Title:

Non-uniform relationship between salt status and aldosterone activity in patients with chronic kidney disease

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ABSTRACT:

Background: Hypertension is prevalent in chronic kidney disease (CKD). Studies suggest that reduction in dietary salt intake reduces blood pressure (BP). We studied relationships between salt intake, BP and renin angiotensin system regulation in order to establish if it is disordered in CKD.

Methods: Mechanistic crossover study of CKD patients versus non-CKD controls. Participants underwent modified saline suppression test prior to randomisation to either low or high salt diet for 5 days and then crossed over to the alternate diet. Angiotensin-II stimulation testing was performed in both salt states. BP, urea and electrolytes, and plasma aldosterone concentration (PAC) were measured.

Results: Twenty-seven subjects were recruited (12 CKD, 15 control). There was no difference in age and baseline BP between the groups. Following administration of intravenous saline, systolic BP increased in CKD but not controls (131 ± 16 mmHg to 139 ± 14 mmHg, p=0.016, vs. 125 ± 20 mmHg to 128 ± 22 mmHg, p=0.38). Median PAC reduced from 184 (124,340) pmol/L to 95 (80,167) pmol in controls (p=0.003), but failed to suppress in CKD (230 (137,334) pmol to 222 (147,326) pmol (p=0.17)). Following dietary salt modification there was no change in BP in either group. Median PAC was lower following high salt compared to low salt diet in CKD and controls. There was a comparable increase in systolic BP in response to angiotensin-II in both groups.

Discussion: We demonstrate dysregulation of aldosterone in CKD in response to salt loading with intravenous saline, but not to dietary salt modification.

Perspectives:

1) This study was undertaken to explore aldosterone regulation and blood pressure response in patients with chronic kidney disease (CKD) under the influence of acute and chronic salt loading, in order to better understand the pathophysiology of hypertension in CKD.
2) Our results show dysregulation of aldosterone in CKD in response to salt loading with intravenous saline, but not to dietary salt modification.
3) Further research is required to explore the possible underlying mechanisms for the differential response to acute versus chronic salt loading in patients with CKD.

INTRODUCTION

Patients with chronic kidney disease (CKD) are at an increased risk of end-stage renal disease (ESRD), cardiovascular disease (CVD) and death.(1,2) Hypertension, which is widely prevalent within CKD populations, is a modifiable risk factor for both CVD and progression of CKD.(3–6) The mechanism of hypertension in CKD is complex and incompletely understood. Over-activation of the renin-angiotensin-aldosterone system (RAAS), in combination with salt and water retention, has been implicated.(7,8) Blockade of RAAS using medications which inhibit angiotensin activity has been shown to reduce the rate of CKD progression, reduce cardiovascular events and reduce proteinuria in patients with CKD, however many of these patients still progress to ESRD and die from CVD.(5,6)

Aldosterone is accepted as having a detrimental effect in the pathogenesis of CVD, contributing to myocardial fibrosis and adverse cardiac modelling.(9) The phenomenon of “aldosterone breakthrough”, whereby excessive aldosterone activity occurs despite angiotensin inhibition, exists and predicts poor outcome.(7,10,11) The addition of drugs that block aldosterone activity at the mineralocorticoid receptor has established survival benefit in patients with congestive heart failure.(12,13) There is emerging evidence for the addition of these drugs for use in patients with CKD, with small trials showing reductions in proteinuria,(14) regression in left ventricular mass (LVM)(15) and potentially slowing the progression of CKD.(14,16,17)

Dietary salt intake is an alternative potentially modifiable risk factor for hypertension and RAAS activation in CKD. The association between dietary salt and hypertension in the general population is well established, with studies showing up to a 25% reduction in CVD risk associated with a low salt diet.(18) In CKD populations, international guidelines(19) advise salt restriction, with some evidence to both support,(20–23) and refute,(24–26) this recommendation. Given these conflicting data, the association between dietary salt intake, RAAS activation and hypertension in CKD merits further study. Animal studies show that aldosterone induced organ damage in CKD is exacerbated in a high sodium environment, with acceleration of renal and cardiac fibrosis.(27,28) In humans, urinary sodium excretion, which is an established method of measuring dietary salt intake,(29,30) has been shown to be an inverse predictor of long-term survival in CKD and hypertensive patients,(31–33) and is also the main determinant of urinary corticosteroid excretion,(34) itself a predictor of LVM and proteinuria in CKD.(35)

