A New Approach Is Needed to Evaluate 24-Hour Urinary Sodium Excretion Using Spot Urines: A Validation Study in a Chinese Child Population

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Background—Accurate assessments of sodium intake in children are important for the early prevention of cardiovascular disease. There is currently no accurate simple and feasible sodium intake approach for children. This study intends to validate the accuracy of 24-hour urinary sodium excretion (UNaV) estimation in children using 3 common formulas: the Kawasaki, INTERSALT (International Cooperative Study on Salt, Other Factors, and Blood Pressure), and Tanaka formulas.

Methods and Results—A hospital-based child population in China was enrolled in the study and completed 24-hour urine sample collection. Concentrations of sodium, potassium, and creatinine in 24-hour urine and spot urine samples were measured. Mean difference as well as absolute and relative differences and misclassification between estimation and measurement of UNaV with 3 commonly used formulas were compared and analyzed. A total of 129 participants aged 5 to 16 years were eligible for analysis. Mean measured UNaV was 2694.9 mg/day. Mean differences between estimated and measured UNaV by the Kawasaki, INTERSALT, and Tanaka formulas were 2367.6, 26.4, and 258.8 mg/day, respectively. Proportions of relative differences of over 40% for the Kawasaki, INTERSALT, and Tanaka formulas were 79.8%, 34.9%, and 38.5%, respectively. Misclassification rates were 73.1% for Kawasaki, 69.0% for INTERSALT, and 62.4% for Tanaka at the individual level.

Conclusions—The results from our study do not support estimation of UNaV for children by the Kawasaki, INTERSALT, and Tanaka formulas using single spot urine samples because of the potential risk for misclassification at the individual level. (*J Am Heart Assoc*. 2020;9:e014575. DOI: 10.1161/JAHA.119.014575.)

Key Words: 24-hour urine • children • hypertension • sodium intake • spot urine

It has been verified that sodium intake plays an important role in regulating blood pressure in children.[1–3] Higher blood pressure in childhood poses an increased risk of hypertension in adulthood.[4–6] Some studies have indicated that the daily salt intake of children is commonly higher than the recommended daily salt intake, and the daily salt intake

also increases with age and is much higher in boys than in girls.[5,7,8] Accurate assessments of sodium intake in children are important for the early prevention of cardiovascular disease. As a "gold standard" for assessing individual levels of sodium intake, the 24-hour urine sodium excretion (UNaV) measurement is extremely inconvenient in clinical research practice.[9] To solve the problem of the low feasibility of 24-hour urine collection in population studies, researchers from Japan and the United States have successively proposed approaches to replace 24-hour urine samples with a single spot urine sample to estimate the UNaV; these methods include the Kawasaki formula (K method using the second morning urine),[10] the INTERSALT (International Cooperative Study on Salt, Other Factors, and Blood Pressure) formula (I method using a single random spot urine),[11] and the Tanaka formula (T method using a single random spot urine).[12] The rationale was based on the assumption that UNaV, with a correction for creatinine excretion, would be proportionate to spot urine excretion. These 3 formulas, explored from the adult population, were also the most commonly used in population studies to estimate UNaV using single spot urine. There are differences in physiological metabolism between children and adults. The applicability of the commonly used

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Clinical Perspective

What Is New?

- Accurate assessments of sodium intake in children are important for the early prevention of cardiovascular disease.
- There is currently no accurate simple and feasible sodium intake approach for children.
- This study indicated that 3 sodium excretion estimation formulas by single spot urine (Kawasaki, INTERSALT [International Cooperative Study on Salt, Other Factors, and Blood Pressure], and Tanaka formulas) were not applicable to a child population with a low accuracy and were prone to be misclassified for sodium intake evaluation.

What Are the Clinical Implications?

- Over 60% of participants in the study were misclassified into the wrong salt intake group, which would be much more significant in practice, especially the misclassification that occurred in the underestimation at a high level, neglecting the real risk to cardiovascular diseases.
- A better approach applied for a population of children is needed to improve the accuracy of 24-hour urinary sodium excretion.

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formulas is unknown. Furthermore, there is currently no accurate simple and feasible sodium intake approach for children. This study intends to compare and evaluate the accuracy of the above 3 formulas in the child population.

Methods

Study Population

A hospital-based cross-sectional study design was conducted to compare the accuracy of the 3 formulas. Sample size was estimated through correlation analysis using PASS software (version 11; NCSS, LLC, Kaysville, UT). Considering the 10% missing data rate, 100 participants with normal renal function who could complete the 24-hour urine sample collection would be needed to obtain 90% power at the 0.05 statistically significant level to obtain an ≈0.35 correlation coefficient between the estimation and measurement of UNaV. Because of the feasibility and compliance of younger children in collecting 24-hour urine samples, 151 inpatient participants aged 5 to 18 years from October 2017 to April 2018 with normal renal function were finally enrolled from the inpatient wards of orthopedics, otolaryngology, and ophthalmology of Beijing Children's Hospital, Capital Medical University (Beijing, China).

The criteria of the study were as follows.

Inclusion criteria: (1) whose age was between 5 and 18 years (including those aged 18 years); (2) who have ability to finish the urine collection procedure before receiving clinical treatment in the inpatient wards of orthopedics, otolaryngology, and ophthalmology. Exclusion criteria: (1) diagnosed with impaired renal function or renal insufficiency; (2) receiving treatment with diuretics; (3) diagnosed with acute or chronic heart failure; (4) diagnosed with infectious diseases, congenital heart diseases, circulatory system diseases, and tumors; and (5) limited dietary intake because of other clinical situations.

All participants were required to have a 24-hour urine collection procedure before receiving surgery treatments without taking intravenous infusion, and were all accommodated in the hospital and eating a nutritional recipe from the nutrition department of the hospital. In addition, participants were reminded of their usual drink or liquids intake as usual and were requested to avoid vigorous physical activity during the urine collection period.

