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# Comparisons of spot vs 24-h urine samples for estimating population salt intake: Validation study in two independent samples of adults in Britain and Italy

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## KEYWORDS

Urinary sodium;  
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Validation

**Abstract** *Objectives:* To assess the reliability and reproducibility of estimations of group mean 24-h urinary sodium (Na) excretion through timed spot urines compared to 24 h urinary Na output in two independent cross-sectional population samples including men and women and different ethnic groups.

*Methods and Results:* Study 1 was carried out in Britain and included 915 untreated 40–59 yrs male and female participants (297 white, 326 of black African origin and 292 South Asian). Study 2 was carried out in Italy and included 148 white men (mean age 58.3 yrs). All participants provided both a 24-h urine collection and a timed urine sample as part of population surveys. Na, creatinine (Cr) and volume (V) were measured in all samples. Age, body mass index (BMI) and blood pressure (BP) were also measured. We compared the daily Na excretion through 24-h urine (gold standard) with its estimate from timed urine samples with two methods: Tanaka's predictions and Arithmetic extrapolations, and assessed them with correlation coefficients, Bland–Altman plot, prediction of quintile position and Receiver Operating Characteristic (ROC) Areas Under the Curve (AUC) for a cut-off of <100 mmol of Na/day. In Study 1 (discovery study) with the Tanaka method there were poor correlations between predicted and measured 24-h Na excretions in different ethnic groups and genders ( $r_{\text{Spearman}}$  from 0.055 [ $R^2 = 0.003$ ] in black women to 0.330 [ $R^2 = 0.11$ ] in white women). The Bland–Altman plots indicated consistent bias with overestimate for low and underestimate for high intakes. ROC AUCs varied from 0.521 to 0.652 with good sensitivity (95–100%) but very poor specificity

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(0–9%). With the Arithmetic extrapolations correlations varied from 0.116 [ $R^2 = 0.01$ ] to 0.367 [ $R^2 = 0.13$ ]. Bias was detected with both Bland–Altman plots and through quintile analyses (underestimate at low levels and overestimate at high levels). Finally, ROC AUCs varied from 0.514 to 0.640 with moderate sensitivity (64–70%) but low specificity (20–53%). In Study 2 (validation study) results were consistent with the discovery phase in white men.

**Conclusion:** Based on these results, 24-h urinary collection for the measurement of Na excretion remains the preferred tool for assessing salt intake when compared with reported methods based on timed spot urine samples.

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## Introduction

In steady state conditions, the kidneys handle most of the sodium (Na) eaten in a day, so that the majority (up to 95%) is eventually excreted in the urine in the subsequent 24-h, the remaining being excreted in sweat, saliva and gastrointestinal secretions. The daily renal excretion rate of Na is not constant throughout the 24-h, depending on variables, such as time of day, individual's posture, and neuro-hormonal influences. A 24-h urinary collection is the gold standard for assessing salt intake through 24-h urinary Na excretion both in individuals and in populations [1]. This method has been used for a long time in physiological, metabolic and epidemiological research. However, particularly for repeated use in large population studies, 24-h urine collections are often deemed inconvenient and alternative methods have been derived [2–4]. Spot and timed urine samples have been suggested as an alternative and several methods have been devised and tested to calculate the daily excretion from partial urine collections [5].

The aim of the present study was to investigate if spot urine can be an acceptable substitute for 24-h urine to estimate group mean 24-h Na in men and women from different ethnic groups (discovery study). The study tested the validity of spot urines applying different methods used in the literature and replicated the analysis in an independent population of white men (validation study).