The aim of this study is to explore aldosterone regulation and BP response in patients with CKD under the influence of acute and chronic salt loading, in order to better understand the pathophysiology of hypertension in CKD. We hypothesised that patients with CKD will fail to suppress aldosterone in response to acute and chronic salt loading. Furthermore, we hypothesised that salt-loaded CKD patients would be more susceptible to stimulation with angiotensin-II than non-CKD controls.

METHODS AND MATERIALS

Study design

This was a mechanistic crossover study with subjects acting as their own control. Two groups of CKD and essential hypertension/control subjects were recruited. Details of each study visit are outlined in figure 1. All study visits were carried out in the Glasgow Cardiovascular Research Facility. To each study visit, subjects attended after a midnight fast with a completed 24-hour urine collection. To minimise the sequence effect subjects were divided in to two groups after visit one. Study visits two and three were identical to each other but were carried out after each 5-day dietary intervention. There was a two-week wash out period between dietary interventions. The study was approved by the West of Scotland ethics committee.

Study population

CKD subjects were recruited from the Western Infirmary (Glasgow) renal unit and its satellite general nephrology clinics. Hypertension subjects were recruited from the Western Infirmary Glasgow hypertension clinic. Control subjects were recruited by means of poster advertisement in the University of Glasgow. Exclusion criteria are listed in supplementary material table 1.

Modified saline suppression test (visit 1)

After 30 minutes of recumbent rest 1000 ml of 0.9% NaCl (3600 mg sodium) was infused over 2 hours with measurement of plasma aldosterone concentration (PAC) and plasma renin concentration (PRC) prior to and on completion of the infusion. Blood pressure (BP) and heart rate (HR) were recorded every 15 minutes throughout the infusion.

Dietary salt manipulation

Each diet was followed for 5 days prior to study visits 2 and 3. Subjects followed a diet sheet to achieve a low sodium intake of <2000 mg/day and were provided with

Slow Sodium® tablets (2 tablets twice daily, additional 920 mg sodium per day) to achieve a high sodium intake of >4600 mg/day.

Angiotensin II stimulation test (visits 2 & 3)

Angiotensin II acetate salt was obtained from BAChem Distribution Services, Weil am Rhein, Germany. After 30 minutes of recumbent rest, blood was obtained for measurement of PAC and PRC prior to and on completion of the infusion. BP and HR were measured every ten minutes during the infusion (60 minutes) and for a further 30 minutes following completion of the infusion. To minimise potential complications a graded dose infusion was used; 1.5 ng/kg/min for 30 minutes then 3 ng/kg/min for a further 30 minutes.

Sample analysis

Measurement of PAC, PRC and urinary excretion of protein and electrolytes was carried out the Biochemistry laboratory, Western Infirmary, Glasgow. For PAC, 5 ml of blood was withdrawn in to an additive-free tube and spun at 3000 RPM for 10 minutes at 4 ^{0}C. The plasma was stored at -80 ^{0}C. PAC was measured in batches utilising a radioimmunoassay (Siemens TKAL2). For PRC, 3.5 ml was withdrawn in to a potassium EDTA tube and then spun at 3000 RPM for 10 minutes at 4 ^{0}C. The plasma was stored at -80 ^{0}C. PRC was measured in batches using the DiaSorin Liason® analyzer.

Outcomes

The primary outcome measure was change in PAC in response to acute salt loading. Secondary outcomes included blood pressure and PAC response to dietary salt manipulation and angiotensin II infusion.