All participants and their families signed informed consent, and the research protocol was approved by the Ethics Committee of Beijing Children's Hospital, Capital Medical University. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Urine Collection

Participants were required to collect spot urine at each void in a 24-hour period and were provided with a detailed instruction sheet and diagram, several independent plastic screw-capped containers (1000 mL each container), stickers for labeling the void time, frequency, and ID number, and a storage handbag for stable holding of the urine sample containers. The steps of the urine collection were explained by 2 specific experienced and trained nurses in each ward. The 24-hour urine collection began after emptying the bladder and discarding the first voided urine. Emphatically, each voided urine sample was collected using 1 container with a sticker, on which the void time, frequency, and ID number were noted. Both the screw-cap and the container were labeled by stickers. Researchers collected all the containers for each participant at the same time the following day and wrote down the time and volume of each spot urine sample from containers by using a graduated cylinder. Then, 3-mL urine samples were extracted from each container and were regarded as spot urine specimens. All the remnant urine in containers was mixed into a 5000-mL plastic container. After fully mixing, the total volume of 24-hour urine was computed, including the volume of all spot urine specimens, and 4-mL aliquots of 24-hour urine specimens were also extracted. The 6 durations were classified according to the voiding time, first morning urine (12:00 AM to 8:00 AM), second morning urine (8:01 AM to 11:00 AM), noon urine (11:01 AM to 1:30 PM), afternoon urine (1:31 PM to 5:00 PM), evening urine (5:01 PM to 9:00 PM), and night urine (9:01 PM to

11:59 PM). Second morning urine was introduced into the Kawasaki formula as applicable. Single randomized urine samples were applied in the INTERSALT and Tanaka formulas, and the specific 24-hour urine collection procedure made the randomization possible in the statistical analysis. Double sampling was conducted for verification and quality control.

Measurements

Urine specimens were stored and frozen at −80°C in the refrigerator. Urinary sodium and potassium were measured using the ion-selective electrode method, and urinary creatinine was measured using the enzymatic method. All specimen measurements were conducted on an automatic biochemical analyzer (Roche 5810; Roche, Basel, Switzerland) in a qualified laboratory.

A physical examination was also conducted for each participant, including height, weight, systolic blood pressure, and diastolic blood pressure. Blood pressure was measured 3 times using an electronic sphygmomanometer (Omron HEM-7200; OMRON Corporation, Tokyo, Japan) in a sitting position. Participants had 5 minutes to rest before measuring blood pressure, and the time interval between each measurement was at least 1 minute.

Relevant information included in-hospital diagnosis, physical activity, leakage or missing volume of voided urine, drinking water volume, medication usage, and ward temperature and humidity during the urine collection period. Serum creatinine laboratory results on the day closest to the urine collection period were extracted from the medicine record system to evaluate the renal function of participants.

Formulas for UNaV from single spot urine.

The measured UNaV was computed by the following formula: UNaV (mg/day)=$23\times$concentration of UNaV (mmol/L)$\times$ 24-hour urine volume (L).

The estimated UNaV from 3 formulas using single spot urine was computed by the formulas listed in Table 1 as follows.

Statistical Analysis

Any 24-hour urine collection with leakage over 5 times or missing voids over 2 times was not regarded as certified and was therefore excluded. Creatinine excretion (mmol/day) was regarded as stable and could be an indicator for completion of 24-hour urine collection.[13] Both suspected incomplete collections (ie, urinary creatinine <4.0 mmol/day for females, urinary creatinine <6.0 mmol/day for males, or total 24-hour urine volume <500 mL) and overcollection (urinary creatinine or volume >3 SDs of the population mean) were also excluded for analysis.[14]

Mean and SD values were obtained for the continuous variables, whereas proportion rates and cases were described for categorical variables. Differences between males and females were compared and tested using the *t* test for normal distribution variables and Wilcoxon's signed-rank test for skewed distributions.

The accuracy of the 3 formulas for UNaV using single spot urine were evaluated from 3 aspects: the population level, individual level, and misclassification analysis. Mean differences between estimated values and measured values obtained with the 3 formulas depicted the total accuracy at the population level. For the skewed distribution of the UNaV, Spearman's correlation analysis was conducted to indicate population-level accuracy. The residual mean square was computed to depict the gap between the estimated and measured values using the predicted formulas. Bland−Altman analysis was also applied to give a visualized assessment for consistency between measured UNaV and estimated values by 3 formulas. The absolute and relative differences between estimated and measured values were compared among the 3 formulas and were computed as follows:

Absolute difference (mg/day) = estimated UNaV (mg/day)
− measured UNaV (mg/day)

Table 1. Three Formulas of 24-Hour Urinary Sodium Excretion Using Single Spot Urine*

Formulas	Population/Single Urine	Formulas for Estimation 24-h Urinary Sodium Excretion (mg/d)[†]
Kawasaki[10]	Japan/SMU	$23\times16.3\times(Na_{spot}/Cr_{spot}\times PrUCr_{24\ h})^{0.5}$ $PrUCr_{24\ h}(male)=15.12\times weight+7.39\times height-12.63\times age-79.9$ $PrUCr_{24\ h}(female)=8.58\times weight+5.09\times height-4.72\times age-74.95$
INTERSALT[11]	Euro, US/RU	$23\times((25.46+0.46\times Na_{spot})-2.75\times Cr_{spot}-0.13\times K_{spot}+4.10\times BMI+0.26\times age)$ (male) $23\times((5.07+0.34\times Na_{spot})-2.16\times Cr_{spot}-0.09\times K_{spot}+2.39\times BMI+2.35\times age-0.03\times age^2)$ (female)
Tanaka[12]	Japan/RU	$23\times21.98\times(Na_{spot}/Cr_{spot}\times PrUCr_{24\ h})^{0.392}$ $PrUCr_{24\ h}=14.89\times weight+16.14\times height-2.04\times age-2244.45$

INTERSALT indicates International Cooperative Study on Salt, Other Factors, and Blood Pressure; RU, random urine; SMU, second morning urine.
*Na_{spot} (mmol/L), K_{spot} (mmol/L), and Cr_{spot} (mmol/L) are concentration of sodium, potassium, and creatinine in spot urine specimens. $PrUCr_{24\ h}$ is the estimation of 24-hour creatinine excretion.
[†]The units of weight, height, and age are applied in kilograms, centimeters, and years, respectively. Body mass index is expressed in kg/m^2.

