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The effect of Spironolactone upon corticosteroid hormone metabolism in patients with early stage chronic kidney disease

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Abstract

Context

Aldosterone has emerged as an important mediator of disease progression and mortality in patients with chronic heart and kidney disease (CKD). Despite the increasing use of mineralocorticoid receptor antagonists (MRAs) in these patients little is known about the effects on corticosteroid hormone secretion and metabolism.

Objective

To assess corticosteroid hormone secretion and metabolism in early stage CKD patients before and after spironolactone (Spiro).

Design

Randomised, double-blind, placebo-controlled interventional study.

Setting

Single tertiary referral center.

Patients

112 patients with stable stage 2/3 CKD.

Interventions

Patients were randomised to receive either Spiro 25mg once daily or placebo for 36 weeks.

Main Outcome measures

Plasma renin activity (PRA), angiotensin II (AngII) and steroid hormones were analysed by standard assays, urinary corticosteroid hormone metabolites (5 α +5 β -tetrahydro cortisol (5 α +5 β -THF), TH-cortisone (THE), 3 α 5 β -TH-aldosterone (TH-Aldo), 5 α +5 β -TH-deoxycorticosterone (5 α +5 β -TH-DOC), TH-11-desoxycortisol (THS)), were analysed by gas chromatography/mass spectrometry (GC/MS).

Results

Plasma aldosterone concentration was inversely correlated with eGFR ($r = -0.331$, $p < 0.001$). Urinary 24h excretion of TH-Aldo was correlated with plasma Aldo concentration (PAC) ($r = 0.214$, $p < 0.05$) and diastolic blood pressure (BP) ($r = 0.212$, $p < 0.05$), whereas total 24h urinary cortisol metabolite excretion was correlated with systolic BP ($r = 0.316$, $p < 0.01$). In addition, 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 1 activity (urinary 5 α +5 β -THF) / THE) ratio) was correlated with PRA ($r = 0.277$, $p < 0.01$). Spiro treatment significantly reduced BP ($123 \pm 11 / 76 \pm 7$ vs $119 \pm 11 / 73 \pm 8$ mmHg, $p < 0.01$) despite RAAS induction, reflected by increased urinary 24h TH-Aldo excretion (17.6 (12,86) vs 26 (18,80) $\mu\text{g}/24\text{h}$, $p < 0.05$). By contrast,

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2 Spiro had no effect on total urinary cortisol metabolite excretion, 11 β -hydroxylase,
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4 11 β -HSD type 1 and 2 activity.

5
6 **Conclusions**

7 Aldo and cortisol are positively associated with BP suggesting that adrenal
8 hyperactivity may in part explain the increased cardiovascular risk in patients with
9 early end-stage CKD. Addition of Spiro had no effect on glucocorticoid metabolism or
10 total 24h corticosteroid production.
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For Peer Review

Introduction

In chronic kidney disease (CKD) the renin-angiotensin-aldosterone system (RAAS) becomes progressively activated with decreasing kidney function¹ and angiotensin II (AngII) and aldosterone (Aldo) have emerged as important drivers of both kidney disease progression and increased cardiovascular mortality²⁻⁴. Consequently, RAAS inhibition by angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) remains the mainstay of treatment. However, RAAS inhibition by ACEi or ARBs is only partial and a recent study suggests that addition of the mineralocorticoid receptor antagonist (MRA) spironolactone (Spiro) in CKD patients already treated with an ACEi and ARB further reduces markers of renal disease progression⁵. Similarly, the use of MRAs in heart failure patients in addition to established treatment regimens including ACEi and ARBs has been shown to dramatically improve survival^{6,7}.

One important mode of action of MRAs in CKD patients is by lowering blood pressure through blockade of renal MR in epithelial cells of the distal nephron. The MR has similar affinities for mineralocorticoids and glucocorticoids *in vitro*⁸ and is only protected from glucocorticoid activation by the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) which inactivates cortisol to cortisone. Reduced activity of 11 β -HSD2 results in glucocorticoid mediated MR activation and has been described in selected patients with salt sensitive essential hypertension^{9,10}. In CKD patients 11 β -HSD2 activity^{11,12} and expression¹³ declines with progressively impaired renal function suggesting that in these patients blood pressure and renal damage may be partly driven by glucocorticoid induced MR activation. Therefore this mechanism provides a rationale for the incremental benefit of MRAs on lowering blood pressure in CKD patients.

Besides MR blockade, animal and *in vitro* studies also suggest that Spiro and its active metabolites can also inhibit steroidogenic enzymes including 11 β -hydroxylase and thereby may impact on corticosteroid hormone synthesis^{14,15}. However, so far this has never been investigated in humans. In addition, studies in healthy subjects suggest that hippocampal MR may play a role in the modulation of hypothalamo-pituitary-adrenal (HPA) drive^{16,17} but so far this has never been demonstrated in patients on long-term low-dose Spiro treatment.

Here we studied corticosteroid hormone secretion and metabolism in CKD patients with mild-moderate renal impairment at baseline and following treatment with low

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dose Spiro. We hypothesised that some of the beneficial effects of Spiro may relate to changes in cortisol secretion and/or metabolism.

