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# Validation of diet and urinary excretion derived estimates of sodium excretion against 24-h urine excretion in a worksite sample



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## **KEYWORDS**

Dietary sodium; Urinary sodium; 24-h dietary recall; 24-h urine collection; Spot urine **Abstract** *Background and aims:* To validate diet and urinary excretion derived estimates of sodium intake against those derived from 24-h urine collections in an Irish manufacturing workplace sample.

*Methods and results:* We have compared daily sodium (Na) excretion from PABA validated 24-h urine collections with estimated daily sodium excretion derived from the following methods: a standard Food Frequency Questionnaire (FFQ), a modified 24-h dietary recall method, arithmetic extrapolations from morning and evening spot urine samples, predicted sodium excretion from morning and evening spot urine samples using Tanaka's, Kawasaki's and the INTERSALT formula. All were assessed using mean differences (SD), Bland–Altman plots, correlation coefficients and ROC Area under the Curve (AUC) for a cut off of  $\geq$ 100 mmol of Na/day. The Food Choice at Work study recruited 802 participants aged 18–64 years, 50 of whom formed the validation sample. The mean measured 24-h urinary sodium (gold standard) was 138 mmol/day (8.1 g salt). At the group level, mean differences were small for both dietary methods and for the arithmetic extrapolations from morning urine samples. The Tanaka, Kawasaki and INTERSALT methods provided biased estimates of 24-h urinary sodium. R<sup>2</sup> values for all methods ranged from 0.1 to 0.48 and AUC findings from 0.57 to 0.76.

*Conclusion:* Neither dietary nor spot urine sample methods provide adequate validity in the estimation of 24-h urinary sodium at the individual level. However, group mean errors from dietary methods are small and random and compare favourably with those from spot urine samples in this population.

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# Introduction

Hypertension is a leading cause of 'death, stroke, myocardial infarction, congestive heart failure and chronic renal impairment' and affects 1 billion people worldwide

http://dx.doi.org/10.1016/j.numecd.2015.04.010 0939-4753/© 2015 Elsevier B.V. All rights reserved. [1]. Observational and experimental research has provided substantial evidence that excess dietary salt intake is a casual factor for hypertension [2]. Irish and UK authorities have set an upper limit for recommended salt intake of 6 g per day while the World Health Organisation (WHO) recommend an upper limit of 5 g per day [3]. Globally, new evidence suggests that the average level of sodium consumption is 3.95 g per day of sodium (approx. 10 g salt) which is almost double the WHO recommendation [4]. There is a compelling need to develop valid and reliable measures of sodium intake that are feasible for use in the study of associations between sodium intake and health

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outcomes and in on-going surveillance population studies of sodium intake.

Current measurements of dietary and urinary sodium are fraught with methodological difficulties [5]. Dietary methods tend to underestimate sodium consumption due to under-reporting of discretionary sources of salt (added at the table, or during cooking) [6]. The 24-h urine collection method which is considered the 'gold standard' is burdensome and potentially limited by under-collection [7]. Several methods have also been used to predict 24h urinary sodium from spot urine samples, including arithmetic extrapolation [8] the INTERSALT formula [9] and the application of predictive formulae based on spot sodium to creatinine ratios as a means of controlling for urinary concentration, including those of Tanaka [10] and Kawasaki [11]. While the latter spot urine methods may be adequate for population level monitoring where the focus is on estimation of mean sodium intake at the group level, their use in analytical epidemiological research, as in recent studies suggesting potential harms from low intakes of dietary sodium [11.12], remains controversial.

The primary aim of this study was to validate a modified 24-h dietary recall method for sodium intakes which used specific verbal prompts for discretionary salt consumption and portion size against the gold standard paraaminobenzoic acid (PABA) validated 24-h urine collections. We also validated a number of other methods for estimation of 24-h sodium excretion: a standard FFQ, arithmetic extrapolations from morning and evening spot urine samples, predicted sodium excretion from morning and evening spot urine samples using Tanaka's formula, Kawasaki's formula and the INTERSALT formula.

# Methods

## Study design

Cross sectional baseline data were obtained from a large clustered controlled trial, the Food Choice at Work (FCW) study which is described elsewhere [14]. Four multinational manufacturing workplaces participated in this trial.