Relative difference $=$ ((estimated UNaV (mg/day)
$-$ measured UNaV (mg/day))
/measured UNaV (mg/day)) $\times$ 100%

The proportional distribution of the absolute and relative differences as well as the residual analysis provided a picture of the accuracy at the individual level. According to the converted amount of salt (mg/day) from the absolute difference (mg/day), which was ≈1000-mg sodium equivalent to 2540 mg of salt, the absolute difference was divided into 5 groups: within ±390 mg/day, ±390 to 780 mg/day, ±780 to 1170 mg/day, ±1170 to 1950 mg/day, and over ±1950 mg/day in sodium amount (approximately equivalent to within ±1000 mg/day, ±1000 to 2000 mg/day, ±2000 to 3000 mg/day, ±3000 to 5000 mg/day, and over ±5000 mg/day in salt amount). A relative difference proportion was defined in 5 groups: within ±10%, ±10% to 19%, ±20% to 29%, ±30% to 39%, and over ±40%.

Furthermore, estimated and measured UNaV were converted into the amount of salt (mg/day), ≈1000-mg sodium equivalent to 2450 mg of salt. Then, misclassification analysis was implemented to assess the applicability of the 3 formulas. We divided salt intake into 4 categories (<4, 4.0–5.9, 6.0–8.9, and ≥9 g/day) because the cutoffs of categories were close to the quartile distribution and more instructive than the quartile. Misclassification rates and the fraction of participants misclassified into the wrong group based on mean salt intake measurements were computed and compared among the 3 formulas.

A 2-tailed $P \leq 0.05$ was applied as being statistically significant for the analysis. All statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., Cary, NC).

Quality Control

Participants were offered a detailed and specific verbal, written, and pictorial instruction on urine collection and the whole procedure of the study by nurses and researchers. The study standard operation procedure was used and trained among all researchers, including recording of the case report form and exacting urine specimens. The case report form was filled by investigators and was checked thoroughly the same day after finishing the urine collection. All specimens were test-centralized in 1 batch, and the instrument was calibrated with standard samples before testing. In addition, 10% blind samples were set and randomly sampled at 10% from double samples for laboratory testing in order to ensure the validity and stability of the measurement.

Results

Participant Characteristics

A total of 151 participants were enrolled in the study, and 129 participants with completed and eligible 24-hour urine collection were included in the analysis after the exclusion of reported leakage of over 1 spot urine sample (n=3) or leakage amount over 200 mL (n=9) and total urine volume <500 mL (n=10; Figure 1). A total of 58.1% of the participants
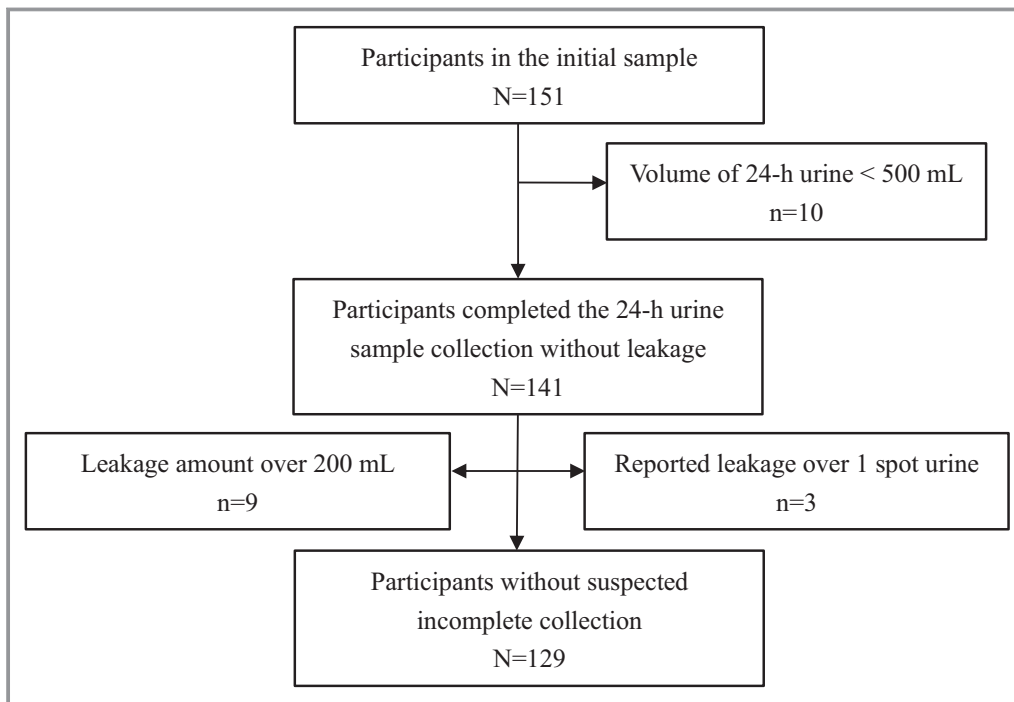

Figure 1. The flowchart.

