Urinary sodium excretion is the main determinant of mineralocorticoid excretion rates in patients with chronic kidney disease

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ABSTRACT

Background. Blockade of the mineralocorticoid receptor (MR) in patients with chronic kidney disease (CKD) improves surrogate cardiovascular outcomes, such as left ventricular mass. Animal models of renal disease support a pathological role of mineralocorticoids, in the context of a high sodium intake. We aimed to assess the regulation of mineralocorticoid biosynthesis in patients with CKD.

Methods. Seventy patients with CKD stages 3/4 and 30 patients with essential hypertension (EH) were recruited. Patients underwent detailed clinical phenotyping, drug history and biochemical assessment. Patients completed a 24-h urine collection for measurement of urinary tetrahydroaldosterone (THALDO) and tetrahydrocorticosterone (THDOC) excretion rates (measured using gas chromatography-mass spectrometry) and urinary electrolytes. The factors which correlated significantly with THALDO and THDOC excretion were entered into linear regression models.

Results. Patients with EH and CKD were well matched with no significant differences in gender, age or weight. The mean estimated glomerular filtration rate (eGFR) in CKD patients was 38.6/min/1.73 m². The mean urinary excretion rates of THALDO, THDOC and 24-h urinary sodium (24-h USod) were not significantly different between CKD and EH patients. The level of renal function did not correlate with THALDO or THDOC excretion. In patients with CKD, 24-h USodium (r = 0.614, P < 0.001) and 24-h UPotassium (r = 0.538, P < 0.001) were positively correlated with THALDO excretion. On multivariate linear regression analysis, 24-h USod was the strongest independent predictor (P = 0.004) of THALDO and THDOC excretion in CKD. In patients with EH, no relationship was seen between mineralocorticoid excretion and 24-h urinary sodium excretion.

Conclusions. In patients with CKD, 24-h urinary sodium excretion is the strongest positive predictor of urinary mineralocorticoid excretion. The nature of this relationship is unexpected, novel, not seen in patients with EH and may explain the association seen between high urinary sodium excretion, mineralocorticoids and poor outcomes in patients with CKD.

INTRODUCTION

Chronic kidney disease (CKD) is associated with a significant risk of end-stage renal disease and cardiovascular mortality [1]. The mechanisms underlying the progression of CKD and the associated cardiovascular risk are, however, poorly understood [2]. Mineralocorticoid excess has been associated with renal and cardiac fibrosis in animal models of CKD [3, 4]. Furthermore, administration of drugs which block the mineralocorticoid receptor (MR) is beneficial in patients with CKD, reducing proteinuria [5], and regressing left ventricular mass [6], while possibly retarding progression of kidney disease [7]. There is some evidence that mineralocorticoid production is inappropriately high in patients with renal failure [8] where
there is evidence of salt and water overload. However, the interpretation of previous studies is limited by small sample sizes and the reliance on single measurements of plasma aldosterone concentration (PAC) as an index of the overall aldosterone production rate. In addition, most studies predate the widespread use of blockers of the renin–angiotensin system.

The aim of the current study was to better understand the mechanism which underlies the beneficial effects of MR blockade in CKD. In particular, we wished to explore the hypothesis that levels of mineralocorticoids are inappropriately elevated in these patients and aimed to identify the factors which are associated with determining mineralocorticoid synthesis in these patients. We did this by measuring the 24-h urinary excretion rate of corticosteroid hormones and their principal regulators in patients with CKD. The principal control group was patients with essential hypertension (EH), to allow for the effects of medications. We focussed on the human mineralocorticoids, aldosterone and its precursor deoxycorticosterone (DOC). While aldosterone has been widely studied in hypertension and cardiovascular disease, the role of DOC has largely been overlooked. However, recent evidence has shown DOC to be associated with left ventricular mass and proteinuria in patients with CKD [9].

** MATERIALS AND METHODS **

**Subject selection**

In a cross-sectional cohort, 70 adult patients with CKD stages 2–4 were recruited from local renal clinics. Patients were included if they had diabetic nephropathy (DMN), IgA nephropathy (IgAN) or membranous nephropathy (MGN). The diagnosis of DMN was clinician defined in the majority of patients, i.e. patients with known diabetes mellitus (type 1 or 2), microvascular complications such as proliferative retinopathy, proteinuria and an absence of alternative explanation for their renal failure. Diagnosis of IgAN or MGN was made from native renal biopsy. Thirty patients with EH requiring drug treatment and normal kidney function were recruited as control subjects from local hypertension clinics.