Statistics and power calculation

This was a study of a continuous response variable from matched pairs of study subjects. Previous data has shown PAC to reduce from 0.12 nmol/l to 0.07 nmol/l in response to a high salt diet in healthy volunteers.(36) In order to reject the null hypothesis (that CKD patients have a similar PAC response to acute salt loading) with 80% power and an alpha level of 0.05 a total of 24 subjects would be required, assuming a standard deviation of 0.05 nmol/l. Mean and standard deviation or median and interquartile range are reported for normally distributed and skewed results, respectively. Paired and independent t-tests were used, as appropriate, to compare normally distributed variables, with Wilcoxon signed rank tests being used

for comparative non-parametric variables. Repeated measures analysis of variance (ANOVA) was used to compare BP response to angiotensin-II between CKD and controls. All analysis were performed using SPSS 22.0 (IBM, NY)

RESULTS

Participants

Twenty-seven subjects were recruited (12 CKD and 15 control). There was no significant difference in mean age, body-mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), PAC or 24-hour urinary sodium (24-h USod) excretion at baseline between the groups (table 1). The CKD group had significantly lower estimated glomerular filtration rate (eGFR), higher serum potassium and higher PRC compared to the control group (table 1). Within the CKD group, primary renal diagnosis consisted of IgA nephropathy (7), granulomatosis with polyangiitis (2), reflux nephropathy (1), chronic pyelonephritis (1) and malignant hypertension (1). Nine patients in the CKD group were on medications that inhibit RAAS activity and 2 patients were on a diuretic (1 loop, 1 thiazide) (supplementary material: table 2). The corresponding figures in the control group were 3 and 1 (thiazide), respectively.

Modified saline suppression test (acute salt loading)

Following administration of intravenous saline, there was a significant increase in mean SBP from baseline in the CKD group but not in the control group (table 2). Median PAC reduced in the control group, but failed to be suppressed in the CKD group (table 2). PRC reduced in both groups in response to saline stimulation (table 2).

Modification of dietary salt intake (chronic salt loading)

A significant difference in 24-h USod excretion was demonstrated following each dietary intervention in both groups (table 3). There was no significant difference in BP across the dietary interventions in either group (table 4). Median PAC was significantly lower following high dietary salt intake compared to low dietary salt intake in both patient groups (table 4). PRC was higher in the CKD group compared to control, but reduced in both groups in response to high dietary salt intake (table 4).

Angiotensin-2 stimulation test

SBP increased in response to angiotensin-2 in both groups on both diets (supplementary material: figure 1). There was no significant difference in SBP or DBP between controls and CKD in response to angiotensin-2 on either diet (2-way

repeated measures ANOVA: SBP low-salt diet p=0.184, SBP high salt diet p=0.242, DBP low salt p=0.239, DBP high salt diet p=0.498). In both groups on both diets, stimulation with angiotensin-2 increased PAC, while reducing median PRC (figure 2). Compared to a low salt diet, a high salt diet suppressed median PAC before and after angiotensin-2 administration in both groups (figure 2). There was no significant difference in PAC between control patients and those with CKD on either diet (p=0.09, p=0.15, p=0.48, and p=0.24 for CKD versus controls on low salt diet pre and post angiotensin II and high salt diet pre and post angiotensin II respectively). PRC was significantly higher in CKD patients than controls before and after angiotensin-2 administration, however the relative change in PRC in response to angiotensin-2 were similar in both groups.

DISCUSSION

The results of this study depict a complex, non-uniform response of RAAS activation following salt loading in patients with CKD (table 5). Firstly, we confirm that there is dysregulation of RAAS activation and BP response in patients with CKD following an acute salt and water load, with a rise in BP and failure to suppress PAC seen in the CKD group, but not controls, following a modified saline suppression test. Secondly, contrary to our hypothesis, a normal physiological response in aldosterone secretion, is maintained in CKD patients in response to dietary modification of salt intake. Finally, we show a comparable response in BP and PAC in response to angiotensin-II stimulation in controls and CKD patients, even in a salt-loaded state.

