Original Article

Formula-led methods using first morning fasting spot urine to assess usual salt intake: a secondary analysis of PURE study data

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Objectives: Observational studies that assess the relationship between salt intake and long-term outcomes require a valid estimate of usual salt intake. The gold-standard measure in individuals is sodium excretion in multiple nonconsecutive 24-h urines. Multiple studies have demonstrated that random spot urine samples are not valid for estimating usual salt intake; however, some researchers believe that fasting morning spot urine samples produce a better measure of usual salt intake than random spot samples.

Methods: We have used publicly available data from a PURE China validation study to compare estimates of usual salt intake from morning spot urine samples and four published formulae with mean of two 24-h urine samples (reference). We estimated the mean and 95% confidence interval of absolute and relative error for each formula-led method and the degree to which estimates were able to be classified into the correct quartile of intake. Bland-Altman plots were used to test the level of agreement.

Results: The results show that compared with the reference method, all formulae-led estimates from spot urine collections have high error rates: both random and systematic. This is demonstrated for individual estimates, as well as by quartiles of reference salt intake. This study conclusively demonstrates the unsuitability of morning spot urine formula-led estimates of usual salt intake.

Conclusion: Our findings support international recommendations to not conduct, fund, or publish research studies that use spot urine samples with estimating equations to assess individuals' salt intake in association with health outcomes.

Keywords: 24-h urine, dietary salt, sodium, spot urine

Abbreviations: ANOVA, Analysis of variance; CVD, Cardiovascular Disease; INTERSALT, International salt study; PURE, Prospective Urban Rural Epidemiology

INTRODUCTION

There is consistent support from national and international organizations for global population dietary salt reduction to reduce blood pressure, cardiovascular and other noncommunicable diseases [1,2]. However, despite extensive evidence of a linear and graded relationship between salt intake and blood pressure and cardiovascular disease outcomes [2,3], a minority of authors continue to question the benefits of the current public health recommendations of reducing population salt intake, thereby threatening the implementation of effective strategies. They often cite data from observational studies such as the Prospective Urban Rural Epidemiology (PURE) study that show a J shaped curve between urinary sodium excretion (as a measure of salt intake) and cardiovascular disease (CVD) outcomes (including mortality) using a single spot urine as the measure of salt intake [4,5]. The J shape is an artefact of the systematic error in the Kawasaki equation which is used to convert a single spot urine into an estimate of usual intake. The fact that multiple studies have demonstrated this error in the measurement of salt intake [6–8], including even the PURE study validation papers [9,10], continues to be ignored by some, and misunderstood by many [11].

Multiple studies have demonstrated that random spot urine samples are not valid for estimating usual salt intake [6,12]. The authors of the PURE study have repeatedly stated that fasting morning spot urine samples produce a better measure of usual salt intake than random spot samples, thereby justifying their use in cohort studies as an accurate measure of exposure [13,14]. They suggest that early morning (first or second) fasting urine samples represent a 'basal level of excretion' [13], and therefore when used with the Kawasaki formula [15], a single fasting spot urine is a valid measure of usual salt intake. They have also stated that previously raised objections to the use of spot urine as a

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valid measure of usual salt intake [16] are 'invalid' because some of the studies cited have used random spot rather than fasting spot urine samples [14].

Here, we aim to investigate whether a single morning fasting urine sample is a valid measure of usual salt intake by re-analysing data from the PURE-China validation study and examining the accuracy and reproducibility of these formula-based methods [9].

MATERIALS AND METHODS

Participants and procedures

We have used publicly available data from a PURE validation study [9] where there is a full description of the methods. Briefly, a subset of participants in the PURE prospective observational study were recruited from urban and rural centres in Shanxi Province China while they were attending their 3 or 6-year follow up visit. Participants were aged 35–70 years, and were excluded if they were taking diuretic medication, pregnant or breastfeeding, had food restrictions due to chronic illness. Participants collected a 24-h urine sample according to standard procedures, and a spot 'morning fasting urine sample' on completion of the 24-h urine collection. This was repeated around 30 days later, so that all participants each collected two 24-h urines and two spot morning urines. Urine samples were analysed for concentrations of sodium, and potassium by emission flame photometry, and creatinine by the Jaffe method [9].

