

Can spot urine measurement be a substitute for 24-hour urine measurement to estimate sodium intake in adolescents?

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ABSTRACT

Importance: Several methods have been established in recent decades that allow use of spot urine to estimate dietary sodium intake. However, their accuracies have been controversial in children.

Objective: To validate the performance of three commonly used methods—the Kawasaki, Tanaka, and International Cooperative Study on Salt, Other Factors, and Blood Pressure (INTERSALT) methods. Additionally, this study explored the accuracies of the Tanaka and INTERSALT methods by using spot urine samples taken at four separate times.

Method: Forty-one adolescents aged 14 to 16 years completed two non-consecutive 24-hour urine collections and their mean values were used as reference data. The second-morning urine was used for assessment with the Kawasaki method; a casual spot urine and spot urine samples taken at four separate times (morning, afternoon, evening, and overnight) were used for assessment with the Tanaka and INTERSALT methods.

Results: The mean differences were 1801 mg, 542 mg, 47 mg, and −31 mg for the Kawasaki, Tanaka, INTERSALT1 (with potassium), and INTERSALT2 (without potassium) methods with their required spot urine, respectively. The proportions of relative difference levels within $\pm 10\%$ were 4.9% for the Kawasaki method, 19.5% for the Tanaka method, 36.6% for the INTERSALT1 method, and 36.6% for the INTERSALT2 method.

Interpretation: The INTERSALT method seemed to provide minimally biased estimations of mean population sodium intake with casual spot urine. However, there is a need to be cautious regarding inconsistencies in estimation among different levels of sodium intake. The methods assessed in this study were unable to accurately estimate sodium intake at the individual level.

KEYWORDS

Adolescents, Spot urine, Sodium, 24-hour urine

INTRODUCTION

A high level of dietary sodium is widely regarded as an important risk factor for elevated blood pressure and the onset of cardiovascular disease.^{1,2} Population mean sodium intake levels in both adults and children are much higher

than recommended in many countries worldwide.^{3–6} The Chinese government and health research organizations have proposed some policies and interventions to promote salt reduction in China, which were proven effective and have been implemented.^{7,8} An accurate and reliable method is vital for evaluating the effects of interventions

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on sodium intake levels. Twenty-four-hour urine collection is widely acknowledged as the gold-standard approach for measuring dietary sodium intake. However, the participant burden and challenges in completing 24-hour urine collection have limited its usage in epidemiological studies. In recent decades, many methods have been established for assessment of adults based on spot urine measurements and some basic information such as sex, age, and weight, which may be useful for estimating 24-hour urinary sodium excretion.⁹⁻¹⁴ However, the accuracies of these methods have been controversial in both adults and children.¹⁵⁻¹⁹

The Kawasaki method was developed in Japanese adults based on the collection of second-morning urine⁹; the Tanaka method was developed by using Japanese-specific items from the International Cooperative Study on Salt, Other Factors, and Blood Pressure (INTERSALT) study between 1987 and 1988¹⁰; and the INTERSALT method was developed by using samples from 29 western populations between 1985 and 1987.^{11,12} Notably, both the Tanaka and INTERSALT methods were established based on the collection of casual spot urine. There are important diurnal variations in urinary sodium excretion, which might substantially influence the results of these methods. Previously, we reported that the Tanaka method was a plausible alternative to 24-hour sodium excretion in a population of children aged 9 to 16 years.¹⁸ However, the first-morning urine was applied in the previous study, rather than the required spot urine used in various methods, which led to controversy regarding the findings.¹⁸ Therefore, the aim of this study was to evaluate the accuracies of three commonly used methods—the Kawasaki,⁹ Tanaka,¹⁰ and INTERSALT^{11,12}—for estimating 24-hour urinary sodium excretion using the required spot urine in adolescents aged 14 to 16 years. This study also aimed to explore the accuracies of the Tanaka and INTERSALT methods by using spot urine samples taken at four separate times (morning, afternoon, evening, and overnight).

METHODS

Ethical approval

This study was approved by the Institutional Review Board and Ethics Committee of Capital Institute of Pediatrics (SHERLL 2016026), Beijing, China. All participants and their guardians provided informed consents.