## Study subjects

Participants were aged 18–64 years. Any full time, permanent employee who consumed one daily meal in the workplace canteen was eligible for the study. The FCW study population comprised of 802 participants and from this 50 participants provided a complete 24-h urine collection for the validation study.

# Data collection

All participants were asked to complete a health, lifestyle and food questionnaire, a physical assessment, a FFQ, a 24h dietary recall, spot urine samples and/or a single 24h urine collection. Participants who did not were excluded from the analysis. Questionnaires were self-completed by participants electronically or in hard-copy format. Physical assessments and 24-h dietary recalls were conducted by trained research assistants. All data was collected during employees working hours.

#### Health, lifestyle and food questionnaire

Socio-demographic indicators included gender, age, ethnicity, education, marital status and work life (job position and usual working hours).

# Physical assessment

All participants underwent a comprehensive physical assessment where body mass index (BMI), midway-waist circumference and resting blood pressure were measured by trained research assistants as per the detailed guide-lines outlined in the Standard Operating Procedures (SOP) manual [15].

## **Dietary information**

## FFQ

The FFQ was an adapted version of the European Prospective Investigation of Cancer (EPIC) FFQ [16]. It was validated for use in the Irish population [17–19]. The average frequency of consumption of each food item over the previous year was recorded by participants. The FFQ was designed to assess extensively the whole diet and included 150 food items arranged into the main food groups.

The food frequency data was analysed using a specifically designed computer program called FFQ Software, Version 2.0, developed by Juzer Lotya of the National Nutrition Surveillance Centre, School of Public Health and Population Science, University College Dublin. The program converted the dietary information provided to food quantities and subsequently to food nutrient values, based on data from the Food Standards Agency [20] and McCance and Widdowson's Food Composition Tables [21].

# 24-h dietary recall

The 24-h dietary recall method was a modified version of the validated UK 24-h dietary recall method [22]. Two dietary recalls were collected within one week to examine on and off duty work dietary patterns. The 3-step method outlined specifically what the participant had to eat and drink in the previous 24-h period.

- 1. Quick list: participants were asked to report everything that they had to eat or drink the day before their appointment (midnight to midnight).
- The nutritionist or research assistant collected detailed information on items named in the quick list (consumption time, place of consumption, brand and recipe), foods likely to be eaten in combination (milk in coffee) and the quantity consumed and any leftovers or second helpings.

3. Recall review: participants had an opportunity to provide additional information or to refer to foods forgotten in the quick list.

Additional modifications to this method included specific prompts for discretionary salt consumption (at the table and while cooking); information on accurate estimations of portion size, eating times, food brands and labels. All recalls were conducted by trained research assistants' and lasted approximately 20 minutes. Each food, drink and portion size was coded according to the 24-h coding instructions based on the validated UK method. Food and nutrient analysis was calculated using NetWISP4<sup>®</sup> (Weighed Intake Software Program; Tinuviel Software, Warrington, UK) [23,24]. The 24-h dietary recall corresponded to the same time period as the 24-h urine collection.

#### Urinary derived estimates

#### Spot urine samples

Each participant provided one sample the evening before and morning of their on-duty or off-duty dietary recall. The urine samples were taken approximately 12 h apart e.g. 8pm—8am either on the evening and morning before the 24-h urine collection commenced or on the opposite appointment to the 24-h urine collection. Urinary electrolyte levels were measured using standard reagents and methods by the biochemistry laboratory of the Mercy University Hospital Cork. To estimate total sodium excretion in the spot urines, the sodium content was converted to mmol per day. To estimate mmol of sodium, we used gender specific PABA validated 24-h mean urinary volume estimations derived from a larger but similar work based population [25].

The INTERSALT formula, Tanaka's and Kawasaki's equations were used to estimate 24-h urinary sodium. The following equations were used:

INTERSALT formula [9]:

$$\begin{split} & \text{Men}: 23 \times \{25.46 + [0.46 \times \text{spot Na}(\text{mmol/L})] \\ & -[2.75 \times \text{spot Cr} \ (\text{mmol/L})] - [0.13 \times \text{spot K} \ (\text{mmol/L})] \\ & +[4.10 \times \text{BMI}(\text{kg}/\text{m2})] + [0.26 \times \text{age}(\text{y})] \} \\ & \text{Women}: 23 \times \{5.07 + [0.34 \times \text{spot Na} \ (\text{mmol/L})] \\ & -[2.16 \times \text{spot Cr} \ (\text{mmol/L})] - [0.09 \times \text{spot K} \ (\text{mmol/L})] \\ & +[2.39 \times \text{BMI}(\text{kg}/\text{m2})] + [2.35 \times \text{age}(\text{y})] - [0.03 \times \text{age}^2 \ (\text{y})] \end{split}$$

