Impact of fractional excretion of sodium on a single morning void urine collection as an estimate of 24-hour urine sodium

Short title: Fractional excretion of sodium and predicting 24-h urine sodium

Caryl A Nowson, PhD[1], Karen Lim, PhD[1], Norm RC Campbell[2], CM, MD, FRCPC, Stella L O'Connell[1], PhD, Feng J He, PhD[3], Robin M. Daly, PhD[1]

[1]Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Geelong, Waurn Ponds Campus, Locked Bag 20000, Geelong Victoria 3220 Australia.

[2]Departments of Medicine, Community Health Sciences, and Physiology and Pharmacology, O'Brien Institute of Public Health, Libin Cardiovascular Institute of Alberta, University of Calgary, Calgary, Canada.

[3] Centre for Environmental and Preventive Medicine, Wolfson Institute of Preventive Medicine, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, London, UK.

Correspondence

Professor Caryl Nowson

Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Geelong, Waurn Ponds Campus, Locked Bag 20000, Geelong Victoria 3220 Australia

E-mail: caryl.nowson@deakin.edu.au

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Abstract. 264 words

The standard for assessing dietary sodium intake is to measure 24-h urine sodium. On average, 93% of daily sodium intake is excreted in 24-h urine. Expense and difficulties in obtaining complete 24-h collections have led to the measurement of sodium concentration in spot and single void urine samples using predictive equations to estimate 24-h urine sodium. Although multiple predictive equations have been developed, in addition to average bias, all the equations overestimate 24-h sodium at lower levels of measured 24-h sodium and underestimate 24-h urine sodium at higher measured 24-h sodium. One of the least-biased estimating equations is the INTERSALT equation, which incorporates a spot urine creatinine concentration. We hypothesized that differential fractional excretion of sodium (derived from a morning void collection) relative to creatinine (FENa) would impact the accuracy of the INTERSALT equation in estimating 24-h urine sodium. In a prospective study of 139 adults aged 65 years and over, three sequential morning void and 24-h urine samples were examined. There was a significant correlation between increasing FENa and the difference between estimated and measured 24-h urine sodium ($r=0.358$, $p<0.01$). In the lowest quartile of FENa, the INTERSALT equation overestimated 24-h urine sodium, but underestimated 24-h urine sodium with greater magnitude in each of the subsequent quartiles of FENa. Differential excretion of sodium relative to creatinine, potentially impacted by renal blood flow and hydration, amongst other factors, affected the accuracy of the INTERSALT equation. Additional research may refine the INTERSALT and other predictive equations to increase their accuracy.

Background

There is increasing interest in utilising spot urine collections to assess population salt intake in countries across the world.[1] The World Health Organisation (WHO) member states have agreed on a voluntary global non-communicable disease (NCD) target for a 30% relative reduction in mean population intake of salt, with the aim of achieving a target of less than 5 grams per day by 2025.[2] Accordingly, a number of countries have set population salt reduction targets to reduce sodium intake. The gold standard method for the assessment of population salt intake is 24-hour urine collection [1,3,4] as it provides a close measure of sodium intake. In order to accurately measure the impact of population-wide salt reduction strategies within different countries, it is necessary to obtain complete 24-h urine collections from a representative sample of people. However, due to the low participation rate, high participant burden, and costs associated with conducting complete 24-h urine collections, considerable efforts are being made to explore the potential of spot urines to predict the mean 24-h urinary sodium output of populations.[5,6]

Single void and spot urine sample collection is relatively easy to perform and have a low subject burden. If population-average daily sodium intake could be derived from single void or spot urine collections, this would significantly increase the survey participation rate across all groups within a population, potentially increasing the representativeness of the measures of sodium intake. A number of predictive equations have been derived in different population groups: Kawasaki[7], INTERSALT [8] and Tanaka.[9] There is some evidence that the INTERSALT equation, using morning spot urine samples might provide the least biased information about the population mean sodium intakes among US adults aged 18-39 years.[10] Furthermore, a recent evaluation of the predictive ability of the estimating equations to

accurately estimate mean population salt intake and mean change found that the INTERSALT equation was one of the two equations that performed best for the estimation of absolute levels of intake. [11]

