless stated otherwise.

3 | Results

3.1 | Recruitment and Participants' Characteristics

A total of 26 patients consented and provided blood and sufficient urine samples for uEV isolation. The diameter of the particles of the uEV pool ranged overall from 181.2 to 208.8 nm (Table 1). uEVs were successfully isolated (both ALIX and TSG101 were detected) from 23 participants (Figure 1a), all of whom were included in the analyses. Participants' characteristics are summarised in Table 2. Briefly, participants' median age was 60 [29, 83] (median [range]) years old, with the median body mass index 29.3 [20.8, 46.7] kg/m^2, median blood pressure 133 [105, 160]/77 [50, 110] mmHg, median eGFR 35 [13, 101] and median creatinine clearance 41 [18, 169] mL/min. Among the 23 [8 females/5 males], 19 participants were on renin-angiotensin-aldosterone system (RAAS) inhibitors, 2 on mineralocorticoid receptor (MR) antagonists, 7 on diuretics, 4 on sodium-glucose cotransporter-2 (SGLT2) inhibitors and 1 on a K^+-binder. There were two participants receiving potassium tablets during sample collection, with one on KCl who had been diagnosed with primary aldosteronism and one on K-citrate. No apparent differences in age, eGFR and creatinine clearance were detected between males and females.

3.2 | Morning Blood and Urine Biochemistry

At sampling, plasma levels of K^+ (4.7 [3.3, 5.6] mmol/L), Na^+ (140 [132, 144] mmol/L), Cl^- (106 [100, 110] mmol/L), HCO_3^- (26 [19, 31] mmol/L) and aldosterone-to-renin ratio (4.3 [0.1, 53.1] pmol/mU) were normal (Table 3). Median random urine NGAL was 31.5 [5.3, 1130.0] mg/dL, median urine total protein was 43.3 [5.4, 277.5] mg/dL and median urine creatinine was 72.9 [40.5, 160.7] mg/dL. There was a significant difference in total urinary protein between females (22.8 [7.5, 67.1] mg/dL) and males (68.8 [5.4, 277.5] mg/dL, $p = 0.04$). After correction for urinary creatinine, this difference became stronger ($p = 0.03$).

3.3 | NCC and Its Phosphorylation in uEV From CKD

NCC and pNCC were successfully detected in uEVs from 23 participants with CKD stages from G1–G5 (Figure 1a).

TABLE 1 | Zetasizer measured particle size of uEV pool from 26 participants.

Type	Sample Name	*T* (°C)	*Z*-ave (d.nm)	PdI	Pk 1 mean int (d.nm)	Pk 2 mean int (d.nm)	Pk 3 mean int (d.nm)	Pk 1 area int (%)	Pk 2 area int (%)	Pk 3 area int (%)
Size	Pool_1000× dilution 1	25	208.8	0.432	115.8	347	5474	51.7	46	2.3
Size	Pool_1000× dilution 2	25	181.2	0.438	278.8	5109	0	97.7	2.3	0

Note: Duplex pool samples were prepared.
Abbreviations: PdI, polydispersity index; Pk 1 (Pk2, Pk3) area int, mean intensity area; Pk1 (Pk2, Pk3) mean int, Peak 1 (Peak 2, Peak 3) mean intensity diameter; *T*, temperature; *Z*-average, intensity weighted mean hydrodynamic size.