Statistical analysis

A total of 116 participants with 24-h urine samples classified as 'complete' at the first urine collection were included in the analysis of the first urine collection. One hundred and seven of the 116 participants provided repeated urine samples 30 days later and thus were included in the analysis of the second urine collection. To estimate usual salt intake, we calculated the average urinary sodium excretion across the two 24-h collections. We utilized the urinary values from the first urine samples for nine out of 116 participants who did not provide a complete second urine collection.

Although Peng *et al.* [9] stated that they excluded participants who provided 24-h urine collections that were regarded as 'incomplete', the criteria for determining completeness are not stated in the article. A variety of methods have been used to assess completeness in other studies, including self-reported missing voids, creatinine excretion and 24-h urine volume [17]. We applied two methods to assess completeness of the 24-h urines: volume (between 500 and 6000 ml) and using a method that excluded urine samples that contained more or less than 15% of the expected creatinine value as predicted by a mathematical formula using the participant's age, sex, weight and height from Kawasaki *et al.* [15]. Our initial assessment showed that using our method, only 62 of the 116 participants first urines could be classified as 'complete'. However, in the main analysis, we used all samples classified as complete in the original dataset, so that our results are directly comparable with the original work, and with the wider PURE study analysis.

The measured 24-h urinary sodium excretion was calculated using the following equation:

$$24h\ urinary\ sodium\ excretion(mmol) = \frac{sodium\ concentration\ of\ urine\ sample\ (mmol/L) * urine\ volume\ (L)}{1,000}$$

The estimated 24-h urinary sodium excretion based on three different methods (i.e. Kawasaki, Tanaka and INTERSALT formulae) was calculated using the formulae shown in below:

Kawasaki formula [15]:

16.3 × (spot sodium concentration[mmol/L] ÷ (spot creatinine concentration[mg/dL] x 10) × estimated 24-h creatinine excretion[mg/24-h]) $^{0.5}$

where the 24-h creatinine excretion is estimated as follows:

For men: (-12.63 × age [year]) + (15.12 × weight [kg]) + (7.39 × height [cm]) - 79.90
For women: (-4.72 × age [year]) + (8.58 × weight [kg]) + (5.09 × height [cm]) - 74.50

Tanaka formula [18]:

21.98 × (spot sodium concentration [mmol/L] ÷ (spot creatinine concentration [mg/dL] × 10) × estimated 24-h creatinine excretion [mg/24-h])$^{0.3925}$

where the 24-h creatinine excretion is estimated as follows for both sexes: (-2.04 × age [year]) + (14.89 × weight [kg]) + (16.14 × height [cm]) - 2244.45

Intersalt with potassium [19]:

Men: (25.46 + (0.46 × spot sodium concentration [mmol/L]) - (2.75 × spot creatinine concentration [mmol/L]) - (0.13 × spot potassium concentration [mmol/L]) + (4.10 × body mass index [kg/m^2*]) + (0.26 × age [years]))* ^{5}
Women: (5.07 + (0.34 × spot sodium concentration [mmol/L]) - (2.16 × spot creatinine concentration [mmol/L]) - (0.09 × spot potassium concentration [mmol/L]) + (2.39 × body mass index [kg/m^2*]) + (2.35 × age [years]) - (0.03 × age*^{2} *[years]))*

The measured or estimated 24-h urinary sodium excretion was then converted to salt intake using the following equation:

*Measured salt intake (g/day) = measured 24 h urinary sodium excretion (mmol) * 58.5/1000*
or

*Formula estimate of salt intake (g/day) = formula estimate of 24 h urinary sodium excretion (mmol) * 58.5/1000*

where 58.5 is the molecular weight of salt (sodium chloride).

To evaluate the accuracy of the formula-led methods, we used the average of two measured salt intake values as the best available measure of usual salt intake (reference method), and calculated absolute error and relative error for each participant using the following equations:

Absolute error (g) = formula estimate of salt intake (g) – averaged measured salt intake (g)(the reference)
Relative error (%) = absolute error (g) x 100 %/averaged measured salt intake (g)(the reference)

We estimated the mean and 95% confidence interval (95% CI) of absolute error and relative error for each formula-led method. We also flagged participants as 'misclassified' if they were classified into different quartile of usual salt intake using measured salt intake and formula estimate of salt intake, and then calculated the number and percentage of 'misclassified' participants.