Table 2. Participant Characteristics (N=129)

	Mean±SD	Range
Male/female	75/54	…
Age, y	9.7±2.4	5.0 to 16.0
Height, cm	140.9±16.4	103.0 to 175.0
Weight, kg	37.6±15.4	17.0 to 88.6
BMI, kg/m^2	18.3±4.5	11.2 to 34.56
SBP, mm Hg	104.1±6.6	86.0 to 130.0
DBP, mm Hg	59.9±6.0	50.0 to 74.0
Serum creatinine, μmol/L	37.8±8.6	27.3 to 62.2
Void frequency, /d	7.5±3.3	2.0 to 21.0
24-h urine		
Sodium concentration, mmol/L	99.7±54.5	30.0 to 264.0
Potassium concentration, mmol/L	25.8±14.3	6.0 to 87.5
Creatininine concentration, μmol/L	5155.1±3580.5	1060.0 to 25 200.0
Volume, mL	1349.0±628.8	530.0 to 3465.0
Sodium excretion, mg/d	2694.9±1220.3	726.6 to 6676.7

BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

were male, aged 9.7±2.4 years, with a void frequency of 7.5±3.3 and a 24-hour urine volume of 1349.0±628.8 mL. All these 129 participants were confirmed to have finished the collection without missing or leakage by themselves, the nurses, and their parents. Among the 129 participants, there were 10 participants taking voids <4 times per day, whose total urine volume ranged from 750 to 2040 mL without missing or leakage, whereas 1 participants had voids 21 times

per day with 1880 mL of total urine volume. Baseline characteristics of the population are listed in Table 2.

Accuracy at the Population Level

Mean measured UNaV was 2694.9±1220.3 mg/day. Mean estimated UNaV values from the Kawasaki, INTERSALT, and Tanaka formulas were 5087.5±1533.1, 2721.3±932.9, and 3000.6±994.2 mg/day, respectively. Mean differences between estimated and measured UNaV are shown in Table 3. All the differences were statistically significant for each estimated value obtained through the 3 formulas compared with the measured values ($P<0.001$).

Correlation analysis on estimation of UNaV using single spot urine by using the 3 formulas indicated a moderate positive correlation with the measured UNaV. The Spearman correlation coefficients between estimated and measured UNaV for the Kawasaki, INTERSALT, and Tanaka formulas were 0.51, 0.47, and 0.52 (all $P<0.001$).

The residual mean square between 2 formulas (INTERSALT and Tanaka) and measurements of the UNaV were 1.3, whereas the residual mean square between the Kawasaki formula and measurement was 7.9.

Accuracy at the Individual Level

Absolute and relative differences between estimated and measured UNaV are presented in Figures 2 and 3. The scatter plot shows estimated and measured UNaV in Figure S1. Proportions of absolute differences at the individual level within ±390 mg/day (approximately ±1 g/day salt) among the Kawasaki, INTERSALT, and Tanaka formulas were 1.9%, 23.3%, and 24.8%, respectively. Absolute differences for the 3 formulas over ±1170 mg/day (approximately ±3 g/day salt) were 79.8%, 23.3%, and 31.6%, respectively. In addition, compared with the measured UNaV, the proportions of relative

Table 3. Comparison Between Estimated and Measured UNaV Using 3 Spot Urine Formulas

Formulas	UNaV (mg/d)	r*	P for r	Mean of Absolute Difference[†] (95% CI) (mg/d)	RMS
Measured value	2694.9±1220.3	Reference	Reference	Reference	Reference
Kawasaki (n=104)[‡]	5087.5±1533.1[§]	0.509	<0.001	2367.6 (2101.0, 2634.1)	7.97
INTERSALT (n=129)[∥]	2721.3±932.9	0.472	<0.001	26.4 (−169.3, 222.1)	1.34
Tanaka (n=117)[¶]	3000.6±994.2[§]	0.521	<0.001	258.8 (57.4, 460.2)	1.36

RMS indicates residual mean square; UNaV, 24-hour urinary sodium excretion.

*r, Spearman correlation coefficient.

[†]Absolute difference (mg/day)=estimated UNaV (mg/day)−measured UNaV (mg/day).

[‡]A total of 104 second morning urine (SMU) samples were analyzed for Kawasaki formula, because 25 participants, without SMU samples, did not void in the time duration.

[§]Estimated values compared with measured values using a paired t test at the 0.05 significance level.

[∥]INTERSALT, International Cooperative Study on Salt, Other Factors, and Blood Pressure.

[¶]A total of 117 randomized spot urine (RU) samples were analyzed for Tanaka formula, because the negative bases (12 cases) occurred in the computation procession so that the estimation cannot be computed.

differences within ±10% for the Kawasaki, INTERSALT, and Tanaka formulas were 0%, 15.5%, and 17.1%, respectively, whereas those >40% were 79.8%, 34.9%, and 38.5%.

The Bland–Altman analysis in Figure 4 shows a further visualized result that the Kawasaki formula obviously overestimated, compared with the plots of INTERSALT and Tanaka formulas, which showed a relatively dispersive distribution with a wide limit range.

Misclassification Analysis

According to the converted individual salt intake, a misclassification analysis was conducted. It showed that only ≈30% of participants was classified into the consistent salt intake categories with measurements using the Kawasaki, INTERSALT, and Tanaka formulas. The misclassification rates with the formulas were 73.1% for Kawasaki, 69.0% for INTERSALT, and 62.4% for Tanaka (Table 4).

Discussion

High blood pressure is a risk factor for cardiovascular disease, which is also a primary cause of death and disability.[13] A large number of studies have confirmed that high sodium intake is closely related to hypertension, and a reasonable reduction in sodium intake can effectively lower blood pressure.[14,15] Therefore, many clinical guidelines, both domestically and internationally, have proposed a salt limitation strategy as 1 of the most important lifestyle interventions to prevent hypertension and cardiovascular diseases and provided the recommended intake of dietary sodium for different populations as well.