Study population

This cross-sectional study was conducted from October 2017 to December 2017 and shared some data with our previous study published elsewhere.¹⁸ Overall, healthy student volunteers from fifth to ninth grade were recruited from a boarding school located in a rural area of Changde, Hunan Province, China. Exclusion criteria were as follows:

(1) current or previous kidney disease; (2) fever; (3) diarrhea; and/or (4) menstruation during the study period. In total, 321 participants were recruited for the collection of two 24-hour urine and two first-morning spot urine samples. For the second 24-hour urine, only students in ninth grade ($n = 51$) collected each urine void separately and were included in a secondary analysis. Data from participants who missed two or more urine voids were excluded from the analysis. During the first 24-hour urine, three participants were excluded due to menstruation, while one participant missed two or more urine voids. During the second 24-hour urine, four participants were excluded due to menstruation and two participants missed two or more urine voids. Finally, 41 participants (20 boys and 21 girls) were included in this data analysis.

Urine collection

Two non-consecutive 24-hour urine samples were collected from each participant. Before the day of collection, detailed face-to-face instruction was provided to all participants during school. During urine collection, participants were required to go to designated washing rooms that were not accessible by non-participants. Each participant was provided with a 2-L jar and a urine collection aid in the washing room during urine collection. During the first-morning urine, each participant was asked to empty their bladder. All subsequent spot urine collections were stored in the 2-L jar during the 24-hour collection period, including the first-morning urine on the following day. For the first 24-hour urine, two 2-mL aliquots were taken for analysis after the completion of collection. For the second 24-hour urine, two 2-mL aliquots were taken for analysis from each urine void; the urine volume was recorded immediately after the participants had voided urine into the jars. The jars were then washed and dried for the next use of the same participants. The headteacher and dormitory managers supervised the entire procedure during the day of urine collection to reduce the risk of missed urine voids. After completion of the 24-hour urine collection period, the headteacher exited the room and all participants were asked to report their missed urine voids honestly to the researchers.

Laboratory testing

After urine samples had been collected, they were temporarily stored at -20°C . All samples were then transported to the researchers' laboratory in Beijing after the completion of the study and were preserved at -80°C until analysis. Urinary sodium and potassium concentrations were measured by the ion-selective electrode method, while urinary creatinine was measured using the enzymatic method.

Calculation and estimation of 24-hour sodium excretion

The first measured 24-hour sodium excretion was calculated using the following equation: 24-hour

sodium excretion (mg/day) = 23×24 -hour urinary sodium concentration (mmol/L) \times 24-hour urine volume (L/day). The second measured 24-hour sodium excretion was calculated as the sum of sodium excretion in each urine void: sodium excretion in each urine void (mg) = $23 \times$ sodium concentration in spot urine (mmol/L) \times spot urine volume (L).

Estimations of 24-hour sodium excretion by spot urine were based on three commonly used methods: Kawasaki, Tanaka, and INTERSALT. The details of these methods are presented in Table S1. As required by each method, we evaluated the accuracies of the Kawasaki method using the second-morning urine, while the Tanaka and INTERSALT methods using a randomly selected spot urine from the second 24-hour urine. Because of the diurnal variation in sodium excretion, we categorized the spot urine of the second 24-hour collection into four time periods: (1) overnight (the first void after the longest period of sleep); (2) morning urine (after the overnight urine and before 12:30); (3) afternoon urine (between 12:31 and 17:30); (4) evening urine (between 17:31 and bedtime). If more than one spot urine was collected during any time period except overnight, the sample in the middle of that period was used for analysis. Accordingly, accuracies of the Tanaka and the INTERSALT methods using spot urine samples taken at four separate times were also assessed.