## Data and methods

### Study 1 (discovery study)

Participants were selected from general practitioners' registers in the South-West London area, as described in detail elsewhere [6,7] ([www2.warwick.ac.uk/go/cappuccio/whss](http://www2.warwick.ac.uk/go/cappuccio/whss)). In brief, the study was a population-based cross-sectional survey of men and women between 40 and 59 years old of three different ethnic groups: northern European origin (whites); West African or Caribbean origin (blacks) and South Asian Indian origin (S Asians). The study was designed so that there were approximately 250 people in each gender and ethnic group stratum. In all 1577 participants were studied between 1994 and 1996. The present analysis includes 915 participants (297 white [131 men], 326 of black African origin [125 men] and 292 South Asian [154 men]) who were untreated and who provided both 24-h urine collections and timed urine samples. The

study had ethical approval (EG/CL/92.5.17 and 10/H1211/29) and participants gave their informed consent. The participants were asked to attend a screening unit between 08.00 am and 12.00 noon. They were requested to fast for the 12-h prior to the visit. All attendees were administered a questionnaire, which was used to determine age and ethnic origin. Height and weight were measured and used to calculate body mass index (BMI) ( $\text{weight}/(\text{height})^2$ ). Supine blood pressure (BP) was measured with standardised procedure using an automatic machine as previously described [7]. After the interview participants were asked to collect 24-h urine sample within a few days. They were given written detailed instructions on how to collect complete 24-h urine sample. Complete urine collections were either returned by the participants or were collected at the participant's address. Time and volume of collections were immediately recorded, aliquots taken and stored at  $-20^\circ\text{C}$  until assayed. A timed urine collection after an overnight fast was also obtained on the morning of the investigation after the participants had drunk one-to-two glasses of tap water in the morning. Volume (in ml) and duration (in min) of the collection were recorded and specimens were aliquoted and stored at  $-20^\circ\text{C}$  until assayed. Urinary Na and creatinine (Cr) concentrations were measured using an automated analyser.

### Study 2 (validation study)

The study started in 1975 with periodical follow-up for 30 years and involved the Olivetti factories male workforce in southern Italy ([www.olivettiheartstudy.org](http://www.olivettiheartstudy.org)). Data presented in this study were collected during the 2002–2004 follow-up examination; a total of 148 healthy men, aged 32–75 years were examined, as described elsewhere [8]. The local ethics committee approved the study protocol, and participants gave their informed consent. Body weight and height were measured on a standard beam balance scale. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest centimeter. The BMI was calculated as in Study 1. BP was taken after the subject had been sitting upright for at least 10 min. Systolic and diastolic (phase V) BP were measured with a random zero sphygmomanometer (Gelman Hawksley Ltd., Sussex, UK) three times, 2 min apart. The first reading was discarded, and the average of the last two readings was recorded. A 24-h urine collection was obtained from each participant for the measurement of Na excretion to estimate daily dietary Na intake. On the day before the visit,

participants were instructed to discard the first urine of the morning of the collection and to save all urines voided for the following 24-h. Urine was collected in polypropylene bottles, and delivered to the laboratory for immediate storage of the samples at  $-80^{\circ}\text{C}$ . A timed urine collection after an overnight fast was also obtained on the morning of the investigation after the participants had drunk one-to-two glasses of tap water in the morning. Volume (in ml) and duration (in min) of the collection were recorded and specimens were aliquoted and stored at  $-80^{\circ}\text{C}$  until assayed. Urinary Na concentration was measured by flame photometry. The 24-h urinary Cr excretion was used as an estimate of the completeness of the collection: the participants whose urinary creatinine fell below the 5th percentile of the distribution (0.6 g Cr/24-h) were excluded from the analysis. Urinary Cr was determined by picric acid colorimetric method (Jaffe's) using Cobas-Mira analyzer.

## Statistical analyses

### Estimation methods

Two methods are used to estimate 24-h urinary Na from timed urinary Na: Tanaka's prediction [9] and Arithmetic extrapolation.

*Tanaka's prediction* is completed in the following three equations:

$$24\text{-h Ur Cr} \approx \text{predicted } 24\text{-h Ur Cr} \quad (1)$$

$$\frac{24\text{-h Ur Na}}{24\text{-h Ur Cr}} \propto \frac{\text{timed Ur Na}}{\text{timed Ur Cr}} \quad (2)$$

$$24\text{-h Ur Na} \propto \frac{\text{timed Ur Na}}{\text{timed Ur Cr}} \times \text{predicted } 24\text{-h Ur Cr} \quad (3)$$

Equation (1) assumes that 24-h urinary Cr excretion at population level can be approximately estimated on the basis of age, weight and height. Equation (2) assumes that 24-h urinary Na to Cr ratio is proportional to timed urinary Na to Cr ratio. Equation (3) is simply obtained by transforming Equation (2) and replacing the measured 24-h urinary Cr with the predicted values. More details of Tanaka's prediction can be found elsewhere [9–11].

