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Original Paper

Relationship between urinary sodium excretion and serum aldosterone in patients with diabetes in the presence and absence of modifiers of the renin–angiotensin–aldosterone system

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Abstract

Although low dietary salt intake has beneficial effects on BP (blood pressure), low 24hUNa (24 h urinary sodium excretion), the most accurate estimate of dietary salt intake, is associated with increased mortality in people with diabetes. In the non-diabetic population, low salt intake is associated with increased RAAS (renin–angiotensin–aldosterone system) activity. In this cross-sectional study, we examined the relationship between 24hUNa, PRA (plasma renin activity), serum aldosterone and BNP (brain natriuretic peptide) in patients with diabetes. Clinical characteristics, 24hUNa, PRA, serum aldosterone and BNP were recorded in 222 consecutive patients (77% with Type 2 diabetes) attending a diabetes clinic at a tertiary hospital. The relationship between 24hUNa, serum aldosterone, PRA, BNP, urinary potassium excretion, serum potassium, serum sodium, eGFR (estimated glomerular filtration rate), urinary albumin excretion and HbA_{1c} (glycated haemoglobin) was examined by a multivariable regression model. Levels of 24hUNa significantly predicted serum aldosterone in a linear fashion ($R^2 = 0.20$, $P = 0.002$). In the subgroup of patients ($n = 46$) not taking RAAS-modifying agents, this relationship was also observed ($R^2 = 0.10$, $P = 0.03$), and the effect of 24hUNa on serum aldosterone was found to be more pronounced than in the whole cohort (coefficient $= -0.0014$, compared with -0.0008). There was no demonstrable relationship between 24hUNa and PRA or BNP. Low 24hUNa is associated with increased serum aldosterone in people with diabetes, in the presence and absence of RAAS-modifying agents. This raises the possibility that stimulation of the RAAS may be a mechanism that contributes to adverse outcomes observed in patients with low 24hUNa.

Key words: diabetes, dietary salt intake, serum aldosterone, sodium excretion

INTRODUCTION

CVD (cardiovascular disease) is the leading cause of death in Australia and individuals with diabetes have an exaggerated risk for the development of CVD. Hypertension is present in approximately 70% of patients with diabetes [1,2], which further increases the risk of CVD in this population by 60–70% [3].

Current guidelines recommend a reduction of dietary salt intake to <100 mmol/24 h [4], particularly in people with hypertension because salt restriction is associated with an approximate 5 mmHg reduction in systolic BP (blood pressure) [5]. We have previously shown in patients with Type 2 diabetes on antihypertensive therapy that salt restriction reduces BP [6] and salt supplementation increases BP [7]. However, the direct evidence

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; AER, albumin excretion rate; Ang-(1–7), angiotensin-(1–7); AngII, angiotensin II; ARB, AT_1 (angiotensin II type 1) receptor blocker; BNP, brain natriuretic peptide; BP, blood pressure; CI, confidence interval; CV, coefficient of variation; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated haemoglobin; 24hUCr, 24 h urinary creatinine; 24hUNa, 24 h urinary sodium; PRA, plasma renin activity; RAAS, renin–angiotensin–aldosterone system; TAG, triacylglycerol.

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regarding the effects of dietary salt intake on cardiovascular outcomes is controversial [8–23]. Previous studies in patients with diabetes have demonstrated an association between adverse outcomes and low 24hUNa (24 h urinary sodium excretion; the most accurate estimate of dietary salt intake) [20,21].

In the general population, low salt intake is associated with increases in PRA (plasma renin activity), plasma aldosterone, plasma adrenaline and plasma noradrenaline levels, as well as total cholesterol and TAG (triacylglycerol) levels [24]. Stimulation of the RAAS (renin–angiotensin–aldosterone system) by a low-salt diet may play a role in the development of atherosclerosis and adverse cardiovascular events by mediating inflammation, thrombosis, oxidative stress and cardiac hypertrophy [25,26]. In an animal study, Tikellis et al. [27] investigated the role of the RAAS in the development of atherosclerotic plaques in ApoE (apolipoprotein E)-knockout mice, and found that a low-salt diet resulted in increased RAAS activity and a 4-fold increase in plaque accumulation. In humans, increased PRA has been associated with increased cardiovascular outcomes and mortality [28–30]. Furthermore, the increased serum aldosterone levels were associated with increased cardiovascular mortality in patients with heart failure [31], myocardial infarction [32] and coronary artery disease outside the setting of acute myocardial infarction or heart failure [33]. Increased cardiovascular events and mortality have also been associated with elevated levels of BNP (brain natriuretic peptide) [34,35] and N-terminal proBNP [36–39] in patients with diabetes. However, there is a lack of studies of dietary salt intake and the degree of activation of the RAAS and BNP levels in people with diabetes. The present cross-sectional study aimed to investigate the relationship between 24hUNa, as a measure of dietary salt intake, with PRA, serum aldosterone and BNP in patients with diabetes.

MATERIALS AND METHODS

Patient recruitment

This was a cross-sectional study of patients with Types 1 and 2 diabetes from diabetes outpatient clinics at Austin Health, Melbourne, Australia. Austin Health is a major tertiary referral centre and a University of Melbourne teaching hospital, serving a population of approximately 700 000 people. The present study has been carried out in accordance with the Declaration of Helsinki, and was approved by the Austin Health Human Research Ethics Committee. Subjects gave written informed consent.