Tanaka's equation [10]:

 $21.98 \times XNa^{0.392}$  where  $XNa = SUNa/SUCr \times PRCr$ 

SUNA = Na concentration (mEq/L) in the spot urine SUCr = creatinine concentration (mg/dl) in the spot urine

Predicted creatinine (PRCr) assumes that 24-h urinary creatinine excretion can be estimated approximately on the basis of age, weight and height at the population level. 773

The predicted creatinine formula as stated by Tanaka was as follows (10):

 $\begin{array}{rl} -2.04 \ \times \ age \ + \ 14.89 \ \times \ weight(kg) \ + \ 16.14 \\ \\ \times \ height(cm) \ - \ 2244.45. \end{array}$ 

Kawasaki's equation for sodium [11]:

 $\begin{array}{l} 16.3\times \sqrt{[\text{Spot Na/Spot Cr}]} \times \text{ predicted } 24-\text{h urinary Cr},\\ \text{where predicted Cr (mg/day) for women is : } -4.72\times\\ \text{age (years)} + 8.58\times \text{weight (kg)} + 5.09\\ \times \text{height(cm)} - 74.5; \text{ and for men is : } -12.63 \end{array}$ 

 $\times$  age (years) + 15.12  $\times$  weight (kg) + 7.39

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\times height (cm) - 79.9.
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#### Single 24-h urine collection

A standard verbal and written explanation of the 24h urine collection process was provided to all participants prior to participation. Eighty nine participants completed a single 24-h urine collection. However, 6 did not complete the FFQ and/or HLFQ and 6 did not wish to take the PABA tablets. Three 80 mg doses of PABA (a biologically inert substance rapidly excreted in urine) were administered to all participants in tablet form the day of urine collection to validate the completeness of the sample. Each participant was provided with 2 three litre storage containers and one 500 ml storage container in a strong opaque carrier bag. Participants were asked to outline whether or not they had accidentally missed a urine collection.

Once 24-h urine collections were returned, the collections were weighed and urinary electrolyte levels were measured in the biochemistry laboratory of the Mercy University Hospital Cork. Urine samples were stored at -20 C once aliquoted. PABA analysis was conducted at the Medical Research Council Human Nutrition Research Laboratory in Cambridge, United Kingdom. The samples were transferred frozen. A colorimetric microplate method was used to assay the PABA samples. Results were reported as a percentage of the PABA dose excreted.

A complete urine sample was assumed when between 70% and 103% of the PABA ingested dose appeared in the urine. Those containing <70% are interpreted as 'incomplete' and >103% are interpreted as 'over'. In this study, 50 participants had >70–102% PABA excretion and detectable sodium in the sample.

#### Statistical analysis

Data was recorded manually and entered electronically into SPSS prior to statistical analysis. Data manipulation and statistical analyses were conducted using SPSS Version 21 and p < 0.05 was considered significant. Unweighted mean (SD), median and 95% CI values were reported for each method. Certain outliers with very high sodium and potassium intakes did remain in the data and analysis of variance (ANOVA) was then used to compare mean nutrient intakes. Outliers were excluded based on z scores for kilocalories, <-3.3 and >3.3. difference between the gold standard and each alternative method was calculated and plotted against the mean of the two measurements. Overall, 95% limits of agreement were calculated as the mean difference  $\pm 1.96$  SD, where SD is the standard deviation of the differences in paired measurements. Agreement between methods was also examined by Pearson's correlation coefficients and by receiver operating characteristic (ROC) area under the curve with a cut -off point of  $\geq 100$  mmol/l for sodium. These levels were chosen as it is the upper tolerable limit for sodium intake in Ireland and the UK.

# Ethics

Ethical approval was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals in the Republic of Ireland May 2012 and amended in March 2013. All participants provided written informed consent.