The *Arithmetic extrapolation* is shown in Equation (4). The estimated 24-h urinary Na is obtained by simply extrapolating the timed 24-h urinary Na excretion level from timed duration to 24-h scale:

$$\begin{aligned} \text{Estimated } 24\text{-h Ur Na} = & (\text{timed Ur naconcentration} \\ & \times \text{timed Ur volume}) \\ & \times (24\text{-h} \div \text{Ur collection time}) \end{aligned} \quad (4)$$

### Statistical methods

In Study 1 the analyses were stratified by gender since men and women differ significantly in weight and height, and therefore have different level in 24-h urinary Cr and Na. Stepwise regression was used to predict 24-h urinary Cr, as

stated by Tanaka et al. [9]. Correlation coefficients were examined to compare the estimated and measured 24-h Na. Bland–Altman plot is generally used to compare agreement of measurements in a graphical way. We used it to validate the agreement between the estimated and measured 24-h values [12]. The agreement was also examined by area under the curve (AUC) of Receiver Operating Characteristic (ROC) curve and sensitivity and specificity with a cut-off of  $<100$  mmol/day of urinary Na [13]. This was chosen as a level comparable to the National population target of 6 g of salt per day set in the United Kingdom. In addition, timed urinary Na was compared by quintiles of 24-h urinary Na. A  $p$ -value  $<0.05$  was considered statistically significant. All analyses were conducted using SPSS v17.

## Results

### Study 1 (discovery study)

The characteristics of the participants in Study 1 are shown in online Appendix 1. Men were heavier and taller than women. Daily urinary Na and Cr excretions were significantly higher in men, whilst daily Na to Cr ratio was higher in women (all  $p$ -values  $\leq 0.001$ ). Similar differences were seen in timed Na and Cr concentrations. Timed Na to Cr ratio was significantly higher in women across all ethnic groups (all  $p < 0.001$ ). The average sodium (salt) intakes estimated with the Tanaka methods were not surprisingly comparable to the measured ones, since they were used to develop coefficients for the Tanaka equations (online Appendix 2). However, the Arithmetic method consistently underestimated the intake.

### Tanaka's prediction

Estimates of daily urinary Na using the Tanaka method were derived for men and women in each ethnic group (online Appendix 2). Correlations between measured and estimated 24-h urinary Na were weak, although significant in whites ( $r_{\text{Spearman}} = 0.251$ ;  $p = 0.004$  in men and 0.330;  $p < 0.001$  in women) and South Asians (0.187;  $p = 0.02$  and 0.310;  $p < 0.001$  in men and women respectively). Low grade and non-significant correlations were seen in men and women of African origin (0.166;  $p = 0.065$  and 0.055;  $p = 0.436$ , respectively)(Table 1).

The Tanaka's prediction produced overestimated values in the low 24-h urinary Na levels and underestimated values in the high 24-h urinary Na levels across genders and ethnic groups indicating consistent bias (Fig. 1 and online Appendix 3). The bias was mainly due to the inaccuracy of age, weight and height to predict 24-h creatinine excretion in the three ethnic groups, particularly in those of African origin (data not shown).

Using Receiver Operating Characteristic (ROC) Areas Under the Curve (AUC) estimated 24-h urinary Na values were poor (all  $<0.750$ ) using measured 24-h urinary Na as gold standard. Using a measured 24-h urinary Na  $>100$  mmol/day as positive test, estimated values had moderate sensitivity and poor specificity in both genders and all ethnic groups (Table 1).

Both estimated and measured 24-h urinary Na plotted against quintiles of estimated 24-h Na are shown in online

**Table 1** Correlations between estimated 24-h urinary Na by Tanaka method (top) and by Arithmetic method (bottom), and measured 24-h urinary Na. Values are expressed as coefficient (*p*-value).