Currently, there are conflicting views on the accuracy and reliability of using spot urine collections to assess the average sodium intake of populations, with some studies advocating the use of spot collections[12-14], some suggesting that they could be useful [8,15] [10,11,16,17] and others indicating that they do not provide a reliable estimate of 24-h urinary sodium excretion of specific populations, e.g. those with kidney disease.[4,18] The TRUE consortium, of international health and scientific organizations, recently concluded that further research is needed to define the role for spot urine sodium estimates of population average 24-h sodium excretion and that there is no current role for spot urine sodium to assess individual sodium 24-h excretion.[4]

There are a number of factors that could reduce the predictive ability of the available equations to estimate 24-h urine sodium from spot collections.[4] We hypothesized that variation in excretion of sodium relative to creatinine would impact the accuracy of estimation of 24-h urine excretion using the INTERSALT equation. Current formulae for converting sodium concentration from spot urine samples to estimates of 24-h urine sodium, including the INTERSALT equation, incorporate urine creatinine concentration and assume the relationship of sodium to creatinine concentration is a constant. However, urine sodium concentration is highly regulated in part as a means to maintain vascular volume and blood pressure, by active net reabsorption in the renal tubules when renal blood flow is reduced.[4] Creatinine concentration is variable depending on glomerular filtration rate, muscle mass,

ingestion of meat, and some drugs that can interfere with the active secretion of creatinine.[4] Changes in hydration state differentially affect sodium (which is actively reabsorbed in dehydration) and creatinine, which is actively secreted.[4] The relationship between sodium and creatinine excretion is assessed by the fractional excretion of sodium (FENa). Therefore, the aim of this study was to examine the impact of FENa on estimates of 24-h urine sodium based on the INTERSALT equation as a potential mechanism to enhance the utility of sodium urine estimates of sodium intake.

Subjects and Methods

This is a prospective study using data from a 6-month randomised controlled trials in which healthy community dwelling men and women who were aged $\geq$65 years, with baseline estimated Glomerular Filtration Rate (eGFR) >45 mL/min/1.73m^2 were entered into an exercise and nutrition intervention referred to as the STEPS study. In brief, STEPS was a 6-month, two-arm randomised controlled trial where all participants undertook progressive resistance training on 3 days per week and half were randomly allocated to consume either two 80g servings of lean red meat on their training days or to consume at least one serving of carbohydrate food on training days.[19] Samples from all participants completing the study from all timepoints were utilised and pooled. Details of the STEPS study protocol and results have been previously published, which include details of measurement techniques and questionnaires to assess medication use and lifestyle practices. [19] Height, without shoes, was measured using a stadiometer to the nearest 0.5 cm. Weight was measured with minimal clothing (gowns) using calibrated electronic digital scales (Seca, model 708), to the nearest 0.1 kg. A lifestyle questionnaire was be used to obtain information on education background, history of disease(s)/illnesses, falls and fractures, smoking history, current use

of medication and dietary supplement use. Information including medication name, dose prescribed, and daily quantity taken was be recorded. Information on any alterations to, or new, medication prescribed by the participants' doctors during the study was also be collected by research staff via the monthly phone calls.

The study was approved by the Deakin University Human Research Ethics Committee (HREC 2013-166) and was registered with the Australian and New Zealand Clinical Trial Registry (ACTRN12613001153707).

Participants' 24-h urine, (including a first morning-void urine collected in a separate bottle) and blood samples were collected three times during the study (baseline, 3 and 6 months). On each occasion, participants delivered their completed urine collections to a local pathology centre where they provided a fasting blood sample within 3 hours of collection of the first morning void urine collection. Participants were instructed to collect their 24-h urine, starting immediately after the first morning void on the day prior to presenting for the fasting blood sample. From that time onwards, all urine passed during the day and during the night up until 5 am the following morning was collected into one bottle. Thereafter (the first morning void within the 24-h period) was collected in a second bottle and its concentrations of sodium, urea, potassium and creatinine were analysed separately. The total volume and total electrolytes and creatinine excreted over the 24-h period were calculated by summing the results for the two urine collections within the 24-h collection period and the morning void collection was used to estimate 24-h excretion of sodium, using the Intersalt equation[8] and FeNa. Participants were provided with both verbal and detailed pictorial instructions for the collection, together with a recording sheet.