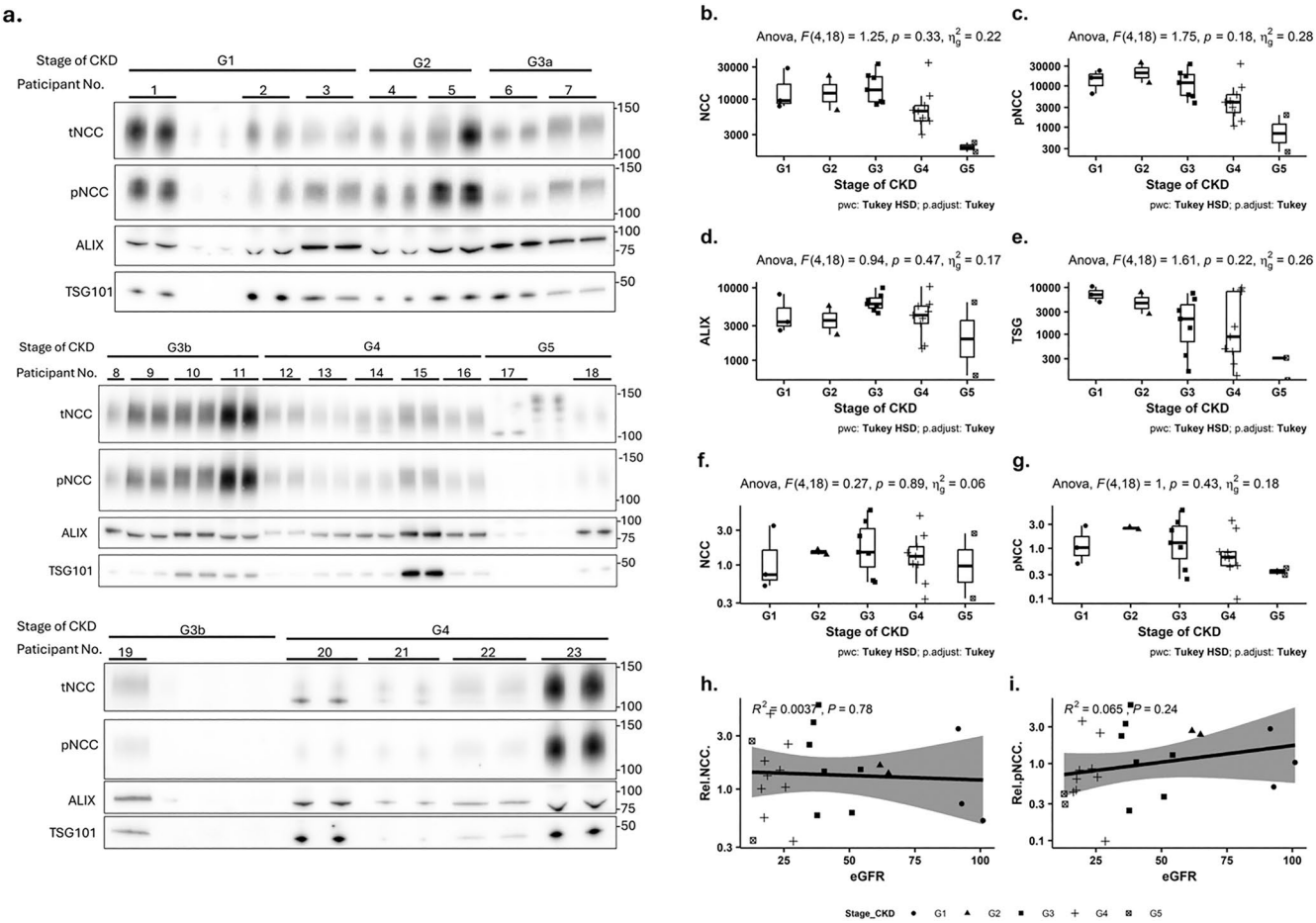

FIGURE 1 | Proteins in urinary extracellular vesicles (uEVs) from participants with chronic kidney disease. (a) Western blotting of uEV levels of total NCC, pNCC, ALIX and TSG101; (b–e) changes in abundances of uEV NCC, pNCC, ALIX and TSG101 with stage of CKD; (f and g) changes in relative abundances of uEV NCC and pNCC with stage of CKD; (h and i) correlations of relative abundances of uEV NCC and pNCC with eGFR. *p* values in (b–g) were based on one-way ANOVA with Tukey's Honestly Significant Difference (HSD) test between each group; Pearson correlation analyses were assessed in (g and h). ALIX, ALG-2-interacting protein X; eGFR, estimated glomerular filtration rate; pNCC, phosphorylated sodium chloride cotransporter; Rel.NCC or Rel.pNCC, NCC or pNCC abundance relative to the sum of ALIX and TSG101; (t)NCC, sodium chloride cotransporter; TSG101, tumour susceptibility gene 101 protein.

TABLE 2 | Participants' characteristics at enrolment.

Variables	Normal range in adults	Overall (*N* = 23)	Female (*N* = 8)	Male (*N* = 15)	Wilcoxon test *p* (female vs. male)
Age, year		60.2 [28.9, 83.1]	62.6 [50.5, 83.1]	57.8 [28.9, 79.7]	0.64
Body mass index, kg/m^2	18.5–24.9	29.3 [20.8, 46.7]	35.0 [20.8, 46.7]	28.2 [25.1, 36.0]	0.10
Systolic blood pressure, mmHg	< 130	133 [105, 160]	130 [110, 149]	134 [105, 160]	0.29
Diastolic blood pressure, mmHg	< 90	77 [50, 110]	78 [62, 83]	77 [50, 110]	0.72
eGFR, mL/min per 1.73 m^2	> 60	35 [13, 101]	37 [19, 101]	27 [13, 93]	0.29
ClCr, mL/min	Male 97–137, female 88–128	41 [18, 169]	61 [25, 117]	36 [18, 169]	0.36

Note: Data are presented in median [range].
Abbreviations: ClCr, creatinine clearance estimated using the Cockcroft-Gault equation; eGFR, estimated glomerular filtration rate using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.

TABLE 3 | Participants' blood and urine biochemistry at sampling.