	Whites	African origins	South Asians
<i>Tanaka's method</i>			
Women	0.330 (<0.001)	0.055 (0.436)	0.310 (<0.001)
Men	0.251 (0.004)	0.166 (0.065)	0.187 (0.020)
<i>Arithmetic method</i>			
Women	0.367 (<0.001)	0.116 (0.102)	0.264 (0.002)
Men	0.282 (0.001)	0.221 (0.013)	0.170 (0.035)

**Appendix 3.** Poor discrimination of measured Na was found between Q3 and Q4 in white men and Q1, Q2 and Q3 in South Asians. The worst estimation of group means was in those of African origin.

### Arithmetic extrapolation

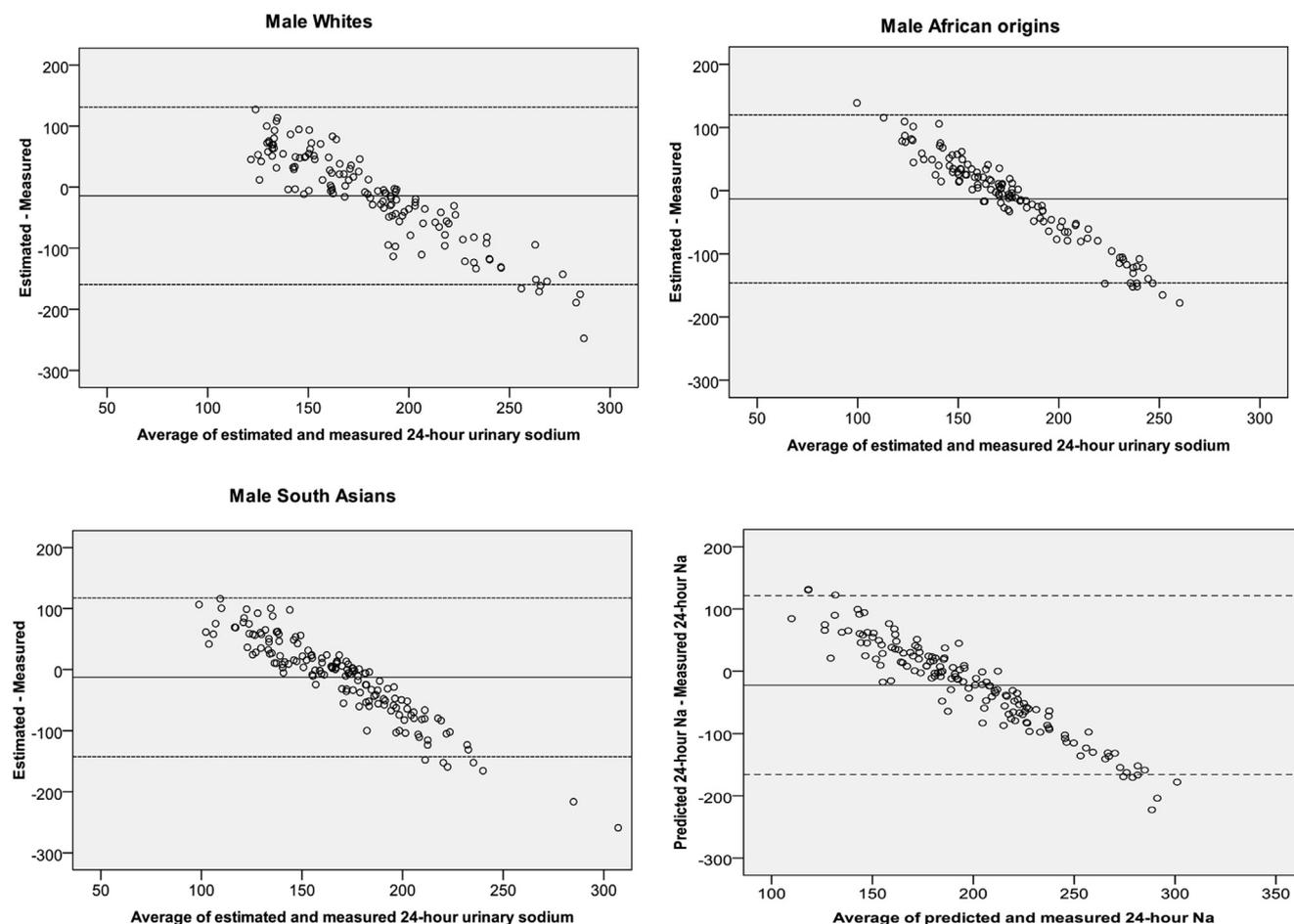
Similar to the Tanaka's method, correlations between measured and estimated 24-h urinary Na were weak,