Previous randomised controlled trials have shown a reduction in BP following dietary salt restriction in patients with CKD.(20,21) However, controversy regarding the role of sodium restriction still exists due to a lack of survival benefit detected on observational data, with some signal that salt restriction may even increase mortality.(24–26,32,37,38) Given the overwhelming evidence for a detrimental role of aldosterone in CVD,(12,13,39) specifically in CKD patients,(15,40) the finding that PAC is suppressed in response to a high salt diet in both controls and CKD patients is difficult to reconcile with advice regarding salt restriction. This leaves the question as to how reduced dietary salt intake might reduce BP if it is not via aldosterone suppression. The possibility of direct salt and water retention resulting from reduced renal tubular excretion of sodium in CKD exists, but in line with previous studies, we did not find evidence of this when measured on 24-h USod.(30) Recent data suggesting that the kidney is not the only organ involved in sodium homeostasis (with large amounts of sodium sequestered in skin) and that sodium excretion is not strictly

diurnal would account for discrepancy between immediate sodium status, blood pressure and relative change in 24-h USod.(41) Furthermore, our results still support the hypothesis that aldosterone homeostasis is dysregulated in CKD, albeit not in response to modification of dietary salt intake.

The observed differential response in CKD patients following acute versus chronic salt loading appears to be mediated by renin-independent aldosterone secretion. In the setting of chronic dietary salt loading, there is a reduction in PRC that results in reduced PAC in both CKD and controls. However, in the acute setting this process is uncoupled in CKD patients. Potential mechanisms underpinning this difference include: i) renin-independent PAC secretion regulated by volume, not salt ii) dopaminergic renin-independent aldosterone regulation, with enhanced tubular dopamine excretion inhibiting PAC in CKD patients in the chronic setting, but not in the acute setting(42,43) iii) delayed aldosterone inhibition in CKD, with compensatory regulation of pro-renin receptors in chronic salt loading for which there is insufficient time to occur in the acute setting (44). There was no difference in serum potassium between the acute and chronic settings to explain the difference. Further research is required to explore the mechanisms behind the differential aldosterone response following acute and chronic salt loading in CKD patients, with monitoring of aldosterone activity following prolonged dietary intervention, measurement of urinary L-DOPA excretion and the addition of body-composition monitoring to inform regarding changes to extracellular fluid volume.

Previous studies have suggested CKD is a particularly salt-sensitive state and salt restriction may augment the benefits of RAAS blockade.(23,45–48) Our results suggest that any adjuvant effect of salt restriction in combination with RAAS inhibition occurs in spite of higher, not lower, aldosterone levels, implying that by paradoxically stimulating RAAS activation with a low salt diet, it is possible to yield enhanced therapeutic effects from RAAS inhibiting medications. Furthermore, the lack of difference in response to angiotensin-II stimulation between controls and CKD patients even when on a high salt diet, contradicts the theory that a high salt state primes CKD patients to be particularly susceptible to angiotensin-II. Similar findings have been shown in normal subjects in whom adrenocorticotrophic hormone therapy resulted in hypertension even in a salt-depleted state.(49,50) PRC was significantly higher in CKD patients than controls at all stages, however this difference is likely to be explained by the differing prevalence RAAS inhibiting medications between the

groups.(51) PRC was higher in control patients receiving RAAS inhibiting medications compared to those who were not (data not shown).

Strengths of this study include its crossover design and variety of relevant experimental conditions (acute salt loading, chronic salt loading, response to angiotensin-II stimulation). The results of this study are limited by small sample size: although adequately powered by our calculations, we cannot exclude the possibility of a smaller treatment effect being present but not detected. We acknowledge the confounding influence of including patients on RAAS inhibiting medications. As these drugs are fundamental in CKD standard of care, our results maintain generalisability, albeit potentially at the expense of data purity. Study of the effect of salt manipulation in CKD independent of use of RAAS inhibition would be required to address this confounding issue, but cessation of RAAS inhibitors in patients with proteinuric renal disease could be considered unethical. Alternatively, recruiting patients with ubiquitous use of RAAS inhibition in both CKD and non-CKD groups may be informative, but more challenging to recruit to. Despite the discrepancy in the prevalence of RAAS inhibiting medications between the groups there was no difference in PAC at baseline, suggesting aldosterone breakthrough had occurred in treated subjects. Furthermore, the fact that PAC varied in response to dietary manipulation in both groups suggests that relative changes within subjects were still possible despite concurrent RAAS inhibiting medications. Importantly we excluded patients on mineralocorticoid receptor antagonists and beta-blockers, and only a total of 3 patients were receiving a diuretic during the study. Inclusion of patients with hypertension as control subjects allows valuable comparison between patients groups, but may mask any difference between CKD and healthy volunteers. The aetiology of CKD within our sample was heterogeneous and different sub-groups of CKD patients may respond differently. Our dietary interventions lasted 5 days each, and it is possible that longer intervention may yield different results. However, we are reassured by confirmation that 24-h USod excretion was altered in response to dietary modification, albeit within the limitations of this method.(52) While increasing 24-h USod has been shown to associate with mortality,(32,33) recent data suggests 24-h USod is insensitive at detecting significant variations in dietary sodium intake.(53) Sodium regulation may not follow strict diurnal regulation and varies over weeks with less dependence on daily sodium intake than previously thought.(52)