Morning blood and urine variables	Normal range in adults	Overall ($N=23$)	Female ($N=8$)	Male ($N=15$)	Wilcoxon test p (female vs. male)
Plasma K^+, mmol/L	3.5–5.2	4.7 [3.3, 5.6]	4.6 [4, 5.5]	4.7 [3.3, 5.6]	0.87
Plasma aldosterone, pmol/L	Adult Ambulatory: 30–800 pmol/L; Supine:0–400 pmol/L	176 [29, 1020]	180 [137, 866]	137 [29, 1020]	0.24
Plasma renin, mU/L	Adult Ambulatory: 3–40 mU/L; Supine 2–29 mU/L	42.9 [7.8, 1510]	46.6 [16.6, 280.6]	42.9 [7.8, 1510]	0.87
Plasma aldosterone/renin, pmol/mU	< 55	4.3 [0.1, 53.1]	6.2 [0.5, 39.4]	3.7 [0.1, 53.1]	0.59
Plasma Na^+, mmol/L	135–145 mmol/L	140 [132, 144]	140 [135, 143]	141 [132, 144]	0.62
Plasma Cl^-, mmol/L	95–110 mmol/L	106 [100, 110]	106 [102, 110]	107 [100, 110]	0.74
Plasma HCO_3^-	22–32 mmol/L	26 [19, 31]	26 [21, 29]	26 [19, 31]	0.77
Urine NGAL, ng/mL		31.5 [5.3, 1130.0]	35.5 [9.3, 70.5]	27.8 [5.3, 1130.0]	0.64
Urine protein, mg/dL		43.3 [5.4, 277.5]	22.8 [7.5, 67.1]	68.8 [5.4, 277.5]	0.04
Urine creatinine, mg/dL		72.9 [40.5, 160.7]	75.5 [41.2, 144.92]	72.9 [40.5, 160.7]	0.93
Urine NGAL/creatinine, ng/mg		0.34 [0.04, 14.79]	0.35 [0.17, 1.21]	0.33 [0.04, 14.79]	0.73
Urine protein/creatinine ratio, mg/mg		0.68 [0.04, 4.04]	0.35 [0.06, 0.80]	0.82 [0.04, 4.04]	0.03

Note: Data are presented in median [range].
Abbreviations: Cl^-, chloride; HCO_3^-, bicarbonate; K^+, potassium; Na^+, sodium; NGAL, neutrophil gelatinase-associated lipocalin.

Participants with CKD stage G5 showed relatively lower levels of NCC and pNCC compared to participants with CKD stages G1–G4, but similar trends were also observed in uEV markers ALIX and TSG101. After correcting uEV NCC and pNCC to uEV markers, the differences between CKD stage G5 and stages G1–4 disappeared (Figure 1b–g). Consistent with these observations, there was no correlation between the relative abundances of NCC and pNCC with eGFR (Figure 1h,i). Levels of NCC tended to be higher ($p=0.06$) and levels of pNCC were significantly higher ($p=0.0015$) in females than in males with CKD (Figure 2a,b).

3.4 | NCC and pNCC Negatively Correlates With Plasma K^+ in Patients With CKD

Plasma K^+ strongly and negatively correlated with NCC ($R^2=0.46$, $p=0.0004$) and pNCC ($R^2=0.30$, $p=0.007$) (Figure 2c,d). Patients with CKD sometimes receive medications that prevent hyperkalaemia, such as SGLT2 inhibitors through compensatory mechanisms and K-binders through direct binding of potassium in the gut to facilitate excretion. We then divided patients into two groups: those who did not receive the medications and those who did. The negative correlations persisted among those who did not receive SGLT2 inhibitors or K-binders (NCC: $R^2=0.5$, $p=0.002$; pNCC: $R^2=0.30$, $p=0.03$). Although there were only five patients who received either an SGLT2 inhibitor or K-binders (NCC: $R^2=0.64$, $p=0.011$; pNCC: $R^2=0.42$, $p=0.24$) (Figure 2g,h), the negative trend remained (NCC: $R^2=0.41$, $p=0.004$; pNCC: $R^2=0.25$, $p=0.036$) (Figure 2e,f). No apparent correlation between NCC or pNCC and plasma aldosterone or urine levels of total protein or NGAL was observed (Figure 3).

4 | Discussion

We had hypothesised that a functional 'renal-K switch' exists in patients with CKD. Our study reveals inverse correlations between NCC abundance and phosphorylation and plasma K^+