According to a study based on data from the China Health and Nutrition Survey with 3 consecutive 24-hour dietary data sets, it was reported that the mean level of daily sodium intake for each age group (from 3148 to 4849 mg/day for 4- to 17-year-old children) is generally almost twice the recommended intake (1600 mg/day).[16] Childhood is a critical stage in developing behaviors and lifestyles, which often become lifelong norms. In particular, dietary habits and tastes in children are gradually formed in the course of their daily life. There is an aggregative effect of the characteristics of cuisine and dining together on Chinese dietary behavioral habits, which is important for the management of sodium intake. Generally, because of the lack of accurate and simple evaluation approaches, parents often do not know their own daily sodium intake or that of their children. For children, given that parents pay more attention to children's health, it is relatively easy to develop healthier dietary habits and tastes in childhood. Therefore, there would be an improved effect on salt intake control and cardiovascular disease prevention if the approach for evaluating individual sodium intake can be more accurate and convenient in practice.

The commonly used approach to evaluate population sodium intake is applying a single spot urine sample instead of a 24-hour urine sample to estimate UNaV by using 3 formulas: the Kawasaki,[10] INTERSALT,[11] and Tanaka[12] formulas. All 3 formulas were established from adults. The inconsistent accuracy of the 3 formulas was reported even in adult population validation studies. With the significant gap
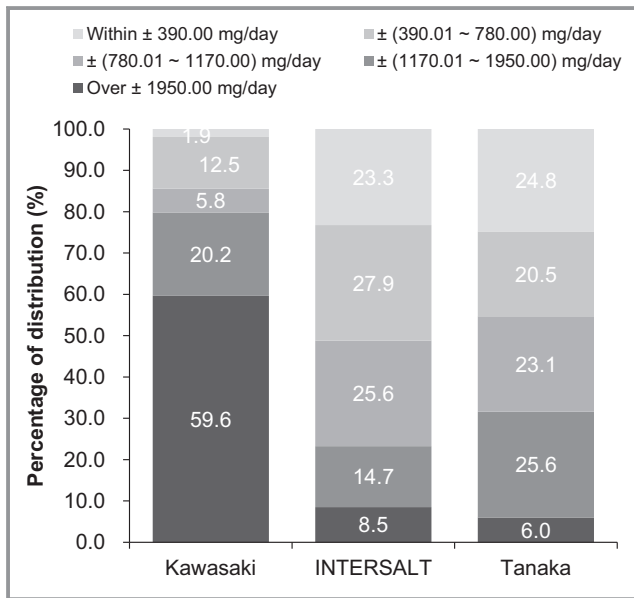

Figure 2. Absolute difference distribution of Kawasaki, INTERSALT, and Tanaka formulas for estimation of 24-hour urinary sodium excretion. INTERSALT indicates International Cooperative Study on Salt, Other Factors, and Blood Pressure.
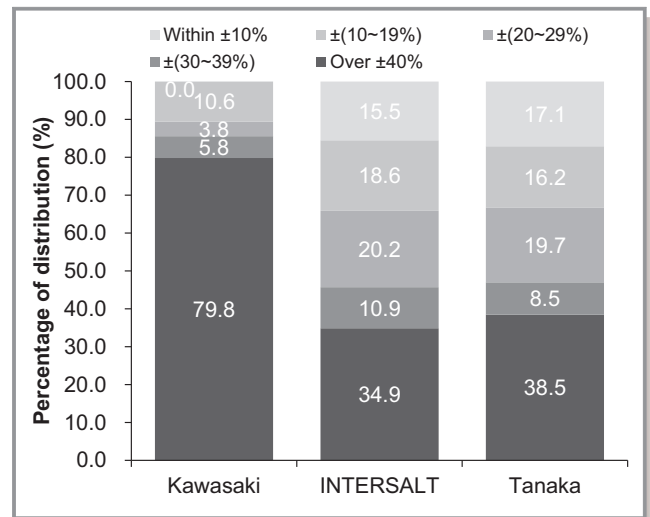

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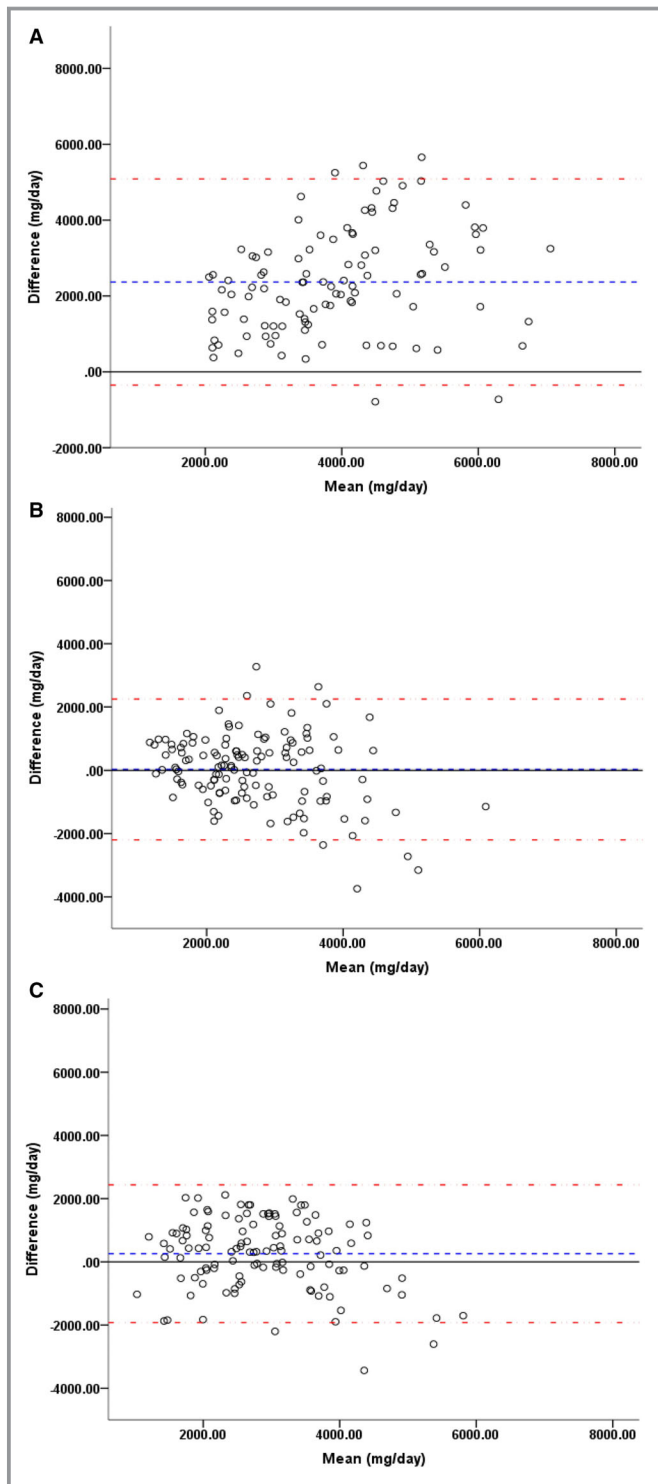
Figure 4. Bland–Altman plots between measured 24-hour urinary sodium excretion and estimation values by Kawasaki, INTERSALT, and Tanaka formulas. **A**, Kawasaki formula with second morning spot urine specimen. **B**, INTERSALT formula with casual spot urine specimen. **C**, Tanaka formula with casual spot urine specimen. The mid-dashed line is the mean difference or bias between measured and estimated values. The dash-point line represents the 95% limits of agreement of the mean difference±1.96 SDs. INTERSALT indicates International Cooperative Study on Salt, Other Factors, and Blood Pressure.

