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Urine spot samples can be used to estimate 24-h urinary sodium excretion in children [i-v]

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[ii] **Abbreviations:** BMI, body mass index; Cre, creatinine; K, potassium; Na, sodium; SD, standard deviation.

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ABSTRACT

Background

The gold standard to assess salt intake is 24-h urine collections. Using a urine spot sample can be a simpler alternative, especially when the goal is to assess sodium intake at the population level. Several equations to estimate 24-h urinary sodium excretion from urine spot samples have been tested in adults, but not in children.

Objective

The objective of this study was to assess the ability of several equations and urine spot samples to estimate 24-h urinary sodium excretion in children.

Methods

A cross-sectional study of children between 6 and 16 years of age was conducted. Each child collected one 24-h urine and three timed urine spot samples, i.e., evening (last void before going to bed), overnight (first void in the morning), and morning (second void in the morning). Eight equations (i.e., Kawasaki, Tanaka, Remer, Mage, Brown with and without potassium, Toft, and Meng) were used to estimate 24-h urinary sodium excretion. The estimates from the different spots and equations were compared with the measured excretion using several statistics.

Results

Among the 101 children recruited, 86 had a complete 24-h urine collection and were included in the analysis (mean age: 10.5 years). The mean measured 24-h urinary sodium excretion was 2.5 g (range: 0.8-6.4). The different spots and equations provided highly heterogeneous estimates of the 24-h urinary sodium excretion. The overnight spots with the Tanaka and Brown equations provided the most accurate estimates (mean bias: −0.20-−0.12 g, correlation: 0.48-0.53, precision: 69.7%-76.5%, sensitivity: 76.9%-81.6%, specificity: 66.7%, and misclassification: 23.0%-27.7%). The other equations, irrespective of the timing of the spot, provided less accurate estimates.

Conclusions

Urine spot samples, with selected equations, might provide accurate estimates of the 24-h sodium excretion in children at a population level. At an individual level, they could be used to identify children with high sodium excretion.

Keywords: sodium, salt, 24-h urine collection, urine spot sample, children

INTRODUCTION

High salt intake is a cause of elevated blood pressure (1, 2) and has been estimated to cause 1.65 million deaths from cardiovascular diseases per year (3). High salt intake has also been associated with other conditions, such as osteoporosis (4), diabetes (5), and cancer (6). While salt intake in adults has been shown to be high in many countries worldwide (7), only few studies have been conducted to evaluate salt intake in children, notably because it is difficult to measure intake in that age group (2, 8). The easiest way to assess sodium intake is by dietary questionnaires, e.g., 24-h dietary recalls and food frequency questionnaires. While these questionnaires can be useful to assess the main dietary sources of sodium intake, they are unable to quantify accurately the actual intake of sodium (9). A standard method to estimate sodium intake is by measuring sodium excretion based on 24-h urine collections (10-14). This method presents however significant practical difficulties, especially among children (15-20).

In adults, urine spot samples could be an alternative to 24-h urine collections to estimate sodium intake at the population level (14, 21, 22). Several equations, such as Kawasaki et al. (23), Tanaka et al. (24), and INTERSALT (25) have been developed and are based on sodium and creatinine concentration in urine spots, while also adjusting for age and sex. Most of these equations were developed in adults, with only a few developed in children (26, 27). To the best of our knowledge, these equations have not been tested in children. Further, at least three types of urine spot samples can be distinguished depending on the timing of the collection of the spot, e.g., evening (last void before going to bed), overnight (first void upon rising in the morning), and morning (second void upon rising in the morning) (22). Given that urinary sodium excretion

76 varies throughout the day (28, 29), timing of the urine spot sample can have an impact on the
77 estimation of the 24-h sodium excretion.