Statistical analysis

The mean of two 24-hour urinary sodium excretion measurements was used as a reference in this analysis. Intraclass correlation coefficients were calculated by using a two-way random effect and single rater model to evaluate the correlation between the estimated 24-hour sodium excretion and the reference value. The mean bias was then calculated as the estimated 24-hour sodium excretion minus the reference value. Paired *t*-tests were used to evaluate differences of mean bias. Bland-Altman plots were used to elucidate the agreement between the estimated 24-hour sodium excretion and the reference value. Regression analysis of the differences between the estimated sodium excretion and the mean reference value was performed to determine whether a bias trend was present in relation to sodium level. At the individual level, the relative difference was calculated as (estimated – reference)/reference and divided into five categories: within $\pm 10\%$, $\pm (10\text{--}19)\%$, $\pm (20\text{--}29)\%$, $\pm (30\text{--}39)\%$, and beyond $\pm 40\%$. Absolute difference was also calculated as (estimated – reference) of 24-hour sodium excretion and categorized into five categories: within ± 393 mg (equal to 1 g salt), $\pm (393\text{--}786)$ mg (equal to 1–2 g salt), $\pm (786\text{--}1179)$ mg (equal to 2–3 g salt), $\pm (1179\text{--}1573)$ mg (equal to 3–4 g salt), and beyond ± 1573 mg (equal to 4 g salt). For most participants ($\geq 75\%$), the relative differences were expected to be within $\pm 20\%$ and the absolute differences were expected to be within ± 786 mg (equal to 2 g salt). A two-tailed *P* value < 0.05 was considered

statistically significant. All analyses were conducted by using SPSS Statistics (version 20.0) and figures were created using GraphPad Prism 7.

RESULTS

The mean age of all participants was 14.8 ± 0.5 years (range, 14–16 years). In the first 24-hour urine, the mean volume and sodium excretion were 865 ± 384 mL and 3264 ± 1108 mg, respectively. In the second 24-hour urine, the mean void frequency was 5.1 ± 1.3 times (range, 3–8 times); the mean urine volume and sodium excretion were 832 ± 350 mL and 3220 ± 1003 mg, respectively. The mean reference 24-hour urinary sodium excretion was 3242 ± 865 mg. Detailed data concerning these measurements are presented in Table 1.

TABLE 1 Basic characteristics of all 41 participants

Variables	Participants
Male/Female	20/21
Age (years)	14.8 ± 0.5
Height (cm)	160.8 ± 5.9
Weight (kg)	53.2 ± 9.1
BMI (kg/m ²)	20.5 ± 3.2
Systolic BP (mmHg)	115.1 ± 7.6
Diastolic BP (mmHg)	66.5 ± 6.9
The first 24-hour urine	
Volume (mL/day)	865 ± 384
Na excretion (mg/day)	3264 ± 1108
K excretion (mg/day)	938 ± 377
Cr excretion (mg/day)	857 ± 217
The second 24-hour urine	
Void frequency	5.1 ± 1.3
Volume (mL/day)	832 ± 350
Na excretion (mg/day)	3220 ± 1003
K excretion (mg/day)	924 ± 285
Cr excretion (mg/day)	951 ± 225
Average of two 24-hour urinary	
Na excretion (mg/day)	3242 ± 865

Data are shown as *n* or mean \pm standard deviation. BMI, body mass index; BP, blood pressure

The Bland-Altman plots in Figure 1A and 1B display the agreement between estimated and reference sodium excretion values. The 95% limit of agreement ranged from 2652 to 3983 mg sodium (equal to 6–10 g salt); this wide range was unacceptable for use in public health applications. When using the second-morning urine

sample for assessment of the Kawasaki method and the random spot urine samples for assessment of the Tanaka and INTERSALT methods, there were no correlations between the means and differences of the estimated and reference 24-hour sodium excretion values. Bland-Altman plots regarding the estimated and first or second 24-hour urine measurements are provided in Figure S1 and S2. For the measurements of spot urine samples taken at four separate times, the means were negatively correlated with the differences when using the overnight spot urine for the Tanaka, INTERSALT1 (with potassium in the formulas), and INTERSALT2 (without potassium in the formulas) methods, and the morning spot urine for the Tanaka method (all $P < 0.05$).

The intraclass correlation coefficients between the reference and the estimated 24-hour urinary sodium excretion varies from 0.311 to 0.622, with the lowest for the INTERSALT1 method by evening urine and the highest for the INTERSALT1 method by morning urine (Table 2). The Kawasaki and Tanaka methods overestimated 24-hour urinary sodium excretion, with differences of 1801 mg (95% confidence interval [CI]: 1498 to 2103, $P < 0.001$) and 542 mg (95% CI: 261 to

824, $P < 0.001$), respectively. The INTERSALT1 and INTERSALT2 methods had similar mean differences of 47 mg (95% CI: -231 to 324, $P = 0.736$) and -31 mg (95% CI: -305 to 244, $P = 0.821$), respectively. Among the measurements of spot urine samples taken at four separate times, the evening spot urine overestimated and the overnight spot urine underestimated sodium excretion in the Tanaka, the INTERSALT1 and INTERSALT2 methods (all $P < 0.05$) (Table 2).