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Methods

Study design

The study protocol and subjects have been reported as part of an evaluation of the effect of Spiro on left ventricular mass and aortic stiffness in CKD patients ¹⁸. In brief, this was a single centre, prospective, double-blind, placebo controlled, randomised interventional trial of patients with early stage CKD of diverse aetiologies including glomerulonephritis (IgA nephropathy, nephrotic disease, focal segmental glomerular sclerosis) (53%), quiescent vasculitis (19%), adult polycystic kidney disease (8%), reflux (8%), calculi (4%) and others (8%) (Henoch-Schoenlein Purpura, sickle cell disease, sarcoidosis, nephrectomy). Patients had stage 2 (GFR 60-89 ml/min/1.73m²) (GFR was calculated using the 4 variable MDRD equation) or stage 3 CKD (GFR 30-59 ml/min/1.73m²) and evidence of kidney damage for ≥3 months) ¹⁹. All patients were treated with an ACE inhibitor and / or ARB for at least 6 months to maximally tolerated dose and had controlled blood pressure (mean daytime 24 hour ambulatory blood pressure monitoring <130/85 mmHg). 24 patients with CKD secondary to vasculitis were on a stable immunosuppressive treatment with glucocorticoids which was not altered during the study period. Patients were excluded if they had a history of diabetes, or symptomatic ischaemic or non-ischaemic heart disease, peripheral vascular or cerebrovascular disease, renovascular disease, anaemia (<12g/dL) or previous documented hyperkalaemia (>5.5mmol/L).

A spironolactone dose of 25mg was chosen in this study as previously low dose spironolactone has been shown to be safe and clinical effective in patients with heart failure ⁷. All patients received a 4 week open label run-in phase of 25mg of spironolactone once daily (or alternate days if potassium levels were between 5.5 and 5.9 mmol/l), after which patients were randomised to continue treatment with 25 mg spironolactone or to placebo for a further 36 weeks. Patients were assessed at baseline (before the run in phase) and at the end of the study (week 40) with a clinical history and examination, 24 hour ambulatory blood pressure monitoring, and collection of a 24h urine sample. Venous blood samples were also collected after 30 minutes supine rest for routine hematology and biochemistry and measurement of plasma renin activity, aldosterone and angiotensin II.

During the open-label run-in phase, 1 patient developed serious hyperkalemia (potassium 6.5 mmol/l) and was withdrawn, 6 (5%) patients had potassium levels between 5.5 and 5.9 mmol/l and were switched to spironolactone on alternate days

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2 as per protocol. On blinded treatment, 4 patients had potassium levels between 5.5
3 and 5.9 mmol/l that required a dose reduction to alternate day treatment. After
4 unblinding, two of these 4 patients were found to have received placebo. After
5 randomization, no patients were withdrawn because of hyperkalemia, and there were
6 no reported side effects, including gynecomastia or menstrual disturbances. The
7 protocol was approved by South Birmingham Local Research Ethics Committee and
8 all patients gave written informed consent.
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16 **Blood Pressure**

17 Office brachial blood pressure was recorded with the subject lying supine after 10, 20
18 and 30 minutes in the non-dominant arm using a validated oscillometric
19 sphygmomanometer (Dinamap® Procare, GE). In addition, all subjects underwent 24
20 hour ambulatory BP monitoring (Meditech® ABPM-04) at baseline and at week 40.
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26 **Biochemical assays**

27 Plasma renin activity (PRA) was measured by an in-house antibody trapping
28 technique in the presence of added excess renin substrate²⁰ (coefficient of variation:
29 3.4%). An in-house radioimmunoassay was used for plasma angiotensin II as
30 previously described²¹. Angiotensin II was pre-extracted from plasma before assay
31 (coefficient of variation: 10%). Plasma aldosterone was measured with a solid-phase
32 (coated tube) radioimmunoassay kit supplied by Diagnostic Products (UK) Ltd
33 (coefficient of variation: <8.3%).
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42 **Urinary steroid metabolites**

43 Mineralocorticoids and glucocorticoids are metabolised by various enzymes and are
44 excreted as metabolites in the urine. The main urinary metabolites of mineralo- and
45 glucocorticoids including their precursors were measured by gas
46 chromatography/mass spectrometry (GC/MS) as described previously²². The sum of
47 total cortisol metabolites (tetrahydrocortisol [THF], tetrahydrocortisone [THE], 5α-
48 THF, α-cortolone, cortisone [E], cortisol [F], β-cortolone, β-cortol, and α-cortol)
49 provides a reflection of cortisol secretion rate. The ratio of tetrahydrometabolites of
50 cortisol (THF + 5α-THF) to those of cortisone (THE) provides a reflection of 11β-
51 HSD1 activity when considered with the ratio of urinary free cortisol (UFF) to
52 cortisone (UFE), which more accurately reflects renal 11β-HSD2 activity²². The
53 ratios of cortols to cortolones and of 11β-hydroxy-etiocholanolone and 11β-hydroxy-
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2 androsterone combined to 11oxo-etiocholanolone also reflect 11 β -HSD1 activity ²³.
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4 The activities of 5 α - and 5 β -reductases can be inferred from measuring the ratio of
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6 5 α -THF to THF and androsterone to etiocholanolone. 3 α 5 β -tetrahydro-aldosterone
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8 (TH-Aldo) is the main urinary aldosterone metabolite and reflects 24h aldosterone
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10 production. The activity of 11 β -hydroxylase can be inferred from measuring the ratio
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12 of total cortisol metabolites (see above) to tetrahydro-11-desoxycortisol (THS) ²⁴.
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14 **Statistical Analysis**