78 The objective of this study was therefore to determine whether urine spot samples can be used
79 to estimate 24-h urinary sodium excretion in children. The specific objectives of this study
80 were: 1) to compare different equations and urine spot samples (collected at different times) to
81 estimate 24-h sodium excretion in children, and; 2) to assess which of these equations and spots
82 are the best to estimate 24-h sodium excretion. Finally, we evaluated if spot estimates are
83 suitable to estimate salt excretion at a population level, for a public health purpose, and at an
84 individual level, for a clinical purpose.

METHODS

Study design

This study was a cross-sectional study with a convenience sample of participants. Children were recruited at the Hospital of Valais, in Sion, and in several pediatric and primary care facilities in Valais between September 2016 and February 2018. Children between 6 and 16 years of age were eligible for inclusion. Children with a condition potentially altering the consumption or excretion of sodium, taking medication that alters sodium excretion, with intravenous fluid infusion during data collection, or with insufficient knowledge of the local language to understand the content of the information forms and questionnaires were not eligible.

Data collection

Upon enrolment, the children were weighed with a weighing scale and measured with a wall mounted stadiometer in light clothes and without shoes by a trained nurse or a research assistant. Urine collection was done at home over three consecutive days (day 1 to day 3), which consisted, consecutively, of 1) one evening spot (last void before going to bed) on day 1, one 24-h urine on day 2, 2) one overnight spot (first void upon rising in the morning) on day 3, and 4) one morning spot (second void upon rising in the morning) on day 3. To ensure a complete urine collection, written and oral instructions were given to the participants and their parents, urine collection times were reported, and special urine collection pots were provided. Moreover, they were instructed to maintain their usual diet and liquid intake during urine collection.

During urine collection, participants and parents were instructed to keep the urine samples in closed containers in the fridge at a temperature between 4-8°C and to bring them to the Sion Hospital laboratory no later than 48 hours after urine collection. The urine samples were stored at -20°C until analysis. Sodium and potassium concentrations were measured with ion-selective electrodes and creatinine concentration with the Jaffe colorimetric method (30), using a Cobas® c-501 Analyzer Roche.

Ethics

The study protocol was approved by the Ethics Committee of Canton de Vaud, Switzerland (CER-VD, identification number: 2015-01178). Information on the study was given to the parents (or legal guardian) and children orally and in writing. Written consent was obtained from the parents (or legal guardians). In addition, children below 14 years of age gave oral consent and children of or above 14 years of age gave written consent. The children received a backpack, a watch and a pen to thank them for their participation.

Statistical analysis

A sample size of 100 children was calculated to be sufficient to detect a difference in sodium excretion between 24-h urine collection and spots with an accuracy of 0.4 g, assuming a correlation of 0.4 between 24-h sodium excretion and estimates based on spots, a standard deviation of 1.5 g for measured and estimated 24-h sodium excretion, an intra-class correlation between children of the same family of 0.7, and a drop-out rate of 15%.

Body mass index (BMI) was calculated by dividing weight (kg) by the squared height (m) and z-scores based on the reference values from the 2000 Centers for Disease Control and Prevention (31). To calculate the 24-h sodium and creatinine urinary excretions (g/24h or mmol/24h), concentrations (g/L or mmol/L) were multiplied by the volume of the 24-h sample (L) and adjusted for self-reported collection times to represent an exact 24-hour duration (as a fraction of 24 hours). A 24-h creatinine excretion of less than 0.1 mmol per kilogram of body weight per day was considered an indication of incomplete 24-h urine collection (32). Children with incomplete 24-h urine collection were excluded from analysis.

We used eight equations to estimate the 24-h urinary sodium excretion from urine spot samples (details are shown in **Table 1**) (23-27, 32-35). These eight equations were used to calculate the estimated 24-h urinary sodium excretion from the three different spots. Scatterplots and Bland-Altman diagrams (36, 37) were plotted for each spot and equation allowing visual comparisons. To compare the estimated 24-h urinary sodium excretion from the different equations and spots with the measured 24-h urinary sodium excretion, several statistics were calculated: mean bias, i.e., mean difference between the estimated and measured 24-h sodium excretion (in grams of sodium); Pearson correlation coefficient between estimated and measured excretion; precision, i.e., proportion of children with a difference within ±1 g between estimated and measured excretion; sensitivity, i.e., the proportion of children who had an estimated 24-h sodium excretion ≥2 g/day among those who had a measured excretion ≥2 g/day; specificity, i.e., the proportion of children who had an estimated 24-h sodium excretion <2 g/day among those who had a measured excretion <2 g/day; and misclassification, i.e., the proportion of children who were incorrectly classified to ≥2 g/day or <2 g/day.