CONCLUSIONS

The results of this study enhance our understanding of the pathophysiology of hypertension in CKD patients by confirming dysregulation of aldosterone in response to acute salt loading. However, the lack of difference in BP and PAC in response to dietary salt modification calls into question the role of salt restriction in patients with CKD, particularly having shown that low salt diet results in higher PAC in both control and CKD participants. While clinical guidance should not change on the basis of these results alone, further research is required to explore the increasingly complex interaction between RAAS activation, dietary salt intake and hypertension in patients with CKD.

DISCLOSURES:

None.

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AUTHOR CONTIBUTION STATEMENT:

All authors contributed substantially to the completion of this project and have read and approved the final manuscript.

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TABLES

Table 1. Baseline demographics

	Control group (n=15)	**CKD group (n=12)**	**p value**
Age (years)	49.6 (14)	56 (9)	0.2
Body mass index (kg/m^2)	26.4 (4)	27.8 (4)	0.4
Creatinine (µmol/L)	72.8 (11)	188.3 (68)	<0.001
eGFR (ml/min/1.73m^2)	96.8 (11)	36.8 (16)	<0.001
Serum K+ (mmol/L)	4.1(0.3)	4.5 (0.5)	0.01
Cortisol (nmol/L)	287(81)	317 (109)	0.4
24hr urinary sodium excretion (mmol/24hr)	122.3(50)	150 (76)	0.3
Plasma Aldosterone (pmol/L)**	184 (124,340)	230 (137,334)	0.4
Plasma Renin Concentration (mlU/L)**	16 (10,26)	73 (23,127)	0.002
Antihypertensive Medication	4 participants	12 participants	

Mean (standard deviation). **Median (inter-quartile range)

eGFR = estimated glomerular filtration rate

Table 2. Response to modified saline suppression test (acute salt loading) in control group and chronic kidney disease (CKD). Tests of significance are paired t-test, or Wilcoxon signed rank test (*), for before and after saline

	CONTROL GROUP			**CKD GROUP**		
	PRE	POST	p value	PRE	POST	p value
Systolic blood pressure (mmHg)	125 (20)	128 (22)	0.4	132 (16)	139 (14)	0.016
Diastolic blood pressure (mmHg)	83 (12)	84 (13)	0.8	86 (13)	88 (12)	0.4
Heart rate (bpm)	62 (10)	57 (11)	0.01	67 (15)	67 (15)	1.0
Plasma Aldosterone* (pmol/L)	184 (124,340)	95 (80,167)	0.003	230 (137,334)	222 (147,326)	0.17
Plasma Renin* Concentration (mIU/L)	16 (10,27)	9 (5.0,15)	<0.001	73 (23,127)	51 (17,125)	0.021

Mean (standard deviation). *Median (inter-quartile range).

Table 3. 24-hour urinary sodium excretion depending on dietary intervention for control group and those with chronic kidney disease (CKD).