Figure 2 show the proportions of relative difference levels for the four methods and the measurements of spot urine samples taken at four separate times for the Tanaka, INTERSALT1, and INTERSALT2 methods, respectively. Figure 2A demonstrates that the Kawasaki method had the least proportion of relative difference levels within $\pm 20\%$ (12.2%) and the greatest proportion of relative difference levels beyond $\pm 40\%$ (73.2%). The INTERSALT1 and INTERSALT2 methods displayed similar findings: 56.1% and 56.1% of relative difference levels within $\pm 20\%$, as well as 19.5% and 9.8% of relative difference levels beyond $\pm 40\%$, respectively. Figure 2B–D indicates that the distributions of relative difference levels varied among spot urine collection times. For the Tanaka method, the

TABLE 2 Comparison between the estimated and the referred 24-hour urinary sodium excretion

Variables	Urinary sodium excretion (mg/day)	ICC		Mean difference	
		ICC	P	Difference (95% CI)	P
Measured value	3242 ± 865	Reference	NA	Reference	NA
Four equations					
Kawasaki	5043 ± 1102	0.532	<0.001	1801 (1498, 2103)	<0.001
Tanaka	3784 ± 804	0.430	0.002	542 (261, 824)	<0.001
INTER-SALT1	3289 ± 927	0.519	<0.001	47 (-231, 324)	0.736
INTER-SALT2	3211 ± 913	0.522	<0.001	-31 (-305, 244)	0.821
Four time-spot urine					
Tanaka					
Morning [†]	3585 ± 563	0.398	0.005	331 (74, 589)	0.013
Afternoon [‡]	4117 ± 624	0.459	0.018	954 (585, 1324)	<0.001
Evening	4262 ± 695	0.503	<0.001	1020 (773, 1267)	<0.001
Overnight	2852 ± 434	0.508	<0.001	-390 (-604, -176)	0.001
INTER-SALT1					
Morning [†]	3181 ± 786	0.622	<0.001	-73 (-303, 158)	0.528
Afternoon [‡]	3228 ± 612	0.407	0.033	65 (-319, 449)	0.726
Evening	3684 ± 866	0.311	0.023	442 (121, 762)	0.008
Overnight	2429 ± 542	0.561	<0.001	-813 (-1026, -599)	<0.001
INTER-SALT2					
Morning [†]	3142 ± 768	0.619	<0.001	-112 (-341, 118)	0.331
Afternoon [‡]	3167 ± 614	0.409	0.033	4 (-379, 388)	0.981
Evening	3600 ± 849	0.325	0.018	358 (44, 673)	0.027
Overnight	2349 ± 544	0.562	<0.001	-893 (-1106, -679)	<0.001

Data are shown as mean ± standard deviation or mean difference (95% CI). [†]n = 40; [‡]n = 20. ICC, intraclass correlation coefficient; INTERSALT, the International Cooperative Study on Salt, Other Factors, and Blood Pressure; INTERSALT1, the INTERSALT with potassium concentration in equation; INTERSALT2, the INTERSALT without potassium concentration in equation; CI, confidential interval; NA, not applicable.

