Analysis of the Association of Urine Sodium Excretion and Serum Aldosterone with Clinical Outcomes

A thesis

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Abstract

The relationship between dietary sodium intake and clinically important outcomes remains controversial. Recent large observational studies have suggested a U-shaped relationship between sodium intake and kidney disease progression, cardiovascular disease, and all-cause mortality, with an increased risk of adverse events occurring in persons consuming high and low levels of sodium. We hypothesized that serum aldosterone, which has known vasculopathic properties, may be higher in individuals who consume lower amounts of sodium and may explain the association of low sodium intake with an increased risk of adverse outcomes.

Using a case-cohort design in the Health Aging and Body Composition (Health ABC) Study, we assayed urine sodium, as a proxy for dietary sodium intake, and serum aldosterone. A subcohort (n=501) was randomly selected from the parent cohort of healthy individuals, and all additional cases who demonstrated progression of kidney disease, heart failure events, and cardiovascular events were added using the case-cohort method. We used linear regression to evaluate the association of urine sodium excretion and serum aldosterone at baseline. We used Cox proportional hazards to examine the associations of serum aldosterone levels and urine sodium levels with progression of kidney disease, incident heart failure, incident atherosclerotic cardiovascular disease (CVD), and all-cause mortality. We evaluated whether adjustment for serum aldosterone attenuated the relationship of urine sodium with these outcomes.

The mean (SD) age of the Health ABC subcohort was 73.6 (2.8) years; 49% were women, and 61% were white. Sixty-four percent of the subcohort had hypertension, and 54% of these individuals were on antihypertensive medications. The mean (SD) estimated urine sodium excretion in the subcohort was 4,174 (1,546) mg/24 hours while

median (25^{th}, 75^{th}) serum aldosterone was 5.12 (3.15, 8.85) ng/dL. There was an inverse relationship between urine sodium excretion and serum aldosterone; each 1 gram higher urine sodium excretion was associated with a 0.93 ng/dL lower serum aldosterone in multivariable adjusted analyses. In the entire case-cohort, there were 420 individuals who demonstrated progression of kidney disease, 220 incident heart failure events, 230 incident atherosclerotic CVD events, and 236 deaths. There was no association between serum aldosterone and progression of kidney disease, incident heart failure, or all-cause mortality in continuous or quartile models. Higher levels of serum aldosterone were associated with a lower risk of incident atherosclerotic CVD [HR per aldosterone doubling 0.85 (0.72, 0.99)] although the relationship was not linear. There was no association between increased urine sodium excretion and progression of kidney disease or all-cause mortality [HR per 1 standard deviation higher urine sodium 1.04 (0.89, 1.22) and 1.05 (0.92, 1.20) respectively], while lower levels of urine sodium showed a trend towards a higher risk of heart failure and atherosclerotic CVD [HR per 1 standard deviation higher urine sodium 0.92 (0.76, 1.10) and 0.87 (0.70, 1.08) respectively]. Serum aldosterone did not attenuate the relationship of low urine sodium with our principal study outcomes when examined as a continuous variable or according to quartiles.

Lower levels of urine sodium were associated with higher levels of serum aldosterone. There was no consistent relationship between serum aldosterone with any of the clinical outcomes examined. Lower levels of urine sodium showed a trend towards higher risk of heart failure and atherosclerotic CVD. Serum aldosterone did not explain the relationship of low urine sodium with the outcomes under study. Further studies are

needed to evaluate the mechanisms by which low urine sodium excretion may be associated with an increased risk of adverse outcomes.

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List of Abbreviations

ACR	Urine albumin to creatinine ratio
BMI	Body Mass Index
BP	Blood Pressure
CVD	Cardiovascular disease
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration rate
Health ABC	Health Aging and Body Composition
HF	Heart Failure
HTN	Hypertension
Na	Sodium

1. Introduction:

1.1: Dietary sodium and clinical outcomes

Sodium intake in the general population is high, with an average sodium intake of 3.4 grams per day (g/day) in the United States [1,2]. Several dietary guidelines have recommended a sodium intake of less than 2.0-2.4 g/day in the general population [3,4], however in a consensus statement in 2013, the Institute of Medicine recognized that while harm exists with excessive sodium intake, there was insufficient evidence to provide a numerical definition for sodium intake [1].

It is well accepted that a high intake of sodium increases blood pressure, and trials of sodium reduction have resulted in decreases in blood pressure and proteinuria [5-12]. Three recent meta-analyses of randomized control trials have observed a decrease in blood pressure after a reduction in sodium intake [13-15]. There have been fewer trial data, however, that have examined the association of low sodium intake with the development of clinically important outcomes including progression of kidney disease, cardiovascular disease and mortality [16]. Several observational studies and one meta-analysis have demonstrated a U-shaped relationship between urine sodium excretion, as a proxy for dietary sodium intake, with these outcomes [17-21] with increased mortality at both extremes of sodium excretion [17,22].

Although there are well-defined mechanisms to explain the relationship of high sodium intake with poor outcomes, there is less supportive evidence to explain the relationship between low sodium intake and an increased risk of adverse outcomes. Several factors have been hypothesized to contribute to this association including the possibility that low sodium intake may be a marker of underlying illness and, therefore,

difficult to adjust for in multivariable analyses [18]. Second, estimates of 24-hour urine sodium excretion may be biased when based on spot urine samples, particularly because studies often use only one urine sodium measurement [23]. Third, the small sample of persons included in prior studies which may have resulted in unreliable estimates of risk of adverse outcomes in those with low salt intake [20]. The U-shaped relationship between sodium intake and clinical outcomes has persisted, however, despite attempting to adjust for these limitations; inasmuch, the mechanisms underlying the association between sodium intake and adverse clinical outcomes remains unknown [17,18,21].

1.2 Serum aldosterone and clinical outcomes

Aldosterone is a mineralocorticoid steroid hormone that is produced by the adrenal glomerulosa. It is the final hormonal activation in the renin-angiontensin-aldosterone system (RAS) and leads primarily to increased distal tubular reabsorption of sodium in the kidney through binding to the mineralocorticoid receptor in the principal cells of the renal collecting tubule. In addition to the effect of increased sodium reabsorption, aldosterone leads to potentiation of angiotensin II, impairment of endothelial function, and reduction in vascular compliance [24-27].

Low sodium intake is known to be a stimulus for aldosterone production through volume mediated mechanisms [28-30]. In turn, elevated serum aldosterone levels may be associated with an increased risk of cardiovascular disease, and, in animal models, pathogenic vascular remodeling [31-34]. Using a population with primary aldosteronism, observational studies have reported increased odds of non-fatal myocardial infarction, as compared to individuals with essential hypertension. In this population, aldosterone

levels were 4-fold higher than those with essential hypertension, and the odds ratio for non-fatal myocardial infarction in the primary aldosterone individuals was 6.5 (95% confidence interval 1.5, 27.4) [34]. There is also emerging evidence in animal models that aldosterone directly contributes to kidney fibrosis, and post hoc analyses of data from clinical trials have suggested that aldosterone blocking agents may reduce albuminuria in individuals with chronic kidney disease (CKD) and proteinuria [35-39]. An observational study in individuals without CKD or hypertension has also described an increased risk of hypertension in those with higher serum aldosterone levels, after adjustment for age, sex, baseline blood pressure, and body mass index (BMI) [40]. This relationship remained significant after adjustment for urine sodium excretion and left ventricular hypertrophy, suggesting an aldosterone-dependent mechanism.

1.3 Relationship between dietary sodium and serum aldosterone with clinical outcomes

In this thesis, we assayed levels of urine sodium and serum aldosterone and evaluated the association between the two variables, and their relationship with clinically important outcomes in a cohort of healthy, elderly individuals enrolled in the Health Aging and Body Composition (Health ABC) Study. In Aim 1, we evaluated the association between levels of urine sodium and serum aldosterone in a cross-sectional analysis, with our working hypothesis being that lower urine sodium excretion is associated with higher levels of serum aldosterone. In Aim 2, we evaluated the association between serum aldosterone levels with progression of CKD, incident heart failure (HF), incident atherosclerotic cardiovascular disease (CVD), and all-cause mortality. We hypothesized that high serum aldosterone levels will be associated with a

higher risk of each of these events. In Aim 3, we evaluated the association between urine sodium excretion and the same outcomes as outlined in Aim 2, but with additional adjustment for serum levels of aldosterone. We hypothesized that there would be a U-shaped relationship between sodium excretion with our pre-specified clinical outcomes; however, the low urine sodium association will be attenuated by adjustment for serum aldosterone levels. We also considered interactions between serum aldosterone and urine sodium.

2. Methods

2.1 Study Population

The Health Aging and Body Composition (Health ABC) Study is a longitudinal cohort study of functional, elderly individuals aged 70 to 79 years old. Participants were enrolled between April, 1997, and July, 1998. Participants were identified from a random sample of Medicare beneficiaries from Pittsburgh, Pennsylvania and Memphis, Tennessee. Criteria for inclusion required self-reported ability to walk ¼ mile, climb ten stairs, perform activities of daily living, and the absence of life threatening diseases. The cohort was followed yearly for the first five years and then biennially for an additional five years. Yearly study visits included collection of demographic and laboratory data to capture overall health status. Baseline serum and urine samples were collected, as was information about risk factors for CVD and CKD, a comprehensive clinical examination and self-reported questionnaire which ascertained additional comorbid conditions and medication use. Written, informed consent was obtained from the University of Pittsburgh and the University of Tennessee, Knoxville. The following analyses using de-identified data were approved by the institutional review boards at the University of California, San Francisco and Tufts Medical Center.

2.2 Study Design

Due to the costs of measuring urine sodium and serum aldosterone, a cost-efficient case-cohort design that could test multiple outcome associations was chosen. A case-cohort study is a type of case-control study that uses a sub-sampling technique from the entire parent cohort in order to identify controls. Selection of controls in the case-

cohort is done at time zero, after baseline inclusion into the cohort and is irrespective of development of the particular outcome. This random selection of controls is known as the sub-cohort and can be used as a control for multiple outcomes. Thus, for an exposure variable of interest, the entire sub-cohort can be used as a control for a variety of outcomes without repeating testing on a newer control population. In this design, cases can arise from the subcohort as well as from the parent cohort.