although significant in whites ( $r_{\text{Spearman}} = 0.282$ ;  $p = 0.001$  in men and  $0.367$ ;  $p < 0.001$  in women) and South Asians ( $0.170$ ;  $p = 0.035$  and  $0.264$ ;  $p = 0.002$  in men and women respectively). In people of African origin the correlation was significant in men ( $0.221$ ;  $p = 0.013$ ) but not in women ( $0.116$ ;  $p = 0.102$ ) (Table 1).

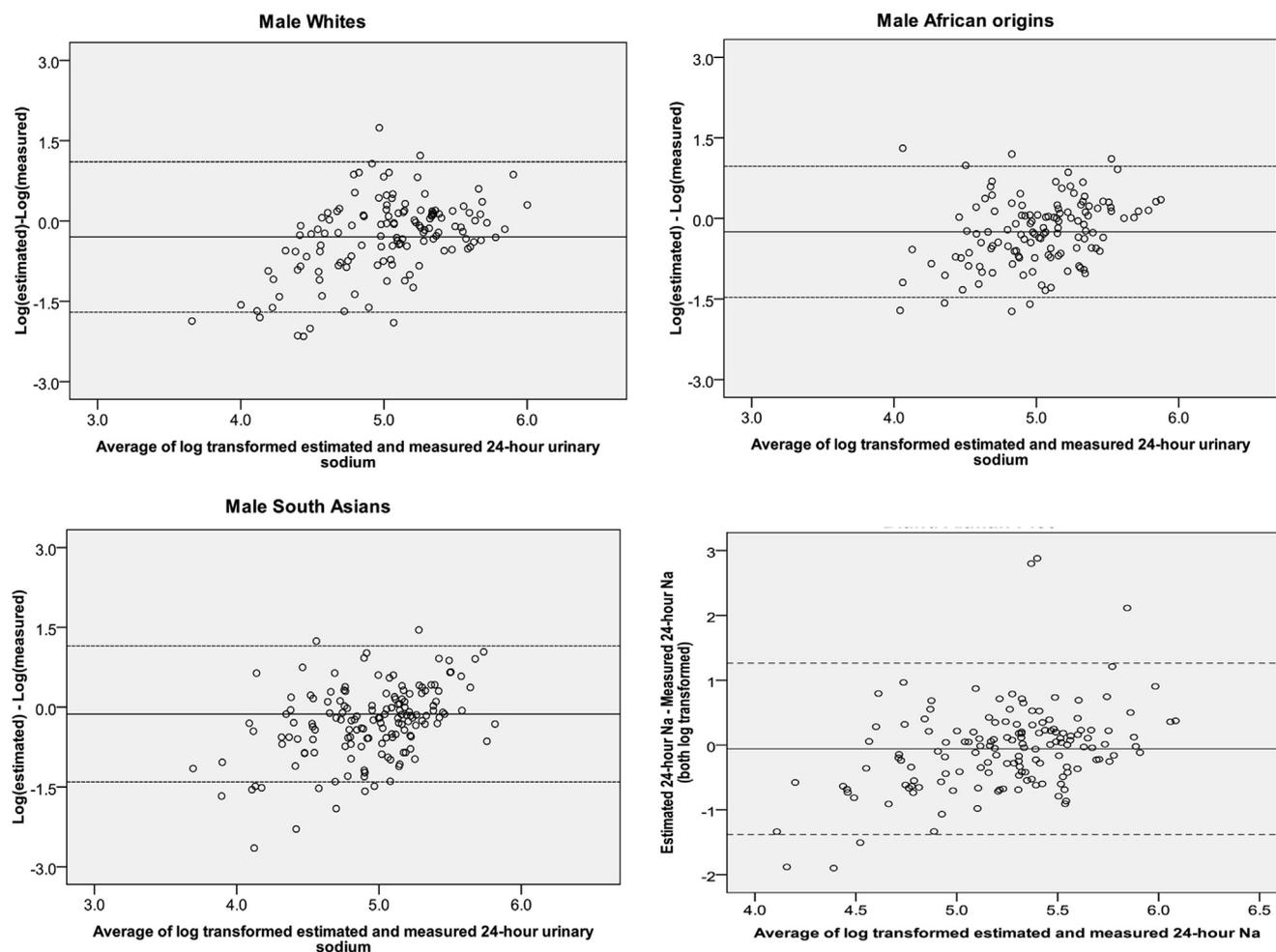
The Arithmetic extrapolation produced underestimated values in the low 24-h urinary Na levels and overestimated values in the high 24-h urinary Na levels across genders and ethnic groups indicating consistent bias (Fig. 2). The bias was in the opposite direction as that described using the Tanaka's method.