## Results

The characteristics of the FCW and validation sample population are summarised in Table 1. Majority of

participants were aged 18–39 years (60%), male (60%) and had a tertiary education (50.7%). Overweight and obesity levels were higher among males (54.3% and 22.3%) in comparison to females (36.9% and 19.6%). More men (22%) than women (7.3%) were classified as hypertensive. Overall, there were no significant differences between the 2 groups with the exception of those in the validation sample group having a higher level of education (p = 0.013) and a lower level of diastolic hypertension (p = 0.003).

Estimations of dietary and urinary sodium for the FCW and validation sample population are presented in Table 2. In the FCW population, mean estimated sodium intake was higher in males than in females for all methods. The mean measured 24-h urinary sodium in the validation was 138 mmol/day (8.1 g), virtually identical to that estimated from the 24-h dietary recall (134 mmol/day), the arithmetic extrapolations from morning spot urine samples (136 mmol/day) and the INTERSALT formula from evening spot samples (132 mmol/day). Group mean estimates from morning spot samples were closer than evening samples to the 24-h estimates of urinary sodium. However for some methods, notably the Kawasaki method both morning and evening samples overestimated sodium excretion relative to the measured 24-h urinary sodium.

Bland–Altman analysis is shown in Fig. 1. The degree of bias (i.e. mean difference between measured and

	Total (n = 50) N (%)	Men (n = 32) N (%)	Women (n = 18) N (%)	p-value	Total (n = 802) N (%)	Men (n = 556) N (%)	Women (n = 246) N (%)	p-value*
Age								
18–39 years	32 (64)	23 (72)	9 (50)	0.122	478 (60)	335 (60)	143 (58)	0.572
40–64years	18 (36)	9 (28)	9 (50)		324 (40)	221 (40)	103 (42)	
Mean	37.7	37.9	37.3		38.7	38.8	38.4	
Education								
Leaving Cert or less	4(8)	3 (9)	1 (6)	0.200	181 (23)	100 (18)	81 (33)	0.000*
Certificate/diploma	16 (32)	8 (25)	8 (44)		214 (27)	143 (26)	71 (29)	
Primary/Degree	17 (34)	14 (44)	3 (17)		241 (30)	192 (35)	49 (20)	
Post Graduate	13 (26)	7 (22)	6 (33)		166 (21)	121 (22)	45 (18)	
Job position/Manage	er							
Manager	5 (10)	4 (13)	1 (6)	0.018	86(11)	73 (13)	13 (5)	0.001*
Supervisor	4 (8)	0	4 (22)		84 (11)	63 (11)	21 (9)	
Not a manager/not a supervisor	41 (82)	28 (88)	13 (72)		632 (79)	420 (76)	212 (86)	
BMI status								
Normal weight	15 (30)	7 (22)	8 (44)	0.239	236 (29)	130 (23)	106 (43)	0.000*
Overweight	29 (58)	21 (66)	8 (44)		393 (49)	302 (54)	91 (37)	
Obese	6(12)	4 (13)	2 (11)		172 (22)	124 (22)	48 (20)	
Mean	26.4	26.9	25.5 <sup>´</sup>		27.2	27.6	26.3	
Hypertensive								
Yes	6(12)	5 (16)	1 (6)	0.293	110 (14)	96 (17)	14 (6)	0.000*
No	44 (88)	27 (84)	17 (94)		692 (86)	460 (83)	232 (94)	
<b>Creatinine:</b> Mean (SD) median	15 (5)15	18 (3)17	12 (5)10					
Blood pressure								
Systolic: Mean (SD) median	120 (16)119	124 (14)121	112 (17)109		121 (15)120	125 (13)123	112 (15)110	
Diastolic: Mean (SD) median	73 (9)72	74 (8)74	70 (9)68		75 (10)75	76 (9)76	72 (10)71	