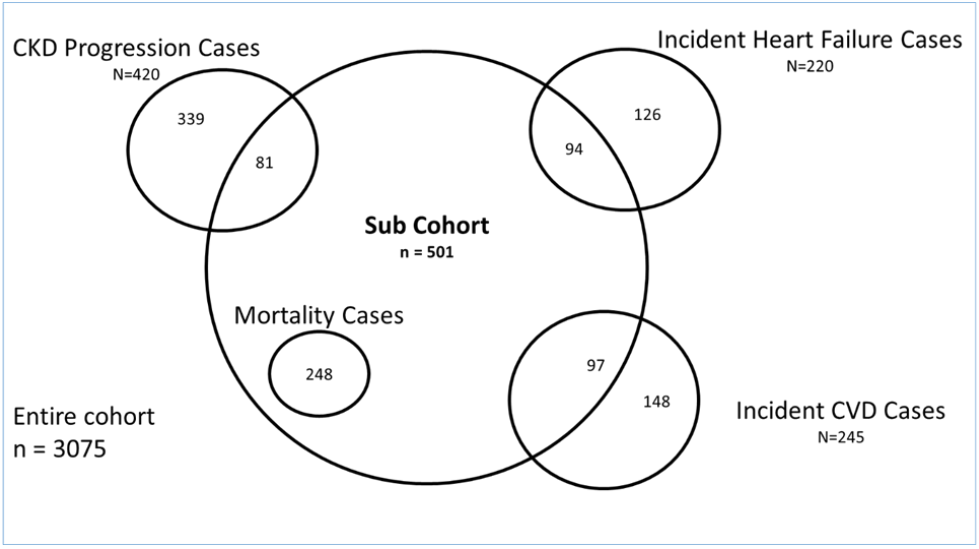
The advantage of using a case-cohort study design includes efficiency in measurement of exposure variables. In this study design, aldosterone measurement, which is expensive and time consuming, was measured in 1/3 of the entire cohort (n=1087), and this subset was used for multiple hypothesis testing, allowing flexibility in post-hoc analyses of large cohort studies. Cases and non-cases are selected from the same population, thus minimizing a potential selection bias because of derivation from the same parent cohort. This particular study design can also provide person-time risk, provided the size of the subcohort is at least 10% of the parent cohort. In prior statistical modeling, it was determined that if a cohort size is >10%, estimates of risk can be comparable to the full cohort [41].

Statistical analysis of a case-cohort study design requires a weighted cox-proportional hazards model for longitudinal study questions. Those arising from the subcohort are given a relative weight at the time the outcome is observed, and additional cases arising from the parent cohort do not contribute to the earlier risk set. This is calculated using a pseudo-likelihood, which is different from a partial likelihood in standard longitudinal analyses because the sum of subjects at risk at a given time point is used instead of the subjects at risk in the entire cohort. Each contribution to the

likelihood of failure is not independent in the pseudo-likelihood calculation, which is different from the partial likelihood used in a nested case control design. Thus, the cases and all available controls at a particular time point (time Tj) are in a risk set that is set by the time of the outcome, or failure time. Cases are not added into the likelihood ratio until this time Tj to avoid bias based on future knowledge of cases becoming cases [42].

Figure 1 depicts the sampling design for this case-cohort study. This sampling strategy has been used in other cohort studies to evaluate associations between kidney biomarkers and multiple outcomes, and this particular case-cohort selection process was done as part of a larger study to measure urine analytes within the Health ABC Study [43,44]. In the entire Health ABC cohort of 3,075 individuals, a subcohort of 502 individuals was randomly selected. One individual was excluded due to missing baseline urine sodium.

Figure 1: Case-cohort study sampling from Health ABC Cohort. Selections of all cases from the parent cohort were added to cases arising from the randomly selected subcohort. Numeric values within diagram depict the number of individuals with the respective outcomes.



In Aim 1, we evaluated the cross-sectional relationship between urine sodium excretion and serum aldosterone levels using the randomly selected subcohort. In this subcohort, 30 individuals did not have a measured aldosterone level due to lack of stored sera. Thus, the total number of participants for Aim 1 was 471.

For the assessment of the associations of urine sodium and serum aldosterone to outcomes, we first evaluated whether we had sufficient power within the subcohort to address these questions. We ascertained that for mortality, a minimum detectable hazard ratio per SD increase was 1.30 and thus no additional cases were required for addition. However, for progression of kidney disease, incident HF, and incident atherosclerotic CVD, we did not have sufficient power within the subcohort and used the case-cohort study design to assess associations. From the entire cohort of Health ABC, 420 individuals met the criteria for progression of kidney disease. Of these, 81 were from the subcohort and 339 were not. Two hundred and twenty individuals developed incident HF. Of these, 94 were from the subcohort, and 126. Two hundred and forty individuals developed incident atherosclerotic cardiovascular disease. Of these, 92 were from the subcohort and 148 were not. From the case-cohort data, 49 individuals had insufficient volume for serum aldosterone measurement and thus were excluded from the analysis. Power calculations were performed using a two-sided α of 0.05 and 80% power to calculate the minimal detectable hazard ratios per standard deviation of exposure variable.

Table 1: A priori power calculation using the case-cohort study design

Outcomes	# events	Minimal detectable HR (per SD increase)
Progression of Kidney Disease	453	1.03
Incident Heart Failure	193	1.21
Incident Atherosclerotic CVD	217	1.17
All-cause mortality	145	1.30
Legend: Power was calculated using information known about the Health ABC cohort at the time of completion. Minimal detectable hazard ratios were calculated per standard deviation increase in exposure variable (urine sodium excretion) for 80% power.		

For each incident HF and CVD events, only half of the cases were included that arose from the parent cohort. Rather, each case was identified from the entire cohort and random sample of these were chosen to augment the cases arising from the subcohort. This random selection of HF and CVD cases does not influence the statistical analysis.

2.3 Analyte measurement of serum aldosterone and urine sodium

Aldosterone was measured using serum samples from baseline. A total of 9 (0.8%) samples had been thawed two times prior to the current assay, and 878 (25%) of samples were thawed once prior to the current assay. Prior data has supported the stability of aldosterone after 3 freeze-thaw cycles [45-47]. Serum aldosterone was measured using AB Sciex 6500 liquid chromatography and tandem mass spectrometry (LC/MS/MS) at the University of Minnesota Pathology Laboratory. Limit of quantification was 4 ng/dL, at which point the intra-assay coefficient of variation was 13.9% (95% CI 7.8, 20.0).

Urine sodium was measured on spot urine samples at baseline and was transformed to estimate 24-hour urine sodium excretion using the Kawasaki formula [48,49]. Spot urine sodium was measured using Siemens QuikLYTE Integrated Multisensor.

Formula 1: Kawasaki formula for predicted creatinine excretion (24hUCr, mg/day):

$$Female\ 24hUCr = (-4.72 * age) + (8.58 * weight) + (5.09 * height) - 74.5$$

$$Male\ 24hUCr = (-12.63 * age) + (15.12 * weight) + 7.39 * height) - 79.9$$

Formula 2: Kawasaki formula for estimated 24-hour urine sodium excretion (UNa, mEq/day):

$$24h\ UNa\ = 16.3 * \sqrt{XNa}$$

$$XNa = \frac{SpotUNa}{SpotUCr} *\ 24h\ UCr$$

24-hour urine sodium excretion was transformed to mg/day as standard units by multiplying by the molecular weight of sodium (22.9897 mg/mmol).

2.4 Definition of Covariates

All covariates were collected at baseline. Age, race, gender, and smoking status were obtained by a questionnaire administered by an interviewer. Smoking status was classified into three categories: never, current, or former smoker, with former smokers requiring exposure to >100 cigarettes within their lifetime. Hypertension was defined by the presence of one of the following: 1) systolic BP ≥ 140 mm Hg; 2) diastolic BP ≥ 90 mm Hg; 3) use of antihypertensive medications and self-report of diagnosis of hypertension. Diabetes mellitus was defined by self-report of diagnosis and use of anti-diabetic medications. Prevalent atherosclerotic CVD was defined as a history of

myocardial infarction, angina, cerebrovascular accident (either stroke or transient ischemic attack), or any vascular surgery. Prevalent HF was considered to be present if a physician had diagnosed the participant with HF. Systolic and diastolic BP was measured by trained and certified clinical staff using a mercury sphygmomanometer while the study participant was in a seated position. An average of two readings was used for systolic and diastolic blood pressures. Height was measured with a Harpenden stadiometer (Holtain, Crosswell, United Kingdom) while the study participant was standing. Weight was measured using a standard clinic scale. BMI was calculated from these measurements. Medications taken within the past two weeks were physically brought into each study visit, recorded, and coded using the Iowa Drug Information System. Diuretic use included thiazide, loop, and other diuretics. Angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blocker (ARB) use was also recorded and defined as renin-angiotensin aldosterone system (RAS) blockade. Low density lipoprotein (LDL) cholesterol was estimated using the Friedwald equation, derived from total and high density cholesterol and serum triglycerides[50]. The latter three measurements were measured using a Johnson & Johnson Vitros 950 analyzer. Urine albumin was measured using a particle-enhanced turbidimetric inhibition immunoassay. Urine albumin: creatinine ratio (ACR) was calculated as the covariate of interest.

2.5 Definition of Clinical Outcomes

Four clinical outcomes were evaluated: progression of kidney disease, incident HF, incident atherosclerotic CVD, and all-cause mortality.

Progression of Kidney Disease

Glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equation using serum cystatin C concentration, age, sex, and race. Cystatin C was measured using a BNII nephelometer (Dade Behring Inc, Deerfield, IL) with a particle-enhanced immunonepholometric assay (N Latex Cystatin C)[51]. Cystatin C-based equations were chosen for assessment of eGFR rather than creatinine-based equations because creatinine measurements were not traceable to isotope dilution mass spectrometry (IDMS) between study visits, therefore the accuracy for estimation of GFR using creatinine based equations is not known. Cystatin C measurements were calibrated between visits, similar to prior experiments [52]. Progression of kidney disease was defined as a 30% decline in estimated GFR (eGFR) at years 3 or 10, as this outcome over 2-3 years has been associated with progression to ESRD and kidney failure [53,54]. There were insufficient events of kidney failure in this population and thus, this outcome was not evaluated.