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16 Normally distributed data were expressed as means \pm SD (unless stated). Non
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18 parametric data were expressed as median (interquartile range) and were log-
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20 transformed where applicable. Treatment groups were compared using t tests or chi-
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22 square tests (at baseline) and repeated measures analysis of variance (for changes
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24 over time). Correlations of non-parametric data were assessed by the Spearman's
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26 correlation coefficient.
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Results

All 112 patients enrolled in this study had stage 2 or 3 CKD, were all on either ACEi or ARB treatment for more than 6 months and had normal office blood pressure levels (**Table 1**). There were no differences in blood pressure or gender distribution between the two groups. However, body weight and BMI was higher in the Spiro compared to the placebo group and in the Spiro group significantly more patients were treated with beta-blockers and statins compared to the placebo group (**Table 1**). As expected, at baseline patients on beta blockers had a significantly lower plasma renin activity (PRA) (median (interquartile range): 23 mU/l (8, 85) vs 82 mU/l (49, 185), $p < 0.001$) and angiotensin II (AngII) levels (4.9 pmol/l (3.1, 8.3) vs 9.9 pmol/l (5.0, 23.1), $p < 0.05$) compared to patients not taking beta-blockers. However by contrast, the plasma aldosterone concentration (PAC) was slightly but significantly higher in patients on beta-blocker treatment (222 pmol/l (152, 300) vs 166 pmol/l (108, 222), $p < 0.05$) suggesting an alternative mechanism of Aldo release in addition to AngII. Analysis of urinary glucocorticoid and mineralocorticoid steroid hormone metabolites between patients on and off beta-blockers did not reveal significant differences suggesting that beta-blockers do not have a major influence on steroid hormone production or metabolism. Comparison of patients on ACEi vs ARB at baseline did not reveal any differences in PRA (64 mU/l (37, 133) vs 81 mU/l (25, 151), $p = 0.676$), PAC (169 pmol/l (114, 247) vs 172 pmol/l (97, 238), $p = 0.627$) or 24h urinary excretion of $3\alpha 5\beta$ -tetrahydro-aldosterone (TH-Aldo) (18.1 $\mu\text{g}/24\text{h}$ (10.5, 26.8) vs 17.5 $\mu\text{g}/24\text{h}$ (13.4, 25.4), $p = 0.984$). However as expected, circulating AngII levels were significantly lower in the ACEi compared to the ARB group (5.3 pmol/l (3.8, 10.5) vs 31.6 pmol/l (12.6, 72.7), $p < 0.001$). No significant differences were found between males and females with regard to PRA, AngII, PAC or urinary TH-Aldo. Total body weight and BMI were correlated with 24h total glucocorticoid excretion ($r = 0.407$, $p < 0.001$; $r = 0.296$, $p = 0.003$) but not with 5α -reductase activity as assessed by urinary metabolite ratios (Etiocolanalone/Androsterone; 5α -tetrahydro cortisol (5α -THF)/THF). PAC levels were significantly correlated with 24h urinary TH-Aldo excretion ($r = -0.214$; $p = 0.036$) and furthermore showed a significant negative association with the estimated glomerular filtration rate (eGFR) ($r = -0.331$, $p < 0.001$). However, 24h urinary TH-Aldo excretion was not associated with eGFR. Mean diastolic 24h ambulatory blood pressure (24h ABP) showed a significant correlation with 24h urinary TH-Aldo excretion (**Figure 1a**) but not with PAC. Moreover, after adjusting for age, BMI and eGFR, systolic 24h ABP was significantly