To investigate the reproducibility of the formula-led methods, we compared the first against the second formula estimate of 24-h salt intake for each participant, and then calculated the absolute error and relative error at individual level using the following equations

Absolute error (g) = formula estimate of 24-h salt intake using the first fasting morning spot urine (g) – formula estimate of 24-h salt intake using the second fasting morning spot urine (g)
Relative error (%) = absolute error (g) x 100 %/ formula estimate of 24-h salt intake using the first fasting morning spot urine (g)

We estimated the mean and 95% CI of absolute error and relative error between the first and second formula estimate of 24-h salt intake for each formula-led method.

ANOVA tests and Chi-square tests are conducted for comparison of continuous outcomes and categorical outcomes, respectively. To further examine how the accuracy and reproducibility of formula-led methods interact with the level of actual salt intake measured by the reference method, we also estimated the above-mentioned outcomes by quartile of the averaged measured salt intake (the reference). Two-way ANOVA tests with interaction effect between the quartile and method are performed for the comparison of continuous outcomes, and the Cochran-Mantel-Haenszel tests are conducted for the comparison of categorical outcomes. All tests are based on two-tailed hypothesis. All analyses were performed using R-4.2.1 (R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

RESULTS

One hundred and sixteen participants completed the first 24-h urine collection and provided a spot urine, while 107 participants completed a second urine collection. Participants characteristics are described in detail in Peng *et al.* [9]. Mean age was 53.16 years, and 68% of participants were women.

Accuracy of formula-led methods

Table 1 shows the mean (SD) salt intake from 24-h measured samples, as well as estimates from the three formulae (Kawasaki, INTERSALT and Tanaka) from first and second urine collections as well as that averaged across the two collections. Mean (SD) measured salt intake was 16.52 (5.8) g/day ($n = 116$) (equivalent to 6608 mg of sodium/day). Estimates from single day spot urine collections ranged from 9.02 g/day (INTERSALT formula first urine collection) to 16.65 g/day (Kawasaki formula second urine collection), and are all lower than the single measured salt intake ($P < 0.0001$). All of the 2-day average salt intake estimates from the formulae were at least 1.5 g/day lower than the average salt intake measured with the 24-h urine collections ($P < 0.0001$). We were unable to reproduce the results reported by Peng *et al.* [9] using the Kawasaki formula. The results reported by Peng *et al.* [9] could be reproduced if errata in the formula were introduced as follows:

(1) The male participants' estimates of 24-h urinary sodium excretion using the Kawasaki equation were inconsistent with ours, and it appeared that the formula applied was that for female participants (Table 1).
(2) The Kawasaki formula for female participants was (8.58 × Weight+5.09 × Height-4.72 × Age-74.95). The correct formula for female should be (8.58 × Weight+5.09 × Height-4.72 × Age-74.5).
(3) Peng *et al.* [9] used incorrect conversion equation (spot CRE in mg/dl = spot CRE in mmol/l * 8.84) when converting the unit of spot creatinine concentration, which led to additional errors in Kawasaki estimate. The correct conversion equation should be spot CRE in mg/dl = spot CRE in mmol/l/0.08842.

TABLE 1. Mean of measured 24-h salt intake and formula estimate of 24-h salt intake

Methods	First urine collection		Second urine collection		Average of first and second collection	
	n	Mean (SD), g/d	*n*	Mean (SD), g/d	*n*	Mean (SD), g/d
Measured 24-h salt intake	116	16.13 (6.28)	107	16.83 (8.25)	116	16.52 (5.8)
Kawasaki estimate	116	13.52 (3.29)	107	16.65 (4.1)	116	14.96 (3.16)
INTERSALT estimate	116	9.02 (2.22)	107	9.95 (2.48)	116	9.43 (2.2)
Tanaka estimate	116	10.27 (1.96)	107	12.08 (2.28)	116	11.11 (1.8)
P *	<0.0001		<0.0001		<0.0001	

*P value of the ANOVA test of difference in the mean across methods.

Table 2 summarizes the mean of measured and estimated salt intake for both first and second spot urine collection by quartile of averaged measured 24-h salt intake (the reference). For Kawasaki method, the mean of formula estimate is higher than measured 24-h salt intake at the lowest level of usual salt intake (Q1), and is lower at the higher level of usual salt intake (Q3, Q4). For INTERSALT and Tanaka methods, the mean of formula estimates are both within ±0.8 g/day of the measured 24-h salt intake at the lowest level of usual salt intake (Q1), but are lower in the medium and high level of usual salt intake (Q2-Q4).