Validation study population					FCW study population			
Method	Total $(n = 50)^d$	$\begin{array}{l} \text{Men} \\ (n = 32) \end{array}$	Women $(n = 18)$	Total (n = 793) <sup>d</sup>	Men (n = 550)	Women $(n = 243)$		
24 h urine PABA validated <sup>a</sup>	138 (53)	147 (46)	121 (61)		_	_		
FFQ <sup>b</sup>	129 (50)	126 (56)	133 (39)	132 (53)	135 (53)	128 (53)		
24 h dietary recall <sup>b</sup>	134 (65)	147 (67)	111 (55)	132 (76)	141 (82)	112 (56)		
Arithmetic extrapolations morning spot <sup>c</sup>	136 (72)	145 (83)	124 (1)	167 (82)	180 (84)	141 (73)		
Arithmetic extrapolations evening spot <sup>c</sup>	168(820	191 (890)	137 (0)	186 (108)	201 (109)	150 (93)		
Tanaka's prediction morning spot 24 h estimate	129 (27)	134 (26)	122 (27)	135 (31)	136 (29)	133 (34)		
Tanaka's prediction evening spot 24 h estimate	147 (32)	148 (33)	122 (27)	157 (32)	159 (32)	154 (33)		
Kawasaki's prediction morning spot 24 h estimate	157 (45)	174 (42)	134 (38)	198 (64)	218 (59)	152 (48)		
Kawasaki's prediction evening spot 24 h estimate	187 (53)	199 (57)	169 (42)	239 (72)	264 (67)	184 (49)		
Intersalt prediction morning spot 24 h estimate	125 (28)	141 (23)	103 (19)	135 (33)	146 (31)	111 (23)		
Intersalt prediction evening spot 24 h estimate	132 (30)	148 (26)	110 (19)	145 (35)	158 (33)	118 (24)		

<sup>a</sup> Gold standard method.

<sup>b</sup> All dietary assessments have been normalised from mg Na to mmol of sodium/day.

<sup>c</sup> Based on single specimen averaged for 24 h collection g/day.

<sup>d</sup> Slight variation to total numbers for different methods.

estimated mean sodium) at the group level was small for both dietary methods and for some but not all of the urine derived methods ranging from 3.8 to -47 mmol sodium. The Kawasaki evening spot prediction had the largest degree of bias (-47 mmol sodium). The Tanaka prediction and INTERSALT prediction tended to underestimate 24-h values at low excretion levels and over estimate at higher levels.

Table 3 presents the findings on the performance of dietary and spot urine derived measures of 24-h sodium excretion versus measured 24-h urinary sodium as assessed by mean difference on Bland–Altman analysis, correlation coefficients and ROC Area under the Curve values. The performance of all methods was relatively poor with  $R^2$  values ranging from 0.07 to 0.48 and AUC values ranging from 0.56 to 0.76.

## Discussion

The findings suggest that at the individual level neither dietary methods nor spot urine samples provide adequate accuracy in the assessment of 24-h urinary sodium relative to the gold standard of measured 24-h urinary sodium. However group mean errors from both dietary methods (FFQ and modified 24-h dietary recall, a novel method that can be completed in under 20 minutes) were small and random and compare favourably with those from spot urine samples in this population.

The findings are consistent with an emerging consensus that spot urinary sodium is a poor predictor of 24h excretion in individuals but may provide adequate mean estimates for population level monitoring [7,26]. Particularly, there was no evidence that the use of the Tanaka [10] and Kawasaki [11] predictive formulae increases the accuracy of estimates of 24-h urinary sodium relative to simple arithmetic extrapolation or the dietary methods. Data on the Tanaka formula which underestimated 24-h values at low excretion levels and overestimated values at higher levels are consistent with the findings from Ji and colleagues who carried out a validation study of spot versus 24-h urine samples in multi-ethnic populations in Britain and Italy [8]. It is also noteworthy that in the latter study, the validity of spot urine estimates varied between men and women and in different ethnic groups. The extent to which the Kawasaki predictive formula overestimates measured 24-h urinary sodium raises concern about the appropriateness of using this formula in analytical epidemiological research [12,13].

Several different formulae have been suggested to estimate spot urinary sodium over 24-h. In this study the INTERSALT formula provided the least bias information regarding mean sodium intake when compared to the Tanaka and Kawasaki formula. This finding is consistent with the findings from Cogswell and colleagues who carried out a validation study of predictive equations for 24h urinary sodium excretion in adults aged 18–64 years [9].