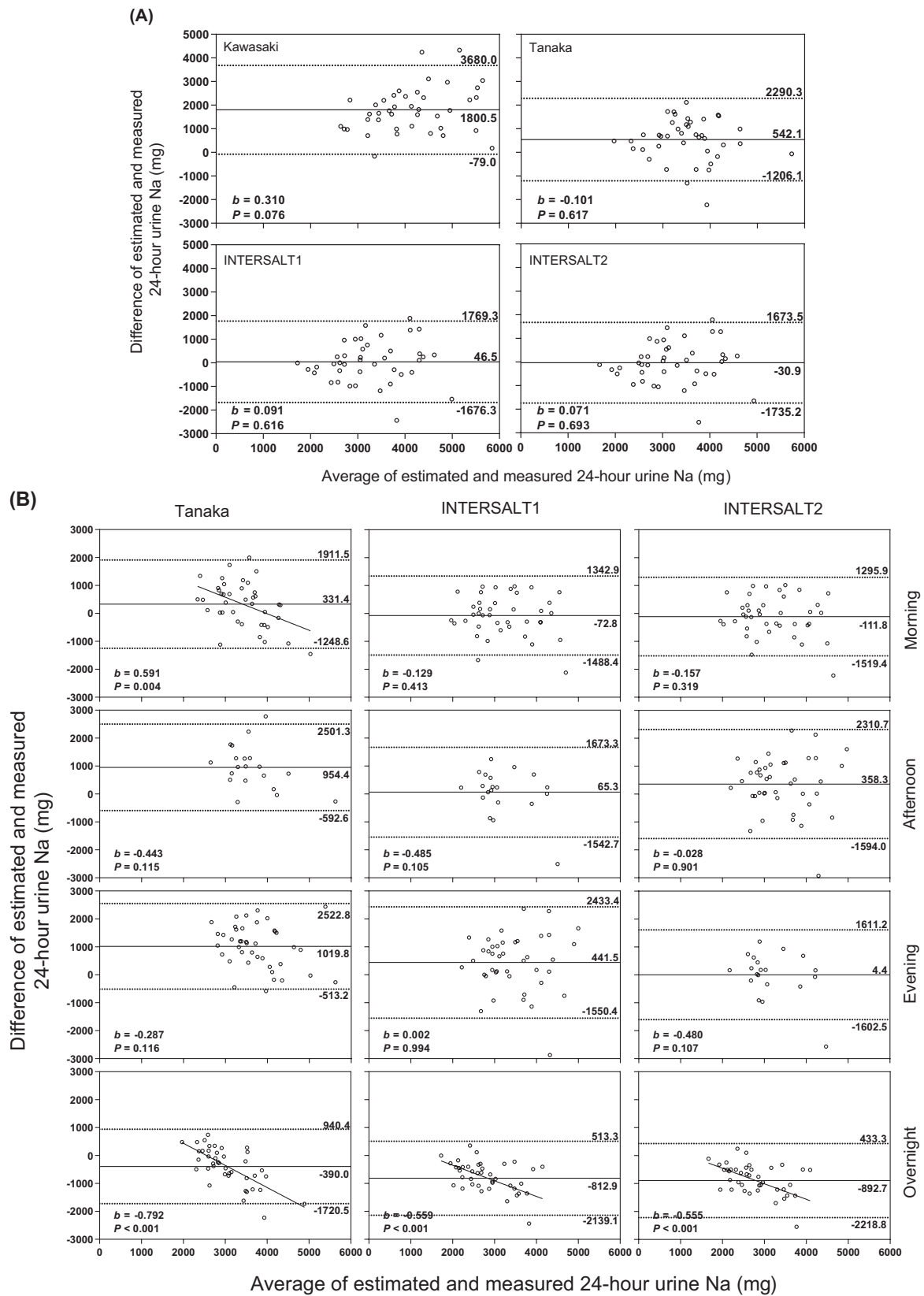


FIGURE 1 (A) Bland-Altman plots of the estimated and the measured 24-hour sodium excretion for the second-morning urine of the Kawasaki method, the random spot urine for the Tanaka, INTERSALT1 and INTERSALT2 methods. (B) Bland-Altman plots of the estimated and the referred 24-hour sodium excretion for four-time spot urine of the Tanaka, INTERSALT1 and INTERSALT2 methods. The x axis indicates the mean of measured and estimated 24-hour sodium excretion; the y axis indicates the difference between measured and estimated 24-hour sodium excretion; the continuous lines indicate the mean differences; and the dashed lines and the numbers indicate the 95% limits of agreement of the mean difference (mean \pm 1.96 SD).