Using Receiver Operating Characteristic (ROC) Areas Under the Curve (AUC), again, estimated 24-h urinary Na values were poor (all  $< 0.750$ ) using measured 24-h urinary Na as gold standard. Using a measured 24-h urinary Na  $> 100$  mmol/day as positive test, estimated values had moderate sensitivity and poor specificity in both genders and all ethnic groups (Table 2).

Both estimated and measured 24-h urinary Na plotted against quintiles of estimated 24-h Na are shown in online Appendix 3. Using this method, there is a poor discrimination of measured Na. Furthermore, a bias is clearly detected with gross underestimates at low intakes and gross



**Figure 1** Bland–Altman plot comparing estimated 24-h urinary Na by Tanaka method and measured 24-h urinary Na in men in the WHSS and in the Olivetti Study. Solid and dashed lines are mean and 95% confidence interval of the difference between estimated and measured 24-h urinary Na. Data for women in online Appendix 3.



**Figure 2** Bland–Altman plot comparing estimated 24-h urinary Na by Arithmetic method and measured 24-h urinary Na in men in the WHSS and in the Olivetti Study. Solid and dashed lines are mean and 95% confidence interval of the difference between estimated and measured log transformed 24-h urinary Na. Data for women in online [Appendix 3](#).

overestimates at high intakes across genders and ethnic groups. Note that natural logarithm transformation was applied in this case on the estimated and measured sodium values in order to remove the dependence of variation on the magnitude of urinary sodium level.

### Study 2 (validation study)

We carried out a validation analysis in an independent population sample of white men, whose characteristics are shown in online [Appendix 4](#).

#### Tanaka's prediction

As the participants were all whites, the formulae for white men obtained in Study 1 were used. The pattern of correlation between measured and estimated 24-h urinary Na was similar to the one obtained in white men in Study 1, although the correlation coefficient was stronger ( $r_{\text{spearman}} = 0.499$ ;  $p < 0.001$ ). Like in Study 1, the Bland–Altman plot indicated overestimated values in the low 24-h urinary Na levels and underestimated values in the high 24-h urinary Na levels indicating consistent bias ([Fig. 1](#)). Although the Area Under the Curve (AUC) of ROC was higher than white men in Study 1 (0.649), it was still below 0.750 with specificity of 100% (sensitivity 0%) for the detection of values  $<100$  mmol/day. Online [Appendix 3](#) shows the comparison between estimated and measured 24-h urinary sodium in Study 2 by quintile. Again the quintile was created on the basis of estimated urinary Na

**Table 2** ROC, sensitivity and specificity of two methods of estimation. Measured 24-h urinary Na 100 mmol/day is defined as positive.<sup>a</sup>

	Whites	African origins	South Asians
<i>Tanaka's method</i>			
Women	0.652 (95.8/8.5)	0.550 (100/0)	0.631 (95.3/6.3)
Men	0.538 (100/0)	0.521 (100/0)	0.582 (100/0)
<i>Arithmetic method</i>			
Women	0.640 (68.1/51.1)	0.533 (64.4/42.1)	0.603 (68.9/53.1)
Men	0.514 (69.0/33.3)	0.575 (70.4/20.0)	0.624 (68.9/45.5)

<sup>a</sup> Results are expressed as area under curve (sensitivity %/specificity%).

values. The estimated values show significant underestimation in the Q3–Q5.