Moreover, to determine the overall performance, we computed a total score combining all these statistics, for each equation and spot. The total score ranged from 0 to 6 and consisted of the sum of points attributed to each measure. A point of 1 was attributed to an absolute mean bias below 0.4 g per day, a correlation above 0.4, a precision above 60%, a sensitivity above 75%, a specificity above 75%, and a misclassification below 30%; for all other values, a point of 0 was attributed. A score of 0 indicated the worst performance and of 6 indicated the best performance. We considered further that estimates could be used to assess salt intake at a population level, for a public health purpose (for example for the surveillance of salt intake in a population, to assess the effectiveness of a population-based intervention to reduce salt intake), if mean bias, correlation, and misclassification were satisfactory. We considered that estimates could be used to assess salt intake at an individual level, for a clinical purpose (for example during a clinical consultation to assess the salt intake of a child with elevated BP), if precision, sensitivity, and specificity were satisfactory.

A heatmap was created to visually represent the different statistics. The color of the cells of this map were determined based on a gradient which ranged from red (worst estimate) through yellow (50^{th} centile) to green (best estimate). Statistical analyses were conducted with R (version 3.3.1) and R Analytic Flow (version 3.0.6).

RESULTS

Of the 101 children enrolled in the study, 94 collected a 24-h urine sample. The 24-h urine collection was complete for 86 children. The study flowchart is shown in **Supplemental Figure 1**. The characteristics of the 86 children included in this analysis are shown in **Table 2**. On average, the children were 10.5 years old (SD: 2.7, range: 6-16) and 41.9% were girls (n=36). The characteristics did not differ significantly between children who collected a complete 24-h urine sample (n=86) and those who did not (n=14). The mean 24-h excretions of sodium, creatinine and potassium were 2.4 g (SD 1.1, range: 0.3-6.4), 6.4 mmol (SD: 3.4, range: 2.5-27.4), and 1.8 g (SD: 0.6, range: 0.6-4.4) respectively.

The scatterplots showing the relation between estimated and measured excretion, for each equation and spot, are shown in **Figure 1**. The scatterplots with the Kawasaki, Toft, Meng and Remer equations tended to be more dispersed than with the Tanaka, Brown, and Mage equations. The Bland-Altman diagrams are shown in **Figure 2**. The diagrams show that the limits of agreement tended to be the largest for the morning spots and that the mean bias tended to be the lowest with the overnight spot. All equations and spots tended to over-estimate very low 24-h excretions of sodium (<1 g) and under-estimate very high 24-h excretions of sodium (>5 g) , except for the equations from Kawasaki, Meng and Toft, who tended to systematically over-estimate 24-h sodium excretion, whatever the level of sodium (**Figures 1 and 2**).

The different statistics used to compare the different equations and spots are shown in **Figure 3**. The mean bias ranged from −0.98 to 2.94 g, the Pearson correlation from 0.40 to 0.54, and the precision from 4.7% to 76.5%. The sensitivity ranged from 36.5% to 100.0%, the specificity

from 0.0% to 93.9%, and the overall percentage of children misclassified from 23.5% to 41.2%. The overall performance score ranged from 1 to 5.