between estimations and measurements of UNaV, the accuracy of the formulas from single spot urine were not ideal, especially at the individual level, and there was a systemic prediction bias, especially an underestimation of the high levels coexisting with an overestimation of the low levels.[17–22] Furthermore, public policy and health management strategies would be misled by some correlation studies between sodium intake and cardiovascular disease outcomes that use these formulas inappropriately because of misclassification at the individual level. With little research in children, whether the commonly used approach could be applicable for children is unknown and not clear. It is necessary to validate and explore the approaches and tools for evaluating sodium intake in children.

Our study showed the least mean difference between estimated and measured UNaV in the INTERSALT formula at 26.4 mg/day; however, this result still cannot be assessed as an acceptable accuracy because the mean differences are affected by both under- and overestimation, which could be neutralized interactively. This has been indicated from the individual accuracy results of counterpart proportions between under- and overestimation both in the absolute and relative difference distribution (ie, $> +30\%$ proportion versus $> -30\%$ proportion for INTERSALT and for Tanaka, respectively). Similarly, in our previous validation study in the adult population, a much smaller mean difference gap between estimated and measured UNaV (-6.0 mg/day using evening spot urine by the Tanaka formula)[22] was observed, and there was no evidence indicating a well-performance accuracy at the individual level. In this study, the difference distribution analysis was conducted to assess accuracy at the individual level. The proportions of relative differences >40% were 79.8%, 34.9%, and 38.5% for the Kawasaki, INTERSALT, and Tanaka formulas, respectively. The proportion within 20%, which might be considered acceptable in practice, was approximately less than half for all 3 formulas (only 10.6% in Kawasaki, 34.1% in INTERSALT, and 36.3% in Tanaka). This is similar to another Chinese population study that investigated 284 teenagers aged 10 to 15 years in a mid-southern province in China. The proportions of relative differences >40% were 65.5%, 17.6%, and 12.7% for the Kawasaki, INTERSALT, and Tanaka formulas, respectively. The biased difference distribution draws a conclusion that the Tanaka formula could offer a plausible alternative for mean UNaV at the population level, rather than at the individual level, for young adolescents.[23] Unlike this school-based population validation study, our research was hospital based, and the population characteristics of non-hospital-based studies, such as age and sex distributions, might lead to different proportion rates, even though the compliance and completeness of 24-hour urine collection might be better controlled in hospital-based populations.

Table 4. Misclassification Analysis for 3 Formulas for Salt Intake at Individual Level*

	Sodium Intake Categories Converted From UNaV				
	<4 g (n=23)	4–5.99 g (n=38)	6–8.99 g (n=40)	≥9 g (n=28)	Total (n=129)
Kawasaki[†]					
<4 g (n=0)	0[‡]	0	0	0	…
4.00 to 5.99 g (n=1)	0	1[‡]	0	0	…
6.00 to 8.99 g (n=19)	8	7	4[‡]	0	…
≥9 g (n=84)	10	21	30	23[‡]	…
Total (n=104)	18	29	34	23	104
Misclassified	18 (100.0)	28 (96.6)	30 (88.2)	0 (0.0)	76 (73.1)
INTERSALT[§]					
<4 g (n=12)	3[‡]	5	4	0	…
4.00 to 5.99 g (n=39)	16	11[‡]	10	2	…
6.00 to 8.99 g (n=53)	2	19	16[‡]	16	…
≥9 g (n=25)	2	3	10	10[‡]	…
Total (n=129)	23	38	40	28	129
Misclassified	20 (87.0)	27 (63.2)	24 (60.0)	18 (64.3)	89 (69.0)
Tanaka[‖]					
<4 g (n=7)	2[‡]	3	2	0	…
4.00 to 5.99 g (n=27)	10	10[‡]	6	1	…
6.00 to 8.99 g (n=46)	8	12	16[‡]	10	…
≥9 g (n=37)	0	9	12	16[‡]	…
Total (n=117)	20	34	36	27	117
Misclassified	18 (90.0)	24 (70.6)	20 (55.6)	11 (40.7)	73 (62.4)

UNaV indicates 24-hour urinary sodium excretion.
*Listed in n (%) for cases and proportion.
[†]A total of 104 second morning urine (SMU) samples were analyzed for the misclassification analysis of Kawasaki formula, because 25 participants, without SMU samples, did not void in the time duration.
[‡]The number of consistent classification between estimation and measurement values.
[§]INTERSALT, International Cooperative Study on Salt, Other Factors, and Blood Pressure.
[‖]A total of 117 randomized spot urine (RU) samples were analyzed for the misclassification analysis of Tanaka formula, because the negative bases (12 cases) occurred in the computation procession so that the estimation cannot be computed.