Baseline characteristics

Between November 2011 and March 2012, data were collected on the clinical and biochemical characteristics of the participants, including a full clinical history, medication use and anthropometric data. In particular, we recorded the use of medications that affect the RAAS {i.e. ACEi (angiotensin-converting enzyme inhibitors), ARB [AT_1 (angiotensin II type 1) receptor blocker] and diuretics}. Pre-existing macrovascular complications of diabetes were defined as a clinical history of myocardial infarction, unstable angina requiring hospitalization, coronary revascularization (including coronary artery bypass grafting, angioplasty and coronary stenting), heart failure, atrial fibrillation, stroke, carotid artery surgery, peripheral revascularization (including bypass grafting, angioplasty or stenting) and amputation for critical limb ischaemia. The presence or absence of retinopathy was ascertained from clinical notes, optometrist or ophthalmologist reports.

Laboratory methods

Prior to each visit, patients attending the diabetes clinic at Austin Health routinely perform a 24 h urine collection to measure the excretion of sodium, potassium, glucose, creatinine and albumin. Fasting blood samples were taken for measurement of glucose, HbA_{1c} (glycated haemoglobin), lipid profile, creatinine, PRA, aldosterone and BNP prospectively at the Department of Laboratory Medicine at Austin Health. Blood samples were taken in the morning after the patients had been in the sitting position for at least 5 min after unrestricted ambulation. eGFR (estimated glomerular filtration rate) was estimated using the MDRD (Modification of Diet in Renal Disease) formula [eGFR (ml/min per 1.73 m^2) $= 175 \times (\text{serum creatinine} \times 0.0113)^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female})$]. PRA was measured in EDTA plasma using the GammaCoat Plasma Renin Activity RIA (radioimmunoassay; Diasorin) with a CV (coefficient of variation) of 16% at levels of 2 and 5.5 μg/l per h. Serum aldosterone was measured with the Count-A-Coat RIA (Siemens), achieving a CV of 10% at 110 pmol/l and 13% at 1499 pmol/l. BNP was measured by immunoassay on the Abbott Architect with CVs of 11% at levels of 90 and 485 ng/l. Microalbuminuria was measured on the Beckman D×C800 by immunoturbidimetry with CVs of 8.5 and 4% at levels of 30 and 100 mg/l respectively. Microalbuminuria was defined as an AER (albumin excretion rate) of 20–200 μg/min and macroalbuminuria as an AER of >200 μg/min.

Statistical methods

The relationship between 24hUNa, serum aldosterone, PRA, BNP, urinary potassium excretion, serum potassium, serum sodium, eGFR, urinary albumin excretion and HbA_{1c} was examined by multivariable regression, using cubic regression splines to model non-linearity in covariate effect [40]. Continuous variables that were normally distributed were analysed using Student's *t* tests. Data that were not normally distributed were logarithmically transformed or analysed using the Mann–Whitney test. χ^2 tests were used for categorical variables. A *P* value of less than 0.05 was considered statistically significant. Minitab version 16 and Stata version 12 were used to perform the statistical analyses.

RESULTS

A total of 222 consecutive patients who had complete data on 24hUNa, PRA, serum aldosterone and BNP formed the study cohort. This included 176 patients who were, and 46 patients who were not, taking medications that could affect the RAAS. Out of the 176 patients who took RAAS-modifying agents, 40% were

Table 1 Baseline clinical characteristics

Data are means ± S.D. unless otherwise indicated. HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDRD, Modification of Diet in Renal Disease

Parameter	All patients	Type 1 diabetes	Type 2 diabetes	*P* value
Patient characteristics				
Number of patients	222	52 (23%)	170 (77%)	<0.001
Age (years)	64 ± 15	50 ± 19	68 ± 10	<0.001
Sex (% male)	62	63	62	0.82
Duration of diabetes (years)	17 ± 11	22 ± 15	15 ± 9	<0.001
BMI (kg/m^2)	31 ± 7	28 ± 6	32 ± 7	<0.001
Prior macrovascular disease (%)	45	19	53	<0.001
Coronary heart disease (%)	34	17	39	0.003
Atrial fibrillation (%)	9	2	11	0.05
Congestive heart failure (%)	8	8	8	0.9
Systolic BP (mmHg)	136 ± 19	131 ± 18	138 ± 19	0.02
Diastolic BP (mmHg)	73 ± 12	74 ± 12	73 ± 12	0.6
Retinopathy (%)	26	33	24	0.22
eGFR<60 ml/min per 1.73 m^2 (%)	32	16	37	0.004
Biochemical results				
HbA_{1c} (mmol/mol,%)	61 ± 11, 7.7 ± 1.1	60 ± 11, 7.6 ± 1	60 ± 14, 7.7 ± 1.1	0.75
Fasting plasma glucose (mmol/l)	8.6 ± 3.4	9.8 ± 5	8.2 ± 2.6	0.2
LDL-cholesterol (mmol/l)	2.2 ± 0.8	2.4 ± 0.7	2.1 ± 0.8	0.016
HDL-cholesterol (mmol/l)	1.2 ± 0.5	1.5 ± 0.5	1.2 ± 0.4	<0.001
TAGs (mmol/l)	1.5 ± 1	1 ± 0.5	1.6 ± 1	<0.001
MDRD eGFR (ml/min per 1.73 m^2)	69 ± 20	78 ± 16	67 ± 20	<0.001
Normoalbuminuria (%)	69	85	65	0.008
Microalbuminuria (%)	25	13	28	0.03
Macroalbuminuria (%)	6	2	7	0.2
Urinary albumin excretion rate (μg/min) (median, interquartile range)	11 (5–27)	9.2 (5–13)	12 (5–35)	0.1
Medication (%)				
RAAS modulator	79	58	86	<0.001
ACEis	40	31	43	0.1
ARB	45	35	48	0.1
Diuretics	47	29	52	0.003
Calcium channel blockers	39	25	43	0.02
β-Blockers	29	13	34	0.004
Other antihypertensives	12	8	14	0.26
Metformin	53	10	66	<0.001
Sulfonylurea	27	0	35	<0.001
Thiazolidinedione	10	0	13	0.006
Gliptin	7	0	9	0.02
Exenatide	3	0	4	0.17
Insulin	62	98	51	<0.001
Statin	79	56	86	<0.001
Ezetimibe	15	12	16	0.4
Fibrates	7	2	8	0.1