Patients were excluded if they had a condition which would interfere with aldosterone metabolism—a proven aldosterone-secreting tumour or adrenal hyperplasia, or treatment with an aldosterone antagonist (spironolactone or eplerenone). Therefore, all patients were screened for aldosterone excess prior to recruitment by measurement of an aldosterone-to-renin ratio; in all cases, this was below the threshold required in our Centre to warrant further investigation for primary aldosteronism.

Patients who were pregnant or lactating and those with an active infection were also excluded. We imposed no proteinuria cut-off level for renal patients.

The protocol was approved by the local research ethics committee and all patients gave written informed consent.

**Patient assessment**

Patients took medications as prescribed. They attended the Glasgow Clinical Research Facility at 9 am with a completed 24-h urine collection from which proteinuria, creatinine and electrolytes were measured. Twenty-four-hour excretion rates of mineralocorticoid metabolites [tetrahydroaldosterone (THALDO), tetrahydrodeoxycorticosterone (THDOC) and cortisol metabolites (THF, THE and aTHE)] were determined from 24-h urine aliquots by gas chromatography-mass spectrometry using the Shackleton method [10]. Urinary free cortisol (F) and urinary free cortisone (E) were measured by high-performance liquid chromatography using ultraviolet absorption quantitation. Height, weight and waist circumference were measured. The lowest of three ‘office’ blood pressure measurements was recorded. After 30 min of supine rest, venous blood was sampled for routine biochemistry and haematology. Plasma renin concentration (PRC) was measured using the DiaSorin radioimmunoassay (normal range undetectable—40 mIU/L) and PAC was measured using a radioimmunoassay (Siemens, TKAL2, Coat-A-Count). Renal function was assessed using the four-variable Modification of Diet in Renal Disease (MDRD4) formula.

**Data analysis**

Data were analysed using SPSS (SPSS Inc., IL) Version 15.0. Data were plotted to assess normality. Comparisons were made between groups using Student’s t-test or one-way ANOVA; non-normally distributed data were compared using the Mann–Whitney U-test or Kruskall–Wallis test as appropriate. Categorical data were compared using the Chi-square test. Correlations were Pearson’s or Spearman’s depending on the distribution of the data. Linear regression analysis was undertaken to assess the relationship between continuous variables.

**RESULTS**

**Demographics**

Baseline demographic data are shown in Table 1. Seventy patients had CKD [34 DMN, 36 primary glomerulonephritis (GN)] and 30 patients had EH. Patients with EH and CKD were well matched with no significant differences in gender, age or weight. Patients with EH had a significantly higher diastolic blood pressure (DBP) (P < 0.001).

Within the CKD cohort, patients with primary GN were significantly younger [54.8 years (11.8) versus 61.8 years (13.0), P = 0.02] and had significantly lower systolic blood pressure (SBP) [138 (21) mmHg versus 157 (21) mmHg, P < 0.001] than patients with DMN.

Biochemical differences between the EH and CKD cohorts are as would be expected and are summarized in Table 1. Renal patients had significantly lower estimated glomerular filtration rate (eGFR) levels (P < 0.001), higher serum concentrations of potassium (P < 0.001) and higher levels of proteinuria (P < 0.001). Twenty-four-hour urinary sodium and potassium excretion rates did not differ between the EH and CKD groups.

Excretion rates of THALDO, THDOC and cortisol metabolites did not differ significantly between patients with EH and patients with CKD (Table 1). There were also no significant differences in excretion rates among primary renal diseases.
(DMN, IgAN or MGN). PAC and PRC did not differ significantly among patient groups.

### Mineralocorticoid excretion and level of renal function

There was no significant association between renal function and steroid measurements (THALDO, THDOC), or PAC (Figure 1). There was a trend towards a higher PRC with poorer renal function ($P = 0.05$).

### Mineralocorticoid excretion and drug therapies

Drug therapies differed between the EH and CKD cohorts; patients with CKD were significantly more likely to be prescribed an angiotensin-converting enzyme inhibitor (ACEi) ($P = 0.01$), combination of ACEi and angiotensin receptor blocker (ARB) ($P = 0.001$) or a loop diuretic ($P < 0.001$). Patients with EH were significantly more likely to be prescribed a thiazide diuretic ($P < 0.001$).