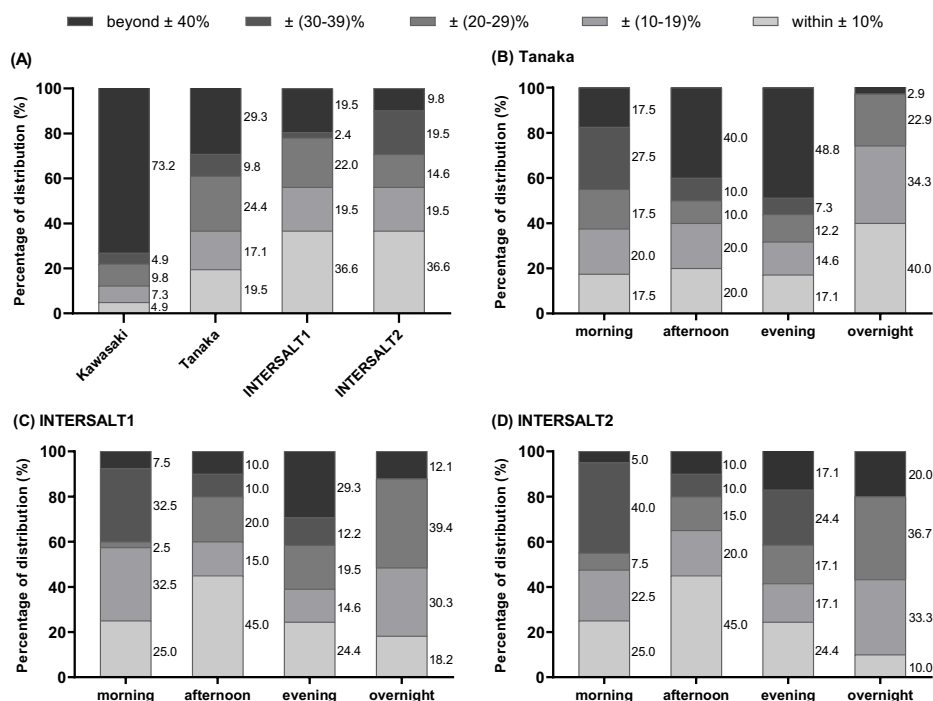


FIGURE 2 (A) Relative difference distributions of the estimated and the referred 24-hour sodium excretion for the second-morning urine of the Kawasaki method, the random spot urine for the Tanaka, INTERSALT1, and INTERSALT2 methods. (B–D) Relative difference distributions of the estimated and the referred 24-hour sodium excretion for four-time spot urine of the Tanaka, INTERSALT1, and INTERSALT2 methods.

proportion of relative difference levels within $\pm 20\%$ substantially increased to 74.3%; only 2.9% of relative difference levels were beyond $\pm 40\%$ when using overnight spot urine sample. For the INTERSALT1 and INTERSALT2 methods, relative difference levels within $\pm 20\%$ by the afternoon spot urine samples were 60.0% and 65.0%, respectively, and by the evening spot urine samples were 39.0% and 41.5%, respectively. Figure 3 showed absolute difference distribution patterns similar to the corresponding distributions of relative difference levels, such that comparatively larger proportions of absolute difference levels were within ± 786 mg (equal to 2 g salt) and smaller proportions of absolute difference levels were beyond ± 1573 mg (equal to 4 g salt). Notably, sodium excretion for the most participants in this study was less than 3932 mg (equal to 10 g salt) in 24 hours.

DISCUSSION

In this study, we assessed the accuracy of the Kawasaki method using the second-morning urine, whereas we assessed the accuracies of the Tanaka and INTERSALT methods using casual spot urine and spot urine samples taken at four separate times to estimate 24-hour sodium excretion in adolescents aged 14 to 16 years. The results suggested that use of the INTERSALT methods for assessment of random spot urine samples had the best performance in estimating mean population sodium intake and differences in distribution patterns, compared with the Kawasaki and Tanaka methods. Minimal differences were

found concerning the inclusion or exclusion of potassium when using the INTERSALT method.