taking ACEi, 45% were taking ARB and 47% were taking diuretics. The clinical characteristics are summarized in Table 1. Complications of diabetes were common, with 45% of patients having pre-existing macrovascular disease. Of the participants, 32% had an eGFR <60 ml/min per 1.73 m^2. Mean 24hUNa and 24 h urinary potassium level were 154 ± 76 and 74 ± 31 mmol/day respectively, in keeping with previous international surveys [41,42].

Serum aldosterone and 24hUNa

In the total study cohort, 24hUNa, 24 h urinary potassium, PRA and plasma sodium concentration significantly predicted log

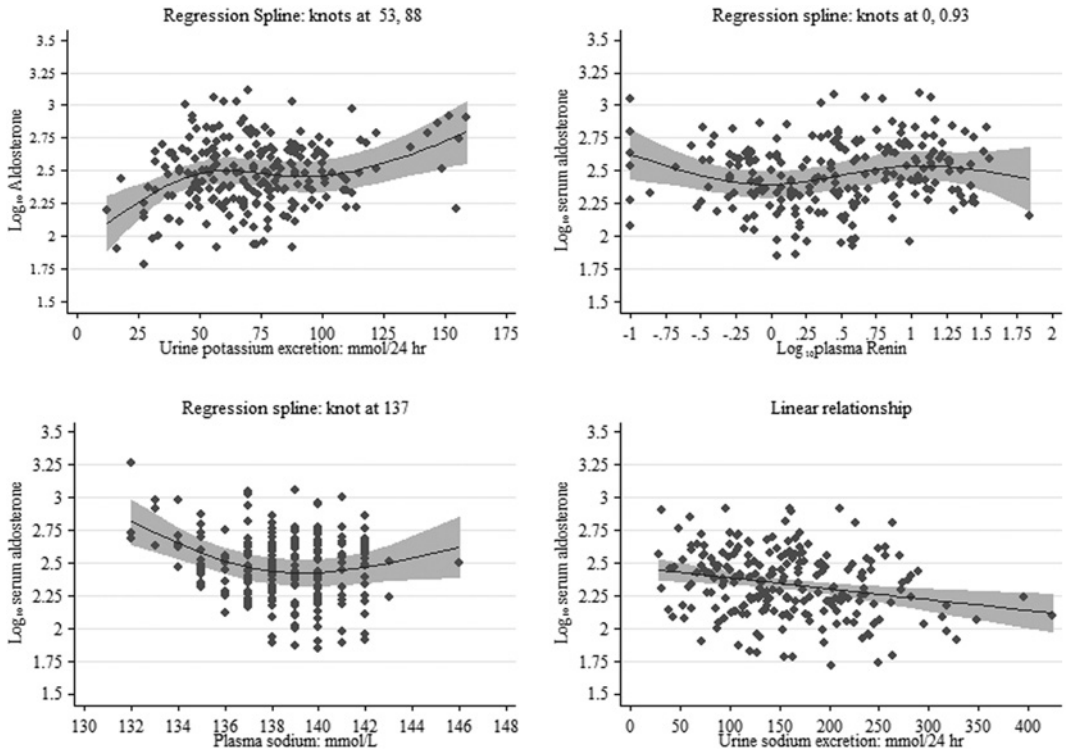

Figure 1 Multivariable spline regression model of 24hUNa, urinary potassium excretion, PRA and plasma sodium, with log serum aldosterone

(Upper left-hand panel) Urine potassium excretion (mmol/24 h) predicted serum aldosterone (pmol/l) with spline transformation. (Upper right-hand panel) PRA (μg/l per h) predicted serum aldosterone (pmol/l) with spline transformation. (Lower left-hand panel) Plasma sodium (mmol/l) predicted serum aldosterone (pmol/l) with spline transformation. (Lower right-hand panel) 24hUNa (mmol/l) predicted serum aldosterone (pmol/l) in a linear fashion. The shaded area indicates the 95% CI.

serum aldosterone levels ($R^2 = 0.2$, $P < 0.001$) (Figure 1 and Table 2). Levels of 24hUNa predicted serum aldosterone in a linear fashion, whereas urinary potassium excretion, PRA and plasma sodium predicted log serum aldosterone in a non-linear fashion, as evidenced by the shape of the regression spline in Figure 1. There was no significant relationship between BNP and serum aldosterone ($P = 0.71$).

In the subgroup of patients not taking agents which interfere with RAAS, 24hUNa also significantly predicted serum aldosterone levels in a linear fashion ($R^2 = 0.1$, $P = 0.03$). The relationship between 24hUNa and log serum aldosterone was more pronounced in this subgroup of patients than in the whole study cohort (coefficient $= -0.0014$, compared with -0.0008) (Figure 2).

PRA and 24hUNa

There was no demonstrable relationship between 24hUNa and PRA in the total study group or in the subgroup of patients not taking RAAS-modifying agents.

BNP and 24hUNa

There was no discernible relationship between BNP with 24hUNa in the total study group or in the subgroup of patients not taking RAAS-modifying agents.