1
2 associated with 24h urinary total cortisol (F) metabolite excretion at baseline (**Figure**
3 **1b**). No correlation was found for systolic ($r=0.156$, $p=0.1$) or diastolic blood pressure
4 ($r=0.105$, $p=0.271$), TH-Aldo ($r=-0.112$, $p=0.284$), eGFR ($r=-0.097$, $p=0.313$) and the
5 aldosterone-renin-ratio. Moreover, neither urinary (tetrahydro-cortisol (THF) + 5 α -
6 THF / tetrahydrocortisone (THE) ratio) reflecting global 11 β -hydroxysteroid
7 dehydrogenase type 1 (11 β -HSD1) activity, nor urinary free cortisol / cortisone
8 (UFF/UFE) reflecting 11 β -HSD type 2 activity were associated with blood pressure or
9 eGFR (**Table 2**). However, PRA was positively associated with the urinary
10 THF+5 α THF/THE ratio (**Figure 2**).
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18 24 patients were on stable glucocorticoid treatment for their underlying kidney
19 disease throughout the study period. However, inclusion or exclusion of
20 glucocorticoid treated patients did not have a significant effect on the analysis of
21 steroid hormone metabolites (total GC excretion, TH-Aldo, enzymatic activities)
22 between the placebo and Spiro group. Treatment with Spiro compared to placebo
23 significantly reduced both systolic and diastolic 24h ABP despite a significant
24 increase in circulating PRA, AngII and aldosterone levels (**Table 2**). Induction of the
25 renin angiotensin aldosterone system (RAAS) was reflected by a significant increase
26 in 24h urinary TH-Aldo excretion, consistent with an increased aldosterone synthase
27 (AS) activity (**Table 2**). By contrast, Spiro had no effect on global 11 β -hydroxylase,
28 11 β -HSD type 1 and 2 activity or total cortisol metabolite excretion as assessed by
29 24h urinary steroid hormone excretion.
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Discussion

In this study we assessed corticosteroid hormone secretion and metabolism in CKD patients on stable treatment with ACEi or ARB before and after addition of Spiro by means of urinary steroid hormone metabolite analysis which allows assessment of cumulative 24h steroid hormone production and, secondly, an estimation of steroid hormone enzyme activities.

As expected, 24h urinary TH-Aldo excretion correlated with PAC although this association was weak. A possible explanation for this weak association could be that the 24h urine collection for TH-Aldo was performed on a different day than blood sampling for circulating aldosterone was done. Additionally, Aldo levels are stimulated by ACTH during stress such as a venous puncture which is likely to vary between individuals. Circulating Aldo concentrations have previously been shown to correlate with blood pressure in black hypertensive²⁵ and PCOS²⁶ patients. However, we did not find a correlation of PAC with systolic or diastolic 24h ABP. In this study lack of association might be explained by the interfering effects of ACEi and ARB on the RAAS and variable stress induced PAC fluctuations during blood sampling. Nevertheless, cumulative 24h urinary TH-Aldo excretion reflecting 24h Aldo production was indeed positively correlated with 24h blood pressure as well as PAC. It was recently reported that Aldo levels in CKD patients treated with an ACEi are lower compared to ARB treated patients²⁷. However, in our study we did not find any significant differences in PAC or urinary TH-Aldo excretion, suggesting that ACEi and ARBs have a similar effect on Aldo secretion.

Total urinary 24h cortisol metabolite excretion is a well established measure to assess daily cortisol production rate and thus reflects activity of the hypothalamo-pituitary-adrenal (HPA) axis. In agreement with previous studies, we found total glucocorticoid metabolite excretion to be correlated with total body weight and BMI²⁸. Furthermore, 24h glucocorticoid secretion rate was positively correlated with 24h systolic blood pressure after correction of potential confounders such as age, BMI, and eGFR, suggesting that increased total glucocorticoid production as a result of increased HPA activity is implicated in blood pressure regulation in CKD patients. Although the vast majority of patients with Cushing's syndrome exhibit hypertension²⁹, increased 24h cortisol production within the physiologic range has to our knowledge not been associated with blood pressure so far. Increased HPA-drive may therefore be a novel risk factor in CKD patients by driving blood pressure levels. These findings are in line with a recent report in which high yet within the normal