Participants recorded the start and end times for their urine collection and reported any missed urine during the 24 h collection period. The reported start and finish times of the collections were used to calculate total daily excretion with all urinary results standardized to a 24 h period. Participants were advised to cease eating and drinking (apart from plain water) from 12 midnight prior to their fasting blood sample, which was drawn between 8am-10am. Blood and urine samples were assayed for sodium, creatinine and urea using standardised methods, by an Australasian pathology laboratory accredited by the National Association of Testing Authorities/Royal College of Pathologists. Urinary sodium and potassium concentration were determined using ion-selective electrodes and urinary creatinine concentration was determined from the kinetic Jaffe reaction using the Roche Cobas 8000 (c701).

Estimated glomerular filtration rate (eGFR) was calculated using the participants' serum creatinine, age and sex. For females with serum creatinine (SCr) ≤ 62 µmol/L: eGFR (mL/min/1.73m2) = 144 × (SCr in µmol/L × 0.0113/0.7) 0.329 × (0.993) age in years: for females with SCr > 62 µmol/L: eGFR (mL/min/1.73m2) = 144 × (SCr in µmol/L × 0.0113/0.7) 1.209 × (0.993)age in years: for males with SCr ≤ 80 µmol/L: eGFR (mL/min/1.73m2) = 141 × (SCr in µmol/L× 0.0113/0.9) 0.411 × (0.993) age in years: for males with SCr > 80 µmol/L: eGFR (mL/min/1.73m2) = 141 × (SCr in µmol/L × 0.0113/0.9) 1.209 × (0.993). [20] Participants were excluded from participating in the study if their eGFR (fasting morning blood sample) was <45 mL/min/1.73m^2. Normal kidney function classified as eGRF >89 (mL/min/1.73m^2), mildly reduced 60-89 (mL/min/1.73m^2) and moderately reduced 45-59 (mL/min/1.73m^2).[21] Fractional excretion of sodium (FENa) is the percentage of the sodium filtered by the kidney

which is excreted in the urine relative to creatinine. FeNa has been used to assess for reduced renal blood flow in the presence of acute renal failure. [22] FENa was calculated by the following equation: FENa = (serum creatinine (umol/L) x urinary sodium (mmol/L) (from morning single void collection) / serum sodium(mmol/L)(collected within 4 hours of single void urine collection) x urinary creatinine (umol/L) (from single void collection) x 100. [23]

Predicted sodium excretion was derived from the sodium concentration obtained from a single void (morning collection) using the INTERSALT equation [8], i.e. for men: 23 x (25.46 + (0.46 * void sodium mmol/L) - (2.75 * void creatinine mmol/L) - (0.13 x void potassium mmol/L) + (4.10 * baseline BMI kg/m^2) + (0.26 * baseline age (yrs)), and for women: 23 x (5.07 + (0.34 * void sodium mmol/L) - (2.16 * void creatinine mmol/L) - (0.09 x void potassium mmol/L) + (2.39 x baseline BMI kg/m^2) + (2.35x baseline age) - (0.03 x (baseline age^2)).

Statistical Methods

Data were analysed using STATA/SE 15.1 for Windows (StataCorp LLC). Descriptive statistics were performed for demographic variables with continuous (mean and standard deviation (SD)) and categorical variables ((n, proportion (%)). We used mixed models to account for repeated biochemical measures at 0, 3 and 6 months in individuals. Multilevel linear regression was used with a random intercept term at the participant level. Pearson's correlations were also assessed separately for women and for men, and by thiazide use. Bland-Altman plots represented the predicted INTERSALT and measured urinary sodium expressed as sodium (mmol/day) as described by published Intersalt equation.[8] Data were adjusted for repeated measures, with mean bias and limits of agreement at 95% confidence interval (CI) presented graphically.