DISCUSSION

The most important finding in the present study was that 24hUNa predicted serum aldosterone in a linear fashion. The serum aldosterone level was greater in those with lower 24hUNa, especially in patients not taking medications that interfere with the RAAS. Medications that interfere with the RAAS can disrupt the RAAS response to salt intake. This potentially contributes to the weaker relationship observed between 24hUNa and serum aldosterone in the subgroup of patients who take RAAS-modifying medications.

One of the earliest studies examining the relationship between dietary salt intake and the RAAS was performed by Brunner et al. [28] in 219 patients with essential hypertension. These investigators reported that PRA was related to 24hUNa and that high PRA increased the risks of myocardial infarction and stroke. Since then, many studies have investigated the relationship between dietary salt intake and the RAAS. A Cochrane review of 167 studies conducted in 2012 concluded that dietary salt reduction resulted not only in a significant decrease in BP, but also a significant increase in PRA and plasma aldosterone [24]. In this review of randomized controlled trials involving people with normal or elevated BP, they reported a standard mean difference of 1.15 [95% CI (confidence interval): 0.99, 1.30] for renin and 1.36 (95% CI: 1.15, 1.57) for aldosterone, following salt reduction.

Table 2 **Multivariable spline regression model of 24hUNa, 24 h urine potassium excretion, PRA and plasma sodium, with $\log_{10}$(aldosterone)**

Note that there are (i) two coefficients for plasma sodium (reflecting the basis function and the knot); (ii) three coefficients for PRA (basis function and two knots); (iii) one coefficient for 24hUNa as it is linear but is modelled with this basis function; and (iv) three coefficients for 24 h urinary potassium. The joint tests of significance for the multiple parameters for each of the non-linear covariate effects are highly significant. The test for plasma sodium: F (2, 212) = 8.76, Prob>F = 0.0002; for PRA: F (3, 212) = 4.10, Prob>F = 0.0075; and for 24 h urinary potassium: F (3, 212) = 5.95, Prob>F = 0.0006.

Variable	Coefficient	*P* value	95% CI
Plasma sodium	0.05	0.002	0.02, 0.09
PRA	−0.05	0.008	−0.08, −0.01
24hUNa	−0.0008	0.002	−0.001, −0.0003
24 h urinary potassium	−0.05	0.001	−0.09, −0.02

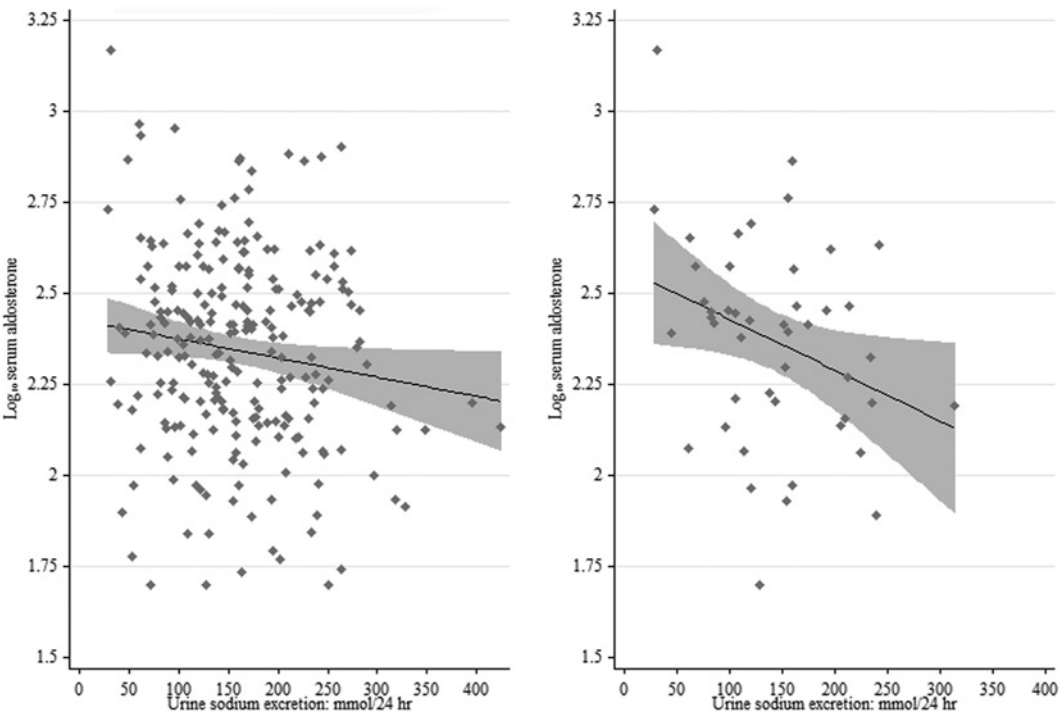

Figure 2 **Regression model of 24hUNa (mmol/24 h) and log serum aldosterone (pmol/l)**

(Left-hand panel) Relationship between serum aldosterone and 24hUNa in the whole cohort (n = 222). Regression line equation: $\log_{10}$(serum aldosterone) = 2.472 − 0.0008×24hUNa. (Right hand panel) Relationship between serum aldosterone and 24hUNa in the subgroup of patients not taking medications that affect RAAS (n = 46). Regression line equation: $\log_{10}$(serum aldosterone) = 2.567 − 0.001×24hUNa. The shaded area indicates the 95% CI.

Despite the abundance of studies of RAAS activity and salt intake, very few have included patients with diabetes. In a study of hypertensive patients with or without diabetes treated with low- or high-salt diet, Price et al. [43] reported that a high-salt diet suppressed PRA in healthy subjects in an expected manner, but patients with Type 2 diabetes showed less PRA suppression with a high-salt diet.