The Kawasaki, Meng and Toft equations provided the least accurate estimates of 24-h sodium excretion (**Figure 3**, score between 1 and 2). They tended to overestimate 24-h sodium excretion (**Figure 3**, mean bias between 0.95 and 2.94 g). The equations from Mage and Remer provided estimates of 24-h sodium excretion of variable accuracy (**Figure 3**, score between 2 and 4). The equations from Tanaka and Brown with and without potassium provided the most accurate estimates of 24-h sodium excretion (**Figure 3**, score between 4 and 5). The overnight spots gave the best estimates with the Tanaka, Brown and Meng equations, the morning spots gave the best estimates with the Remer equation, and the evening spots gave the best estimates with the Mage equation. The most accurate estimates were provided with the Tanaka and Brown equations and the overnight spot (**Figure 3**, score 5).

To estimate salt intake at a population level, i.e., with the lowest bias, highest correlation, and lowest misclassification, the Tanaka and Brown equations with the overnight spot and the Mage equation with the evening spot provided the best estimates (**Figure 3**). To estimate salt intake at an individual level, i.e., with the highest precision, sensitivity, and specificity, the Tanaka equation with any of the spots, the Brown equations with the overnight or morning spot and the Toft equation with the evening spot provided the best estimates (**Figure 3**).

DISCUSSION

Summary of findings

In this study, we found that the different equations and spots provided highly heterogeneous estimates of 24-h sodium excretions in children. The equations from Tanaka et al. and Brown et al. with the overnight spot provided the most accurate estimates. The accuracy of these equations and spot was sufficient to estimate salt intake at a population level, for a public health use. At an individual level, for a clinical use, they might be used as an alternative to a single 24-h urine collection to identify children with high sodium excretion. Other equations and spots were less satisfactory.

Comparison with other studies

In adults, several studies have compared 24-h urinary sodium excretion with estimates from different equations and spots. In a systematic review by Huang et al (22), 29 studies comparing estimates from spots and measures from 24-h collections were identified, but none involved children. According to the latter review, the Tanaka and Brown equations with the morning and evening spots provided the best estimates, while the Kawasaki equation largely over-estimated 24-h sodium excretion. This is consistent with our findings, where the Tanaka and Brown equations provided the most accurate estimates of 24-h sodium excretion, while the Kawasaki equation over-estimated 24-h sodium excretion. These equations tended to overestimate 24-h sodium excretion at low levels of excretion and under-estimate excretion at high levels of excretion (22), in line with our findings.

Overnight urine spot samples were shown to under-estimate 24-h sodium excretion in adults (22). In our study, the overnight spot also provided the lowest estimates, but these estimates provided the best agreements with the 24-h urine collection. Overnight urine spots usually represent a longer period of collection (around 8 hours) than the other spots, which could be a reason why this spot tended to provide better estimates than the evening or morning spots. Moreover, they are potentially less influenced by hydration and physical activity than the other spots. Overnight spots might be however less convenient to collect than the morning spots, which can be collected during the consultation at the doctor's office.

The two equations which were developed specifically for children, i.e., Remer et al. (32) Meng et al. (26), did not perform well in our sample. There are different reasons why these equations might have not performed well. The equation from Remer et al. (32) was developed to estimate the 24-h excretion of analytes in urine spot samples of healthy white children. The equation was derived from 24-h urine samples indirectly, without collecting any separate urine spot sample (32), contrarily to other equations developped in adults such as the one from Brown et al. Further, in this study, the equation was developed to predict the excretion of several analytes in 24-h urine from spots, and not specifically for sodium (32); it is possible that the equation should have been refined for sodium. The equation from Meng et al. (26) was developed using hospital data from children who had a 24-h urine and urine spot sample. It is unclear what was the time lapse between the 24-h urine and urine spot sample and how many children were included in this study. It is possible that this equation did not fit well our sample because our study population was different (e.g., healthy instead of sick and predominantly white instead of Asian), as well as our urine sampling (i.e., 24-h urine sequential to urine spot samples).

Nevertheless, whatever the reason of this discrepency, it confirms that it is always key to test such equation in various populations before making any recommendation.