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2 range serum cortisol levels were found to be associated with an adverse outcome in
3 heart failure patients ³⁰. Further clinical studies are urgently needed to better define
4 and understand the mechanisms of high-normal as opposed to low-normal cortisol
5 levels on blood pressure and ultimately cardiovascular risk in these patients.
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9 Previous studies on glucocorticoid metabolism in CKD patients suggest that 11 β -
10 HSD2 activity ^{11, 12} and expression ¹³ declines with reduced GFR which in turn may
11 lead to increased MR activation by glucocorticoids leading to increased sodium
12 retention and blood pressure. In our study we did not find a correlation between renal
13 function and the UFF / UFE ratio reflecting renal 11 β -HSD2 activity. This is most
14 likely explained by the relatively mild impairment in renal function in our cohort,
15 whereas cohorts in previous studies included patients with more severe renal
16 impairment as well as patients on haemodialysis.
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19 Following Spiro treatment all components of the RAAS were significantly up-
20 regulated including PRA, AngII and PAC levels. Consistent with a RAAS activating
21 effect, total 24h urinary TH-Aldo excretion was also significantly increased in the
22 Spiro but not the placebo group. Our findings are in good agreement with a recent
23 report on the neurohormonal effects of Spiro in patients with congestive heart failure
24 and on stable ACEi treatment, which showed increased AngII and Aldo levels
25 following Spiro treatment ³¹. These findings suggest that MR blockade by Spiro
26 results in a compensatory stimulation of the RAAS despite tonic inhibition by ACEi
27 and ARBs. However, the degree of compensation inflicted by Spiro is not complete
28 as both diastolic and systolic 24h ABP were reduced compared to baseline.
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31 Previous reports suggested that Spiro and its metabolites exert direct inhibitory
32 effects on steroidogenic enzymes ^{14, 15}. Here, using urinary steroid hormone
33 metabolites as a surrogate Spiro did not exert an obvious direct inhibitory effect on
34 11 β -hydroxylase. The most likely explanation for this finding may be that the Spiro
35 dose used in this study was too low to exert a measurable effect on 11 β -hydroxylase
36 compared to previous studies.
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39 However, by contrast, we found an increased aldosterone synthase (AS) activity
40 following Spiro treatment. AS is the final enzyme involved in Aldo production by
41 converting 11-deoxycorticosterone (DOC) into aldosterone in the *zona glomerulosa*
42 and is regulated by circulating AngII which was also significantly increased following
43 Spiro. This finding is consistent with *in vitro* studies which show increased expression
44 of AS following AngII treatment ³².
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2 Compared to baseline, Spiro treatment did not alter total urinary 24h cortisol
3 metabolite excretion in this study. The main conclusion that can be drawn from this
4 observation is that MR blockade does not seem to interfere with the negative
5 feedback mechanism of cortisol on the HPA axis. Although the hypothalamus mainly
6 expresses the glucocorticoid receptor (GR) but not the mineralocorticoid receptor
7 (MR)³³, the hippocampus readily expresses both receptors and is implicated in HPA
8 axis modulation^{34, 35}. It is therefore conceivable that the negative feedback of cortisol
9 is not solely mediated through the GR, particularly as the MR *in vitro* shows a 10 fold
10 higher affinity for cortisol than the GR³⁶. A number of studies have addressed the
11 role of the MR in mediating negative cortisol feedback. Most studies have shown a
12 short term stimulatory effect on the HPA axis following canrenoate infusion³⁷⁻³⁹ or
13 spironolactone treatment^{16, 17}. However, a recent study assessing the effects of the
14 glucocorticoid receptor (GR) antagonist RU28486 and Spiro alone or in combination
15 on the HPA axis⁴⁰ showed that RU28486 and Spiro by themselves had no effect but
16 in combination showed a significant compensatory activation of the HPA axis. It is
17 difficult to compare the findings of these studies with our own results for a number of
18 reasons. First of all we assessed HPA axis activity indirectly by means of urinary
19 steroid metabolite excretion. Moreover, treatment duration and doses of MRAs were
20 considerably different. Our study does not support a major effect of low dose Spiro
21 on HPA axis modulation but equally does not rule out more subtle changes.

22 Indirect analysis of global 11 β -HSD type 1 and 2 activity by means of urinary steroid
23 hormone metabolite ratios did not reveal any significant differences following Spiro
24 treatment. It has been suggested that the ratio of urinary free cortisol to free
25 cortisone (UFF/UFE) more precisely reflects renal 11 β -HSD2 activity than the
26 tetrahydro-cortisol (THF) + 5 α -THF / tetrahydrocortisone (THE) ratio which is a better
27 surrogate marker for global 11 β -HSD1 activity^{22, 41}. In the kidney the enzyme 11 β -
28 HSD2 protects the MR from illicit cortisol binding and thereby ensures Aldo
29 specificity. Loss of function mutations are the underlying cause of severe
30 hypertension and hypokalaemia in AME patients, whereas polymorphisms in
31 HSD11B2 encoding for 11 β -HSD2 have been associated with salt-sensitive essential
32 hypertension²⁹. Spiro did not result in downregulation of 11 β -HSD type 2 activity,
33 which would allow cortisol to activate the MR and thereby lead to sodium and fluid
34 retention, suggesting that only RAAS activation but not modulation of 11 β -HSD2 in
35 the kidney is involved in compensating MR antagonism.

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2 Interestingly, global 11β -HSD1 activity was correlated with PRA in this study. We are
3 not aware of a direct interaction of 11β -HSD1 activity and PRA but one could
4 speculate that 11β -HSD1 activity and PRA are regulated by a common factor.
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7 In summary, total 24h production of aldosterone and cortisol are positively associated
8 with BP in CKD patients suggesting that adrenal hyperactivity may contribute to the
9 hypertension and increased cardiovascular risk. Addition of Spiro resulted in
10 compensatory RAAS activation but did not affect glucocorticoid production or
11 metabolism.
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Table 1. Patient characteristics at baseline

	Placebo (n=56)	Spirolactone (n=56)
Male (%)	33 (59)	32 (57)
Age (years)	53 ± 12	54 ± 12
Body weight (kg)	74 ± 14	81 ± 14†
BMI (kg/m ²)	25.9 ± 3.8	28.0 ± 4.4†
Office blood pressure (mmHg)		
Systolic	130 ± 19	130 ± 16
Diastolic	77 ± 10	77 ± 10
Creatinine (µmol/l)	123 ± 35	132 ± 29
eGFR (ml/min/1.73m ²)	53 ± 12	49 ± 12
	Medication	
ACE Inhibitors (%)	39 (70)	38 (68)
Angiotensin receptor blockers (ARBs) (%)	19 (34)	19 (34)
Beta blockers (%)	8 (14)	15 (27)
Calcium channel blockers (%)	17 (30)	13 (23)
Diuretics (%)	13 (23)	18 (32)
Statins (%)	17 (30)	27 (48)