We report a significant relationship between 24hUNa and serum aldosterone in patients with diabetes; however, there was no demonstrable relationship between 24hUNa and PRA. Essential hypertension is associated with reduced PRA [44,45] and 70% of patients with diabetes have hypertension [1,2]. It is possible that an overall reduction in PRA activity in patients with diabetes partly contributed to the lack of a relationship between 24hUNa and PRA in the present study. In the present study, the mean 24hUNa was 154 ± 76 mmol/day. In the original studies by Brunner et al. [28], the increases in PRA were non-linear with much greater levels of PRA seen when 24hUNa was < 100 mmol/24 h. Given that a minority of patients with diabetes had a 24hUNa of < 100 mmol/24 h (24% in the present study), this may explain the lack of a relationship seen between 24hUNa and PRA. Furthermore, Price et al. [43] have reported reduced suppression of PRA with a high-salt diet in patients with diabetes, which may also explain the lack of correlation between 24hUNa and PRA in the present study.

Studies of patients with Type 2 diabetes have reported an association between raised BNP/NT-proBNP (N-terminal proBNP fragment) levels and excess-all cause and cardiovascular mortality [34–39]. Low salt intake has been associated with both a reduction [46] and an increase [18] in BNP levels in patients

with heart failure. In the present study involving patients with diabetes, we found no demonstrable relationship between BNP and 24hUNa. Differences in the sample population, size and methods make comparison of results between the existing studies challenging. Furthermore, 79% of patients in our study were taking either ARB or ACEi, and as a result may have increased the ACE2/ACE ratio and a subsequent increase in Ang-(1–7) [angiotensin-(1–7)]. Ang-(1–7) has been shown to attenuate cardiac remodelling [47], and may potentially contribute to the lack of association seen between BNP and 24hUNa in the present study. Further studies are needed to determine the relationship, or lack thereof, between BNP and salt.

Although the relationship between salt and hypertension is well established [5], the relationship between salt intake and cardiovascular outcome or mortality is controversial, with studies showing no significant association [15,17], increased [8,20–23] or decreased [12–14,48] cardiovascular outcomes and mortality with a low-salt diet. We have previously reported that low 24hUNa was associated with increased all-cause and cardiovascular mortality in a cohort of 638 patients with Type 2 diabetes after 10 years of follow-up [20]. The FinnDiane study [21], which involved 2807 patients with type 1 diabetes, reported that mortality was highest in patients with both high and low sodium excretion, suggesting a U-shaped relationship between salt intake and outcome. However, the mechanisms linking salt with mortality in patients with diabetes have not been defined.

Increased RAAS activation has been suggested as the mechanism that potentially links low salt intake with less favourable cardiovascular outcomes. In humans, PRA has been shown to be independently associated with increased risk of myocardial infarction [29,30,49]. However in the present study, we did not find an association between high PRA levels and low 24hUNa. Serum aldosterone levels have also been independently associated with increased mortality and cardiovascular outcomes [31–33]. Animal studies demonstrated that accelerated atherosclerosis is associated with dietary salt restriction in apoE-knockout mice, through a mechanism mediated by AngII [27,50]. The latter study demonstrated that apoE$^{-/-}$ mice receiving a low-salt diet had increased plasma aldosterone levels and developed fatty streaks [27]. In another study, dogs that were fed a low-salt diet had a 3-fold increase in plasma AngII and a 60% reduction in flow-induced dilation in coronary arterioles compared with dogs on a normal salt diet [51]. Observational studies have previously suggested the possibility of less favourable cardiovascular outcomes with low-salt intake, and these animal studies suggest potential mechanisms for these findings. However, we also recognize the difficulties in translating animal studies to humans.

The major strength of the present study is the use of 24hUNa to estimate dietary salt intake. Up to 90% of salt intake is renally excreted [52]; therefore, 24hUNa provides a more accurate estimate of salt intake compared with dietary recall, which can underestimate dietary salt intake by up to 50% [53]. Patients with Type 2 diabetes attending our diabetes clinics have their urine collected two or three times per year and are routinely given advice on how to accurately and completely collect 24 h urine. In a previous longitudinal study, the mean 24hUCr (24 h urinary creatinine excretion) per subject over the study duration was used to correct for the completeness of urine collections for each participant [54]. The mean intra-individual CV of the corrected 24hUNa, the uncorrected 24hUNa and 24hUCr were 21 ± 1, 22 ± 1 and 13 ± 1% respectively. The finding of very similar CVs for the corrected and uncorrected 24hUNa, and a higher CV for 24hUNa compared with 24hUCr, suggests that the variation in 24hUNa is likely to be secondary to dietary variation in sodium intake rather than 24 h urine collection error [54], validating the use of a single 24hUNa in the present study to estimate dietary intake.

We recognize the limitations of the present study. As with any observational non-randomized studies, the present study is hypothesis-generating. The other limitation is that there are a relatively smaller number of participants in the present study, especially in the subgroup without RAAS modulation. Another limitation is that most of our patients from the clinic took medications that interfere with RAAS. However, despite this, there still remained a significant relationship between 24hUNa and serum aldosterone.

In conclusion, in patients with diabetes, low 24hUNa, as an index of low dietary salt intake, was associated with increased serum aldosterone levels. This raises the possibility that RAAS modification is one mechanism that links low salt intake with adverse outcomes.