Table 3 summarizes the means (95% CIs) of absolute error and relative error between estimates from the first and second spot urine collections and the mean of two measured 24-h salt intake (the reference). The mean (95% CI) of absolute error and relative errors of formula estimate of 24-h salt intake are grouped by quartile of averaged measured salt intake (the reference). The Kawasaki formula overestimates usual salt intake by 2.82 (1.4, 4.25) - 6.76 (4.92, 8.6) g/day at the lowest level (Q1), and underestimate by 6.24 (4.74, 7.75) - 9.51 (7.87, 11.15) g/day at the highest level (Q4). The INTERSALT and Tanaka formulae underestimate usual salt intake by 11.1 (9.96, 12.25) - 15.16 (13.95, 16.36) g/day at the highest level (Q4). The percentage of mean error (95% CI) ranges from -61.95 (-64.97, -58.94)% (INTERSALT estimate, Q4, first spot urine) to 104.34 (48.26, 160.42)% (Kawasaki estimate, Q1, second spot urine).

Table 4 summarizes the percentage of misclassified participants by quartile of averaged measured 24-h salt intake (the reference). In each quartile of usual salt intake as measured by the reference method, only ≈20%∼38% of participants are classified into the correct quartile using formula-led methods. Participants with low level of usual salt intake (Q1, Q2) are more likely to be misclassified into higher quartile with formula-led methods, and participants with high level of usual salt intake (Q3, Q4) are likely to be misclassified into lower quartile with formula-led method. There is no significant difference in the percentage of misclassified participants across quartile of usual salt intake and across different formula-led methods ($P > 0.05$), indicating that all the three formulae are inaccurate in classifying individual estimates into quartiles of intake.

Figure 1 shows the Bland-Altman plots [20] comparing average measured reference and estimated salt intake from single spot urine measures from first and second collection spot urines using the mean difference method. The limits of agreement are wide for all plots, indicating that there is poor agreement for individual measures against the reference method. The Bland-Altman plots also demonstrate that all formulae systematically overestimate 24-h salt intake at low average levels of measured intake, and under-estimate intake at high average levels of measured intake. This is also demonstrated in scatter plots between formula estimates and the reference method in Supplementary Figure 1, http://links.lww.com/HJH/C539.

Reproducibility and variability of measured and formula-led methods

Supplentary Table 1, http://links.lww.com/HJH/C540 and Supplementary Figure 2, http://links.lww.com/HJH/C539 show that there is a much greater variability in the measured

TABLE 2. Mean of measured 24-h salt intake and formula estimate of 24-h salt intake by quartile of averaged measured 24-h salt intake (the reference)

Quartile of averaged measured salt intake	Methods	First urine collection ($n = 116$)		Second urine collection ($n = 107$)		Average of first and second collection	
		n	Mean (SD), g/d	*n*	Mean (SD), g/d	*n*	Mean (SD), g/d
Q1	Measured 24-h salt intake	29	10.12 (4.11)	27	9.43 (3.56)	29	9.82 (2.95)
	Kawasaki estimate	29	12.64 (2.39)	27	16.51 (4.66)	29	14.44 (3.07)
	INTERSALT estimate	29	8.75 (1.96)	27	9.75 (1.9)	29	9.16 (1.83)
	Tanaka estimate	29	9.64 (1.36)	27	11.78 (2.43)	29	10.65 (1.59)
	P *		<0.0001		<0.0001		<0.0001
Q2	Measured 24-h salt intake	29	15.01 (3.28)	27	13.28 (3.68)	29	14.18 (1.04)
	Kawasaki estimate	29	13.04 (2.85)	27	15.32 (3.19)	29	14.16 (2.38)
	INTERSALT estimate	29	8.64 (2.24)	27	9.4 (2.57)	29	8.98 (2.25)
	Tanaka estimate	29	10.08 (1.69)	27	11.43 (1.84)	29	10.75 (1.35)
	P *		<0.0001		<0.0001		<0.0001
Q3	Measured 24-h salt intake	29	17.96 (3.14)	27	17.7 (4.01)	29	17.77 (1.28)
	Kawasaki estimate	29	13.56 (3.5)	27	16.98 (3.81)	29	15.09 (3.11)
	INTERSALT estimate	29	9.51 (2.65)	27	10.81 (3)	29	10.11 (2.65)
	Tanaka estimate	29	10.18 (2.08)	27	12.16 (2.11)	29	11.06 (1.78)
	P *		<0.0001		<0.0001		<0.0001
Q4	Measured 24-h salt intake	29	21.44 (7.29)	26	27.3 (7.58)	29	24.33 (3.14)
	Kawasaki estimate	29	14.82 (3.96)	26	17.84 (4.42)	29	16.15 (3.7)
	INTERSALT estimate	29	9.17 (1.97)	26	9.83 (2.19)	29	9.48 (1.93)
	Tanaka estimate	29	11.19 (2.35)	26	12.98 (2.53)	29	11.97 (2.17)
	*P**		<0.0001		<0.0001		<0.0001
P-interaction**			<0.0001		<0.0001		<0.0001