Strengths and limitations

Our study has several strengths. Firstly, we tested within the same sample of children a large number of equations built to estimate 24-h sodium urinary excretion from spots, including two equations specifically designed for children. Secondly, we used a comprehensive set of statistics and plots (i.e. mean bias, Pearson correlation coefficients, precision, sensitivity, specificity, misclassification, scatterplots, and Bland-Altman plots) to compare the different equations and spots and to assess their validity to estimate 24-h sodium urinary excretion. It allowed us to assess the possibility of using these equations and spots not only at a population level, for a public health use, but also at an individual level, for a clinical use, in comparison to a single 24-h urine collection. Thirdly, our study design allowed to compare three different timings of urine spot samples (evening, overnight and morning) and to determine which one was the best suited to estimate 24-h urinary sodium excretion.

Our study, however, has also several limitations. The main limitation is that only one 24-h urine collection was collected per individual. It has been shown that 24-h urinary sodium excretion varies not only due to the fluctuations in the diet, but also due to infradian fluctuations in sodium excretion that happen even at constant sodium intake (38). It is therefore better to have multiple 24-h urine collections to reliably estimate the average sodium intake at the individual level (39, 40). With our study, we were able to show that a urine spot sample can estimate 24-h urinary sodium excretion from one 24-h urine collection, but we could not determine whether a urine

spot samples can estimate average sodium intake estimated from multiple 24-h urine collections. Another weakness of our study is that the external validity of our findings might be limited as our study sample was comprised of a small number of children from one region of Switzerland and between 6 and 16 years of age.

Future research

In order to increase the external validity of our findings, other studies should test the different spots and equations in other populations of children. Furthermore, a study collecting multiple 24-h urine samples and urine spot samples over several weeks would be useful to evaluate the validity of using either a unique spot or multiple spots to estimate the true average sodium intake at the individual level. Further, many different equations have been developed to estimate 24-h urinary sodium excretion from urine spot samples. When clinicians or researchers are faced with so many possibilities, it can be difficult to choose which equation is best (41). Instead of developing a new equation for each different population group, it would be useful to pool all the individual data together and develop a universal equation. A systematic review and meta-analysis of individual participant data (42) is currently ongoing, with this same aim.

Conclusions

Our study suggests that urine spot samples, with appropriate equations, might provide fairly accurate estimates of 24-h sodium excretion in children. The overnight urine spot sample with the Tanaka and Brown equations provided the most accurate estimates of 24-h urinary sodium excretion.

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429 **TABLES**

430 **Table 1. Equations to estimate 24-h sodium excretion from urine spot samples**