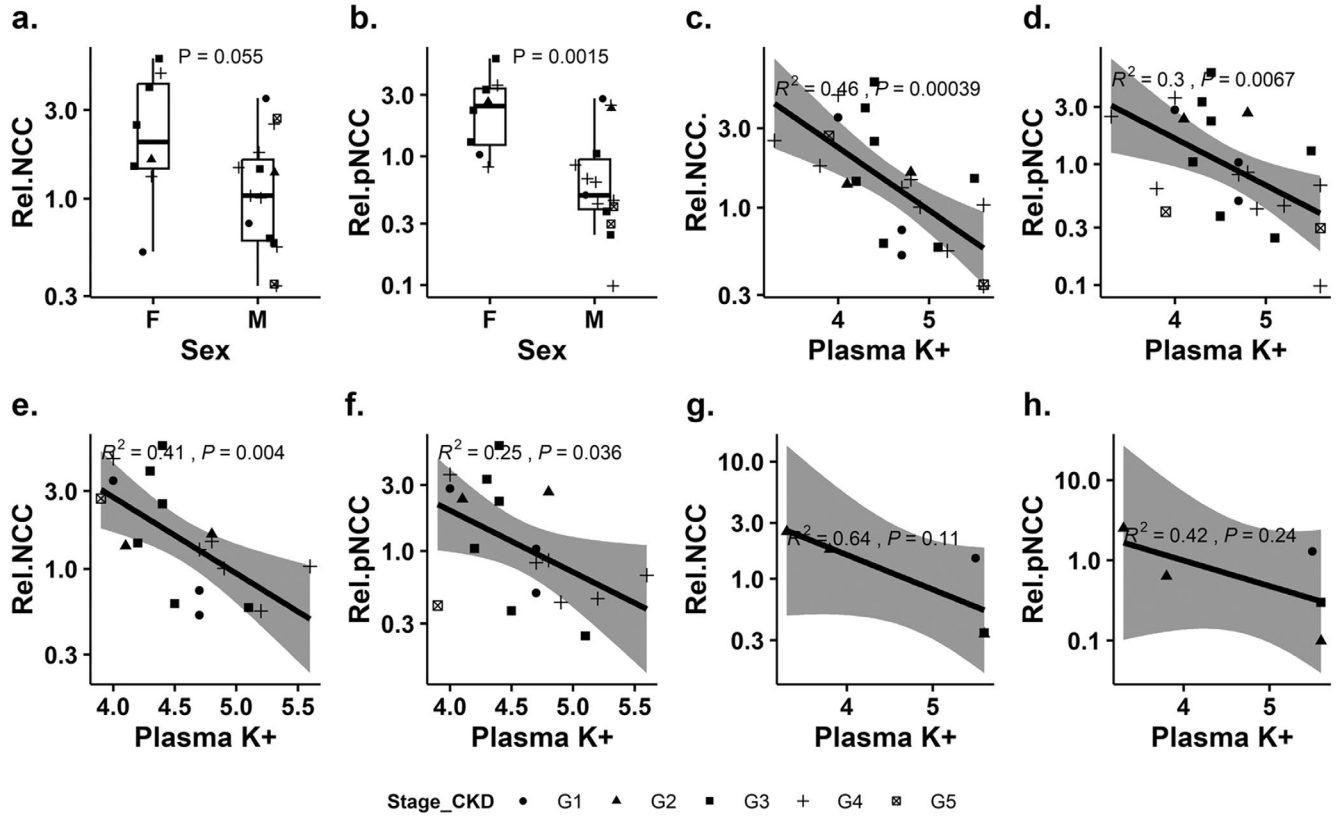

FIGURE 2 | Differences in and correlations of NCC and pNCC. (a and b), differences in relative abundances of NCC and pNCC between females and males; (c and d), negative correlations of plasma K^+ with relative abundances of NCC and pNCC in uEVs; (e and f), negative correlations of plasma K+ with relative abundances of NCC and pNCC among those who did not receive SGLT2 inhibitors or K-binders; (g and h), correlations of plasma K+ with relative abundance of NCC and pNCC in patients who received SGLT2 inhibitors or K-binders.