Values are Mean ± SD

† p<0.01 spironolactone vs. placebo

Table 2. Changes following placebo / spironolactone treatment

	Placebo		Spironolactone	
	Week 0	Week 40	Week 0	Week 40
24 ABP (mmHg)				
Systolic	125 ± 12	124 ± 11	124 ± 11	119 ± 11†
Diastolic	77 ± 9	76 ± 7	76 ± 8	73 ± 8 †
Na+ (mmol/l)	139 (138,141)	140 (139,142)	140 (139,142)	140 (138,141)
K+ (mmol/l)	4.3 (4.1,4.6)	4.3 (4.2,4.5)	4.4 (4.1,4.7)	4.5 (4.3,5.0)
Creatinine (mmol/l)	123 ± 35	126 ± 35	132 ± 29	140 ± 32††
eGFR (mmol/min/1.73m ²)	53 ± 12.3	52 ± 12.1	49 ± 12	46 ± 15.9
ACR (mg/mmol)	8.2 ± 48.4	9.5 ± 34.9	17.8 ± 48.6	5.4 ± 34.9†
Renin Angiotensin Aldosterone System				
PRA (mU/l)	83 (34,138)	68 (42,148)	75 (36,146)	130 (72,260)††
Angiotensin II (pmol/l)	9.0 (4.6,17.0)	7.9 (4.5,20.1)	7.5 (4.5,18.2)	13.6 (6.2, 22.4)††
PAC (pmol/l)	186 (114,252)	166 (114,219)	133 (83,230)	360 (233, 557)††
24h urinary steroid hormone metabolite excretion				
Total F metabolites (µg/24h)	5218 (2510,7370)	4938 (2992,7327)	6108 (3928,8663)	6024 (4040,8218)
5β-TH-DOC (µg/24h)	10.1 (7.0, 18.0)	12.0 (7.1,19.9)	14.4 (8.2,20.8)	10.4 (6.8,16.6)††
5α-TH-DOC (µg/24h)	3.1 (1.7,4.4)	3.1 (1.5,5.3)	2.8 (2.1,5.7)	2.7 (1.6,5.5)
TH-Aldo (µg/24h)	16.8 (13.3,26.5)	18.3 (12.8, 23.3)	18.9 (10.6, 26.3)	27.3 (17.4, 42.3)††
Steroid hormone metabolite ratios				
5α-reductase (THF/5α-THF)	1.12 (0.82,2.13)	1.16 (0.83, 2.06)	1.26 (0.91,1.74)	1.10 (0.80,1.76)††
11β-HSD1 (5αTHF+THF/THE)	1.1 (0.8,1.3)	1.1 (0.83,1.37)	1.0 (0.7, 1.4)	1.1 (0.8, 1.5)
11β-HSD2 (F/E)	0.69 (0.59,0.87)	0.68 (0.55,0.94)	0.62 (0.51,0.84)	0.69 (0.54,0.83)
11β-hydroxylase (total F metabolites/THS)	98 (66, 137)	86 (50, 121)	105 (80, 151)	110 (79, 145)

Values are median (interquartile range). eGFR: estimated glomerular filtration rate; PRA: Plasma renin activity; PAC: Plasma aldosterone concentration; ACR: albumine creatinine ratio; TH-Aldo: tetrahydro-aldosterone; Andro: Androsterone; Etiochol:

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2 Etiocholanolone; THF: tetrahydrocortisol; THE: tetrahydrocortisone; F: cortisol; E:
3 cortisone; THS: tetrahydro-11-desoxycortisol.

4 Normally distributed values are presented as mean \pm SD; the remainder as mean
5 (inter quartile range). To compare changes in the two groups repeated measures
6 analysis of variance with the time point (week 0, week 40) as the 'within subjects'
7 factor and the group (spironolactone and placebo) as the 'between subjects' factor
8 were used. † $p < 0.01$, †† $p < 0.01$ spironolactone vs. placebo at week 40
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For Peer Review

Figure Legends

Figure 1 **A** Correlation of 24h urinary $3\alpha5\beta$ -tetrahydro-aldosterone (TH-Aldo) excretion with diastolic 24h ambulatory blood pressure (24h ABP); **B** Correlation of 24h urinary total cortisol (F) metabolite excretion with systolic blood pressure.

Figure 2 Plasma renin activity (PRA) is positively correlated with the (THF+5 α -THF)/THE) ratio reflecting global 11β -hydroxysteroid dehydrogenase type I activity.