Results

The characteristics of the study participants are shown in Table 1. Complete biochemical data were available for 150 participants at baseline, 139 at 3 months and 136 at 6 months. Urine samples were excluded if classified as under-collected, based on expected minimum range for 24-hurinary excretion of creatinine[24], or over-collected, if urinary creatinine was greater than 3 SD from mean for gender. One sample was excluded from a female with urinary creatinine <4 mmol/24-hr; 2 samples from females and 1 sample from a male were excluded for urinary creatinine >3 SD above the gender-specific mean. The median collection was 24.0 ((IQR): 23.6, 24.8)) hours. A further 26 samples where participants underwent blood sampling >4 hours after final void were also excluded. Therefore, valid biochemical data were available for 139 participants at baseline, 126 participants at 3 months, and 130 participants at 6 months. Valid biochemical data at all three timepoints were available for 105 participants.

Of the 139 participants with an average age of 70 years, less than one quarter had normal renal function based on eGFR, with the majority having mildly reduced kidney function (71%) and less than 5% having moderately reduced function. Just over three quarters of participants had FENa values <1%. The 24-hurinary excretion indicated that men had a 32% greater excretion of sodium than women, with no other notable differences between different subgroups with respect to other measured urinary or serum metabolites (Table 1).

Overall, there was a significant correlation between predicted sodium (mmol/d)(INTERSALT equation) and the actual measured sodium excretion over 24 hrs, with no gender difference (overall r=0.57, p<0.001, men r=0.58, p<0.001; women r=0.49, p<0.001). There were also significant correlations between predicted sodium (mmol/d)(INTERSALT equation) and the measured sodium excretion over 24 hrs in the subgroups taking anti-hypertensive therapy and those not, those with normal renal function or impaired function, and those with FENa <1% or > 1% (range r=0.45 - 0.59, p<0.001).

There was a significant association between FENa and the difference between estimated 24-h urine sodium by the INTERSALT equation and that measured in 24-h urine (r=0.36, p<0.01). The higher the FENa, the greater the INTERSALT equation underestimated 24-h sodium excretion. When assessing the mean 24-h sodium excretion across quartiles of FENa (Table 2), the INTERSALT equation overestimated measured 24-h urinary sodium in the lowest quartile, and in each higher quartile there was successive larger underestimates of 24-h sodium by the INTERSALT equation (Figure 1). There was a significant trend for increasing sodium excretion from a mean of 106 mmol/d sodium in quartile 1(Q1) to 172 mmol/d in Q4 and a similar trend for predicted sodium excretion using the INTERSALT equation.

Of the 48 participants who provided valid urine samples at baseline, 3 months and 6 months, 46% (22/48) consistently had FENa <1% and 8% (4/48) consistently had FENa $\geq$1% throughout the study. FENa categorisation of 54% participants changed throughout the study.

Discussion

Consistent with known differences in renal excretion of sodium and creatinine, we found that variation in FENa had an impact on the INTERSALT equations prediction of measured 24-h urine sodium. At lower end of the distribution of FENa, the INTERSALT equation overestimated measured 24-h urine sodium whilst the second to fourth quartile of FENa, the INTERSALT equation increasing underestimated of 24-h urine sodium. It is likely the bias of other predictive equations will also be impacted by variation in FENa, as all current predictive equations also incorporate spot urine creatinine concentrations.

Our study included older people with some age-related impairment of renal function, but most had normal function or mild impairment (average eGFR was 79 mL/min/1.73m^2), as having an eGRF >45 mL/min/1.73m^2 was an exclusion criterion for entry into the study. Accordingly, only 4% of samples were classified as moderately impaired renal function 45-59 (mL/min/1.73m^2) and none had values <44 (mL/min/1.73m^2). A recent study conducted in 2777 chronic kidney disease patients with a median GFR of 56 mL/min/1.73m^2 found that there was an association between urine urea and sodium excretion.[25] The authors then developed new predictive equations, based on the Kawasaki, INTERSALT, and Tanaka equations, to estimate 24-h urinary sodium excretion from spot urine collections. The equations which then additionally included urea, reduced bias within the limits of agreement in Bland-Altman plots.

Accounting for differences in FENa in predictive equations of 24-h urine sodium might therefore result in the development of more accurate predictive equations. Procedures to standardize FENa (e.g. providing volunteers with standard hydration instructions) before collecting spot urine samples might also reduce variation in the predictive ability of

equations related to differences in renal excretion in creatinine and sodium caused by varying degrees of dehydration (e.g. when fasting or in hot dry environments).