Our study results indicated a low accuracy for estimating UNaV by using the Kawasaki, INTERSALT, and Tanaka formulas in children at the individual level. From the results of the difference distribution analysis, a higher proportion of underestimations at high levels still existed, which is similar to the Chinese adult population.[17,22] In some adult population studies, the inconsistency of accuracy was reported based on correlation analysis or mean differences between estimated and measured UNaV using spot urine formulas,[24,25] which provided a clue that the accuracy of the formulas might be affected by the population. Whether the modeling method was inclined to bring some bias or not would require further exploration and research.

In addition, we found that over 60% of participants in the study were misclassified into the wrong salt intake group, which would be much more significant in practice, especially

the misclassification that occurred in the underestimation at a high level, neglecting the real risk to cardiovascular diseases. Another population validation study enrolled 101 children aged 6 to 16 years to compare several equations, including the Kawasaki, INTERSALT, and Tanaka formulas, using spot urine samples. The misclassification rate (only taking the sodium intake cutoff at 2 g/day) in the Tanaka formula was much lower, and the formulas using single spot urine samples were concluded as able to be used to identify children with high sodium excretion.[26] The studies all indicated that single spot urine was limited as applied in evaluation sodium intakes, with unstable accuracy attributed to the characteristics of the population, dietary custom, and lifestyle. From our misclassification analysis results, the misclassification rate in Tanaka was 62.4%, although it was the least among that of the 3 formulas. A significant misclassification bias, underestimation

at high level, and overestimation at low level were also observed. Generally, all the results suggest that it is not supportive to regard the Kawasaki, INTERSALT, and Tanaka formulas using single spot urine to estimate UNaV as an alternative with acceptable performance and accuracy. Directly applying these formulas in children might not be an appropriate method to evaluate salt intake at the individual level.

Two limitations of this study should be noted. First, the participants were hospital based from the wards of orthopedics, otolaryngology, and ophthalmology because we considered the influence of pharmaceutical medication on kidney function, inducing abnormal urine metabolism. Compliance and convenience for the urine sample collection were also considered in the hospital-based population. Other confounders, such as dietary intake, physical activity, drinking water amount, environmental temperature and humidity, and rest circadian rhythm, could be relatively controlled as well because of the in-hospital accommodation and nursery management. However, generalization of the results and conclusions to a community child population would be limited, and further research in community-based child populations is needed. Furthermore, considering the compliance and convenience of urine collection, we did not collect multiple 24-hour urine on continuous days, and some stability and time delay in metabolism could not be analyzed. This would be considered in our future studies. The highlight of this study was the 24-hour urine collection procedure; each void was collected in an independent container, based on which we will continue to conduct a circadian rhythm analysis and screen multispot urine and further explore the modeling approach to improve accuracy.

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Disclosures

None.

References

1. Aparicio A, Rodríguez-Rodríguez E, Cuadrado-Soto E, Navia B, López-Sobaler AM, Ortega RM. Estimation of salt intake assessed by urinary excretion of sodium over 24 h in Spanish subjects aged 7–11 years. *Eur J Nutr*. 2017;56:171–178.

2. Okuda M, Asakura K, Sasaki S, Shinozaki K. Twenty-four-hour urinary sodium and potassium excretion and associated factors in Japanese secondary school students. *Hypertens Res*. 2016;39:524–529.

3. Grimes CA, Riddell LJ, Campbell KJ, He FJ, Nowson CA. 24-h urinary sodium excretion is associated with obesity in a cross-sectional sample of Australian schoolchildren. *Br J Nutr*. 2016;115:1071–1079.

4. Leyvraz M, Chatelan A, da Costa BR, Taffe P, Paradis G, Bovet P, Bochud M, Chiolero A. Sodium intake and blood pressure in children and adolescents: a systematic review and meta-analysis of experimental and observational studies. *Int J Epidemiol*. 2018;47:1796–1810.

5. Yang Q, Zhang Z, Kuklina EV, Fang J, Ayala C, Hong Y, Loustalot F, Dai S, Gunn JP, Tian N, Cogswell ME, Merritt R. Sodium intake and blood pressure among US children and adolescents. *Pediatrics*. 2012;130:611–619.

6. Rosner B, Cook NR, Daniels S, Falkner B. Childhood blood pressure trends and risk factors for high blood pressure: the NHANES experience 1988–2008. *Hypertension*. 2013;62:247–254.

7. He FJ, Marrero NM, MacGregor GA. Salt and blood pressure in children and adolescents. *J Hum Hypertens*. 2008;22:4–11.

8. Lava SA, Bianchetti MG, Simonetti GD. Salt intake in children and its consequences on blood pressure. *Pediatr Nephrol*. 2015;30:1389–1396.

9. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of urine biomarkers to assess sodium intake: challenges and opportunities. *Annu Rev Nutr*. 2015;35:349–387.

10. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. *Clin Exp Pharmacol Physiol*. 1993;20:7–14.

11. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, Elliott P; INTERSALT Co-Operative Research Group. Estimating 24-h urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. *Am J Epidemiol*. 2013;177:1180–1192.

12. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens*. 2002;16:97–103.