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Figure 1

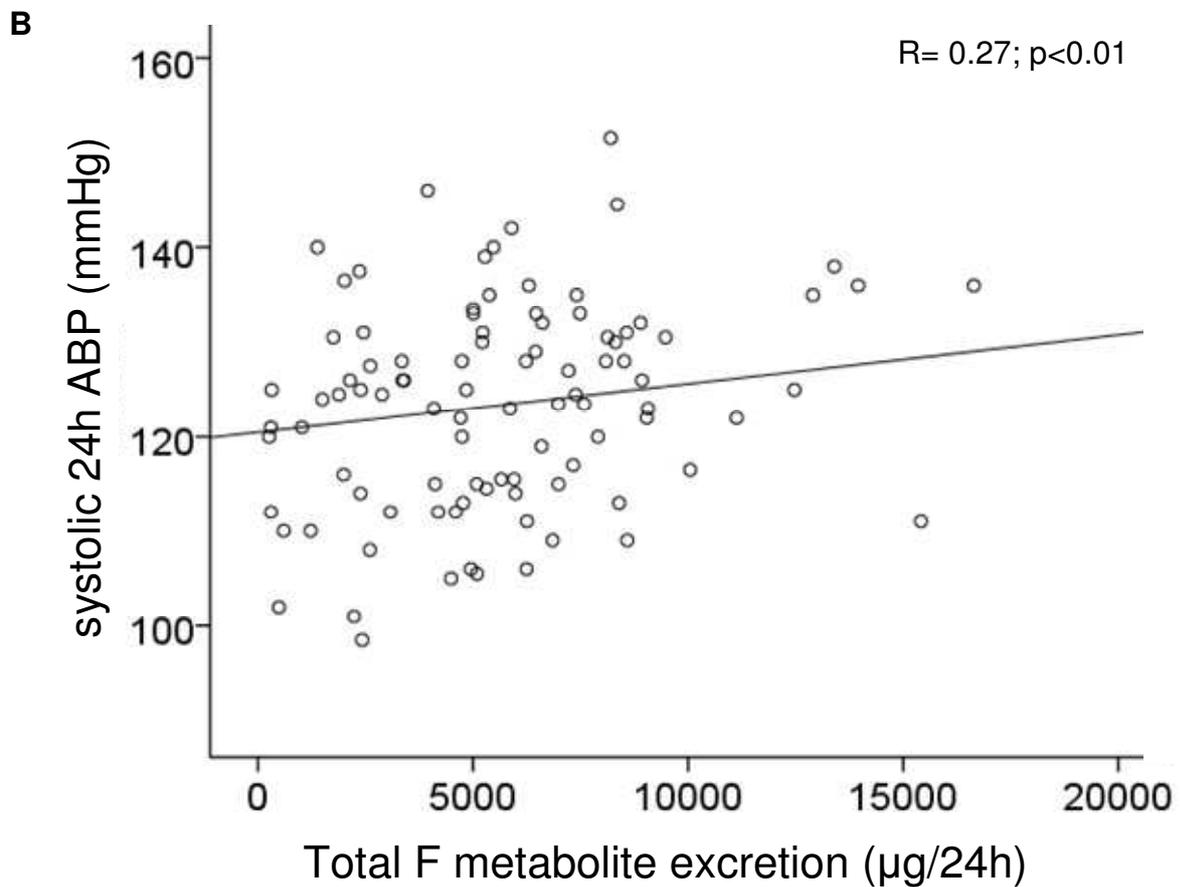
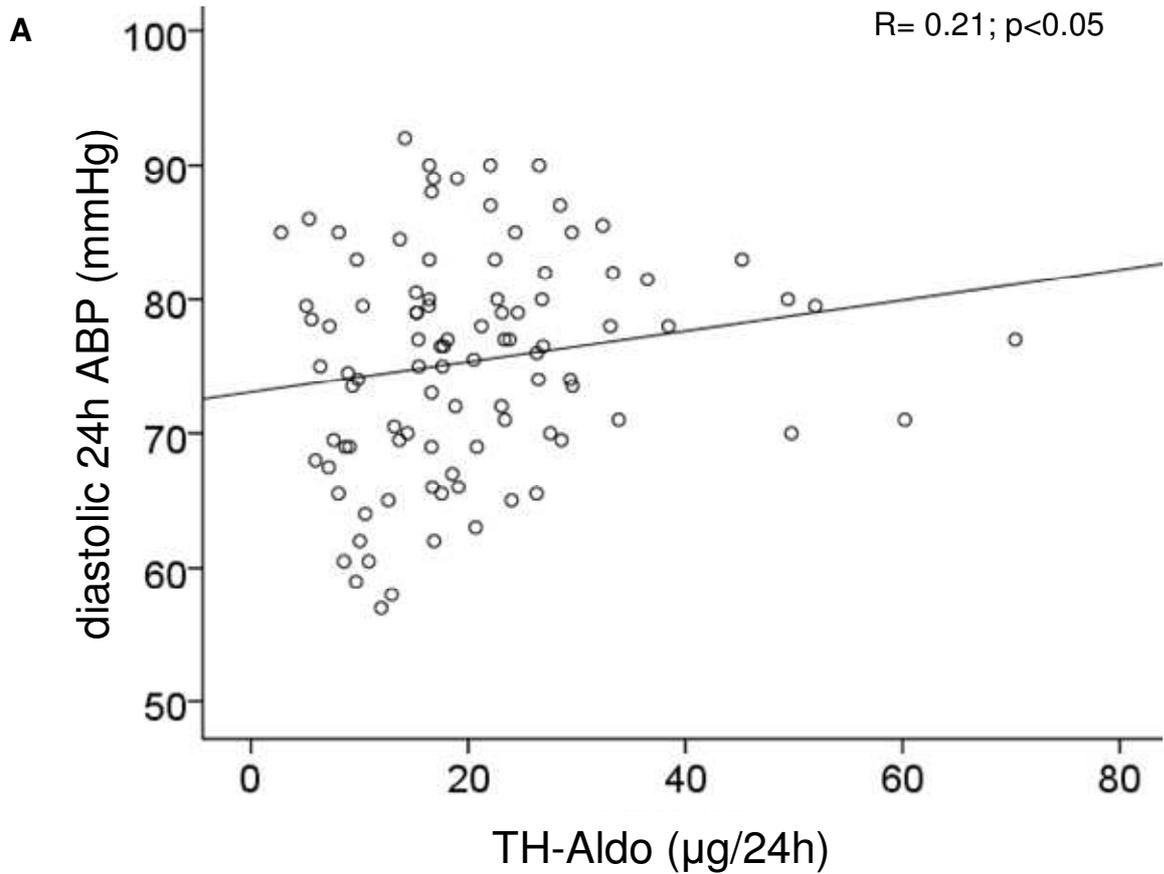