### Arithmetic extrapolation

Similar to the Tanaka's method, correlations between measured and estimated 24-h urinary Na were weak, although significant ( $r_{\text{Spearman}} = 0.329$ ;  $p < 0.001$ ).

The Arithmetic extrapolation produced underestimated values in the low 24-h urinary Na levels and overestimated values in the high 24-h urinary Na levels indicating consistent bias (Fig. 2). The bias was in the opposite direction as that described using the Tanaka's method, consistent with the results of Study 1.

Using Receiver Operating Characteristic (ROC) Areas Under the Curve (AUC), like in Study 1, estimated 24-h urinary Na values were poor (0.576) using measured 24-h urinary Na as gold standard. Using a measured 24-h urinary Na  $>100$  mmol/day as positive test, estimated values had moderate sensitivity (85.5%) and poor specificity (20.0%).

Online Appendix 3 shows both estimated and measured 24-h urinary Na plotted against quintiles of estimated 24-h Na. Using this method, there is poor discrimination of measured Na. Furthermore, a bias is clearly detected with underestimate at low intakes and overestimates at high intakes. Note that, as in Study 1, natural logarithm transformation was applied in this case on the estimated and measured sodium values in order to remove the dependence of variation on the magnitude of urinary Na level.

## Discussion

### Main findings

This study investigated the reliability and reproducibility of two methods of assessing daily sodium excretion (hence salt intake) alternative to 24-h urinary sodium, based on spot timed urine collections, using two independent population samples. The results show that simple and less expensive alternative methods using spot and timed urine samples produce biased estimations and have low-grade agreement with 24-h urine collections in both populations. This is in spite of the average value in sex and ethnic groups were highly comparable with one method. The methods show different performance in male and female white, black and South Asian groups, with the worst estimation in people of black African origin. The results suggest that whilst alternative methods using spot and timed urine samples may be able to approximate average true levels in population samples, they are unreliable and not reproducible for estimating salt intake between groups within the same population and they are therefore unsuitable for monitoring group changes over time.

The estimations by the two methods across all ethnic groups show consistently low-grade correlation with measured 24-h urinary sodium. The weak correlations of the estimation by the Tanaka's method are different from other studies. Tanaka et al. [9] reported a correlation coefficient of 0.54 in a Japanese population sample but a coefficient of 0.32 in a validation Japanese sample, both using random spot urines. Kawasaki et al. [10] adopted a

similar estimation method. They produced a correlation coefficient of 0.73 with measured values in a Japanese sample using second morning voiding urines. In the United States, Mann also employed this method and used measured 24-h urinary creatinine in a group of patients with unstated illness. Correlation coefficients were 0.17, 0.31 and 0.86 in random spot, morning and evening urines. The variation of reported coefficients indicates that Tanaka's method performs inconsistently in different populations.

### What is new?

Our conclusions are in keeping with those of a recent systematic review of the world literature carried out in over 1.3 M participants in adults and children [5]. The present study adds to previous evidence in that it provides a systematic validation study repeated in an independent population sample to show the presence of bias. This is important since the assessment of population's salt intake and its changes over time underpin current policies for population salt reduction and they are a major pillar of such programs globally [14].