CLINICAL PERSPECTIVES

- We have previously reported an increase in mortality associated with low 24hUNA excretion, which is the best marker of dietary salt intake, in people with diabetes. The RAAS has been implicated in the development of atherosclerosis as well as in cardiovascular mortality in the non-diabetic population.
- The present study demonstrated an association between low 24hUNa and increased serum aldosterone levels in people with diabetes.
- The activation of the RAAS may potentially contribute to the adverse outcomes observed in patients with diabetes and low 24hUNa.

AUTHOR CONTRIBUTION

Elif Ekinci, George Jerums and Richard MacIsaac designed the research. Renata Libianto, Elif Ekinci, Angela Chen, Sara Baqar and Felicity Pyrlis performed the data collection. Renata Libianto, Elif Ekinci and George Jerums drafted the paper. Que Lam provided pathology support and John Moran provided statistical support. All authors approved the final version of the paper.

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REFERENCES

1 Tarnow, L., Rossing, P., Gall, M. A., Nielsen, F. S. and Parving, H. H. (1994) Prevalence of arterial hypertension in diabetic patients before and after the JNC-V. Diabetes Care **17**, 1247–1251
2 Gee, M. E., Janssen, I., Pickett, W., McAlister, F. A., Bancej, C. M., Joffres, M., Johansen, H. and Campbell, N. R. (2012) Prevalence, awareness, treatment, and control of hypertension among Canadian adults with diabetes, 2007 to 2009. Can. J. Cardiol. **28**, 367–374
3 Chen, G., McAlister, F. A., Walker, R. L., Hemmelgarn, B. R. and Campbell, N. R. (2011) Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure. Hypertension **57**, 891–897
4 Appel, L. J., Frohlich, E. D., Hall, J. E., Pearson, T. A., Sacco, R. L., Seals, D. R., Sacks, F. M., Smith, S. C. Jr, Vafiadis, D. K. and Van Horn, L. V. (2011) The importance of population-wide sodium reduction as a means to prevent cardiovascular disease and stroke: a call to action from the American Heart Association. Circulation **123**, 1138–1143
5 He, F. J. and MacGregor, G. A. (2004) Effect of longer-term modest salt reduction on blood pressure. Cochrane Database Syst. Rev. **3**, CD004937
6 Houlihan, C. A., Allen, T. J., Baxter, A. L., Panangiotopoulos, S., Casley, D. J., Cooper, M. E. and Jerums, G. (2002) A low-sodium diet potentiates the effects of losartan in type 2 diabetes. Diabetes Care **25**, 663–671
7 Ekinci, E. I., Thomas, G., Thomas, D., Johnson, C., Macisaac, R. J., Houlihan, C. A., Finch, S., Panagiotopoulos, S., O'Callaghan, C. and Jerums, G. (2009) Effects of salt supplementation on the albuminuric response to telmisartan with or without hydrochlorothiazide therapy in hypertensive patients with type 2 diabetes are modulated by habitual dietary salt intake. Diabetes Care **32**, 1398–1403
8 Alderman, M. H., Madhavan, S., Cohen, H., Sealey, J. E. and Laragh, J. H. (1995) Low urinary sodium is associated with greater risk of myocardial infarction among treated hypertensive men. Hypertension **25**, 1144–1152
9 Kagan, A., Popper, J. S., Rhoads, G. G. and Yano, K. (1985) Dietary and other risk factors for stroke in Hawaiian Japanese men. Stroke **16**, 390–396
10 Sasaki, S., Zhang, X. H. and Kesteloot, H. (1995) Dietary sodium, potassium, saturated fat, alcohol, and stroke mortality. Stroke **26**, 783–789
11 Tunstall-Pedoe, H., Woodward, M., Tavendale, R., A'Brook, R. and McCluskey, M. K. (1997) Comparison of the prediction by 27 different factors of coronary heart disease and death in men and women of the Scottish Heart Health Study: cohort study. Br. Med. J. **315**, 722–729
12 He, J., Ogden, L. G., Vupputuri, S., Bazzano, L. A., Loria, C. and Whelton, P. K. (1999) Dietary sodium intake and subsequent risk of cardiovascular disease in overweight adults. JAMA, J. Am. Med. Assoc. **282**, 2027–2034
13 Tuomilehto, J., Jousilahti, P., Rastenyte, D., Moltchanov, V., Tanskanen, A., Pietinen, P. and Nissinen, A. (2001) Urinary sodium excretion and cardiovascular mortality in Finland: a prospective study. Lancet **357**, 848–851
14 Cook, N. R., Cutler, J. A., Obarzanek, E., Buring, J. E., Rexrode, K. M., Kumanyika, S. K., Appel, L. J. and Whelton, P. K. (2007) Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). BMJ **334**, 885
15 Geleijnse, J. M., Witteman, J. C., Stijnen, T., Kloos, M. W., Hofman, A. and Grobbee, D. E. (2007) Sodium and potassium intake and risk of cardiovascular events and all-cause mortality: the Rotterdam study. Eur. J. Epidemiol. **22**, 763–770
16 Umesawa, M., Iso, H., Date, C., Yamamoto, A., Toyoshima, H., Watanabe, Y., Kikuchi, S., Koizumi, A., Kondo, T., Inaba, Y. et al. (2008) Relations between dietary sodium and potassium intakes and mortality from cardiovascular disease: the Japan collaborative cohort study for evaluation of cancer risks. Am. J. Clin. Nutr. **88**, 195–202
17 Cohen, H. W., Hailpern, S. M. and Alderman, M. H. (2008) Sodium intake and mortality follow-up in the Third National Health and Nutrition Examination Survey (NHANES III). J. Gen. Intern. Med. **23**, 1297–1302
18 Paterna, S., Gaspare, P., Fasullo, S., Sarullo, F. M. and Di Pasquale, P. (2008) Normal-sodium diet compared with low-sodium diet in compensated congestive heart failure: is sodium an old enemy or a new friend? Clin. Sci. **114**, 221–230
19 Parrinello, G., Di Pasquale, P., Licata, G., Torres, D., Giammanco, M., Fasullo, S., Mezzero, M. and Paterna, S. (2009) Long-term effects of dietary sodium intake on cytokines and neurohormonal activation in patients with recently compensated congestive heart failure. J. Card. Failure **15**, 864–873
20 Ekinci, E. I., Clarke, S., Thomas, M. C., Moran, J. L., Cheong, K., MacIsaac, R. J. and Jerums, G. (2011) Dietary salt intake and mortality in patients with type 2 diabetes. Diabetes Care **34**, 703–709
21 Thomas, M. C., Moran, J., Forsblom, C., Harjutsalo, V., Thorn, L., Ahola, A., Wadén, J., Tolonen, N., Saraheimo, M., Gordin, D. and Groop, P. H. (2011) The association between dietary sodium intake, ESRD, and all-cause mortality in patients with type 1 diabetes. Diabetes Care **34**, 861–866
22 Stolarz-Skrzypek, K., Kuznetsova, T., Thijs, L., Tikhonoff, V., Seidlerová, J., Richart, T., Jin, Y., Olszanecka, A., Malyutina, S., Casiglia, E. et al. (2011) Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. JAMA, J. Am. Med. Assoc. **305**, 1777–1785
23 O'Donnell, M. J., Yusuf, S., Mente, A., Gao, P., Mann, J. F., Teo, K., McQueen, M., Sleight, P., Sharma, A. M., Dans, A. et al. (2011) Urinary sodium and potassium excretion and risk of cardiovascular events. JAMA, J. Am. Med. Assoc. **306**, 2229–2238
24 Graudal, N. A., Hubeck-Graudal, T. and Jurgens, G. (2012) Effects of Low-Sodium diet vs. High-Sodium diet on blood pressure, renin, aldosterone, catecholamins, cholesterol, and triglyceride (Cochrane Review). Am. J. Hypertens. **25**, 1–15
25 O'Keefe, J. H., Lurk, J. T., Kahatapitiya, R. C. and Haskin, J. A. (2003) The renin–angiotensin–aldosterone system as a target in coronary disease. Curr. Atheroscler. Rep. **5**, 124–130
26 Loot, A. E. (2012) With a pinch of salt: does reduced dietary sodium consumption promote atherosclerosis? Hypertension **60**, 15–16
27 Tikellis, C., Pickering, R. J., Tsorotes, D., Huet, O., Chin-Dusting, J., Cooper, M. E. and Thomas, M. C. (2012) Activation of the renin–angiotensin system mediates the effects of dietary salt intake on atherogenesis in the apolipoprotein E knockout mouse. Hypertension **60**, 98–105
28 Brunner, H. R., Laragh, J. H., Baer, L., Newton, M. A., Goodwin, F. T., Krakoff, L. R., Bard, R. H. and Bühler, F. R. (1972) Essential hypertension: renin and aldosterone, heart attack and stroke. N. Engl. J. Med. **286**, 441–449
29 Campbell, D. J., Woodward, M., Chalmers, J. P., Colman, S. A., Jenkins, A. J., Kemp, B. E., Neal, B. C., Patel, A. and MacMahon, S. W. (2005) Prediction of myocardial infarction by N-terminal-pro-B-type natriuretic peptide, C-reactive protein, and renin in subjects with cerebrovascular disease. Circulation **112**, 110–116
30 Volpe, M., Battistoni, A., Chin, D., Rubattu, S. and Tocci, G. (2012) Renin as a biomarker of cardiovascular disease in clinical practice. Nutr. Metab. Cardiovasc. Dis. **22**, 312–317