In the EH and CKD cohorts, excretion rates of THALDO and THDOC were not significantly different in patients prescribed different classes of medications (ACEi, ARB, Bblocker, CCB, thiazide diuretic, loop diuretic, alpha blocker) when compared with those who were not. PAC was significantly lower in patients prescribed an ACEi (median 227 versus 300 pmol/L, $P = 0.022$) than in those not.

### Associations with urinary steroid excretion

In patients with CKD, the factors which correlated positively with urinary THALDO and THDOC excretion were 24-h USod excretion ($r = 0.614$, $P < 0.001$; $r = 0.526$, $P < 0.001$) and 24-h UPot excretion ($r = 0.538$, $P < 0.001$; $r = 0.481$, $P < 0.001$). THDOC and THALDO were significantly correlated with each other ($P < 0.001$). No significant relationship was seen between PAC or THALDO and serum potassium concentration, even when patients prescribed with ACEi/ARB were excluded.

Univariate linear regression confirmed the association between THALDO, THDOC and urinary sodium excretion in CKD patients ($R^2 = 0.38$, $P < 0.001$, THDOC $R^2 = 0.28$, $P < 0.001$) (Figure 2). This relationship persisted when looking at patients with diabetes or primary renal disease separately. Twenty-four-hour USod and UPot excretion rates were also both significant multivariate predictors of THALDO and THDOC excretion in patients with CKD (Tables 2 and 3). Adding gender, primary renal disease, presence of diabetes, drug treatment with ACEi/ARB or diuretics, blood pressure and eGFR to the model did not alter the relationship.

Importantly, in patients with EH, no relationship was seen between THALDO or THDOC excretion and urinary sodium...

### Table 1. Baseline demographics, stratified by group

<table>
<thead>
<tr>
<th>Variable</th>
<th>EH controls $N = 30$</th>
<th>CKD patients $N = 70$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.4 (9.2)</td>
<td>58.2 (12.8)</td>
<td>0.28</td>
</tr>
<tr>
<td>% Male</td>
<td>83.3</td>
<td>75.7</td>
<td>0.45</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.3 (4.8)</td>
<td>29.3 (5.8)</td>
<td>0.99</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>152 (20)</td>
<td>147 (23)</td>
<td>0.37</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>93 (11)</td>
<td>82 (12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>58 (13)</td>
<td>65 (19)</td>
<td>0.004</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m$^2$)</td>
<td>90.4 (18.2)</td>
<td>38.6 (24.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum sodium (mmol/L)</td>
<td>139 (2)</td>
<td>139 (3)</td>
<td>0.19</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>3.8 (0.3)</td>
<td>4.6 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAC (pmol/L)</td>
<td>236.5 (163.2–331.3)</td>
<td>254.5 (162.3–442.5)</td>
<td>0.52</td>
</tr>
<tr>
<td>PRC (uIU/mL)</td>
<td>32.4 (17.7–91.2)</td>
<td>62.4 (17.0–213.9)</td>
<td>0.31</td>
</tr>
<tr>
<td>USod (mmol/24 h)</td>
<td>155.7 (79.7)</td>
<td>162.9 (70.4)</td>
<td>0.66</td>
</tr>
<tr>
<td>UPot (mmol/24 h)</td>
<td>86.4 (31.1)</td>
<td>61.0 (24.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UPr (g/24 h)</td>
<td>0.1 (0.1–0.1)</td>
<td>1.0 (0.3–2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>THALDO (mcg/24 h)</td>
<td>62.3 (24.0)</td>
<td>56.7 (22.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>THDOC (mcg/24 h)</td>
<td>75.2 (32.5)</td>
<td>66.1 (23.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>CORT METAB (mcg/24 h)</td>
<td>100 049 (4985)</td>
<td>8507 (5050)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; eGFR, estimated glomerular filtration rate (MDRD4); PAC, plasma aldosterone concentration; PRC, plasma renin concentration; USod, urine sodium; UPot, urine potassium; UPr, urinary proteinuria excretion; Cort Metab, Cortisol metabolites.

Data expressed as mean (SD) or median (IQR). Significant results highlighted in bold. Comparison made by the $t$-test or Mann–Whitney $U$-test, as appropriate.
**FIGURE 1:** Scatterplots of THALDO (A), THDOC (B), plasma aldosterone (C) and plasma renin concentrations (D) versus eGFR (measured using the MDRD4 formula). Significance assessed using linear regression.