The findings from this study suggest that specific dietary intake methods can usefully estimate mean sodium intakes at the population level. This is consistent with reports from the USDA Automated Multiple Pass Method Validation study which uses a 24-h dietary recall method [27]. The latter study reported that sodium intake was underestimated by less than 9% in comparison to the sodium biomarker and the authors suggest that dietary intake methods are an acceptable measure at the population/group level for estimating sodium intakes. Failure to capture discretionary salt or salt added during cooking or at the table is a major factor in the underestimation of daily sodium intake [23]. However, one of the unique features of the 24-h dietary recall method used in this study is the use of prompts for discretionary salt and the careful questioning by trained research assistants regarding actual portion size consumed, eating times and food labels.

Strengths of the study include that all workplaces had similar characteristics as they were all manufacturing workplaces with similar shift patterns and work schedules.



**Figure 1** Bland–Altman analysis for dietary and urinary sodium based on the validation study population (n = 50).



**Table 3** Performance of dietary and spot urine derived measures of 24-h sodium excretion versus measured 24-h urinary sodium as assessed by mean difference on Bland–Altman analysis, correlation coefficients and ROC Area under the Curve values based on the validation study population (n = 50).

Method	Mean difference (SD)	95% CI on mean difference	95% limits of agreement	R <sup>2</sup>	p-value	AUC	95% CI
FFQ	9.1 (52.4)	-5.7, 24	-95.7, 113.9	0.48	0.000	0.76	0.6, 0.9
24-h dietary recall	3.8 (69.4)	-15, 23	-135, 142.6	0.32	0.023	0.71	0.5, 0.8
Arithmetic extrapolations morning spot	3.8 (77.4)	-20, 27	-151, 158.6	0.28	0.075	0.57	0.4, 0.7
Arithmetic extrapolations evening spot	-28.3 (94.7)	-57, 10	-217.7, 161.1	0.07	0.066	0.56	0.4, 0.7
Tanaka's prediction morning spot 24 h estimate	10.9 (54)	-5, 27	-94.9, 116.7	0.24	0.114	0.60	0.4, 0.8
Tanaka's prediction evening spot 24 h estimate	-7.8 (52)	-23, 8	-109.7, 94.1	0.35	0.022	0.64	0.4, 0.8
Kawaski's prediction morning spot 24 h estimate	-17.1 (61)	-36, 1.1,	-136.3, 102.5	0.24	0.122	0.63	0.4, 0.8
Kawaski's prediction evening spot 24 h estimate	-47.0 (61)	-65, -28	-166.6, 72.6	0.34	0.025	0.68	0.5, 0.9
Intersalt prediction morning spot 24 h estimate	15.1 (52)	-0.8, 31	-74, 163.6	0.32	0.033	0.70	0.5, 0.8
Intersalt prediction evening spot 24 h estimate	7.8 (51)	-7.9, 24	-89, 158	0.36	0.019	0.71	0.5, 0.9

Employees that participated in the validation study had comparable demographics and health status characteristics when compared to the overall FCW study population. This is one of the few studies to compare both diet and spot urine estimates of 24-h sodium in the same population.

Limitations associated with this study include the small sample size of the validation population (n = 50). It may also be objected that the generalisability of the findings is limited by the fact that the participants are a nonrepresentative group of healthy employees in a workplace setting where dietary exposures are relatively stable. This may have contributed to the relative accuracy of the dietary recall methods versus the spot urine sample estimates in this setting. However as there is no accepted alternative to a 24-h urine collection suitable for use in all settings, the findings highlight the need, in specific settings such as the workplace, to compare and calibrate methods of estimating 24-h sodium excretion against 24h collections. The findings also suggest that in some settings, dietary methods, in addition to providing valuable information on the sources of dietary sodium, may also provide estimates of 24-h intake of adequate accuracy at the group level.

# Conclusion

Although the 24-h urine collection is burdensome for use in large scale studies it remains the gold standard for work addressing the impact of sodium intake on health outcomes. The present study demonstrated that neither dietary nor urinary methods based on morning or evening spot samples provide adequate validity in the estimation of dietary sodium intake at the individual level. However the dietary methods and some of the urinary methods may be applied at the population level for estimations of mean dietary sodium intake.

## **Contributions of authors**

CK was responsible primarily for the final content of the paper. CK, FG, GB, IJP worked on the study design and cowrote the final manuscript. CK, FG, TF, GB, were responsible for data analysis and interpretation of results. All authors approved the final version of the paper for publication.

# **Declarations of interest**

The authors declare that there are no conflicts of interest.

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