This preliminary study has several limitations. The study was conducted in an older population, many of whom had some small degree of impaired renal function, and/or were taking medications that influence renal excretion of sodium. Additionally, 32% of the samples were derived from participants who were consuming two 80g servings of meat, three days per week. Larger servings of meat (225g) at one sitting have been found to acutely increase serum creatinine and urinary creatinine. [26] However, although two 80g servings of meat have been found to increase 24 hr urinary excretion of urea in a similar subject group participating in a similar study, we found that there was no impact on serum or urinary creatinine. [27] The calculation of FeNa is based on single serum sodium and creatinine values as well the spot urine sodium and creatinine values. Creatinine and sodium, in serum and urine, will fluctuate under independent regulation during a 24-h. period. Hence the calculation of FeNa can only be considered an estimate of the relationship of excretion of sodium and creatinine over 24-h. Our study also excluded people with an eGFR < 45ml/min. Investigating FeNa on estimates of sodium excretion using spot samples in people with an eGFR < 45 ml/min would be useful to assess in a further study.

In conclusion, differential excretion of sodium relative to creatinine, as assessed by FeNa, appears to alter the capacity of the Intersalt equation to predict 24-h urinary sodium excretion from a single void collection. Further study is needed to examine the impact of FENa on the currently available predictive equations in healthy populations and under

varying environmental conditions likely to affect FENa (e.g. hot dry conditions). Introducing FENa into predictive equations, potentially in combination with urinary urea, should be explored as a potential factor which may reduce equation bias when predicting 24-h urinary sodium excretion from spot and single void urine collections. Currently, the role of spot urine samples in estimating average population sodium excretion requires more research and is recommended not to be used to assess individual sodium excretion.[4] Our findings do not impact recommendations that further research is needed to define the role for spot urine sodium estimates of population average 24-h sodium excretion.[4]

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Conflicts of Interest

NRCC was a paid consultant to the Novartis Foundation (2016-2017) to support their program to improve hypertension control in low to middle income countries, which includes travel support for site visits and a contract to develop a survey. NRCC has provided paid consultative advice on accurate blood pressure assessment to Midway Corporation (2017) and is an unpaid member of World Action on Salt and Health (WASH). RMD received a grant from Fonterra Co-operative Group Ltd, outside the submitted work. FJH is a member of Consensus Action on Salt & Health (CASH) and World Action on Salt & Health (WASH). Both CASH and WASH are non-profit charitable organisations and FJH does not receive any financial support from CASH or WASH. CN is a member of WASH and does not receive any financial support from WASH.

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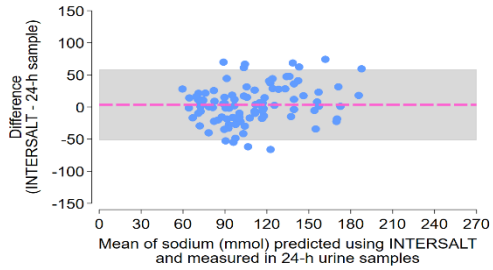
Figure 1a, b, c, d legend

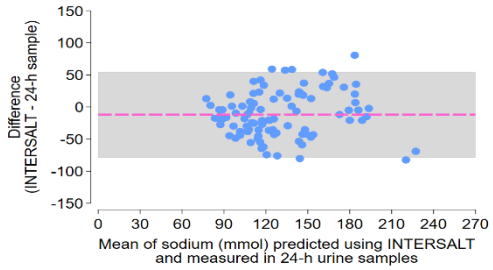
Bland Altman plots of the difference in predicted salt (Intersalt equation) and measured sodium (24-h urine) (mmol/24-h), by quartiles Fractional Excretion of Sodium (FENa) as independent variable). The horizontal line indicates mean difference between predicted and measured sodium (mmol/24-h) and shaded bar denotes 95% limits of agreement.