Incident Heart Failure

Incident HF was defined as a physician diagnosis of heart failure and (1) documentation of signs and symptoms in medical record (eg, dyspnea, orthopnea, paroxysmal nocturnal dyspnea, fatigue, edema, adventitious lung sounds, or cardiovascular gallop); (2) diagnostic imaging studies of either chest x-ray showing evidence of pulmonary edema or echocardiographic evidence of heart failure; (3) therapeutic intervention for heart failure, including a diuretic and either a vasodilator or digitalis. Incident HF was analyzed separately from atherosclerotic CVD because of previous studies had suggested that measures of kidney function are more strongly associated with HF as compared with myocardial infarction [55,56]. This outcome was ascertained by interviews between each

participant and site coordinators every 6 months. When a hospitalization was reported by a study participant, all medical records were reviewed by each site investigator. Clinical outcomes were ultimately adjudicated by the Health ABC Study Diagnosis and Disease Ascertainment Committee.

Incident Atherosclerotic Cardiovascular Disease

Incident atherosclerotic CVD was defined as receipt of elective coronary revascularization (surgical or percutaneous), hospitalization for myocardial infarction or angina pectoris with use of anti-anginal medications, and stroke (both fatal and nonfatal). All cardiovascular outcomes were adjudicated using criteria adapted from the Cardiovascular Health Study [57,58]. Atherosclerotic CVD events were ascertained from bi-annual interviews between each participant and site coordinators, review of all medical records if a hospitalization occurred, and were adjudicated by the Health ABC Study Diagnosis and Disease Ascertainment Committee.

All-cause Mortality

All-cause mortality was adjudicated by the Health ABC Study Diagnosis and Disease Ascertainment Committee after review of all hospital records, death certificates, and autopsy data to ascertain the underlying cause of death.

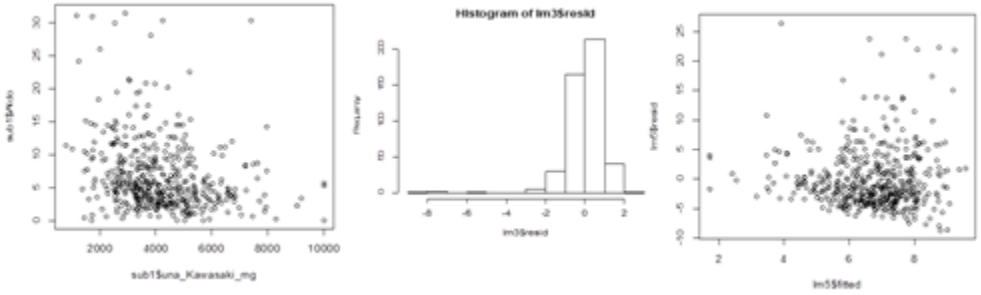
2.6 Statistical Analysis

Using the subcohort, summary statistics were calculated using means and standard deviations for normally distributed baseline covariates and median (Interquartile Range (IQR) as 25^{th}, 75^{th} quartiles) for those that had a skewed distribution.

Using only the subcohort, the association between urine sodium excretion and serum aldosterone was evaluated in a cross-sectional analysis. The distribution of covariates was compared across quartiles of urine sodium excretion using analysis of variance (ANOVA) and χ^2 testing for continuous and categorical covariates, respectively. For covariates with skewed distribution, Kruskal-Wallis test was performed for continuous variables and Fisher's Exact Test was performed for categorical variables. Pearson correlation was used to calculate a correlation coefficient for the linear relationship between urine sodium excretion and serum aldosterone. Linear regression was used to model the relationship between urine sodium excretion (per 1 gm higher) and serum aldosterone, after adjustment for age, race, gender, BMI, diabetes mellitus, hypertension, systolic BP, LDL cholesterol, smoking status, estimated GFR, ACR, any use of RAS blockade, and any diuretic use. A linear relationship was observed between urine sodium excretion and serum aldosterone. Assumptions of normality of residuals and homoscedasticity were met as well, so no transformation of either variable was performed.

In order to use a linear regression model to statistically evaluate the relationship between urine sodium excretion and serum aldosterone, linear regression assumptions were tested. A linear relationship was observed between urine sodium excretion and serum aldosterone (Figure 2). The assumption of normality of residuals was also met (Figure 2). Homoskedasticity assumption was met, showing that thee variance of serum aldosterone at every value of urine sodium excretion is the same (Figure 2). Finally, all observations were independent.

Figure 2: Assumptions of linear regression were evaluated and confirmed. (a) Scatter plot showing the linear relationship between urine sodium excretion (x axis) and serum aldosterone (y axis). (b) Histogram of residuals using linear modeling confirmed a normal distribution. (c) Scatter plot showing the random relationship between residual and fitted values of the linear regression model. All assumptions were tested using the fully adjusted model (Model 3).



The distribution of covariates was compared across quartiles of serum aldosterone using similar methods described for Aim 1. Event rates were calculated using incidence rates from the subcohort only for each clinical outcome. Sequentially adjusted multivariable models were fitted using a weighted Cox proportional hazards model, which weights the hazard of development of an outcome at the time of inclusion into the cohort and thus does not contribute risk prior to the addition of that particular case. Cases arising from the subcohort were weighed using the inverse of the sampling fraction. Since all-cause mortality events arose exclusively from the subcohort (with no additional case sampling) we analyzed the mortality outcome using standard Cox proportional hazards regression. Serum aldosterone was log transformed due to the skewed distribution, and analyses were performed per doubling of serum aldosterone in order to provide meaningful interpretation of the association with outcomes. Transformation was employed to eliminate the risk of bias introduced from outliers as

well as to provide a more meaningful interpretation of results. Models were constructed a priori based on clinical knowledge and included the following: Unadjusted: serum aldosterone (log transformed); Model 1: Age, Sex, Race; Model 2: Model 1 + BMI, diabetes, hypertension, systolic BP, LDL cholesterol, and smoking status; Model 3: Model 2 + eGFR + ACR + diuretic use + RAS blockade; Model 4: Model 3 + urine sodium excretion. Assumptions were tested using validated methods for weighted Cox proportional hazards models. Each model was evaluated using a continuous and quartile analysis between serum aldosterone and each outcome. In exploratory analysis, we evaluated nonlinear relationships between serum aldosterone and clinical outcomes using model 3 and modeling using restricted cubic splines.

The assumptions of the Cox proportional hazards were assessed for the outcome of mortality (Figure 3). Mortality is the only outcome assessed using the standard Cox proportional hazards model due to the study design, as described previously. The proportional hazards assumption assumes that the hazard is constant over time. This was addressed by evaluating Schoenfeld residuals against time. There was no correlation between Schoenfeld residuals and time, thus the proportional hazards assumptions were met using log-transformed aldosterone (global p =0.61). Schoenfeld residuals for each predictor variable were also plotted. Log transformation was done to provide a more meaningful outcome interpretation. Using nontransformed aldosterone, linear relationship was also not entirely met, therefore additional analysis using categories of aldosterone must be explored in future analyses (Figure 4.

Figure 3: Representative Schoenfeld Plots for (a) serum aldosterone and (b) urine sodium excretion with age.

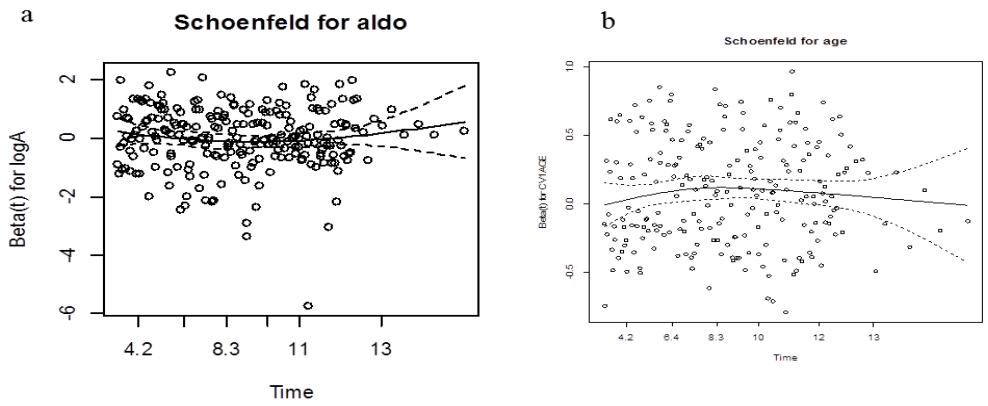
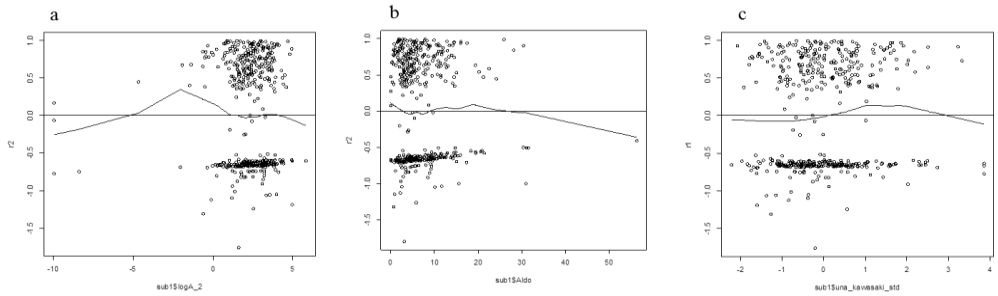


Figure 4: Martingale residuals for (a) log transformed aldosterone (b) non-transformed aldosterone (nontransformed) and (c) urine sodium excretion as a continuous variable.



The following sensitivity analyses were performed to evaluate serum aldosterone levels above and below the accurate limit of quantification: (a) Inclusion of only samples with serum aldosterone levels greater than 4 ng/dL; (b) Stratifying serum aldosterone levels into tertiles, with tertile 1 comprised of all 35% of samples below the limit of quantification.