**P* value of the ANOVA test of difference in the mean across methods.
***P* value of the two-way ANOVA with interaction between method and quartile of averaged measured 24-h salt intake.

TABLE 3. Mean error and relative error between estimates from the first and second spot urine collections and the mean of two measured 24-h salt intake (the reference)

Quartile of averaged measured salt intake	Methods	Mean error (mean and 95% CI), g/d	Relative error (mean and 95% CI), %
First spot urine formula estimate vs. averaged measured 24-h salt intake			
Q1	Kawasaki estimate	2.82 (1.4, 4.25)	58.54 (13.81, 103.27)
	INTERSALT estimate	-1.06 (-2.31, 0.18)	5.31 (-17.66, 28.28)
	Tanaka estimate	-0.18 (-1.41, 1.05)	20.48 (-12.08, 53.03)
Q2	Kawasaki estimate	-1.13 (-2.17, -0.1)	-7.78 (-15.35, -0.21)
	INTERSALT estimate	-5.54 (-6.33, -4.75)	-39.07 (-44.71, -33.44)
	Tanaka estimate	-4.1 (-4.75, -3.44)	-28.67 (-33.18, -24.17)
Q3	Kawasaki estimate	-4.21 (-5.65, -2.76)	-23.01 (-30.89, -15.14)
	INTERSALT estimate	-8.26 (-9.3, -7.23)	-46.3 (-51.62, -40.98)
	Tanaka estimate	-7.6 (-8.56, -6.64)	-42.32 (-47.07, -37.57)
Q4	Kawasaki estimate	-9.51 (-11.15, -7.87)	-38.6 (-44.71, -32.49)
	INTERSALT estimate	-15.16 (-16.36, -13.95)	-61.95 (-64.97, -58.94)
	Tanaka estimate	-13.14 (-14.46, -11.83)	-53.53 (-57.39, -49.66)
Second spot urine formula estimate vs. averaged measured 24-h salt intake			
Q1	Kawasaki estimate	6.76 (4.92, 8.6)	104.34 (48.26, 160.42)
	INTERSALT estimate	0.01 (-1.04, 1.06)	15.43 (-5.37, 36.22)
	Tanaka estimate	2.04 (0.75, 3.32)	46.36 (7.94, 84.78)
Q2	Kawasaki estimate	1.15 (-0.13, 2.44)	8.98 (-0.14, 18.1)
	INTERSALT estimate	-4.78 (-5.74, -3.82)	-33.51 (-40.13, -26.9)
	Tanaka estimate	-2.75 (-3.58, -1.91)	-18.75 (-24.34, -13.16)
Q3	Kawasaki estimate	-0.92 (-2.46, 0.62)	-4.46 (-13.11, 4.19)
	INTERSALT estimate	-7.09 (-8.18, -6)	-39.52 (-45.25, -33.8)
	Tanaka estimate	-5.74 (-6.69, -4.78)	-31.62 (-36.51, -26.73)
Q4	Kawasaki estimate	-6.24 (-7.75, -4.74)	-25.75 (-31.92, -19.58)
	INTERSALT estimate	-14.25 (-15.43, -13.07)	-58.82 (-62.19, -55.46)
	Tanaka estimate	-11.1 (-12.25, -9.96)	-45.75 (-49.63, -41.87)

Absolute error (g) = formula estimate of salt intake (g) – averaged measured salt intake (g)(the reference); Relative error (%) = absolute error (g) x 100 %/averaged measured salt intake (g)(the reference).