In our previous validation study, we found that the mean differences for the Kawasaki, Tanaka, INTERSALT1, and INTERSALT2 methods were 1613 mg (95% CI: 1491 to 1735), -12 mg (95% CI: -97 to 73), -214 mg (95% CI: -319 to -110) and -289 mg (95% CI: -394 to -185), respectively.¹⁸ The corresponding values were higher in this study: 1801 mg (95% CI: 1498 to 2103), 542 mg (95% CI: 261 to 824), 47 mg (95% CI: -231 to 324) and -31 mg (95% CI: -305 to 244), respectively. Sodium and potassium concentrations were lower in the overnight urine samples, compared with spot urine samples taken at other times, both in healthy adults²⁰ and our participants (Table S2), which may have contributed to these inconsistent results. However, these methods had lower mean bias when using the same spot urine (overnight) in the present study, compared with our previous study: -390 mg for the Tanaka, -813 mg for the INTERSALT1 and -893 mg for the INTERSALT2 method (Table 2). This may have been partly caused by underestimation at high levels of sodium intake when using the Tanaka and INTERSALT methods in the previous study.¹⁸ Findings from another validation study conducted in Beijing Children’s Hospital were similar to those of our study, with mean differences of 2368 mg for the Kawasaki formula, 259 mg for the Tanaka formula, and 26.4 mg for the INTERSALT1 method.¹⁹

Our findings are also in agreement with those of other

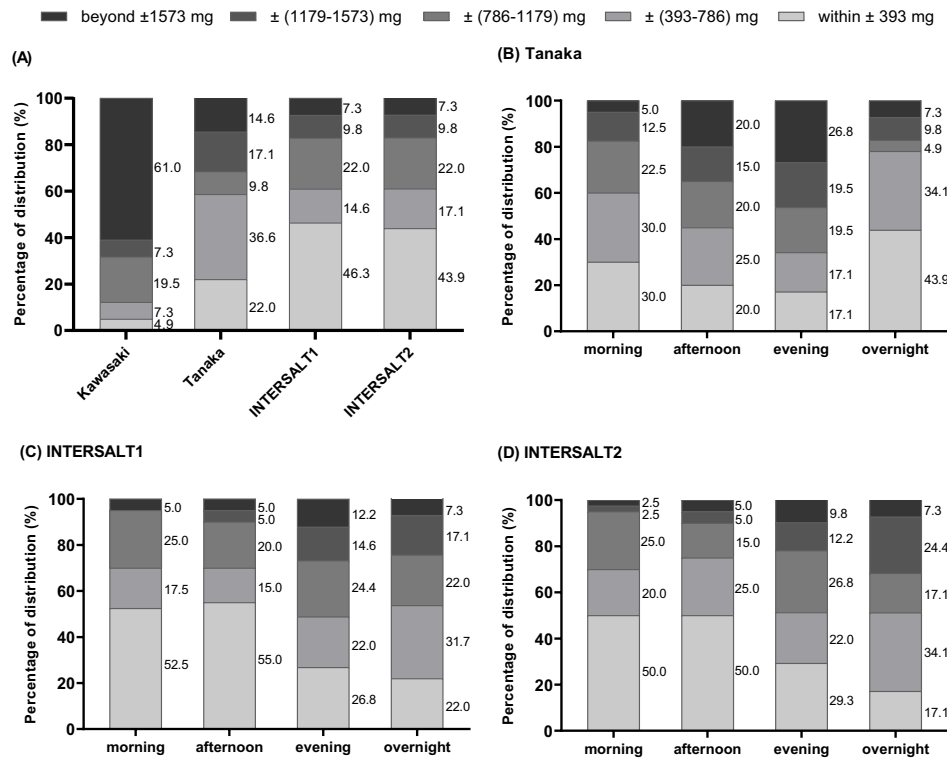


FIGURE 3 (A) Absolute difference distributions of the estimated and the referred 24-hour sodium excretion for the second-morning urine of the Kawasaki method, the random spot urine for the Tanaka, INTERSALT1, and INTERSALT2 methods. (B–D) Absolute difference distributions of the estimated and the referred 24-hour sodium excretion for four-time spot urine of the Tanaka, INTERSALT1, and INTERSALT2 methods.

studies conducted in adults at the population level. A systematic review and meta-analysis published in 2016 by Huang et al,¹⁵ which included 29 studies and 10 414 participants from 34 countries, found that the Kawasaki method was most biased in terms of overestimating salt intake by 2.5 g (95% CI: 1.5 to 3.4, $P < 0.001$); the mean differences when using the Tanaka and INTERSALT methods were 0.2 g (95% CI: -0.2 to 0.5, $P = 0.30$) and -0.2 g (95% CI: -0.5 to 0.0, $P = 0.10$), respectively. Several other studies published in recent years have also supported this finding.^{16,21,22} Daily variations in sodium intake and urinary sodium excretion, or inconsistent estimation by the Tanaka and INTERSALT methods among different levels of sodium intake, could partly explain the slight differences between the previous studies and our study.