For the final aim to evaluate urine sodium excretion and clinical outcomes, modified by aldosterone, we employed similar statistical techniques as above. The exposure variable of interest was urine sodium excretion. Analyses were again performed using weighted Cox proportional hazards models for progression of kidney

disease, HF, atherosclerotic CVD, and standard Cox proportional hazards models for mortality. The final model (model 4) was adjusted for serum aldosterone, as specified in the hypothesis for Aim 3. Pre-specified interactions were performed between urine sodium excretion and serum aldosterone levels. In an exploratory analysis, we used restricted cubic splines to graphically evaluate the association between urine sodium excretion and the hazard for each clinical outcome, using Model 3 adjustments. The reference point chosen was the mean urine sodium excretion of the subcohort and used only data from the subcohort for outcomes.

The assumptions of the Cox proportional hazards were assessed for the outcome of mortality. Mortality is the only outcome assessed using the standard Cox proportional hazards model due to the study design, as described previously. The proportional hazards assumption assumes that the hazard is constant over time. This was addressed by evaluating Schoenfeld residuals against time, similar to Aim 2. There was no correlation between schoenfeld residuals and time, thus the proportional hazards assumptions were met using log-transformed aldosterone (global p =0.86). Schoenfeld residuals for each predictor variable was also plotted (Figure 3). Figure 4c shows the Martingale plots evaluating the linearity assumption for urine sodium excretion in all-cause mortality. The assumption of linearity was met. Additional transformations using polynomial function did not improve the shape of the Martingale plot. All statistics were done using R version 3.1.1 (Vienna, Austria, URL: http://www.R-project.org).

3. Results

3.1 Baseline Characteristics

The mean (SD) age of the subcohort was 73.6 (2.8) years, 49% were women, and 61% were white. The mean (SD) estimated urine sodium excretion was 4,174 (1,546) mg/24 hours while the median (IQR) serum aldosterone level was 5.12 (3.15, 8.85) ng/dL. Thirty-five percent of aldosterone values were measured below the limit of quantification. Mean (SD) eGFR was 73.1 (18.3) mL/min/1.73m2 and median (IQR) ACR was 8.4 (3.88, 21.60) mg/gm. Baseline characteristics across quartiles of sodium excretion are summarized in **Table 2**. Higher sodium excretion was noted among men as well as individuals with cardiovascular disease. Greater sodium intake was also significantly associated with higher baseline systolic BP and higher levels of albuminuria.

Table 2: Participants characteristics, according to quartiles of baseline urine sodium excretion within subcohort

	Q1 (n=125)	**Q2 (n=126)**	**Q3 (n=124)**	**Q4 (n=126)**
Urine Na excretion*	796-3,134	3,134-3,998	3,998-5,030	5,030-10,000
Age, years	74 (3)	74 (3)	74 (3)	74 (3)
Male, No. (%)	49 (42)	55 (48)	65 (58)	70 (59)
Black race, No. (%)	52 (44)	42 (35)	44 (37)	47 (40)
BMI (kg/m^2)	26.8 (4.2)	26.8 (4.7)	27.2 (4.0)	28.0 (4.8)
Diabetes mellitus, No. (%)	30 (26)	30 (26)	22 (20)	28 (24)
Hypertension, No. (%)	75 (63)	70 (63)	73 (61)	85 (71)
Prevalent CVD, No. (%)	31 (27)	21 (19)	29 (26)	45 (38)
Prevalent HF, No. (%)	5 (4.1)	2 (2.4)	3 (3.2)	5 (4.0)

LDL (mg/dL)	121.3 (35.1)	121.2 (36.6)	119.4 (34.8)	120.8 (32.9)
Smoking history, No. (%)				
Never	50 (41)	56 (45)	42 (36)	49 (39)
Former	58 (50)	50 (46)	67 (56)	63 (55)
Current	10 (9)	11 (9)	9 (8)	6 (5)
Systolic BP (mm Hg)	132 (19)	135 (20)	132 (20)	139 (23)
Diastolic BP (mm Hg)	70 (11)	73 (12)	68 (12)	71 (12)
eGFR (mL/min/1.73m^2)	72.6 (18.7)	71.5 (17.8)	74.1 (18.2)	74.2 (18.5)
Urine ACR (mg/g)*	6.9 (4.0, 15.2)	7.6 (3.8, 20.4)	7.5 (4.5, 14.7)	13.4 (5.8, 33.4)
Aldosterone (ng/dL) *	7.2 (4.8, 11.6)	5.5 (3.6, 8.6)	4.5 (2.8, 8.0)	4.0 (2.4, 6.2)
Diuretic use, %	33 (28)	26 (22)	27 (23)	32 (28)
Thiazide	25 (21)	17 (14)	21 (18)	24 (20)
Loop	6 (5)	8 (7)	6 (5)	6 (5)
Other	16 (14)	9 (8)	8 (7)	13 (11)
RAS Blockade, No. (%)	12 (10)	21 (18)	18 (15)	19 (16)
Any BP med, No. (%)	71 (57)	55 (44)	58 (47)	70 (56)

Legend: Urine sodium excretion was estimated using the Kawasaki Equation (mg/day); BMI = body mass index; LDL = Low density lipoprotein cholesterol; Systolic BP = mean (SD) systolic blood pressure; Diastolic BP = mean (SD) diastolic blood pressure; eGFR = estimated glomerular filtration rate using CKD-EPI equation for cystatin; Urine ACR = urine albumin-to-creatinine ratio; Diuretic use = Any diuretic use; RAS blockade = Use of medications blocking renin-angiotensin-system; BP = blood pressure.
All values are represented as % or mean +/- s.d. except those marked with *. * Values represent median (25^{th}, 75^{th} interquartile ranges)

3.2: Cross-sectional association between urine sodium excretion and serum aldosterone

Urine sodium excretion was inversely associated with serum aldosterone, with a correlation of -0.27 (p=<0.001) **(**Figure 5**)**. For each 1 gram of higher urine sodium excretion, there was a 0.93 ng/dl lower serum aldosterone in multivariable adjusted analyses (Table 3**)**. One individual had dramatically elevated serum aldosterone levels (56 ng/dL). Removal of this individual did not significantly change the results.

Figure 5: Graph of linear regression model for urine sodium excretion and serum aldosterone relationship, adjusted for age, race, gender, BMI, diabetes, hypertension, systolic BP, LDL, smoking history, eGFR, ACR, any diuretic use, and RAS inhibition.

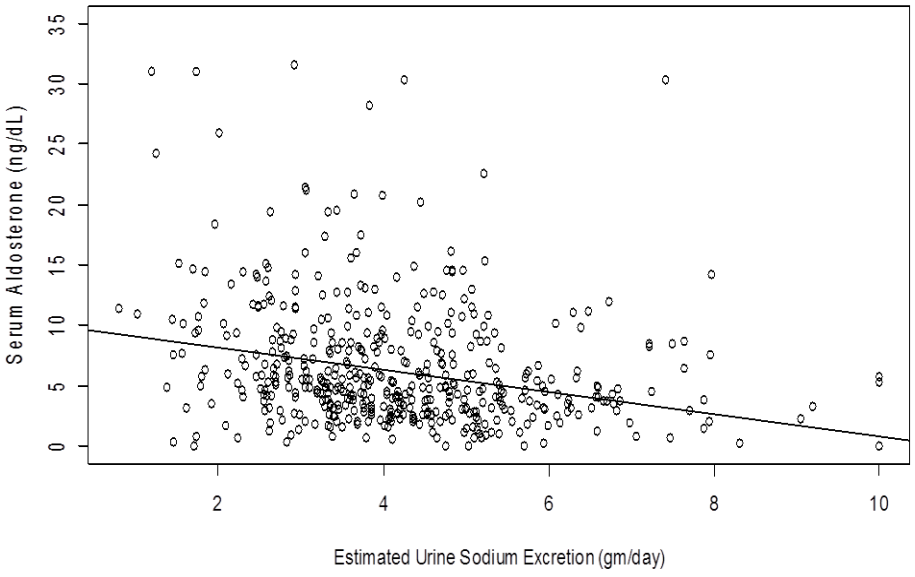


Table 3: Linear relationship between urine sodium excretion and serum aldosterone

	B-coefficient (per 1 gm ↑Una)	**P value**
Unadjusted	-0.89 (-1.22, -0.56)	<0.001
Model 1	-0.89 (-1.23, -0.56)	<0.001
Model 2	-1.00 (-1.33, -0.67)	<0.001
Model 3	-0.96 (-1.31, -0.63)	<0.001
Model 4	-0.93 (-1.24, -0.60)	<0.001
Legend: B-coefficients presented per 1 gram higher urine sodium excretion Model 1=Urine sodium excretion + age + race + gender Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN+ systolic BP + LDL + smoking history Model 3 = Model 2 + eGFR+ urine ACR Model 4 = Model 3 + RAS blockade + any diuretic use		

3.3: Association between serum aldosterone and clinical outcomes

Higher levels of aldosterone were associated with lower use of RAS blockade, lower sodium intake, increased use of diuretics, and lower eGFR in the subcohort (Table 4**)**.