31 Güder, G., Bauersachs, J., Frantz, S., Weismann, D., Allolio, B., Ertl, G., Angermann, C. E. and Störk, S. (2007) Complementary and incremental mortality risk prediction by cortisol and aldosterone in chronic heart failure. Circulation **115**, 1754–1761

32 Beygui, F., Collet, J. P., Benoliel, J. J., Vignolles, N., Dumaine, R., Barthélémy, O. and Montalescot, G. (2006) High plasma aldosterone levels on admission are associated with death in patients presenting with acute ST-elevation myocardial infarction. Circulation **114**, 2604–2610

33 Ivanes, F., Susen, S., Mouquet, F., Pigny, P., Cuilleret, F., Sautière, K., Collet, J. P., Beygui, F., Hennache, B., Ennezat, P. V. et al. (2012) Aldosterone, mortality, and acute ischaemic events in coronary artery disease patients outside the setting of acute myocardial infarction or heart failure. Eur. Heart J. **33**, 191–202

34 Bhalla, M. A., Chiang, A., Epshteyn, V. A., Kazanegra, R., Bhalla, V., Clopton, P., Krishnaswamy, P., Morrison, L. K., Chiu, A., Gardetto, N. et al. (2004) Prognostic role of B-type natriuretic peptide levels in patients with type 2 diabetes mellitus. J. Am. Coll. Cardiol. **44**, 1047–1052

35 Onodera, M., Nakamura, M., Tanaka, F., Takahashi, T., Makita, S., Ishisone, T., Ishibashi, Y., Itai, K., Onoda, T., Ohsawa, M. et al. (2012) Plasma B-type natriuretic peptide is useful for cardiovascular risk assessment in community-based diabetes subjects: comparison with albuminuria. Int. Heart J. **53**, 176–181