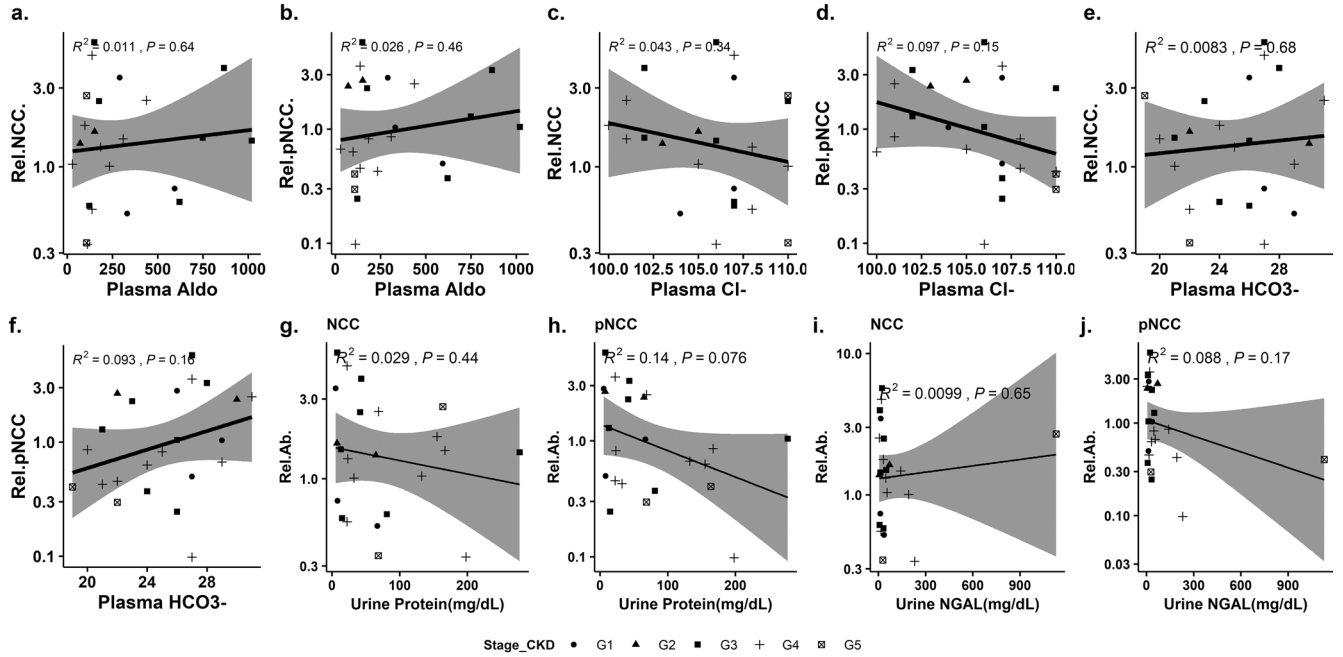

FIGURE 3 | Correlations of plasma and urine parameters with NCC and pNCC. Correlations of NCC and pNCC with plasma aldosterone (a and b), plasma Cl^- (c and d), plasma HCO_3^- (e and f), urine total protein (g and h) and urine NGAL (i and j). Aldo, aldosterone; Cl^-, chloride; HCO_3^-, bicarbonate; NGAL, neutrophil gelatinase-associated lipocalin; Rel.NCC or Rel.pNCC, NCC or pNCC abundance relative to the sum of ALIX and TSG101.

levels in patients with CKD, and these correlations appear independent of eGFR, indicating that the mechanism at play is likely distinct from the overall kidney filtration function.

In patients with CKD, kidney function and potassium handling are closely intertwined. While the kidneys typically maintain potassium balance in CKD until the GFR drops below 10 mL/min/1.73m^2 or in cases of hypoaldosteronism or secretory defects [28], it is widely recognised that impaired kidney function as CKD progresses can disrupt normal potassium handling [29, 30]. Our data reveal that, although all trends are not significant, the levels of NCC and pNCC are relatively similar across CKD stages 1 to 3, with stages 2 and 3 even exhibiting higher levels than stages 1, 4 and 5 (Figure 1b,c). This suggests that in intermediate stages of CKD, compensatory mechanisms may upregulate NCC expression and phosphorylation, potentially as a response to altered electrolyte dynamics. It was initially hypothesised that reductions in NCC and pNCC with increased CKD stage might be attributable to altered uEV excretion, as uEV markers were expected to decline with worsening kidney damage [31]. Our observations reveal that ALIX levels remain consistent across CKD stages 1 to 4, but TSG101 levels decrease with CKD progression (Figure 1d,e). Emerging evidence suggests that uEV markers may be differentially regulated in the context of CKD [32]. The reason for these differences is unclear, but there are reports showing that TSG101 might be exosome-specific [33], and that the ALIX presents on exosomes and microvesicles [34]. After correction of NCC and pNCC to ALIX and TSG101, the trends toward decreased NCC and pNCC with CKD stage disappeared, with corrected levels being similar across all stages, and no correlations were detected between corrected levels of NCC and eGFR (Figure 1). These findings imply that NCC abundance and phosphorylation in CKD patients are more strongly influenced by local regulatory mechanisms, such as K^+-dependent modulation in the distal convoluted tubule, than by global renal function assessed by eGFR.

Our observation of decreased uEV markers ALIX and TSG101 with CKD stage, although not reaching significance, suggests that the reductions in NCC and pNCC may be due to altered uEV excretion. As expected, after correction of NCC and pNCC levels to uEV markers, the trends toward decreased NCC and pNCC with CKD stage disappeared, and no correlations were detected between corrected levels of NCC and pNCC with eGFR. This suggests that NCC abundance and phosphorylation in CKD patients are influenced by factors other than the overall filtration capacity of the kidneys. Local regulatory mechanisms within the distal convoluted tubule, such as K^+ dependent regulation of NCC, likely play a more critical role in modulating its activity than global renal function assessed by eGFR.

The negative association between uEV corrected levels of NCC and pNCC with plasma K^+ aligns with the 'renal-K switch' mechanism (WNKs-SPAK-NCC pathway). The independence of NCC regulation from eGFR in patients with CKD suggests that a high K^+ diet that deactivates NCC to increase K^+ excretion may be effective regardless of CKD stage, as indicated by eGFR. These observations suggest that lower K^+ levels are associated with increased sodium reabsorption, which can contribute to hypertension in CKD patients. Clinically, this finding reinforces the importance of adequate dietary potassium intake as a modifiable factor to help control blood pressure in this population. By modulating NCC activity, relatively higher K^+ intake can promote natriuresis, thereby reducing extracellular fluid volume and blood pressure. As a result, these data support the potential revision of dietary recommendations for CKD patients to include moderate potassium supplementation, particularly in the early stages of the disease [12–18]. However, in patients with late-stage CKD, K^+ excretion is significantly impaired, leading to a risk of hyperkalaemia. It is essential to balance these benefits with the risk of hyperkalaemia by carefully monitoring plasma K^+ levels and tailoring supplementation to individual patient profiles. These suggest that a personalised dietary approach, potentially incorporating controlled K^+ supplementation along with sodium restriction, could optimise cardiovascular and renal outcomes in CKD.