Table 4: Baseline participant characteristics, according to serum aldosterone level quartiles

	Q1 (n=118)	**Q2 (n=118)**	**Q3 (n=118)**	**Q4 (n=117)**
Aldosterone (ng/dL)	0-3.15	3.15-5.12	5.12-8.88	8.88-57
Age, years	74 (3)	73 (3)	74 (3)	74 (3)
Male, No. (%)	63 (54)	68 (58)	44 (38)	62 (53)
Black race, No. (%)	54 (46)	39 (33)	51 (44)	41 (35)
BMI (kg/m^2)	26.8 (4.7)	26.3 (3.9)	27.6 (4.7)	27.8 (4.3)

Diabetes mellitus, No. (%)	29 (25)	24 (20)	27 (23)	33 (28)
Hypertension, No. (%)	68 (58)	72 (61)	80 (68)	81 (69)
Prevalent CVD, No. (%)	30 (25)	30 (25)	34 (29)	29 (25)
Prevalent HF, No. (%)	4 (3)	2 (2)	4 (3)	5 (4)
LDL	122.3 (34.0)	120.3 (33.0)	116.5 (33.9)	119.2 (35.0)
Smoking history, No. (%)				
Never	54 (46)	45 (38)	47 (40)	51 (44)
Former	57 (48)	65 (55)	55 (47)	60 (51)
Current	7 (6)	8 (7)	15 (13)	5 (5)
Systolic BP (mm Hg)	136 (23)	134 (19)	136 (19)	133 (22)
Diastolic BP (mm Hg)	68 (12)	71 (11)	71 (12)	70 (10)
eGFR (mL/min/1.73m^2)	74.3 (18.2)	75.6 (18.1)	73.7 (17.3)	69.6 (18.9)
Urine ACR (mg/g)*	7.4(4.3,20.0)	7.9 (4.4,16.5)	8.2(4.4,18.8)	8.2 (4.3,23)
Urine sodium, mg/day	4,534 (1,410)	4,429 (1,448)	4,056 (1,553)	3,579 (1,380)
Diuretic Use, No. (%)	13 (11)	20 (17)	37 (31)	49 (42)
Thiazide	8 (7)	15 (13)	24 (20)	41 (35)
Loop	4 (3)	4 (3)	11 (10)	7 (6)
Other	2 (2)	5 (4)	14 (12)	23 (21)
RAS Blockade use, No. (%)	25 (21)	20 (17)	17 (14)	9 (8)
Any BP Medication, No. (%)	52 (44)	52 (44)	68 (58)	80 (68)

Legend: BMI = body mass index; LDL = Low density lipoprotein cholesterol; Systolic BP = mean (SD) systolic BP; Diastolic BP = mean (SD) diastolic BP; eGFR = estimated glomerular filtration rate using CKD-EPI equation for cystatin; Urine ACR = urine albumin-to-creatinine ratio; Urine sodium = estimated urine sodium excretion; RAS blockade = Use of medications blocking renin-angiotensin-system; BP = blood pressure.
All values are represented as % or mean +/- s.d. except those marked with *. * Values represent median (25^{th}, 75^{th} interquartile ranges)

Progression of Kidney Disease

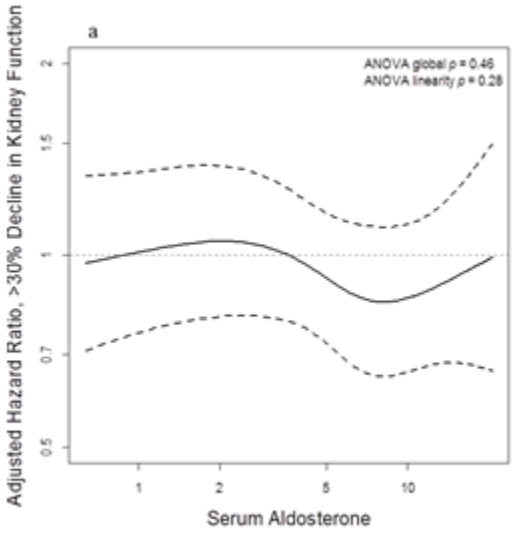
There were 2.85 progression of kidney disease events per 100 person-years. In models adjusted for baseline demographics, risk factors for CVD and progression of kidney disease, and urine sodium excretion, there was no association between serum aldosterone and progression of kidney disease **(**Table 5**)**. In a fully adjusted quartile analysis, there was a trend towards a lower hazard of progression of kidney disease in the highest quartile of serum aldosterone in comparison with lowest quartile [HR 0.73 per doubling of serum aldosterone (0.46, 1.17)], although a non-linear relationship was not observed between quartiles (Table 5, Figure 6). Interaction with urine sodium excretion did not significantly change the association between levels of serum aldosterone and progression of kidney disease (p=0.67).

Table 5: Association between serum aldosterone and progression of kidney disease

	Continuous Model*	**Q1**	**Q2**	**Q3**	**Q4**
	Progression of Kidney Disease				
Number at risk	471	198	200	198	199
Events	399	97	97	108	97
Event Rate	2.85	1.38	1.35	1.66	1.83
Unadjusted	1.00 (0.91, 1.09)	1.00 [Ref]	0.75 (0.48, 1.16)	1.07 (0.70, 1.64)	0.80 (0.51, 1.25)
Model 1	1.00 (0.92, 1.10)	1.00 [Ref]	0.75 (0.48, 1.16)	1.08 (0.70, 1.67)	0.84 (0.54, 1.31)
Model 2	1.00 (0.92, 1.10)	1.00 [Ref]	0.80 (0.52, 1.25)	1.03 (0.66, 1.59)	0.73 (0.46, 1.17)
Model 3	0.99 (0.91, 1.09)	1.00 [Ref]	0.77 (0.50, 1.18)	1.05 (0.69, 1.60)	0.72 (0.45, 1.14)
Model 4	1.01 (0.92, 1.10)	1.00 [Ref]	0.77 (0.50, 1.18)	1.06 (0.70, 1.61)	0.73 (0.46, 1.17)

Legend:
*Hazard ratios are reported per doubling serum aldosterone. Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Model outcomes are reported as HR (95% confidence intervals) and are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + urine sodium excretion

Figure 6: Restricted cubic splines for serum aldosterone (ng/dL) levels and progression of kidney disease (30% decline in eGFR), modeled using model 3. Dotted lines represent 95% confidence intervals.



Heart Failure

There were 1.92 incident heart failure events per 100 person-years. In models adjusted for baseline demographics, risk factors for CVD and progression of kidney disease, and urine sodium excretion, there was no significant relationship between aldosterone and incident HF. In comparison with the lowest quartile of serum aldosterone, there was a

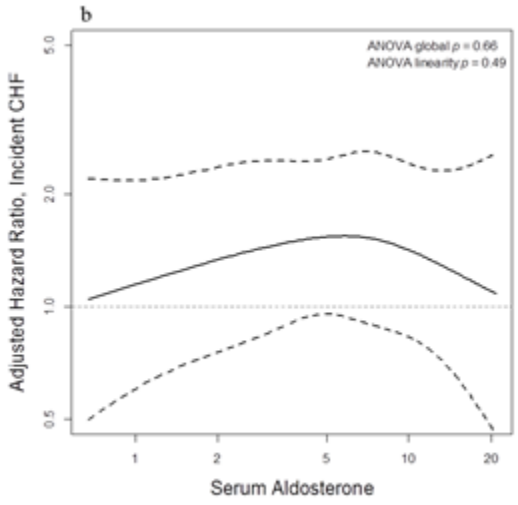
nonlinear relationship among higher quartiles of serum aldosterone (Table 6, Figure 7). Interaction with urine sodium excretion did not significantly change the association between levels of serum aldosterone and incident heart failure (p=0.81).

Table 6: Association between serum aldosterone and incident heart failure

	Continuous Model*	**Q1**	**Q2**	**Q3**	**Q4**
	Incident Heart Failure				
Number at risk	567	134	147	184	138
Events	223	42	49	86	46
Event Rate	1.92	1.62	2.06	3.02	1.62
Unadjusted	1.08 (0.97, 1.22)	1.00 [Ref]	1.05 (0.64, 1.73)	1.87 (1.16, 3.03)	1.06 (0.64, 1.76)
Model 1	1.09 (0.97,1.23)	1.00 [Ref]	1.04 (0.63, 1.72)	2.00 (1.23, 3.27)	1.04 (0.63, 1.74)
Model 2	1.05 (0.93, 1.18)	1.00 [Ref]	1.03 (0.61, 1.74)	1.83 (1.09, 3.05)	0.88 (0.51, 1.52)
Model 3	1.04 (0.93, 1.17)	1.00 [Ref]	0.98 (0.56, 1.66)	1.60 (0.96, 2.66)	0.79 (0.45, 1.40)
Model 4	1.03 (0.92, 1.17)	1.00 [Ref]	0.98 (0.58, 1.66)	1.56 (0.93, 2.61)	0.76 (0.42, 1.36)

Legend:
*Hazard ratios are reported per doubling serum aldosterone. Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Model outcomes are reported as HR (95% confidence intervals) and are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + urine sodium excretion

Figure 7: Adjusted restricted cubic splines for serum aldosterone (ng/dL) and incident heart failure, modeled using model 3. Dotted lines represent 95% confidence intervals.



Atherosclerotic Cardiovascular Disease

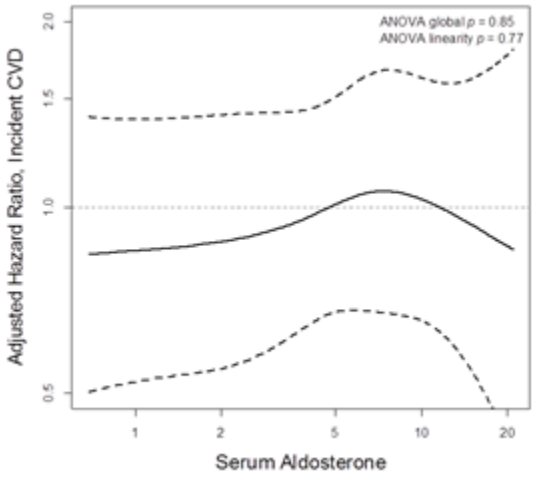
There were 2.63 incident CVD events per 100 person-years. In models adjusted for baseline demographics, risk factors for CVD and progression of kidney disease, and urine sodium excretion, levels of serum aldosterone were inversely associated with incident atherosclerotic CVD [HR 0.85 per doubling of serum aldosterone (0.72, 0.99)]. In quartile analyses, the highest quartile was associated with a lower risk of atherosclerotic CVD in comparison with the lowest quartile [HR 0.43 per doubling serum aldosterone (0.23, 0.79)] although the relationship was not linear across quartiles (Table 7, Figure 8). A non-linear relationship was also observed between serum aldosterone per doubling and the adjusted hazard ratio for incident atherosclerotic CVD (Figure 8). Interaction with urine sodium excretion did not significantly change the association between levels of serum aldosterone and incident atherosclerotic CVD (p=0.43).