Because most participants (75.6%) in our study excreted less than 4 g sodium (equal to 10 g salt) in 24 hours, the relative differences might be more suitable than the absolute differences for evaluation of method performance at the individual level. The proportions of relative difference levels within $\pm 20\%$ in our previous study¹⁸ were 15.2% for the Kawasaki method, 58.8% for the Tanaka method, 46.8% for the INTERSALT1 method, and 45.8% for the INTERSALT2 method, respectively; the corresponding values in this study were 12.2%, 36.6%, 56.1%, and 56.1%, respectively, indicating no substantial

improvement was associated with using the required spot urine for each of the four methods. The validation study conducted in Beijing Children's Hospital¹⁹ also had similar results concerning the proportion of relative difference level within $\pm 20\%$: 10.6% for the Kawasaki method, 33.3% for the Tanaka method, and 34.1% for the INTERSALT1 method. Based on the poor performance on Bland-Altman plots regarding the differences between estimated and measured 24-hour sodium excretion values, these methods could not accurately evaluate sodium intake at the individual level and thus are not suitable for use in clinical or epidemiological studies concerning associations between individual sodium intake and other health outcomes in children.

One problem needed to be pointed out was that many validation studies used the INTERSALT1 method that included measurement of potassium concentration.^{17,19, 23–25} However, the INTERSALT Co-Operative Research Group revised their model because they confirmed that the results were not meaningfully improved by the inclusion of urinary potassium concentrations.¹² In our study, we found minimal differences between INTERSALT1 and INTERSALT2 assessments in terms of mean bias, Bland-Altman plots, and proportions of relative difference level; our findings were similar to the results in other studies involving both adults²¹ and children.²⁶ Considering the potential applications of spot urine in estimating

population sodium intake and other methods that did not include assessment of potassium concentration, we suggest that the INTERSALT method without potassium should be applied in subsequent investigations to reduce expenses.

There were several limitations in this study. First, the small sample size might have led to inconsistent study results. Only five participants excreted less than 2300 mg sodium in 24 hours, which limited the ability to perform misclassification analysis at the individual level. Second, because of limited funding and human resources, only one class from the participating boarding school was able to collect each urine void separately for the second 24-hour urine. These participants were similar in age, lifestyle, and dietary habits. Therefore, caution is needed when generalizing the results to other populations. Third, the exclusion criteria in this study did not include 24-hour urinary volume or 24-hour creatinine excretion, which generally have involved 24-hour urinary volume less than 300 mL or creatinine excretion less than 0.1 mmol/kg of weight in many other studies.^{4,26-29} However, we conducted strict quality control throughout the urine collection procedure to ensure complete collection. The 24-hour urinary volume ranged from 320 to 1960 mL for all participants. Only three participants (3/41, 7.3%) had creatinine excretion of 0.09 mmol/kg in the first 24-hour urine; all other 24-hour creatinine excretion values were equal to or greater than 0.1 mmol/kg. Notably, we found similar results after exclusion of the three participants with 24-hour creatinine excretion less than 0.1 mmol/kg of weight (data not shown). Additionally, a systematic review concluded that no current method can reliably and accurately assess the completeness of 24-hour urine collection.³⁰

Overall, the prediction performance varied among methods and times of spot urine collection. Although the agreement between estimated and reference values was unsatisfactory, the INTERSALT method seemed to provide the least biased estimations of 24-hour sodium intake with random spot urine at the population level. This finding may be helpful when evaluating sodium intake and the effectiveness of salt reduction interventions at the population level. There is a need for caution when evaluating mean sodium intake at the population level by means of spot urine measurements, given the inconsistencies in estimation among levels of sodium intake. Further studies are needed to explore other methods (e.g., multiple spot urine) that more accurately reflect daily sodium intake.

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CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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