Reference	Description	Equations[1]
Kawasaki et al. (23)	Developed with a sample of 159 Japanese of 20-79 years of age, with a morning spot sample followed by 3-5 24-h urine collection.	Male: $Est24hNa \approx 23 \times [16.3 \times (SpNa / (SpCre \times 10))^{0.5} \times (-12.63 \times age + 15.12 \times weight + 7.39 \times height - 79.9)] / 1000$ Female: $Est24hNa \approx 23 \times [16.3 \times (SpNa / (SpCre \times 10)^{0.5} \times (-4.72 \times age + 8.58 \times weight + 5.09 \times height - 74.5)] / 1000$
Tanaka et al. (24)	Developed with a sample of 336 Japanese of 20-69 years of age, with a casual spot sample followed by a 24-h urine collection.	Both sexes: $Est24hNa \approx 23 \times [21.98 \times [(SpNa / (SpCre \times 10)) \times (-2.04 \times age + 14.89 \times weight + 16.14 \times height - 2244.45)]^{0.392} / 1000]$
Remer et al. (32)	Developed with a sample of 454 healthy white children 3-18 years, with 24-h urine samples to predict 24-h creatinine excretion.	Both sexes: $Est24hNa \approx 23 \times ((SpNa / SpCre)) \times 10^{(0.0102 \times height - 0.6854)}) / 1000$
Mage et al. (34)	Also known as the NHANES equation, developed with a sample of 267 adults 18-92 years of age, originally developed to predict urine pesticide and chemical exposure.	Male: $Est24hNa \approx 23 \times [(SpNa / (SpCre \times 10)) \times 0.00179 \times (140 - age) \times weight^{1.5} \times height^{0.5} \times 1.366 - 0.0159 \times BMI] / 1000$ Female: $Est24hNa \approx 23 \times [(SpNa / (SpCre \times 10)) \times 0.00163 \times (140 - age) \times weight^{1.5} \times height^{0.5} \times 1.429 - 0.0198 \times BMI] / 1000$
Brown et al. (25) with K	Also known as the INTERSALT equation, developed with a subsample of the INTERSALT study of 5,693 individuals of 20-59 years from various Western countries, with a casual spot sample followed by 24-h urine collection. Two versions of this equation exists, one using potassium (K) and another without potassium.	Male: $Est24hNa \approx 23 \times [25.46 + 0.46 \times SpNa - 2.75 \times SpCre - 0.13 \times SpK + 4.10 \times BMI + 0.26 \times age] / 1000$ Female: $Est24hNa \approx 23 \times [5.07 + 0.34 \times SpNa - 2.16 \times SpCre - 0.09 \times SpK + 2.39 \times BMI + 2.35 \times age - 0.03 \times age^2] / 1000$
Brown et al. (25) without K		Male: $Est24hNa \approx 23 \times [23.51 + 0.45 \times SpNa - 3.09 \times SpCre + 4.16 \times BMI + 0.22 \times age] / 1000$ Female: $Est24hNa \approx 23 \times [3.74 + 0.33 \times SpNa - 2.44 \times SpCre + 2.42 \times BMI + 2.33 \times age - 0.03 \times age^2] / 1000$
Toft et al. (27)	Developed with a sample of 473 Danish adults 28-74 years, with a casual spot sample followed by a	Male: $Est24hNa \approx 23 \times [33.56 \times ((SpNa / SpCre \times 10) \times (-7.54 \times age + 14.15 \times weight + 3.48 \times height + 423.15))^{0.345}]$

	24-h urine sample within 0-14 days.	Female: $Est24hNa \approx 23 \times [52.65 \times ((SpNa / SpCre \times 10) \times (-6.13 \times age + 9.97 \times weight + 2.45 \times height + 342.73))^{0.196}]$
Meng et al. (26)	Developed with children from a Chinese hospital database with a casual spot sample and a 24-h urine sample.	Both sexes: $Est24hNa \approx 23 \times [12.3 \times (SpNa / (SpCre \times 10))^{0.5} \times (-11.53 \times age + 14.12 \times weight + 8.39 \times height - 68.9)] / 1000$

[1]Abbreviations and units: K: Potassium; Est24-hNa: estimated 24-h sodium urinary excretion in g/day; SpNa: concentration of sodium in spot in mmol/L; SpCre: concentration of creatinine in spot in mmol/L; SpK: concentration of potassium in spot in mmol/L; age in years; weight in kg; height in cm; BMI: body mass index in kg/m^2.

436 **Table 2. Sample characteristics (n=86).** Values are mean ± standard deviation (range), or
437 percentages.

	Values
Age (years)	10.5 ± 2.7 (6-16)
Female (%)	41.9%
Height (cm)	141 ± 16 (113-186)
Weight (kg)	34.8 ± 12.0 (17.4-72.0)
Body mass index (kg/m^2)	17.0 ± 3.4 (12.5-37.2)
Overweight (%)	11.6%
24-h sodium excretion (g/d)	2.4 ± 1.1 (0.3-6.4)
24-h creatinine excretion (mmol/d)	6.4 ± 3.4 (2.5-27.4)
24-h potassium excretion (g/d)	1.8 ± 0.6 (0.6-4.4)