Table 7: Association between serum aldosterone and incident atherosclerotic CVD

	Continuous Model*	**Q1**	**Q2**	**Q3**	**Q4**
	Incident Atherosclerotic Cardiovascular Disease				
Number at risk	475	119	118	119	119
Events	230	61	54	68	47
Event Rate	2.63	2.96	2.00	3.46	2.26
Unadjusted	0.92 (0.79, 1.07)	1.00 [Ref]	0.83 (0.50, 1.37)	1.28 (0.78, 2.12)	0.63 (0.38, 1.04)
Model 1	0.94 (0.81, 1.10)	1.00 [Ref]	0.85 (0.50, 1.42)	1.35 (0.80, 2.26)	0.64 (0.38, 1.08)
Model 2	0.91 (0.77, 1.07)	1.00 [Ref]	0.98 (0.56, 1.69)	1.29 (0.74, 2.24)	0.57 (0.32, 1.01)
Model 3	0.87 (0.74, 1.03)	1.00 [Ref]	0.80 (0.46, 1.41)	1.25 (0.72, 2.16)	0.48 (0.27, 0.87)
Model 4	0.85 (0.72, 0.99)	1.00 [Ref]	0.80 (0.45, 1.39)	1.19 (0.68, 2.08)	0.43 (0.23, 0.79)

Legend:
*Hazard ratios are reported per doubling serum aldosterone. Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Model outcomes are reported as HR (95% confidence intervals) and are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + urine sodium excretion

Figure 8: Adjusted restricted cubic splines for serum aldosterone (ng/dL) and incident CVD, modeled using model 3. Dotted lines represent 95% confidence intervals.



All-Cause Mortality

There were 4.73 mortality events per 100 person-years. In models adjusted for baseline demographics, risk factors for CVD and progression of kidney disease, and urine sodium excretion, there was no significant relationship between levels of serum aldosterone and all-cause mortality [HR 0.99 per doubling serum aldosterone (0.91, 1.07)]. Similarly, there was no consistent relationship in quartile analysis (Table 8, Figure 9). Interaction with urine sodium excretion did not significantly change the association between levels of serum aldosterone and all-cause mortality (p=0.58).

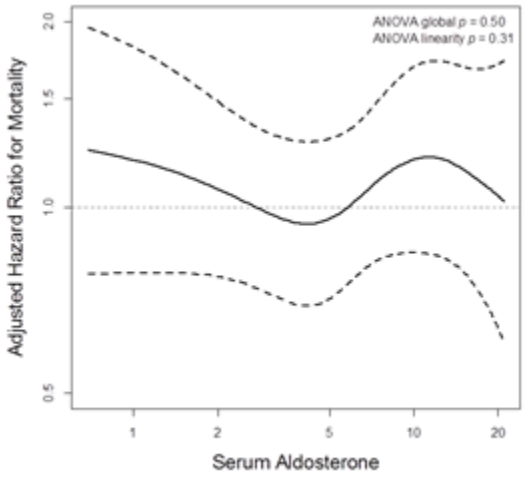
Table 8: Association between serum aldosterone and all-cause mortality

	Continuous Model*	**Q1**	**Q2**	**Q3**	**Q4**
	All-Cause Mortality				
Number at risk	471	118	120	110	123

Events	236	64	57	58	57
Event Rate	4.73	5.31	4.26	5.09	4.37
Unadjusted	0.98 (0.92, 1.05)	1.00 [Ref]	0.80 (0.56, 1.15)	0.97 (0.68, 1.37)	0.85 (0.59, 1.21)
Model 1	1.00 (0.93, 1.07)	1.00 [Ref]	0.83 (0.58, 1.20)	1.07 (0.75, 1.53)	0.92 (0.64, 1.33)
Model 2	0.98 (0.91, 1.06)	1.00 [Ref]	0.80 (0.55, 1.16)	0.99 (0.68, 1.44)	0.90 (0.62, 1.30)
Model 3	0.98 (0.91, 1.07)	1.00 [Ref]	0.75 (0.52, 1.10)	1.02 (0.70, 1.49)	0.90 (0.61, 1.34)
Model 4	0.99 (0.91, 1.07)	1.00 [Ref]	0.76 (0.52, 1.11)	1.02 (0.70, 1.50)	0.94 (0.63, 1.40)

Legend:
*Hazard ratios are reported per doubling serum aldosterone. Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Model outcomes are reported as HR (95% confidence intervals) and are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + urine sodium excretion

Figure 9: Adjusted restricted cubic splines for serum aldosterone (ng/dL) and all-cause mortality, modeled using model 3. Dotted lines represent 95% confidence intervals.



Sensitivity Analysis

Relationships with outcomes were similar in analyses comparing those below the detection limit versus those above the detection limit (Table 9).

Table 9: Sensitivity analysis using tertiles of serum aldosterone and clinical outcomes

	Continuous	**Tertile 1**	**Tertile 2**	**Tertile 3**
	Progression of Kidney Disease			
Unadjusted	1.13 (0.82, 1.55)	1.00 [Ref]	0.71 (0.43, 1.19)	0.77 (0.51, 1.15)
Model 4	1.01 (0.71, 1.40)	1.00 [Ref]	0.69 (0.41, 1.16)	0.79 (0.52, 1.21)
	Incident Heart Failure			
Unadjusted	0.90 (0.67, 1.20)	1.00 [Ref]	0.85 (0.47, 1.53)	0.89 (0.55, 1.46)
Model 4	0.65 (0.45, 0.94)	1.00 [Ref]	0.89 (0.47, 1.70)	1.10 (0.63, 1.91)
	Incident Atherosclerotic Cardiovascular Disease			
Unadjusted	0.84 (0.61, 1.17)	1.00 [Ref]	0.85 (0.47, 1.53)	0.89 (0.55, 1.46)
Model 4	0.55 (0.36, 0.85)	1.00 [Ref]	0.73 (0.38, 1.39)	0.79 (0.45, 1.39)
	All-Cause Mortality			
Unadjusted	0.97 (0.72, 1.32)	1.00 [Ref]	0.84 (0.47, 1.51)	0.82 (0.51, 1.31)
Model 4	0.98 (0.69, 1.41)	1.00 [Ref]	0.67 (0.35, 1.29)	0.84 (0.49, 1.45)
Legend: Association of serum aldosterone and clinical outcomes of interest were evaluated first in continuous models by removing all data points less than the limit of detection of 4 ng/dL for serum aldosterone. Associations were tested per doubling of				

serum aldosterone. Tertile analysis was also performed, with all individuals with serum aldosterone levels below the limit of detection categorized as Tertile 1, and Tertiles 2 and 3 were equally divided from the remainder of the group. All models were tested, although only the unadjusted and Model 4 are presented in this table. Model 4 includes maximal adjustment, including age + race + gender + BMI + prevalent DM + prevalent HTN + systolic BP+ LDL + smoking history + eGFR + urine ACR + RAS blockade + any diuretic use + urine Na excretion

3.4: Association between urine sodium excretion and clinical outcomes

Progression of Kidney Disease

There was no association between urine sodium excretion and progression of kidney disease in fully adjusted continuous models or quartile analyses (Table 10). There was no attenuation in this relationship with adjustment for aldosterone (Model 3 vs Model 4) in both continuous and quartile analyses. In an exploratory analysis, we did not observe a non-linear relationship between urine sodium excretion and progression of kidney disease in fully adjusted models (Figure 10). Interaction with levels of serum aldosterone did not significantly change the relationship between urine sodium excretion and progression of kidney disease (p=0.58).

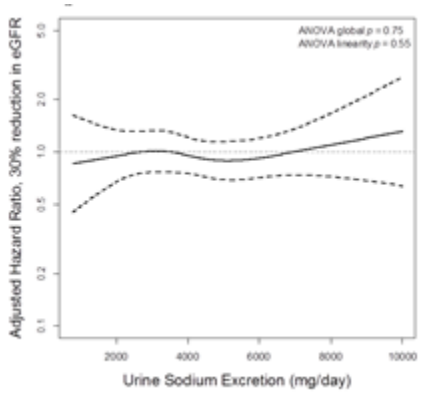
Table 10: Association between urine sodium excretion and progression of kidney disease

	Continuous Model*	**Q1**	**Q2**	**Q3**	**Q4**
	Progression of Kidney Disease				
Number at risk	840	211	208	206	213
Events	420	107	112	100	101
Event Rate	2.85	2.62	3.49	2.25	3.03
Unadjusted	1.15 (0.98, 1.34)	1.00 [Ref]	1.29 (0.82, 2.02)	1.09 (0.69, 1.72)	1.51 (0.97, 2.35)

Model 1	1.17 (1.00, 1.36)	1.00 [Ref]	1.39 (0.89, 2.17)	1.11 (0.69 1.77)	1.63 (1.05, 2.54)
Model 2	1.14 (0.97, 1.13)	1.00 [Ref]	1.41 (0.90, 2.20)	1.10 (0.69, 1.77)	1.51 (0.96, 2.38)
Model 3	1.05 (0.90, 1.22)	1.00 [Ref]	1.31 (0.86, 2.00)	1.02 (0.66, 1.60)	1.09 (0.70, 1.69)
Model 4	1.04 (0.89, 1.22)	1.00 [Ref]	1.31 (0.86, 2.00)	1.02 (0.65, 1.60)	1.08 (0.69, 1.69)

Legend:
Model outcomes are reported as hazard ratios (95% confidence intervals) and are reported as urine sodium excretion per SD increase (SD = 1,546 mg/day). Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Models are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + urine ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + serum aldosterone

Figure 10: Restricted cubic splines for estimated 24-hour urine sodium excretion and progression of kidney disease (30% decline in eGFR), adjusted for age, race, gender, body mass index, prevalent diabetes mellitus, prevalent hypertension, systolic BP, LDL cholesterol, smoking history, eGFR, ACR, RAS blockade, and diuretic use. Dotted lines represent 95% confidence intervals.