13. Cappuccio FP, Beer M, Strazzullo P; European Salt Action Network. Population dietary salt reduction and the risk of cardiovascular disease. A scientific statement from the European Salt Action Network. *Nutr Metab Cardiovasc Dis*. 2018;29:107–114.

14. Mills KT, Chen J, Yang W, Appel LJ, Kusek JW, Alper A, Delafontaine P, Keane MG, Mohler E, Ojo A, Rahman M, Ricardo AC, Soliman EZ, Steigerwalt S, Townsend R, He J; Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. Sodium excretion and the risk of cardiovascular disease in patients with chronic kidney disease. *JAMA*. 2016;315:2200–2210.

15. Cook NR, Appel LJ, Whelton PK. Sodium intake and all-cause mortality over 20 years in the trials of hypertension prevention. *J Am Coll Cardiol*. 2016;68:1609–1617.

16. Wang H, Wang D, Ouyang Y, Huang F, Ding G, Zhang B. Do Chinese children get enough micronutrients? *Nutrients*. 2017;9:E397.

17. Peng Y, Li W, Wang Y, Chen H, Bo J, Wang X, Liu L. Validation and assessment of three methods to estimate 24-h urinary sodium excretion from spot urine samples in Chinese adults. *PLoS One*. 2016;11:e0149655.

18. Zhou L, Tian Y, Fu JJ, Jiang YY, Bai YM, Zhang ZH, Hu XH, Lian HW, Guo M, Yang ZX, Zhao LC. Validation of spot urine in predicting 24-h sodium excretion at the individual level. *Am J Clin Nutr*. 2017;105:1291–1296.

19. Allen NB, Zhao L, Loria CM, Van Horn L, Wang CY, Pfeiffer CM, Cogswell ME, Wright J, Liu K. The validity of predictive equations to estimate 24-hour sodium excretion: the MESA and CARDIA Urinary Sodium Study. *Am J Epidemiol*. 2017;186:149–159.

20. Whitton C, Gay GM, Lim RB, Tan LW, Lim WY, van Dam RM. Evaluation of equations for predicting 24-hour urinary sodium excretion from casual urine samples in Asian adults. *J Nutr*. 2016;146:1609–1615.

21. Vidal-Petiot E, Joseph A, Flamant M. Estimation of populational 24-h urinary sodium and potassium excretion from spot urine samples: evaluation of four formulas in a large national representative population. *J Hypertens*. 2017;35:1119–1120.

22. Zhang Y, Peng Y, Li K, Peng X. Assessing whether a spot urine specimen can predict 24-h urinary sodium excretion accurately: a validation study. *J Hypertens*. 2019;37:99–108.

23. Dong J, Yan Y, Fan H, Zhao X, Mi J. Accuracy validation of eight equations to estimate 24-h sodium by spot urine in young adolescents. *Am J Hypertens*. 2019;32:257–264.

24. Mente A, O'Donnell MJ, Dagenais G, Wielgosz A, Lear SA, McQueen MJ, Jiang Y, Xingyu W, Jian B, Calik KB, Akalin AA, Mony P, Devanath A, Yusufali AH, Lopez-Jaramillo P, Avezum A Jr, Yusoff K, Rosengren A, Kruger L, Orlandini A, Rangarajan S, Teo K, Yusuf S. Validation and comparison of three formulae to estimate sodium and potassium excretion from a single morning fasting urine compared to 24-h measures in 11 countries. *J Hypertens*. 2014;32:1005–1014.

25. Mercado CI, Cogswell ME, Loria CM, Liu K, Allen N, Gillespie C, Wang CY, de Boer IH, Wright J. Validity of predictive equations for 24-h urinary potassium excretion among adults: the MESA and CARDIA Urinary Sodium Study and NHANES Urinary Sodium Calibration Study. *Am J Clin Nutr*. 2018;108:532–547.

26. Rios-Leyvraz M, Bovet P, Tabin R, Genin B, Russo M, Rossier MF, Bochud M, Chiolero A. Urine spot samples can be used to estimate 24-hour urinary sodium excretion in children. *J Nutr*. 2018;148:1946–1953.

SUPPLEMENTAL MATERIAL

Figure S1. Scatter plots between measured 24-h urinary sodium excretion and estimation values by Kawasaki, INTERSALT, and Tanaka formulas.
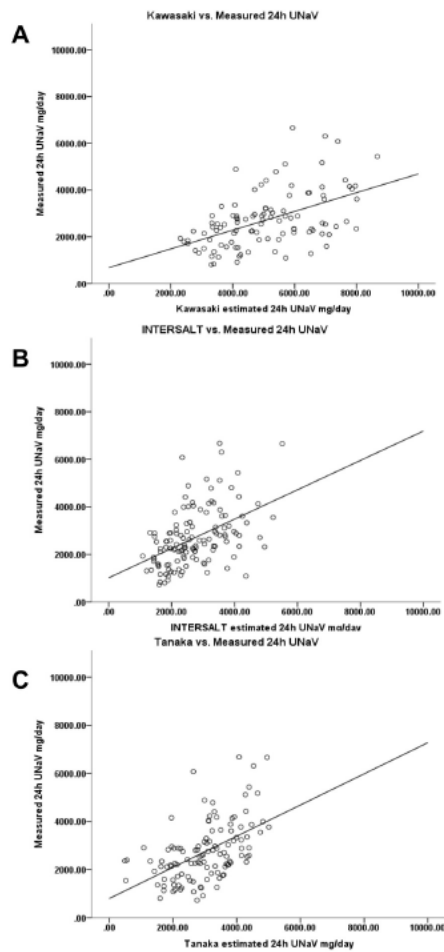
A. Kawasaki formula with second morning spot urine specimen. **B.** INTERSALT formula with casual spot urine specimen. **C.** Tanaka formula with casual spot urine specimen. The solid line was the linear fitting line between measured and estimated values.