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FIGURE

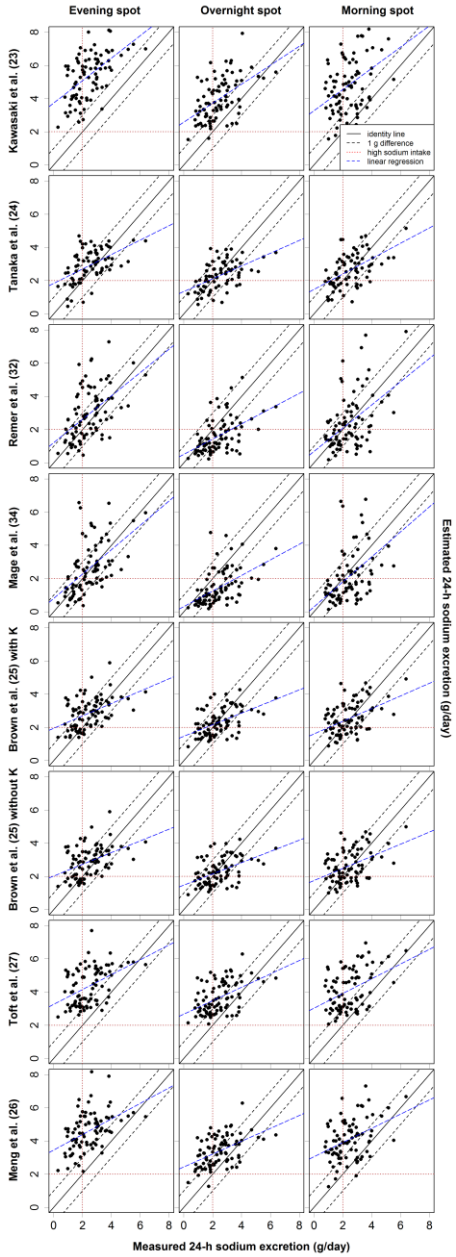
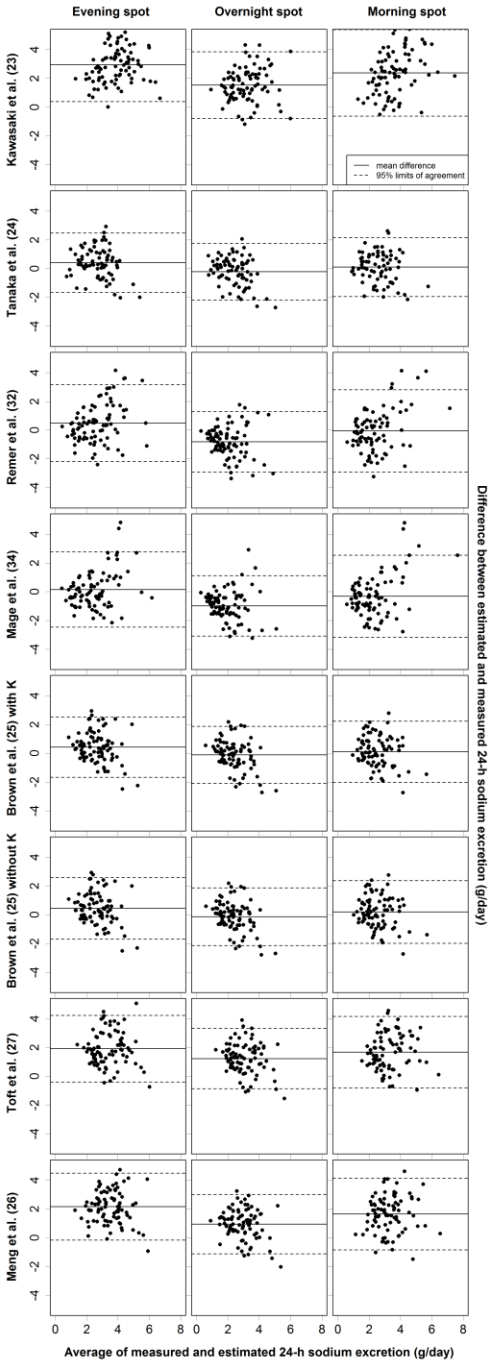


Figure 1. Scatterplot of measured 24-h sodium excretion versus estimated 24-h sodium excretion from urine spot samples using different equations. Legend: Black continuous line: identity line, i.e. perfect correlation; black dashed lines: 1 g difference between measured and estimated excretion; red dotted lines: threshold for high sodium intake, i.e. 2 g; blue dashed line: linear regression.