TABLE 4. Percentage of participants misclassified into different quartile of formula estimates of 24-h salt intake

Quartile of averaged measured salt intake	Methods	Same quartile (*n* and %)	Higher quartile (*n* and %)	Lower quartile (*n* and %)	*P**
First formula estimate vs. averaged measured 24-h salt intake					
Q1	Kawasaki estimate	9 (31.03)	20 (68.97)	–	0.947
	INTERSALT estimate	8 (27.59)	21 (72.41)	–	
	Tanaka estimate	9 (31.03)	20 (68.97)	–	
Q2	Kawasaki estimate	7 (24.14)	14 (48.28)	8 (27.59)	0.596
	INTERSALT estimate	7 (24.14)	11 (37.93)	11 (37.93)	
	Tanaka estimate	10 (34.48)	12 (41.38)	7 (24.14)	
Q3	Kawasaki estimate	7 (24.14)	8 (27.59)	14 (48.28)	0.254
	INTERSALT estimate	12 (41.38)	6 (20.69)	11 (37.93)	
	Tanaka estimate	7 (24.14)	7 (24.14)	15 (51.72)	
Q4	Kawasaki estimate	10 (34.48)	–	19 (65.52)	0.858
	INTERSALT estimate	9 (31.03)	–	20 (68.97)	
	Tanaka estimate	11 (37.93)	–	18 (62.07)	
P_{MH}**	0.970				
Second formula estimate vs. averaged measured 24-h salt intake					
Q1	Kawasaki estimate	11 (37.93)	16 (55.17)	–	0.492
	INTERSALT estimate	7 (24.14)	20 (68.97)	–	
	Tanaka estimate	10 (34.48)	17 (58.62)	–	
Q2	Kawasaki estimate	11 (37.93)	10 (34.48)	6 (20.69)	0.761
	INTERSALT estimate	10 (34.48)	7 (24.14)	10 (34.48)	
	Tanaka estimate	10 (34.48)	10 (34.48)	7 (24.14)	
Q3	Kawasaki estimate	9 (31.03)	6 (20.69)	12 (41.38)	0.661
	INTERSALT estimate	10 (34.48)	8 (27.59)	9 (31.03)	
	Tanaka estimate	6 (20.69)	7 (24.14)	14 (48.28)	
Q4	Kawasaki estimate	8 (27.59)	–	18 (62.07)	0.650
	INTERSALT estimate	6 (20.69)	–	20 (68.97)	
	Tanaka estimate	9 (31.03)	–	17 (58.62)	
P_{MH}**	0.941				

**P* value of the Chi-square test of difference in the percentage of misclassification across three formula-led methods.
***P* value of the Cochran-Mantel-Haenszel Chi-Squared test of difference in the percentage of misclassification across three formula-led methods and quartile of usual salt intake (the reference).