a) **Quartile 1 of FENa:** Mean difference: +8.6 mmol sodium, limits of agreement: -51.9, 58.8 mmol sodium
b) **Quartile 2 of FENa:** Mean difference: -7.4 mmol sodium, limits of agreement: -78.8, 54.7 mmol sodium
c) **Quartile 3 of FENa:** Mean difference: -17.3 mmol sodium, limits of agreement: -89.9, 53.1 mmol sodium
d) **Quartile 4 of FENa:** Mean difference: -33.4 mmol sodium, limits of agreement: -105.2, 57.9 mmol sodium

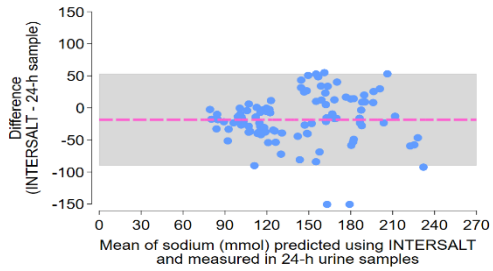
Quartile 1 of FENa



Quartile 2 of FENa



Quartile 3 of FENa



Quartile 4 of FENa

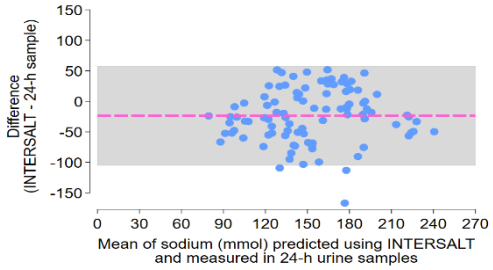


Table 1 Age, anthropometric measurements, use of antihypertensive medication at baseline (mean (95% CI) or n (%)) by medication use

	Overall (n=139)	Women (n=85)	Men (n=54)	Anti-hypertensive medication excl. thiazide (n=55)	[a] Thiazides + anti-hypertensive medication (n=15)	No anti-hypertensive medication (n=68)
Women / Men	61%/38%			34 (62%) women 21 (38%) men	9 (60%) women 6 (40%) men	42 (62%) women 26 (38%) men
Age (y)	70.4 (69.7, 71.0)	69.7 (69.0, 70.4)	71.5 (70.3, 72.7)	70.6 (69.5, 71.7)	70.4 (68.4, 72.5)	70.1 (69.2, 71.0)
Height (m)	1.66 (1.64, 1.67)	1.61 (1.59, 1.62)	1.74 (1.72, 1.76)	1.65 (1.63, 1.68)	1.69 (1.63, 1.75)	1.65 (1.63, 1.67)
Weight (kg)	75.8 (72.8, 78.7)	69.9 (66.6, 73.2)	85.0 (80.4, 89.7)	79.9 (75.1, 84.6)	87.8 (76.3, 99.2)	69.7 (66.2, 73.2)
BMI (kg/m2)	27.4 (26.6, 28.3)	27.0 (25.9, 28.1)	28.1 (26.8, 29.4)	29.2 (29.7, 30.6)	30.4 (27.8, 32.9)	25.4 (24.4, 26.3)
FENa[b]	0.80 (0.73, 0.87)	0.77 (0.68, 0.86)	0.84 (0.75, 0.94)	0.83 (0.74, 0.93)	0.82 (0.50, 1.15)	0.76 (0.67, 0.85)
- <1%	105 (76%)	70 (82%)	35 (65%)	38 (69%)	13 (87%)	54 (79%)
- ≥1%	34 (24%)	15 (18%)	19 (35%)	17 (31%)	2 (13%)	14 (21%)
eGFR[c] (mL/min/1.73m^2)	80.2 (78.5, 82.0)	81.4 (79.3, 83.6)	78.4 (75.4, 81.3)	78.3 (75.3, 81.2)	79.5 (72.9, 86.1)	82.0 (79.7, 84.4)
- Normal kidney function[d]	28 (20%)	21 (25%)	7 (13%)	8 (15%)	3 (20%)	17 (25%)
- Mildly reduced kidney function[d]	107 (77%)	62 (73%)	45 (83%)	45 (82%)	11 (73%)	50 (74%)
- Moderately reduced kidney function (3A)[d]	4 (3%)	2 (2%)	2 (4%)	2 (4%)	1 (7%)	1 (1%)