	Urinary sodium excretion (mmol/24 hours)		
	Baseline	**Low Sodium**	**High Sodium**
Control Group	98 (92,154)	80 (60,145)	179 (134,224)
CKD Group	123 (98,210)	91 (69,120)	174 (114,220)

Table 4. Response to low dietary salt intake versus high dietary salt intake in control group and chronic kidney disease (CKD). Tests of significance are paired t-test, or Wilcoxon signed rank test (*), compared to baseline

	CONTROL GROUP			**CKD GROUP**		
	LOW	HIGH	p value	LOW	HIGH	p value
Systolic blood pressure (mmHg)	122 (19)	124 (20)	0.5	125 (12)	132 (12)	0.07
Diastolic blood pressure (mmHg)	79 (13)	82 (13)	0.2	82 (9)	86 (8)	0.1
Heart rate (bpm)	60 (12)	62 (13)	0.06	64 (12)	67 (13)	0.04
Serum Na+ (mmol/L)	139 (2.0)	139 (1.6)	0.3	139 (1.9)	140 (0.8)	0.2
Serum K+ (mmol/L)	4.2 (0.4)	4.1 (0.3)	0.5	4.7 (0.6)	4.6 (0.45)	0.7
Plasma Aldosterone* (pmol/L)	309 (184,380)	162 (84,225)	0.007	424 (253,739)	188 (138, 257)	0.012
Plasma Renin* Concentration (mIU/L)	23 (17,34)	15 (6,27)	0.005	90 (37, 234)	79 (24,132)	0.003

Mean (standard deviation). *Median (inter-quartile range).

Table 5. Summary of relative blood pressure (BP), plasma aldosterone concentration (PAC) and plasma renin concentration (PRC) response to acute salt loading (modified saline suppression test), chronic modification of dietary salt intake and angiotensin II stimulation (both low and high salt diets) in controls versus chronic kidney disease (CKD).

	CONTROL	**CKD**
Acute salt and water load	BP → PRC ↓ PAC ↓	BP ↑ PRC ↓ PAC →
Chronic salt - LOW	BP → PRC ↑ PAC ↑	BP→ PRC ↑ PAC ↑
Chronic salt – HIGH	BP → PRC ↓ PAC ↓	BP → PRC ↓ PAC ↓
Angiotensin II stimulation (both diets)	BP ↑ PRC ↓ PAC ↑	BP ↑ PRC ↓ PAC ↑

FIGURES

Figure 1: Flow chart of study visits.