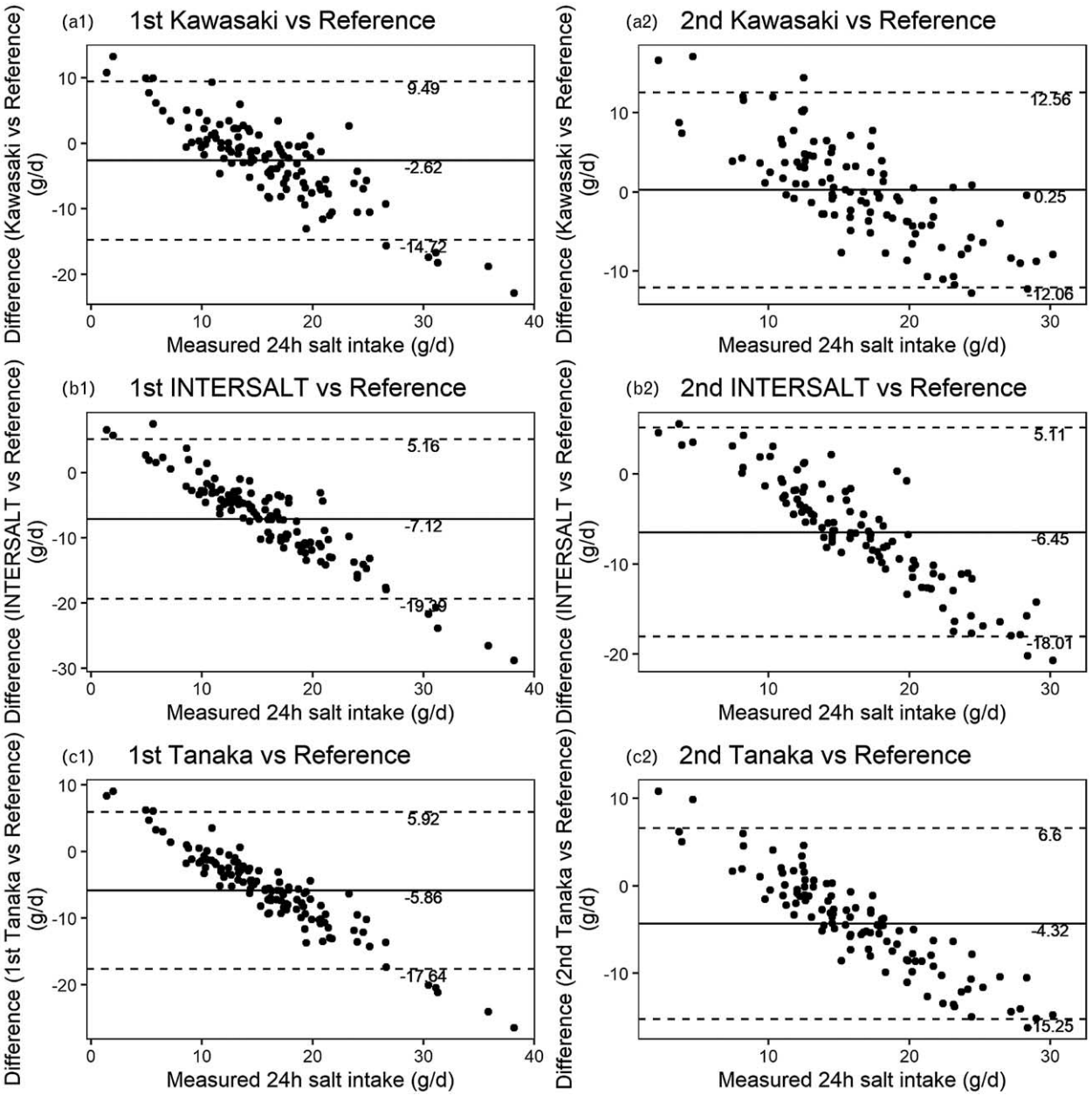

FIGURE 1 Bland-Altman plots of formulae estimates from the spot urine collection and the averaged measured 24-h salt intake. The solid line indicates the mean of difference between formula estimate and measured 24-h salt intake. The dashed lines are the upper and lower bounds of the 95% confidence interval of the mean of difference between formula estimate and measured 24-h salt intake.

24-h urine collection from first to second urine collection (indicated by the wider scatter) compared with the spot urine formul estimates.

DISCUSSION

Our results demonstrate that a single spot urine sample is unsuitable for estimating usual salt intake in individuals from an independent analysis using PURE study data. This secondary data analysis of publicly available data from a sub-sample of PURE-China study participants [9] confirms that estimates of 24-h urinary sodium excretion from fasting morning spot urine samples are highly inaccurate compared with the mean sodium excretion from two 24-h urine collections (our reference method for usual salt intake). Compared with the reference method, all formulae-led estimates have high error rates: both random and systematic. This is apparent when we examined individual estimates of 24-h salt intake in g/day, as well as when we assessed participant's excretion by quartiles of measured intake, with only around one-third of individuals classified into the correct quartile for all three formulae. Individual differences between spot urine estimates and measured mean salt intake vary by several grams (up to 13.09 g) with systematic bias at low and high levels of mean 24-h salt intake. Bland-Altman plots show that for each formula, salt intake was substantially overestimated at low levels and underestimated at higher levels of actual intake. Analyses

using Bland-Altman methods for assessing agreement [20] between the two measures show with wide limits of agreement based on between formula-led methods and the reference method for both first and second spot urine samples, and for all three formulae. These data confirm the lack of validity of using spot urine samples with formula to estimate an individual's usual salt intake when the spot sample are collected in the fasting state as in the PURE China study, consistent with the literature on the inaccuracy of spot urine samples taken under other conditions [6].