36 Tarnow, L., Gall, M. A., Hansen, B. V., Hovind, P. and Parving, H. H. (2006) Plasma N-terminal pro-B-type natriuretic peptide and mortality in type 2 diabetes. Diabetologia **49**, 2256–2262

37 Gaede, P., Hildebrandt, P., Hess, G., Parving, H. H. and Pedersen, O. (2005) Plasma N-terminal pro-brain natriuretic peptide as a major risk marker for cardiovascular disease in patients with type 2 diabetes and microalbuminuria. Diabetologia **48**, 156–163

38 Huelsmann, M., Neuhold, S., Strunk, G., Moertl, D., Berger, R., Prager, R., Abrahamian, H., Riedl, M., Pacher, R., Luger, A. and Clodi, M. (2008) NT-proBNP has a high negative predictive value to rule-out short-term cardiovascular events in patients with diabetes mellitus. Eur. Heart J. **29**, 2259–2264

39 Bruno, G., Landi, A., Barutta, F., Ghezzo, G., Baldin, C., Spadafora, L., Schimmenti, A., Prinzis, T., Cavallo Perin, P. and Gruden, G. (2013) NH_2-terminal probrain natriuretic peptide is a stronger predictor of cardiovascular mortality than C-reactive protein and albumin excretion rate in elderly patients with Type 2 diabetes: The Casale Monferrato population-based study. Diabetes Care, doi:10.2337/dc13-0353

40 Royston, P. and Sauerbrei, W. (2008) Multivariable modelling with cubic regression splines: a principled approach. Stata J. **7**, 45–70

41 Brown, I. J., Tzoulaki, I., Candeias, V. and Elliott, P. (2009) Salt intakes around the world: implications for public health. Int. J. Epidemiol. **38**, 791–813

42 Intersalt Cooperative Research Group (1988) Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 h urinary sodium and potassium excretion. Br. Med. J. BMJ **297**, 319–328

43 Price, D. A., De'Oliveira, J. M., Fisher, N. D., Williams, G. H. and Hollenberg, N. K. (1999) The state and responsiveness of the renin–angiotensin–aldosterone system in patients with type II diabetes mellitus. Am. J. Hypertens. **12**, 348–355

44 Thomas, G. W., Ledingham, J. G., Beilin, L. J. and Stott, A. N. (1976) Reduced plasma renin activity in essential hypertension: effects of blood pressure, age and sodium. Clin. Sci. Mol. Med. **3** (Suppl.), 185–188

45 Durukan, M., Guray, U., Aksu, T., Guray, Y., Demirkan, B. and Korkmaz, S. (2012) Low plasma renin activity and high aldosterone/renin ratio are associated with untreated isolated systolic hypertension. Blood Pressure **21**, 320–325

46 Sadanaga, T., Ando, K., Hirota, S., Mitamura, H., Tsuchihashi, T., Kohsaka, S., Fukuda, K. and Ogawa, S. (2013) B-type natriuretic peptide levels are decreased by reducing dietary salt intake in patients with compensated heart failure with preserved ejection fraction. Int. Med. J. **43**, 663–667

47 McCollum, L. T., Gallagher, P. E. and Tallant, E. A. (2012) Angiotensin-(1–7) attenuates angiotensin II-induced cardiac remodeling associated with upregulation of dual-specificity phosphatase 1. Am. J. Physiol. Heart Circ. Physiol. **302**, H801–H810

48 Heerspink, H. J., Holtkamp, F. A., Parving, H. H., Navis, G. J., Lewis, J. B., Ritz, E., de Graeff, P. A. and de Zeeuw, D. (2012) Moderation of dietary sodium potentiates the renal and cardiovascular protective effects of angiotensin receptor blockers. Kidney Int. **82**, 330–337

49 Alderman, M. H., Madhavan, S., Ooi, W. L., Cohen, H., Sealey, J. E. and Laragh, J. H. (1991) Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. N. Engl. J. Med. **324**, 1098–1104

50 Ivanovski, O., Szumilak, D., Nguyen-Khoa, T., Dechaux, M., Massy, Z. A., Phan, O., Mothu, N., Lacour, B., Drueke, T. B. and Muntzel, M. (2005) Dietary salt restriction accelerates atherosclerosis in apolipoprotein E-deficient mice. Atherosclerosis **180**, 271–276

51 Huang, A., Yan, C., Suematsu, N., Cuevas, A., Yang, Y. M., Kertowidjojo, E., Hintze, T. H., Kaley, G. and Sun, D. (2010) Impaired flow-induced dilation of coronary arterioles of dogs fed a low-salt diet: roles of ANG II, PKC, and NAD(P)H oxidase. Am. J. Physiol. Heart Circ. Physiol. **299**, H1476–H1483

52 Holbrook, J. T., Patterson, K. Y., Bodner, J. E., Douglas, L. W., Veillon, C., Kelsay, J. L., Mertz, W. and Smith, Jr, J. C. (1984) Sodium and potassium intake and balance in adults consuming self-selected diets. Am. J. Clin. Nutr. **40**, 786–793

53 Leiba, A., Vald, A., Peleg, E., Shamiss, A. and Grossman, E. (2005) Does dietary recall adequately assess sodium, potassium, and calcium intake in hypertensive patients? Nutrition **21**, 462–466

54 Ekinci, E. I., Cheong, K. Y., Dobson, M., Premaratne, E., Finch, S., Macisaac, R. J. and Jerums, G. (2010) High sodium and low potassium intake in patients with Type 2 diabetes. Diabetes Med. **27**, 1401–1408

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