[a]taking both thiazide diuretics and antihypertensive medications at baseline

[b]Fractional Excretion of Sodium (FENa) = FENa = (serum creatinine (µmol/L) x urinary sodium (mmol/L) (from morning void collection)/serum sodium(mmol/L) x urinary creatinine (µmol/L) (from morning void collection) x 100

[c]estimated glomerular filtration rate

[d]Normal kidney function classified as eGRF >89 (mL/min/1.73m^2), mildly reduced 60-89 (mL/min/1.73m^2) and moderately reduced 45-59 (mL/min/1.73m^2)

Table 2 Subject characteristics and sodium excretion (Mean (95% CI)) by quartile of Fractional Excretion of Sodium (FENa)

FENa[a]	**Quartile 1 (n=99) (0.17 - <0.54)**	**Quartile 2 (n=99) (≥0.54 - <0.74)**	**Quartile 3 (n=99) (≥0.74 – <1.00)**	**Quartile 4 (n=98) (≥1.00 - <2.80)**	**P value for trend**
Women (n (%))	68 (68.7%)	64 (64.7%)	61 (61.6%)	51 (52.0%)	0.099
Age (y)	70.9 (70.2, 71.6)	70.9 (70.2, 71.6)	71 (70.3, 71.6)	70.9 (70.2, 71.5)	0.797
No antihypertensive medication	39 (56.5%)	32 (47.8%)	30 (45.5%)	29 (43.9%)	0.460
Height (m)	1.65 (1.64, 1.67)	1.65 (1.64, 1.67)	1.65 (1.64, 1.67)	1.66 (1.64, 1.67)	0.072
Weight (kg)	76.1 (73.2, 79)	75.9 (73, 78.8)	75.5 (72.6, 78.4)	75.6 (72.7, 78.5)	0.344
24-h salt excretion[b] g/d	6.3 (5.9, 6.6)	7.9 (7.6, 8.3)	8.9 (8.5, 9.4)	9.7 (9.2, 9.4)	<0.0001
Sodium excretion mmol/day	106.3 (96.1, 116.4)	133.7 (124, 143.3)	150.5 (140.9, 160)	172.1 (161.9, 182.3)	<0.0001
Predicted sodium excretion[b] mmol/d	114.8 (107.9, 121.7)	126.3 (119.5, 133.1)	133.2 (126.3, 140.0)	138.7 (131.8, 145.6)	<0.0001
Difference between 24h Sodium excretion & predicted sodium excretion mmol/d	8.6 (2.8, 14.4)	-7.4 (-13.2, -1.6)	-17.3 (-23.1, -11.5)	-33.4 (-39.2, -27.6)	<0.0001
Creatinine mmol/day	10.6 (10, 11.2)	10.9 (10.3, 11.5)	11 (10.4, 11.6)	10.9 (10.2, 11.5)	0.560
Urea mmol/d	382.7 (358.6, 406.8)	406.9 (383.9, 429.8)	423.9 (401.2, 446.6)	416.5 (392.3, 440.7)	0.032
Urinary volume ml/d	1886 (1738, 2034)	2041 (1900, 2183)	2084 (1944, 2223)	2201(2053, 2350)	0.006
FENa[a]	0.41 (0.38, 0.45)	0.65 (0.62, 0.69)	0.87 (0.84, 0.91)	1.31 (1.27, 1.34)	<0.0001
- <1%	99 (100%)	99 (100%)	99 (100%)	1 (1.0%)	<0.0001
eGFR[c] mL/min/1.73m^2	80.6 (77.9, 83.2)	79.1 (76.9, 81.2)	76.4 (73.6, 79.2)	75.1 (71.7, 78.5)	0.297

[a]Fractional Excretion of Sodium (FENa) = FENa = (serum creatinine (µmol/L) x urinary sodium (mmol/L) (from morning void collection) / serum sodium(mmol/L) x urinary creatinine (µmol/L) (from morning void collection) x 100

[b]Mixed linear regression with participant ID as the random effect, and main model was dependent variable 24-h sodium and independent variable was quartiles of FENa [c]Estimated glomerular filtration rate[20]