Sex-related differences in the urine protein-to-creatinine ratio and NCC expression and activity were observed in patients with CKD. Many studies have attributed sex differences to the influence of oestrogen. The sex-related difference in random urine protein-to-creatinine ratio may reflect the stages of CKD, as most females in the current study were in G1–G3, while most males were in G4–G5. Oestrogen may protect women against cardiovascular and renal diseases before menopause [35, 36]. It has been observed that oestrogen restores NCC abundance in ovariectomized female rats and stimulates NCC [36–38]. However, our female patients were postmenopausal (according to age), suggesting that sex- or oestrogen-related differences in uEV NCC and pNCC may be minimal. Potassium is now recognised as the primary regulator of NCC, and the lower levels of uEV NCC and pNCC in men may be because the larger muscle mass in men is associated with a higher plasma potassium level compared to women [39, 40]. However, in the current study, the sex difference in plasma K^+ was minimal (females: 4.6 [4.5, 5.5] vs. males: 4.7 [3.3, 5.6], $p = 0.87$). Dietary K^+ intake may also have contributed to these differences.

This study has some limitations. It is important to note that the relatively small sample size of 26 patients may limit the statistical power of our study. This limitation increases the risk of type II errors, whereby true associations might not be detected, and may affect the generalisation of the findings. Therefore, while our preliminary results provide insights into the association between plasma K^+ and NCC activity in CKD patients, these findings should be interpreted with caution. Future studies with larger patient cohorts are warranted to validate and expand upon the results. Moreover, the results were obtained mainly from men (> 60%) and sex-related differences in NCC activity were observed in our study. While hormonal factors may contribute to differential NCC regulation, a significant disparity in sample sizes between males and females can lead to an inaccurate representation. Such sex-specific variations could influence the overall findings, with potential implications for personalised treatment strategies. Therefore, further research is warranted to elucidate the impact of sex on NCC activity and response to dietary K^+ interventions in CKD patients through a larger, more controlled studies that can stratify results by sex. In addition, the current study is an observational study, and most of the participants were on medications that

affect RAAS and K^+ excretion. Furthermore, because only a small number of patients on SGLT2i or K^+ binders were recruited, the effects of these medications on the switch mechanism are unclear. K^+ binders are sometimes used to treat chronic hyperkalaemia in patients with CKD and it is possible that reducing dietary K^+ reabsorption by a K^+ binder will impact the 'switch' mechanism.

In conclusion, our study demonstrates a negative correlation between plasma K^+ and both NCC and pNCC in uEVs from patients with CKD, suggesting that lower K^+ intake may contribute to increased sodium reabsorption and higher blood pressure. These findings enhance our understanding of the 'renal-K switch' mechanism and its implications for blood pressure regulation and cardiovascular risk in CKD. Future research should include longitudinal studies to assess the long-term effects of dietary K^+ interventions on CKD progression and cardiovascular health. Studies involving larger and more diverse patient populations are needed to further validate the renal-K^+ switch in CKD patients, including potential sex-specific differences in NCC regulation. Such investigations will be critical in developing personalised dietary strategies to optimise both renal and cardiovascular outcomes in CKD patients.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. P. E. Stevens, A. Levin, and Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group M, "Evaluation and Management of Chronic Kidney Disease: Synopsis of the Kidney Disease: Improving Global Outcomes 2012 Clinical Practice Guideline," *Annals of Internal Medicine* 158, no. 11 (2013): 825–830.

2. N. J. Aburto, S. Hanson, H. Gutierrez, L. Hooper, P. Elliott, and F. P. Cappuccio, "Effect of Increased Potassium Intake on Cardiovascular Risk Factors and Disease: Systematic Review and Meta-Analyses," *BMJ* 346 (2013): 378–396.

3. L. D'Elia, G. Barba, F. P. Cappuccio, and P. Strazzullo, "Potassium Intake, Stroke, and Cardiovascular Disease a Meta-Analysis of Prospective Studies," *Journal of the American College of Cardiology* 57, no. 10 (2011): 1210–1219.

4. M. J. O'Donnell, S. Yusuf, A. Mente, et al., "Urinary Sodium and Potassium Excretion and Risk of Cardiovascular Events," *JAMA* 306, no. 20 (2011): 2229–2238, https://doi.org/10.1001/jama.2011.1729.

5. D. H. Ellison, A. S. Terker, and G. Gamba, "Potassium and Its Discontents: New Insight, New Treatments," *Journal of the American Society of Nephrology* 27, no. 4 (2016): 981–989.