### Explanation of findings

Although the alternative methods can provide a convenient and less expensive way to estimate 24-h urinary sodium, the estimations are dependent on the variability of urinary sodium concentration. Sodium concentration in spot and timed urines has high inter-individual and intra-individual variability, which are caused by various factors, including time of collection (e.g. morning vs afternoon), dietary consumption (e.g. high-salt-content meal vs low-salt-content meal), hydration and residual bladder volume [5]. Using quintile analysis and Bland–Altman plots, the estimation by the Arithmetic method shows also a large discrepancy with measured 24-h sodium in both samples. It is mainly caused by the sodium variability. If sodium concentration in the timed urine is close to 0, the estimated 24-h urinary sodium will also be close to 0. Therefore, the Arithmetic method is very likely to produce extreme estimates. Compared to the Arithmetic method, Tanaka's method estimates 24-h urinary sodium with adjustment for 24-h urinary creatinine. Although the adjustment somewhat reduces the variability of sodium concentration, its 24-h sodium estimation is still constrained by the accuracy in prediction of 24-h urinary creatinine excretion. The prediction assumes that participants' age, weight and height can approximately produce accurate 24-h creatinine excretion. Our results, however, indicate that this assumption is population-specific. The correlation between predicted and measured 24-h urinary creatinine is weak (results not shown). Although the quintile comparison shows underestimation across all ethnic groups, which is consistent with Tanaka and colleagues' results [9–11], poor discrimination is presented in some ethnic groups, e.g. African men and women and White women. Consistent bias is shown in Bland–Altman plots with poor diagnostic performance presented in sensitivity and specificity tests. Tanaka's method produced underestimations at high levels of salt intake and overestimations at low levels.

## Strengths and limitations

Timed urine samples were used in our analysis instead of spot urine samples. It is known that sodium concentration varies considerably in spot urines. The variability in timed urine samples may be stabilised by the collection time. In addition, our analysis was conducted in participants from populations rather than from patients' groups. Different ethnic representation enabled us to investigate the reproducibility and reliability in different groups. Moreover, our analysis used not only correlation coefficient and quintile comparison, but also Bland–Altman plot, Receiver Operating Characteristic curve and sensitivity/specificity test for comparing agreement and diagnostic performance.

Our analysis has limitations. The validation sample only includes Italian White men. Hence validations in other ethnic groups should be implied. This may limit our validation in both genders and people of African origin and South Asian groups. We relied on a single 24-h urine collection for each participant. This may not be adequate for accurate measurement as salt intake differs largely from day to day [15]. Our analysis tested two methods. Other forms of estimation, such as Na/K ratio, are not analysed and discussed here. The inaccuracy of completeness (both under- and over-collections) is also a limitation. We did not use the para-aminobenzoic acid method [16,17] as it would not be feasible for population studies, especially in low- and middle-income countries, would reduce response rate, and would increase resources needed for surveillance programs [5].

## Implications

The use of alternative methods based on spot and timed urines has become increasingly popular, as it is less onerous for participants, allows faster screening time and requires less training of staff. Although these methods are convenient, they are likely to produce biased estimations of salt intake in populations. Moreover, they are not reproducible. The estimated coefficients for equations used in both Tanaka's methods and the Arithmetic method are different across ethnic groups. This indicates that for each specific population, the sodium to creatinine ratio, sodium concentration, and anthropometric information have to be collected each time in order to obtain the estimation equations for the population.

## Conclusions

Urinary sodium concentration in spot and timed urines does not provide reliable and reproducible estimates of 24-h urinary sodium excretion. Twenty-four-hour urinary collection for measurement of sodium excretion remains the preferred tool for assessing salt intake in populations.

## Disclosure

The publication does not necessarily represent the decisions or the stated policy of W.H.O. and the designations employed and the presentation of material do not imply the expression of any opinion on the part of W.H.O.

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FPC and MAM (Wandsworth Heart and Stroke Study) and PS and FPC (The Olivetti Heart Study) were responsible for the setting up and conduction of the population studies. CJ and AV carried out statistical analyses. CJ and FPC drafted the manuscript and all authors made significant intellectual contribution to the manuscript. The studies were supported over the years by the SWT Regional Health Authority, the NHS Directorate, the British Heart Foundation, the British Diabetic Association, the Stroke Association, the University of Warwick RDF, the Ministry of Health of the Italian Government. No conflict of interest declare

## Appendix A Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2013.06.011>.

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