**FIGURE 2:** Scatterplot of urinary sodium excretion (mmol/24 h) versus THALDO or THDOC excretion, with fitted linear regression line and an estimate of significance. A and B = CKD patients, C and D = EH patients.
Table 2. Univariate and multivariate linear regression models in CKD patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate B</th>
<th>95% CI</th>
<th>P</th>
<th>Multivariate B</th>
<th>95% Confidence interval (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>THDOCa</td>
<td>0.765</td>
<td>0.681–0.849</td>
<td>&lt;0.001</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>USod (mmol/24 h)</td>
<td>0.167</td>
<td>0.113–0.221</td>
<td>&lt;0.001</td>
<td>0.102</td>
<td>0.034–0.171</td>
<td>0.004</td>
</tr>
<tr>
<td>UPot (mmol/24 h)</td>
<td>0.428</td>
<td>0.259–0.597</td>
<td>&lt;0.001</td>
<td>0.235</td>
<td>0.04–0.43</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Predicted variable THALDO.

*a*R² for the multivariate model 0.492, P < 0.001. USod = 24-h urinary sodium excretion; UPot = 24-h urinary potassium excretion. Due to high collinearity, THDOC was not included in the multivariate model.

Table 3. Univariate and multivariate linear regression models in CKD patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate B</th>
<th>95% CI</th>
<th>P</th>
<th>Multivariate B</th>
<th>95% Confidence interval (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>THALDOa</td>
<td>1.099</td>
<td>0.979–1.22</td>
<td>&lt;0.001</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>USod (mmol/24 h)</td>
<td>0.171</td>
<td>0.102–0.241</td>
<td>&lt;0.001</td>
<td>0.091</td>
<td>0.003–0.179</td>
<td>0.044</td>
</tr>
<tr>
<td>UPot (mmol/24 h)</td>
<td>0.459</td>
<td>0.248–0.67</td>
<td>&lt;0.001</td>
<td>0.285</td>
<td>0.035–0.535</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Predicted variable THDOC.

*a*R² for the multivariate model 0.417, P < 0.001. USod = 24-h urinary sodium excretion; UPot = 24-h urinary potassium excretion. Due to high collinearity, THDOC was not included in the multivariate model.

Discussion

These novel data suggest that, in patients with CKD, the qualitative relationship between aldosterone and sodium is reversed; 24-h excretion of mineralocorticoids positively correlates with 24-h excretion of urinary sodium, a surrogate for dietary sodium intake. Moreover, it would appear that the factors controlling mineralocorticoid production differ in patients with CKD compared with patients with EH or normal controls. Additionally, mineralocorticoid excretion rate is not influenced by the level of renal function. The findings described in this paper support the notion that failure to appropriately suppress mineralocorticoid production, in the presence of salt and water excess, may be a major contributory factor to cardiovascular risk in this population.

Analysis of urinary steroid metabolites over 24 h is a more representative indication of the aldosterone status as it eliminates the variation associated with single plasma measurements of aldosterone including posture, episodic secretion of the hormone and diurnal variation [11]. The metabolites of aldosterone and DOC, THALDO and THDOC have a molecular weight of 365 and 334 Daltons, respectively, and will be freely filtered at the glomerulus. As our patients had preserved liver function and were well out-patients, 24-h excretion of urinary mineralocorticoids is likely to accurately reflect the rate of hormone synthesis. In this study, there was no relationship between THALDO excretion and PAC, perhaps reflecting the snapshot view PAC provides, rather than an integrated 24-h measure.

The conventional view of hypertension in patients with CKD is that salt and water retention occurs in conjunction with inappropriate renin–angiotensin–aldosterone (RAAS) activation. Additionally, in the presence of therapies which should suppress the RAAS (e.g. ACEi), it is noted that around 30–40% of patients will exhibit ‘aldosterone escape’, elevated plasma aldosterone levels around a year after commencing treatment [12]. Contrary to this, there is no evidence from our study that levels of urinary steroid excretion or plasma aldosterone or plasma renin concentrations relate to levels of renal function in patients with CKD stages 2–4, or patients with EH. Our study also demonstrated that there were no differences in steroid levels between patients with diabetic renal disease and primary glomerular disease.

The main recognized trophins for mineralocorticoid production are potassium, intravascular volume status and angiotensin II. We have shown that associations between conventional regulators of steroid synthesis and steroid levels...
appear to differ in CKD from those with EH. In patients with
CKD, the strongest independent determinant of a high
THALDO or THDOC excretion rate was a high 24-h urinary
sodium excretion rate, a measure of dietary sodium intake.
This is a novel and unanticipated finding and was not seen in
EH patients, where no relationship was found, as has been re-
ported in other studies [13].