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448 **Figure 2. Bland-Altman plots.** Legend: x-axis: average of measured and estimated 24-h
449 sodium excretion; y-axis: mean difference between estimated and measured 24-h sodium
450 excretion; continuous line: mean difference; dashed lines: 95% limits of agreement of the mean
451 difference (mean ±1.96 SD).

Equation	Spot	Mean bias[1] (g/day)	Pearson correlation	Precision[2] (%)	Sensitivity[3] (%)	Specificity[4] (%)	Misclassification[5] (%)	Score[6]
Kawasaki	Evening	2.94	0.52	4.7	100.0	0.0	38.8	2
	Overnight	1.53	0.51	29.4	100.0	6.1	36.5	2
	Morning	2.38	0.43	22.4	100.0	6.1	36.5	2
Tanaka	Evening	0.41	0.52	61.8	95.9	40.7	23.7	4
	Overnight	-0.20	0.53	69.7	81.6	66.7	23.7	5
	Morning	0.12	0.52	64.5	83.7	55.6	26.3	5
Remer	Evening	0.49	0.52	58.8	82.7	57.6	27.1	3
	Overnight	-0.81	0.51	52.9	42.3	87.9	40.0	2
	Morning	-0.05	0.48	56.5	100.0	75.8	30.6	4
Mage	Evening	0.15	0.54	60.0	78.8	72.7	23.5	4
	Overnight	-0.98	0.51	45.9	36.5	93.9	41.2	2
	Morning	-0.32	0.51	54.1	53.8	87.9	32.9	3
Brown with potassium	Evening	0.42	0.46	65.9	94.2	42.4	25.9	4
	Overnight	-0.12	0.49	76.5	78.8	66.7	25.9	5
	Morning	0.10	0.45	67.1	76.9	54.5	31.8	4
Brown without potassium	Evening	0.44	0.44	65.9	92.3	42.4	27.1	4
	Overnight	-0.14	0.48	75.3	76.9	66.7	27.1	5
	Morning	0.19	0.43	62.4	76.9	51.5	32.9	4
Toft	Evening	1.91	0.45	21.2	100.0	0.0	38.8	2
	Overnight	1.22	0.48	37.6	100.0	0.0	38.8	2
	Morning	1.67	0.41	32.9	100.0	0.0	38.8	2
Meng	Evening	2.16	0.46	15.3	100.0	0.0	38.8	2
	Overnight	0.95	0.48	44.7	100.0	9.1	35.3	2
	Morning	1.65	0.40	27.1	100.0	6.1	37.6	1

Legend : best estimate : 50th percentile : worst estimate

Figure 3. Heat map comparison of the different equations with the evening, overnight and morning urine spot samples to estimate 24-h urinary sodium excretion. Colors: Gradient from red (worst estimate), through yellow (50th centile) to green (best estimate). 1) Mean bias: mean difference between estimated and measured 24-h sodium excretion; 2) Precision: proportion of children with a difference between estimated and measured sodium excretion of less than 1 g sodium; 3) Sensitivity, i.e., the proportion of children who had an estimated 24-h sodium excretion ≥2 g/day among those who had a measured excretion ≥2 g/day; the specificity; 4) Specificity: the proportion of children who had an estimated 24-h sodium excretion <2 g/day among those who had a measured excretion <2 g/day; 5) Misclassification: proportion of children misclassified to ≥2 g or <2 g sodium intake per day; 6) Score: overall score ranges from 0 to 6 and is the sum of measures above/below threshold values (i.e., a score of 1 was

464 attributed to absolute mean bias below 0.4 g per day, a Pearson correlation above 0.4, a
465 precision above 60%, a sensitivity above 75%, a specificity above 75%, and a misclassification
466 below 30%; otherwise a score of 0 was attributed to the measure).