6. A. S. Terker, C. Zhang, J. A. McCormick, et al., "Potassium Modulates Electrolyte Balance and Blood Pressure Through Effects on Distal Cell Voltage and Chloride," *Cell Metabolism* 21, no. 1 (2015): 39–50, https://doi.org/10.1016/j.cmet.2014.12.006.

7. E. J. Hoorn, M. Gritter, C. A. Cuevas, and R. A. Fenton, "Regulation of the Renal NaCl Cotransporter and Its Role in Potassium Homeostasis," *Physiological Reviews* 100, no. 1 (2020): 321–356.

8. A. A. McDonough, L. C. Veiras, C. A. Guevara, and D. L. Ralph, "Cardiovascular Benefits Associated With Higher Dietary K(+) Vs. Lower Dietary Na(+): Evidence From Population and Mechanistic Studies," *American Journal of Physiology. Endocrinology and Metabolism* 312, no. 4 (2017): E348–E356.

9. C. M. Rebholz, D. C. Crews, M. E. Grams, et al., "DASH (Dietary Approaches to Stop Hypertension) Diet and Risk of Subsequent Kidney Disease," *American Journal of Kidney Diseases* 68, no. 6 (2016): 853–861.

10. B. Neal, Y. Wu, X. Feng, et al., "Effect of Salt Substitution on Cardiovascular Events and Death," *New England Journal of Medicine* 385, no. 12 (2021): 1067–1077, https://doi.org/10.1056/NEJMoa2105675.

11. A. A. McDonough and R. A. Fenton, "Potassium Homeostasis: Sensors, Mediators, and Targets," *Pflügers Archiv* 474, no. 8 (2022): 853–867.

12. S. Araki, M. Haneda, D. Koya, et al., "Urinary Potassium Excretion and Renal and Cardiovascular Complications in Patients With Type 2 Diabetes and Normal Renal Function," *Clinical Journal of the American Society of Nephrology* 10, no. 12 (2015): 2152–2158.

13. L. M. Kieneker, S. J. Bakker, R. A. de Boer, G. J. Navis, R. T. Gansevoort, and M. M. Joosten, "Low Potassium Excretion but Not High Sodium Excretion Is Associated With Increased Risk of Developing Chronic Kidney Disease," *Kidney International* 90, no. 4 (2016): 888–896.

14. H. W. Kim, J. T. Park, T. H. Yoo, et al., "Urinary Potassium Excretion and Progression of CKD," *Clinical Journal of the American Society of Nephrology* 14, no. 3 (2019): 330–340, https://doi.org/10.2215/CJN.07820618.

15. T. Nagata, H. Sobajima, N. Ohashi, et al., "Association Between 24h Urinary Sodium and Potassium Excretion and Estimated Glomerular Filtration Rate (eGFR) Decline or Death in Patients With Diabetes Mellitus and eGFR More Than 30 Ml/Min/1.73m2," *PLoS One* 11, no. 5 (2016): e0152306.

16. A. Smyth, D. Dunkler, P. Gao, et al., "The Relationship Between Estimated Sodium and Potassium Excretion and Subsequent Renal Outcomes," *Kidney International* 86, no. 6 (2014): 1205–1212, https://doi.org/10.1038/ki.2014.214.

17. N. Van Noordenne, R. H. G. O. Engberink, T. C. denVan Hoek, B. J. H. denVan Born, and L. Vogt, "Associations of 15-Year Average Potassium Intake With Long-Term Cardiovascular and Renal Outcome in the Outpatient Clinical Setting," *Journal of Hypertension* 34 (2016): E6.

18. A. K. Leonberg-Yoo, H. Tighiouart, A. S. Levey, G. J. Beck, and M. J. Sarnak, "Urine Potassium Excretion, Kidney Failure, and Mortality in CKD," *American Journal of Kidney Diseases* 69, no. 3 (2017): 341–349.

19. M. Gritter, R. D. Wouda, S. M. H. Yeung, et al., "Effects of Short-Term Potassium Chloride Supplementation in Patients With CKD," *Journal of the American Society of Nephrology* 33 (2022): 1779–1789.

20. Q. Wu, S. B. Poulsen, S. K. Murali, et al., "Large-Scale Proteomic Assessment of Urinary Extracellular Vesicles Highlights Their Reliability in Reflecting Protein Changes in the Kidney," *Journal of the American Society of Nephrology* 32, no. 9 (2021): 2195–2209.

21. A. Wu, M. J. Wolley, H. L. Mayr, et al., "Randomized Trial on the Effect of Oral Potassium Chloride Supplementation on the

Thiazide-Sensitive Sodium Chloride Cotransporter in Healthy Adults," *Kidney International Reports* 8, no. 6 (2023): 1201–1212.

22. A. Wu, M. J. Wolley, R. A. Fenton, and M. Stowasser, "Using Human Urinary Extracellular Vesicles to Study Physiological and Pathophysiological States and Regulation of the Sodium Chloride Cotransporter," *Frontiers in Endocrinology* 13 (2022): 981317.