Plasma potassium is a powerful aldosterone agonist and
this relationship was clearly seen in patients with CKD. This
was not so in EH patients, possibly because serum potassium
levels were lower in this group and spanned a more narrow
range. There was a trend towards an inverse relationship
between THALDO excretion and cortisol metabolites [a surro-
gate for adrenocorticotropic hormone (ACTH)] in patients
with CKD and no significant relationship in patients with EH.
Furthermore, we found that THALDO excretion did not cor-
relate with PRC (a surrogate for angiotensin II) in patients
with EH or CKD, likely reflecting the effects of concomitant
drug therapy.

Data with which to compare our findings relating to steroid
levels and determinants in CKD are sparse. In a historical
study, Hene et al. [8] showed that PAC was higher in patients
with primary renal disease and very low levels of creatinine
clearance (3–10 mL/min) on no medication. Our study did
not include patients with renal function as low as this which
may explain why this association was not found. A more
recent study by Hammer et al. [14] is the only comparable
study which reports urinary steroid excretion in patients with
CKD. Hammer assessed 112 patients with non-diabetic, mini-
mally proteinuric renal disease with well-preserved renal func-
tion (CKD 3). Our patients had lower baseline renal function,
a median of 1 g proteinuria per day and half had DMN. They
also found that THALDO excretion was not associated with
eGFR, SBP or DBP. In their cohort, PAC correlated weakly
with THALDO excretion, which was not seen in our cohort.
In their cohort, ACEI/ARB had no effect on PAC, PRA or
THALDO excretion, whereas in our cohort PAC was lower in
patients prescribed an ACEI, likely reflecting the differing
underlying patient cohorts. Hammer et al. did not investigate
determinants of steroid excretion.

Most studies of the control of aldosterone levels in CKD
used patients with more advanced renal disease and describe
aldosterone responses to administered stimuli. Berl et al. in
1978 [15] showed that eight patients with advanced renal
failure responded to marked sodium restriction or the assump-
tion of an upright posture with a rise in PAC. More recently,
Bomback et al. [16] found that in patients requiring haemodia-
lysis, aldosterone levels were suppressed by increasing extra-
cellular volume, although to a much lesser degree than in
normal controls. Serum potassium did not influence serum
aldosterone levels in haemodialysis patients. No previous
studies report determinants of THDOC excretion in patients
with CKD and no studies have analysed the relationship
between urinary electrolytes and mineralocorticoids in patients
with CKD.

This is also the first study to report associations with pro-
duction of DOC, a largely overlooked mineralocorticoid which
is known to bind and activate the MR with equal avidity to
aldosterone. Levels of this hormone have recently been linked
to left ventricular mass and proteinuria excretion in patients
with CKD [9], suggesting that further study is required for
which the ligand is inhibited in studies of MR receptor antag-
onism.

There are a number of potential mechanistic explanations
underlying our novel finding of the positive association
between urinary sodium excretion and urinary steroid
excretion. As a cross-sectional study, it is not clear where the
primary abnormality lies, and whether sodium intake drives
aldosterone or aldosterone drives sodium excretion. It may be
that aldosterone production is increased to compensate for ex-
cessive natriuresis seen in CKD. Alternatively, it may be that
aldosterone production in CKD is more closely regulated by
other factors, such as catecholamines, endothelin and arginine
vasopressin, than traditional trophins [17]. Or is the adrenal
response to stimuli altered in the presence of lower plasma pH
and high levels of inflammation in patients with CKD [18]?

Limitations

The study findings are limited by the fact that this was a
‘free range’ population and patients were taking multiple
different medications, a number of which are known to impact
upon aldosterone or renin production, but this is the everyday
reality of treating patients with CKD. It is difficult to comment
on the role of intravascular volume in determining steroid
excretion as this is difficult to measure. Similarly, measure-
ment of total body sodium would be of value.
CONCLUSIONS

The novel findings in this study are of importance in understanding how regulation of steroid metabolism varies in the uraemic milieu and may help explain the pathological consequences of renal disease. The usual regulatory mechanisms of serum potassium, angiotensin II and ACTH appear to be uncoupled in patients with CKD and the strong association between urinary sodium excretion and THALDO and THDOC excretion suggests that alternative regulatory mechanisms are involved. This novel association demands further study, expands on the interplay between different mineralocorticoids and sodium and may help understand the therapeutic benefits seen with blockade of the MR in CKD.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


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