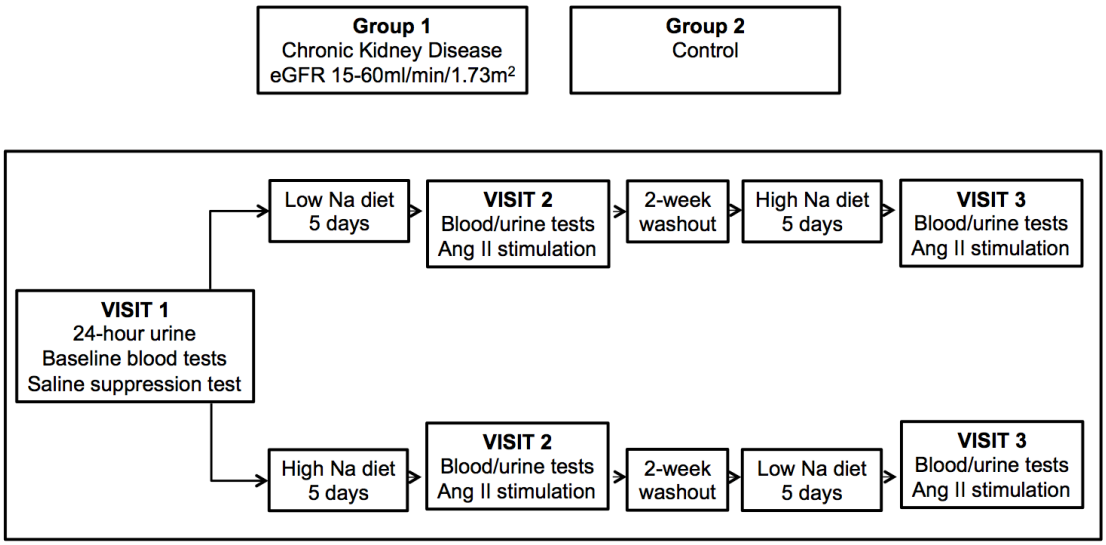
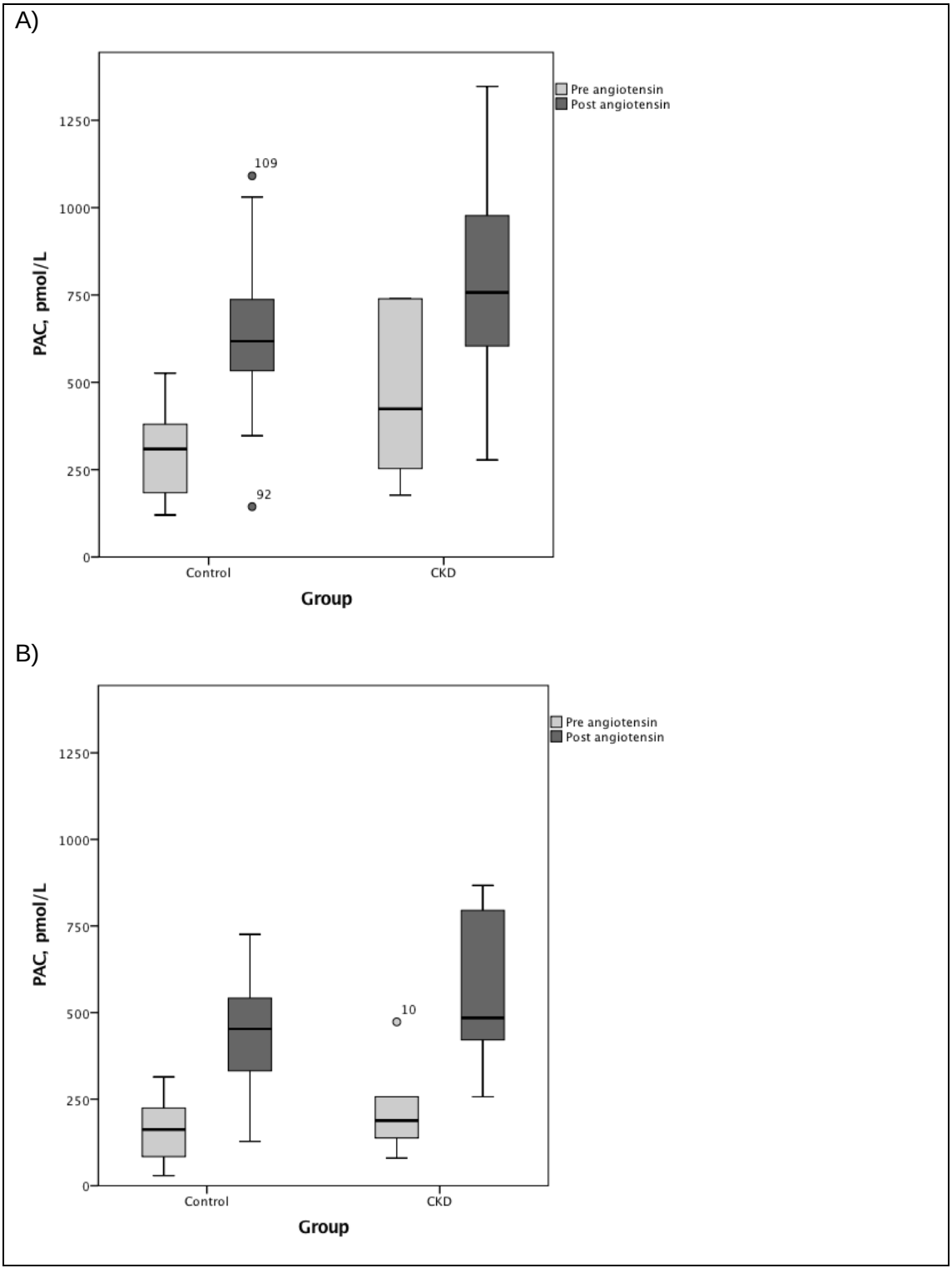


Figure 2. Box plot of plasma aldosterone concentration before and after an infusion of angiotensin 2 in control group and chronic kidney disease (CKD) on low salt (A) and high salt (B) diets.



SUPPLEMENTARY MATERIAL

Non-uniform relationship between salt status and aldosterone activity in patients with chronic kidney disease

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Running title: Salt loading and aldosterone in CKD

Supplementary files:

- Table1: Study exclusion criteria
- Table 2: Full list of participant medications.
- Figure 1: Line graph comparing mean systolic blood pressure over time during the angiotensin-2 stimulation test in control group and those with chronic kidney disease (CKD) following low salt diet (A) and high salt diet (B).