It is widely accepted that where an accurate measure of an individual's usual salt intake is required, such as in cohort studies that examine associations between salt intake and health outcomes, several nonconsecutive 24-h urinary collections should be undertaken [7]. This is because of large day-to-day variability of salt intake and sodium excretion. This finding was confirmed in our analysis with substantial variability between the first and second 24-h salt excretion, and only a quarter of participants classified as being in the same quartile of salt intake in the first and second 24-h urine collection. The variability of the spot urine estimates appears lower, with between 35 and 57% of participants classified as being in the same quartile of salt intake for the first and second spot urine estimates. However, this apparent lower variability is inaccurate (compared with the reference method) and artificial because it is a result of the presence of variables in the formulae other than salt intake (age, sex and BMI) that are fixed over the 30-day study. The effect of including these fixed variables in the formulae also leads to concerns that they may introduce a strong confounding effect into cohort studies, where age, sex and body size are also potent, independent risk factors to the outcomes being examined (such as for CVD). Further, in trials of dietary salt interventions, the use of spot urine to estimate 24-h excretion at baseline and at follow up is likely to systematically underestimate the magnitude of change in intake, due to the impact of these fixed measures being included at both time points, as well as the systematic bias inherent in the formulae to under and overestimate actual urinary sodium excretion (hence salt intake) at the upper and lower end of the range, respectively [17]. Conversely, in epidemiological population surveys where estimates of population mean salt intake are assessed, a single 24-h urine collection can be used, because day-to-day variability in individuals is aggregated at the group (population level).

The systematic errors in single spot urine estimates demonstrated here, and in other studies, [6] have shown to be responsible for the so-called J shaped curve between estimated salt intake and mortality outcomes [21]. Of concern, the PURE study has been used to challenge dietary recommendations based on high-quality evidence, generating a false 'controversy' in some publications [22,23]. Further, the inaccuracy of individual-level estimates, especially at low levels of intake, means that interpretations about the effects of specific levels of intake are inaccurate at best.

We were unable to exactly reproduce the results reported by Peng *et al.* [9]. The original PURE validation study publication has a similar error outlined in the erratum which indicates possible errors in the different formulae for men and women [10]. The principal PURE investigators have indicated their errata were only in the published formula and not in their analyses. Several WHO protocols now recommend more stringent criteria of quality control to assess completeness of 24-h urine collections [24–26], which have not been applied in the validations of the PURE study. Given the likelihood of errata in the formula used to estimate 24-h urinary sodium excretion in the Chinese PURE validation study [9], and lack of complete and appropriate analyses in the main PURE validation study [10], it is important to independently verify and complete the main PURE validation study analyses.

The main strength of this study is that the use of PURE study data reinforces our previous criticism that the use of spot urine sample with formula to estimate 24-h urinary sodium excretion is inappropriate in the PURE study and in other cohorts where these formulas have been applied. There are a number of potential weaknesses. All participants are from PURE China, which may limit generalizability to other populations and ethnic groups. The use of secondary data means that we have had no control over data quality including completeness of 24-h urine collection, and timing of spot urine collection. Indeed, we have concerns (outlined above) about the completeness of many of the 24-h urine collections included in this analysis. Nevertheless, the data from the PURE China study are consistent with the extensive evidence of gross systematic and random errors when using this methodology to estimate 24-h urinary sodium excretion.

In conclusion, this study conclusively demonstrates the unsuitability of spot urine formula-led estimates of 24-h sodium excretion and usual salt intake. Our findings support international recommendations to not conduct, fund or publish research studies that use spot urine samples with estimating equations to assess individuals' salt intake in association with health outcomes [7,16,27].

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J.S. and C.W. designed and undertook analysis of data.

All authors contributed to interpretation of data analysis.

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Conflicts of interest

R.M.M. is an unpaid member of the NZ Heart Foundation Scientific Advisory Committee.

J.S. reports no conflicts of interest.

C.W. reports no conflicts of interest.

F.J.H. and G.A.M. are unpaid members of Action on Salt and World Action on Salt, Sugar and Health (WASSH).

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