23. M. Thuzar, K. Young, A. H. Ahmed, et al., "Diagnosis of Primary Aldosteronism by Seated Saline Suppression Test-Variability Between Immunoassay and HPLC-MS/MS," *Journal of Clinical Endocrinology and Metabolism* 105, no. 3 (2020): e477–e483.

24. Z. Guo, M. Poglitsch, B. C. McWhinney, et al., "Measurement of Equilibrium Angiotensin II in the Diagnosis of Primary Aldosteronism," *Clinical Chemistry* 66, no. 3 (2020): 483–492.

25. M. J. Wolley, A. H. Wu, S. X. Xu, R. D. Gordon, R. A. Fenton, and M. Stowasser, "In Primary Aldosteronism, Mineralocorticoids Influence Exosomal Sodium-Chloride Cotransporter Abundance," *Journal of the American Society of Nephrology* 28, no. 1 (2017): 56–63.

26. N. B. Pedersen, M. V. Hofmeister, L. L. Rosenbaek, J. Nielsen, and R. A. Fenton, "Vasopressin Induces Phosphorylation of the Thiazide-Sensitive Sodium Chloride Cotransporter in the Distal Convoluted Tubule," *Kidney International* 78, no. 2 (2010): 160–169.

27. Team RC, "RA Language and Environment for Statistical Computing, 2020." R Foundation for Statistical Computing.

28. D. C. Batlle, J. A. Arruda, and N. A. Kurtzman, "Hyperkalemic Distal Renal Tubular Acidosis Associated With Obstructive Uropathy," *New England Journal of Medicine* 304, no. 7 (1981): 373–380.

29. M. Wieers, J. Mulder, J. I. Rotmans, and E. J. Hoorn, "Potassium and the Kidney: A Reciprocal Relationship With Clinical Relevance," *Pediatric Nephrology* 37, no. 10 (2022): 2245–2254.

30. S. M. H. Yeung, L. Vogt, J. I. Rotmans, E. J. Hoorn, and M. H. de Borst, "Potassium: Poison or Panacea in Chronic Kidney Disease?," *Nephrology, Dialysis, Transplantation* 34, no. 2 (2019): 175–180.

31. C. Delrue, S. De Bruyne, R. Speeckaert, and M. M. Speeckaert, "Urinary Extracellular Vesicles in Chronic Kidney Disease: From Bench to Bedside?," *Diagnostics (Basel)* 13, no. 3 (2023): 443–469.

32. S. Oshikawa-Hori, N. Yokota-Ikeda, H. Sonoda, Y. Sasaki, and M. Ikeda, "Reduced Urinary Release of AQP1- and AQP2-Bearing Extracellular Vesicles in Patients With Advanced Chronic Kidney Disease," *Physiological Reports* 9, no. 17 (2021): e15005.

33. E. H. Koritzinsky, J. M. Street, R. R. Chari, et al., "Circadian Variation in the Release of Small Extracellular Vesicles Can Be Normalized by Vesicle Number or TSG101," *American Journal of Physiology. Renal Physiology* 317, no. 5 (2019): F1098–F1110.

34. M. Colombo, G. Raposo, and C. Thery, "Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles," *Annual Review of Cell and Developmental Biology* 30 (2014): 255–289.

35. J. F. Reckelhoff, "Gender Differences in the Regulation of Blood Pressure," *Hypertension* 37, no. 5 (2001): 1199–1208.

36. G. Cobo, M. Hecking, F. K. Port, et al., "Sex and Gender Differences in Chronic Kidney Disease: Progression to End-Stage Renal Disease and Haemodialysis," *Clinical Science (London, England)* 130, no. 14 (2016): 1147–1163.

37. L. Rojas-Vega, L. A. Reyes-Castro, V. Ramirez, et al., "Ovarian Hormones and Prolactin Increase Renal NaCl Cotransporter Phosphorylation," *American Journal of Physiology. Renal Physiology* 308, no. 8 (2015): F799–F808.

38. J. W. Verlander, T. M. Tran, L. Zhang, M. R. Kaplan, and S. C. Hebert, "Estradiol Enhances Thiazide-Sensitive NaCl Cotransporter Density in the Apical Plasma Membrane of the Distal Convoluted Tubule in Ovariectomized Rats," *Journal of Clinical Investigation* 101, no. 8 (1998): 1661–1669.

39. H. Lee, M. B. Fessler, P. Qu, J. Heymann, and J. B. Kopp, "Macrophage Polarization in Innate Immune Responses Contributing to Pathogenesis of Chronic Kidney Disease," *BMC Nephrology* 21, no. 1 (2020): 270.

40. M. I. Lindinger and S. P. Cairns, "Regulation of Muscle Potassium: Exercise Performance, Fatigue and Health Implications," *European Journal of Applied Physiology* 121, no. 3 (2021): 721–748.