Heart Failure

In models adjusted for baseline demographics, risk factors for CVD and progression of kidney disease, there was a trend towards an association between higher levels of urine sodium excretion and a lower risk of incident HF, although these results were not statistically significant in either continuous or quartile analyses (Table 11). Adjustment for serum aldosterone (model 4 vs model 3) did not attenuate the relationship between urine sodium excretion and hazard of incident HF. When continuous urine sodium excretion was modeled using restricted cubic splines (Figure 11), there was a non-linear, albeit nonsignificant, relationship between urine sodium excretion and incident heart failure. Interaction with levels of serum aldosterone did not significantly change the relationship between urine sodium excretion and incident heart failure (p=0.91).

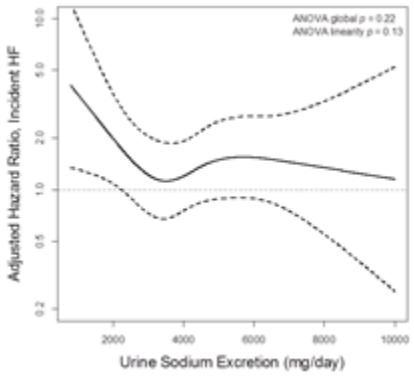
Table 11: Association between urine sodium excretion and incident heart failure

	Continuous Model	**Q1**	**Q2**	**Q3**	**Q4**
	Incident Heart Failure				
Number at risk	603	151	149	152	151
Events	220	63	50	51	56
Event Rate	1.91	2.32	1.26	1.54	2.52
Unadjusted	0.96 (0.81, 1.14)	1.00 [Ref]	0.73 (0.46, 1.15)	0.75 (0.47, 1.18)	0.89 (0.57, 1.39)
Model 1	0.95 (0.80, 1.13)	1.00 [Ref]	0.74 (0.46, 1.18)	0.73 (0.46, 1.17)	0.88 (0.56, 1.39)
Model 2	0.92 (0.77, 1.10)	1.00 [Ref]	0.78 (0.48, 1.27)	0.71 (0.43, 1.16)	0.83 (0.51, 1.35)
Model 3	0.94	1.00	0.72	0.72	0.87

	(0.79, 1.13)	[Ref]	(0.44, 1.19)	(0.43, 1.18)	(0.53, 1.43)
Model 4	0.92 (0.76, 1.10)	1.00 [Ref]	0.71 (0.43, 1.16)	0.68 (0.41, 1.14)	0.81 (0.48, 1.35)

Legend:
Model outcomes are reported as hazard ratios (95% confidence intervals) and are reported as urine sodium excretion per SD increase (SD = 1,546 mg/day). Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Models are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + urine ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + serum aldosterone

Figure 11: Restricted cubic splines for estimated 24-hour urine sodium excretion and incident heart failure, adjusted for age, race, gender, body mass index, prevalent diabetes mellitus, prevalent hypertension, systolic BP, LDL cholesterol, smoking history, eGFR, ACR, RAS blockade, and diuretic use. Dotted lines represent 95% confidence intervals.



Atherosclerotic Cardiovascular Disease

In models adjusted for baseline characteristics, risk factors for CVD and progression of kidney disease, there was a trend towards an association between higher levels of urine sodium excretion and lower risk of atherosclerotic CVD in continuous models [HR per

SD higher urine sodium 0.91 (0.73, 1.12)] although this was not statistically significant. In quartile analysis adjusted for baseline characteristics, risk factors for CVD and progression of kidney disease, the highest quartile of urine sodium excretion was associated with a lower hazard of atherosclerotic CVD, as compared to the lowest quartile, although this was not significant [HR 0.62 per SD higher urine sodium (0.35, 1.11) in highest quartile]. Adjustment for serum aldosterone (model 4 vs model 3) did not attenuate the differences between the top 3 quartiles and the lowest quartile (Table 12). When continuous urine sodium excretion was modeled using restricted cubic splines (Figure 12), there was a suggestion of a non-linear relationship, with a higher risk of incident CVD with lower urine sodium excretion, although this was not significant. Interaction with levels of serum aldosterone did not significantly change the relationship between urine sodium excretion and incident atherosclerotic CVD (p=0.89).

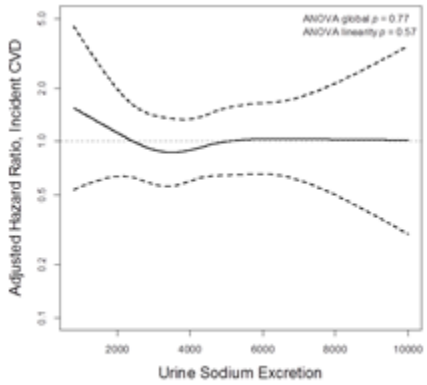
Table 12: Association between urine sodium excretion and atherosclerotic CVD

	Continuous Model	**Q1**	**Q2**	**Q3**	**Q4**
	Incident Atherosclerotic Cardiovascular Disease				
Number at risk	475	119	119	119	118
Events	230	62	52	60	56
Event Rate	2.63	2.80	2.24	2.76	2.74
Unadjusted	1.00 (0.83, 1.20)	1.00 [Ref]	0.75 (0.45, 1.24)	0.99 (0.60, 1.63)	0.90 (0.54, 1.49)
Model 1	0.96 (0.79, 1.16)	1.00 [Ref]	0.70 (0.42, 1.19)	0.92 (0.54, 1.54)	0.80 (0.48, 1.35)
Model 2	0.89 (0.73, 1.09)	1.00 [Ref]	0.57 (0.32, 0.99)	0.71 (0.41, 1.26)	0.62 (0.35, 1.09)

Model 3	0.91 (0.73, 1.12)	1.00 [Ref]	0.53 (0.30, 0.94)	0.71 (0.40, 1.26)	0.62 (0.35, 1.11)
Model 4	0.87 (0.70, 1.08)	1.00 [Ref]	0.50 (0.28, 0.88)	0.65 (0.36, 1.15)	0.55 (0.31, 1.00)

Legend:
Model outcomes are reported as hazard ratios (95% confidence intervals) and are reported as urine sodium excretion per SD increase (SD = 1,546 mg/day). Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Models are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking history
Model 3 = Model 2 + eGFR + urine ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + serum aldosterone

Figure 12: Restricted cubic splines for estimated 24-hour urine sodium excretion and incident atherosclerotic CVD, adjusted for age, race, gender, BMI, prevalent DM, prevalent HTN, systolic BP, LDL cholesterol, smoking history, eGFR, ACR, RAS blockade, and diuretic use. Dotted lines represent 95% confidence intervals.



All-cause Mortality

In models adjusted for baseline characteristics, risk factors for CVD and progression of kidney disease, there was no significant association between urine sodium excretion and

all-cause mortality in either continuous or quartile analyses (Table 13). Adjustment for serum aldosterone (Model 3 vs Model 4) did not attenuate the association between urine sodium excretion and all-cause mortality. In exploratory analysis, there was no observed non-linear trend in the association between urine sodium excretion and all-cause mortality (Figure 13). Interaction with levels of serum aldosterone did not significantly change the relationship between urine sodium excretion and all-cause mortality (p=0.74).

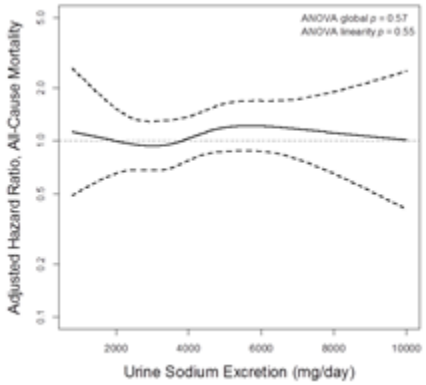
Table 13: Association between urine sodium excretion and all-cause mortality

	Continuous Model	**Q1**	**Q2**	**Q3**	**Q4**
	All-Cause Mortality				
Number at risk	471	118	120	110	123
Events	236	53	57	57	69
Event Rate	4.73	4.13	4.61	4.69	5.47
Unadjusted	1.11 (0.98, 1.25)	1.00 [Ref]	1.10 (0.76, 1.60)	1.15 (0.79, 1.67)	1.52 (1.06, 2.18)
Model 1	1.08 (0.95, 1.22)	1.00 [Ref]	1.14 (0.78, 1.67)	1.06 (0.72, 1.54)	1.42 (0.99, 2.04)
Model 2	1.06 (0.93, 1.21)	1.00 [Ref]	1.15 (0.78, 1.69)	0.99 (0.67, 1.47)	1.41 (0.96, 2.05)
Model 3	1.06 (0.93, 1.21)	1.00 [Ref]	1.13 (0.77, 1.66)	0.96 (0.64, 1.43)	1.36 (0.93, 1.99)
Model 4	1.05 (0.92, 1.20)	1.00 [Ref]	1.09 (0.74, 1.62)	0.96 (0.64, 1.44)	1.36 (0.92, 2.01)

Legend:
Model outcomes are reported as hazard ratios (95% confidence intervals) and are reported as urine sodium excretion per SD increase (SD = 1,546 mg/day). Events for each outcome include addition of supplemental cases. Event rates are calculated from subcohort only and written as per 100 person-years
Models are defined as follows:
Model 1= Adjusted for age + race + gender
Model 2 = Model 1 + BMI + prevalent DM + prevalent HTN + systolic BP + LDL + smoking

history
Model 3 = Model 2 + eGFR + urine ACR + RAS blockade + any diuretic use
Model 4 = Model 3 + serum aldosterone

Figure 13: Restricted cubic splines for estimated 24-hour urine sodium excretion and all-cause mortality, adjusted for age, race, gender, body mass index, prevalent diabetes mellitus, prevalent hypertension, systolic BP, LDL cholesterol, smoking history, eGFR, ACR, RAS blockade, and diuretic use. Dotted lines represent 95% confidence intervals.



4. Discussion

4.1: Association between urine sodium excretion and serum aldosterone levels

We observed that lower urine sodium excretion was associated with higher levels of serum aldosterone. It has been hypothesized that the mechanism underlying this association is volume-mediated hormonal regulation, with lower sodium intake leading to activation of RAS and thus higher levels of serum aldosterone. An extreme example of this relationship can be seen in patients with severe HF, where lower urine sodium levels are associated with elevated aldosterone levels.