Supplementary material: Table 1. Study exclusion criteria

Exclusion criteria	
Age <18 years or >85 years	
Pregnancy/breast feeding	
Medication prescription:	Aldosterone antagonist
	Direct renin inhibitor
	Corticosteroids
(including those prescribed treatment in preceding 3 months)	
Medical History:	Conns syndrome
	Addisons disease
	Cushings disease
	Type 1 diabetes mellitus
	Severe coronary artery disease
	Left ventricular systolic dysfunction
CKD group:	Decline in eGFR >5 ml/min/1.73m^2 in preceding 6-months
	Uncontrolled BP (>160/90 mmHg)
	Urinary PCR >300 mg/mmol
Control group:	Uncontrolled BP (>160/90 mmHg)
	Urinary PCR >20 mg/mmol

Abbreviations: eGFR = estimated glomerular filtration rate; BP = blood pressure; PCR = protein:creatinine ratio.

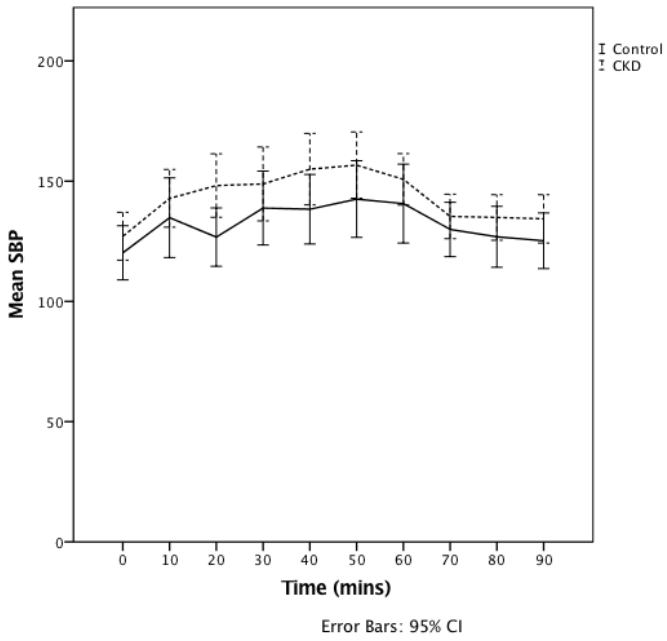
Supplementary material: Table 2. Full list of participant medications.

Participant	Control group	Participant	CKD group
1	Amlodipine, Ramipril, Salbutamol.	1	Allopurinol, Amlodipine, Bendroflumethiazide, Ramipril .
2	Amlodipine, Valsartan.	2	Allopurinol, Felodipine, Sodium Bicarbonate.
3	Bendroflumethiazide, Nifedipine, Perindopril, Simvastatin.	3	Allopurinol, Lisinopril, Pravastatin, Salbutamol.
4	Natur Nuriatum (Homeopathy).	4	Allopurinol, Ramipril.
5	Ranitidine.	5	Allopurinol, Vitamin D, Ramipril.
6	Ramipril, Simvastatin, Symbicort, Ventolin.	6	Amlodipine, Atorvastatin.
7	Symbicort.	7	Amlodipine, Doxazosin, Ramipril.
8		8	Amlodipine, Hydroxychloroquine, Nefopam.
9		9	Aspirin, Furosemide, Levothyroxine, Mycophenolate mofetil, Ramipril, Simvastatin.
10		10	Candesartan, Hormone-replacement therapy, Simvastatin, Sodium Bicarbonate.
11		11	Ezetemibe, Lisinopril, Paroxetine.
12		12	Felodipine, Quinine, Ramipril, Rinzolamide, Travoprost.
13			
14			
15			

Supplementary material: Figure 1

Line graph comparing mean systolic blood pressure over time during the angiotensin-2 stimulation test in control group and those with chronic kidney disease (CKD) following low salt diet (A) and high salt diet (B).

A)



B)