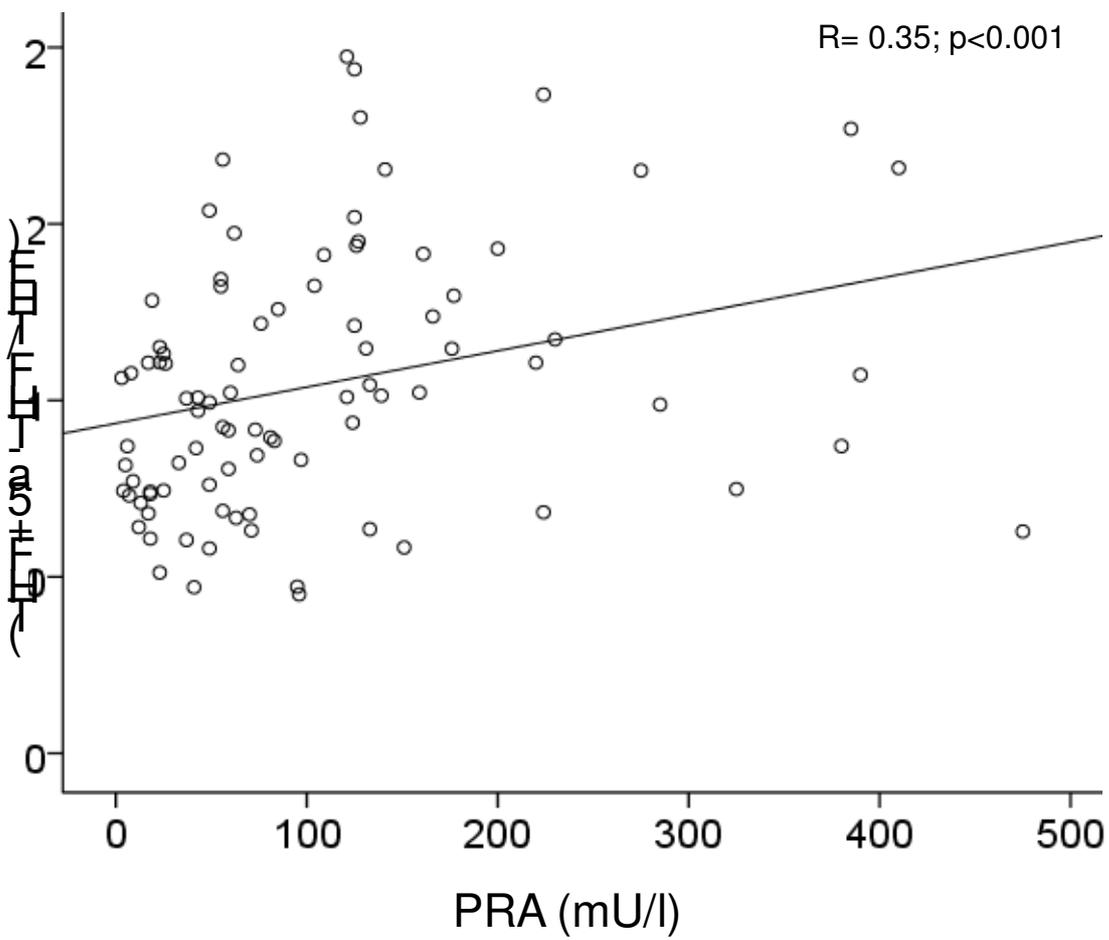


Figure 2



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