The results of our study are consistent with prior analyses among individuals with diabetes and in meta-analyses of randomized control trials of lower versus higher sodium intake, where inverse associations have been observed between sodium intake, or urine excretion as a proxy for intake, with serum aldosterone levels. [59] [14]. Our study adds to the current literature by including an older population who may be at particular risk for adverse outcomes related to both extremes of sodium intake and high levels of serum aldosterone. These results are also consistent with the hypothesis that low sodium intake/excretion may be associated with adverse outcomes through increasing serum aldosterone levels. We recognize, however, that this is a cross sectional observational study and, therefore, the direction of the association is unknown. Furthermore, although the relationship between urine sodium excretion and serum aldosterone was statistically significant, the observed relationship was small and its clinical relevance remains unclear.

4.2: Serum aldosterone levels and clinical outcomes

We noted no significant association between levels of serum aldosterone with progression of kidney disease, incident HF, or all-cause mortality. We did however note that high levels serum aldosterone was associated with a lower risk of incident atherosclerotic CVD.

Several clinical trials have shown that blockade of aldosterone by mineralocorticoid receptor antagonism is associated with reduced mortality in patients with HF and reduction in albuminuria in individuals with proteinuric kidney disease [28,31]. Similarly, higher aldosterone levels have been associated with a greater risk of hypertension and CVD events in some, but not all observational studies [31,32,40].

In a recent post-hoc analysis of the Chronic Renal Insufficiency Cohort (CRIC) Study, serum aldosterone was not an independent risk factor for atherosclerotic CVD, all-cause mortality, or kidney related outcomes, but was associated with an increased risk of developing incident HF [31]. Our results were consistent with CRIC in regards to the kidney progression, CVD, and mortality, but in contrast with the finds with regards to HF. Potential reasons for this discrepancy include differences in the sociodemographic and clinical characteristics of the study populations. For example, the CRIC study included individuals with a mean eGFR of 45 mL/min/1.73m^2 with a median serum aldosterone level of 10.9 ng/dL, while in our study, the mean eGFR was 73 mL/min/1.73m^2, and the median serum aldosterone was lower. An alternative explanation includes a false positive result in our study due to multiple testing. Additional data in support of the false positive result is the lack of known mechanism to explain the result as well as the lack of a linear relationship between aldosterone and

CVD. Of note, we intentionally did not adjust for multiple testing due to the hypothesis generating nature of our study.

The strengths of our analysis include measuring aldosterone using the LC/MS/MS technique, which is considered more accurate than prior ELISA methods, which have been used in the CRIC study and other analyses evaluating serum aldosterone. Second, detailed medication use was known, which can affect serum aldosterone levels and was adjusted for in the final models. We were also able to include and adjust for other physiologically relevant covariates, including blood pressure measurements and presence of diabetes. Despite these strengths, this aim has important limitations. Although the aldosterone measurement was accurate, 35% of the measurements were less than the limit of detection. We realize that these lower aldosterone measurements that cannot be accurately measured below the limit of detection may lead to exposure misclassification bias in the overall results. Specifically, the direction of association between low levels of serum aldosterone and clinical outcomes may have been biased towards the null, although high levels of serum aldosterone would be at less risk of bias from this exposure misclassification. Additional sensitivity analyses were done using those measurements less than the limit of detection vs those above the limit of detection, and the general direction of risk for each clinical outcome did not change.

In addition, serum aldosterone was measured only once and at one time point, thus assessment of long-term trends and changes in levels was not possible. Last, variations in aldosterone concentration can occur with changes in positioning, dietary intake (particularly sodium), and other physiologic parameters. Although we adjusted for

sodium excretion, residual confounding of the exposure variable measurement may be present.

4.3: Urine sodium excretion and clinical outcomes

We failed to observe any statistically significant or consistent associations between urine sodium and the clinical outcomes under study, although higher levels of urine sodium excretion showed a trend towards reduced risk for incident HF and atherosclerotic CVD. In both quartile analyses and exploratory analyses using restricted cubic splines, we observed a nonlinear relationship between urine sodium excretion and incident atherosclerotic CVD events, with higher risk of outcomes in those in the lowest quartile of urine sodium. We noted no attenuation of the relationship of low sodium with HF and CVD after adjustment for aldosterone.

We noted no association or overt U-shaped relationship between urine sodium and all-cause mortality over the 10-year follow-up of this study. This is consistent with several older observational studies, although more recent studies have observed U-shaped relationships between urine sodium and all-cause mortality [17-20,61]. These more recent studies have generated several hypotheses in order to explain the relationship between extremes of sodium intake and adverse outcomes including the possibility that low sodium intake may simply be a marker of illness or that estimates of dietary intake (either by food frequency questionnaire or by spot urine samples) may be biased or inaccurate. Low sodium intake has been associated with activation of RAS, which has in turn been associated with increased glomerular fibrosis and higher intraglomerular pressure [62]. Additionally, in animal models, stimulation of RAS may lead increased atherosclerosis,

oxidative stress, and cardiac hypertrophy, which in turn may associate with adverse outcomes. We had hypothesized that serum aldosterone may attenuate the relationship of low urine sodium with the outcomes under study. Our results, however, demonstrated that adjustment of serum aldosterone did not significantly weaken any estimates of low urine sodium with these outcomes. In this older cohort with relatively low aldosterone levels, it seems less likely that aldosterone levels are an important confounder in the relationship of low urine sodium to an increased risk of adverse health outcomes.

This aim has several strengths, including the study cohort, which was a healthy population free from baseline disease. The latter is particularly relevant given prior hypotheses which have suggested that individuals with lower urine sodium excretion may be a sicker population. Second, this study is the first analysis, to our knowledge, that incorporates urine sodium and serum aldosterone at the same time; therefore the simultaneous measurements are more likely to be reflective of the steady state of sodium and aldosterone levels. Despite these strengths, our study also has important limitations. Urine sodium excretion was estimated using the Kawasaki formula from a single spot urine sodium measurement. This formula was recently used and validated in the Prospective Urban Rural Epidemiology (PURE) study, where comparisons were made between 24-hour urine collections and the Kawasaki formula as well as two other estimating equations. The Kawasaki formula had the highest intra-class correlation coefficient among the estimating equations. However, the PURE study population included a younger, international cohort and thus the assumption that the Kawasaki equation is valid in the use of an older cohort is subject to question. Lastly, the measurement of urine sodium excretion has not been fully evaluated in the elderly

population. There may be a functional effect of aging on the kidney (in addition to reduction of GFR) in terms of the kidney's ability to regulate sodium and water [63].

4.4: Final conclusions

In a large, community-dwelling cohort of older adults free of life-threatening illnesses, we found an inverse association between levels of urine sodium excretion and serum aldosterone. For each 1-gram higher urine sodium excretion, serum aldosterone levels decreased by 0.93 ng/dL. We also demonstrated that higher serum aldosterone is associated with a lower risk of atherosclerotic CVD; however we did not find an association between serum aldosterone levels and progression of chronic kidney disease, incident HF, or all-cause mortality. Similarly, there was no statistically significant association between urine sodium excretion and clinical outcomes of progression of kidney disease, incident HF, incident atherosclerotic CVD, and all-cause mortality, although there were trends which suggested that higher quartiles of urine sodium excretion were associated with a lower risk of developing new onset HF and atherosclerotic CVD. We found no attenuation of these relationships after adjusting for serum aldosterone, and there was no interaction of urine sodium with serum aldosterone.

The results of this analysis confirm an inverse relationship between urine sodium excretion and levels of serum aldosterone. Higher serum aldosterone was associated with a lower risk of atherosclerotic CVD, although there was no significant relationship between aldosterone and other clinical outcomes. Lower urine sodium was associated with a higher risk of atherosclerotic CVD in quartile analysis, although this was not attenuated after adjustment for serum aldosterone levels.

The strengths of the study include a large cohort of individuals with well-described baseline characteristics as well as clinical outcomes adjudicated by a Health ABC Diagnosis and Disease Ascertainment Committee. This independent assessment of outcomes by a committee is superior to use of hospital coding mechanisms or patient self-reported medical complications, strengthening the ascertainment of outcomes. Additionally, multiple comorbidity measures were assessed and adjusted for, including the presence of hypertension, diabetes, and baseline cardiovascular disease in attempts to minimize confounding by baseline condition. Finally, due to the case-cohort study design, we were also able to test multiple hypotheses and outcomes in an efficient manner. Using this design, there was a large number of events for the principal outcome, which provided statistical strength to detect small differences in these outcomes.

Our study has several limitations, however, that must be kept in mind in the interpretation of our results. First, the study includes an elderly cohort of individuals free of known life-threatening diseases. This may limit the generalizability of the results to a younger cohort and may also introduce competing risk biases. We did not test for the competing risk of death in the current analysis. Second, the associations between urine sodium, serum aldosterone, and clinical outcomes assume that elderly individuals have the ability to regulate sodium and volume, which may introduce additional biases towards a null conclusion if these mechanisms are, in fact, altered in an aging kidney. Finally, although our study was powered to detect a meaningful hazard ratio in continuous models, we may not have been adequately powered to detect nonlinear relationships.

Our hypotheses assumed that high levels of serum aldosterone irrespective of their cause may be associated with adverse outcomes. However it is possible that

aldosterone levels that are elevated due to appropriate activation of the RAS system due to volume depletion do not lead to adverse effects. Rather, it may only be when maladaptive activation of RAS occurs in clinical situations of hypertension, HF, CKD and primary hyperaldosteronism, which adverse outcomes ensue.

In conclusion, lower levels of urine sodium are associated with higher levels of serum aldosterone. There was no consistent relationship between serum aldosterone with any of the clinical outcomes. Higher levels of urine sodium showed a trend to higher risk of progression of kidney disease, while lower levels of urinary sodium showed a trend towards higher risk of heart failure and atherosclerotic CVD. Serum aldosterone did not explain the relationship of low urine sodium with outcomes. Results may have been partially limited by insufficient statistical power. Further studies are necessary to evaluate the mechanism by which low urine sodium excretion associates with adverse outcomes.

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