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ORIGINAL CONTRIBUTION

Population biomonitoring of micronutrient intakes in children using urinary spot samples

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Abstract

Purpose Urinary spot samples are a promising method for the biomonitoring of micronutrient intake in children. Our aim was to assess whether urinary spot samples could be used to estimate the 24-h urinary excretion of potassium, phosphate, and iodine at the population level.

Methods A cross-sectional study of 101 children between 6 and 16 years of age was conducted. Each child collected a 24-h urine collection and three urinary spot samples (evening, overnight, and morning). Several equations were used to estimate 24-h excretion based on the urinary concentrations of each micronutrient in the three spot samples. Various equations and spot combinations were compared using several statistics and plots.

Results Ninety-four children were included in the analysis (mean age: 10.5 years). The mean measured 24-h urinary excretions of potassium, phosphate, and iodine were 1.76 g, 0.61 g, and 95 µg, respectively. For potassium, the best 24-h estimates were obtained with the Mage equation and morning spot (mean bias: 0.2 g, correlation: 0.27, precision: 56%, and misclassification: 10%). For phosphate, the best 24-h estimates were obtained with the Mage equation and overnight spot (mean bias: − 0.03 g, correlation: 0.54, precision: 72%, and misclassification: 10%). For iodine, the best 24-h estimates were obtained with the Remer equation and overnight spot (mean bias: − 8 µg, correlation: 0.58, precision: 86%, misclassification: 16%).

Conclusions Urinary spot samples could be a good alternative to 24-h urine collection for the population biomonitoring of iodine and phosphate intakes in children. For potassium, spot samples were less reliable.

Keywords Iodine · Potassium · Phosphate · Urinary excretion · Children · Urinary spots

Introduction

Monitoring of micronutrient intake at the population level is essential to adjust food supply and policies for the optimal health of populations and to assess the effectiveness of nutrition interventions [1]. The monitoring of micronutrient intake is most commonly done with dietary questionnaires. However, this method is prone to several biases, such as reporting, recall, misclassification, and measurement biases [2]. In children, the monitoring of micronutrient intake is especially important due to its impact on growth. However, biases with dietary questionnaires are highly prevalent in children. It is even more difficult to complete dietary questionnaires in this population and therefore biases can be strong. Therefore, alternatives to dietary questionnaires, such as urinary biomonitoring [3, 4], are of interest for this population group to overcome these biases.

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A standard biomonitoring method is the collection of 24-h urine in which biomarkers, such as sodium, are measured [5]. This method presents however major practical challenges, especially for children. To allow a better and easier biomonitoring of micronutrient intake, urinary spot samples have been proposed as an alternative. For sodium, urinary spot samples have been compared to 24-h urinary excretion in adults in several studies [5–7] and in children only in very few studies [8, 9]. For other micronutrient intake, such as potassium, phosphate or iodine, even less studies comparing urinary spot samples and 24-h urine have been conducted [10–12].

Taking advantage of a study in which 24-h urinary collection and several spot samples were collected, our aim was therefore to determine whether urinary spot samples can be used to estimate 24-h urinary excretion of iodine, potassium, and phosphate as quantitative biomarkers in dietary intake.

Methods

A biomonitoring study was conducted among children between 6 and 16 years of age in canton of Valais between September 2016 and February 2018. The main objective of this study was to assess whether 24-h urinary sodium excretion could be estimated with urinary spot samples. The detailed methods of this study and the results have been published elsewhere [8, 13]. We used these samples for the present study.

Children between 6 and 16 years of age, without any disease potentially altering the consumption and excretion of sodium and with sufficient knowledge of the local language to understand the content of the information forms, and visiting the local hospital or pediatric health centers were invited to participate in the study. Upon enrollment, the children were weighed and measured with light clothes and without shoes by a trained nurse or a research assistant.

Ethical considerations

Ethical approval was obtained by the Ethics Committee of canton of Vaud, Switzerland (CER-VD, identification number: 2015-01178). Written informed consent was obtained from the parent (or legal guardian) of the child. Children below 14 years of age gave oral consent and children above 14 years of age gave written consent.

Urine samples

Urine collection was done at home over three consecutive days (day 1 to day 3), which consisted, consecutively, of (a) one evening spot (last void before going to bed on day 1), (b) one 24-h urine (starting after the last void before going to bed on day 1 and finishing with the last void before going to bed on day 2), (c) one overnight spot (first void upon rising in the morning on day 3), and (d) one morning spot (second void upon rising in the morning on day 3). To ensure a complete urine collection over 24 h, written and oral instructions were given to the participants and their parents, and urine collection pots were provided. Participants 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 and 8 °C and to bring them to the laboratory no later than 48 h after urine collection. The urine samples were stored at −20 °C until analysis. Potassium, phosphorus, and creatinine concentrations were measured using a Cobas® c-501 analyzer (Roche). Iodine concentrations were measured with an isotope dilution, inductively coupled plasma-mass spectrometry method [14].

Statistical analysis

The total 24-h urinary excretions of potassium, phosphate, iodine, and creatinine were calculated by multiplying the concentration in the 24-h urine sample by the total volume of the sample and by adjusting for self-reported collection times to represent an exact 24-h duration. A 24-h creatinine excretion of less than 0.1 mmol per kilogram of body weight was considered an indication of incomplete 24-h urine collection [15] and was corrected to equal to 0.1 mmol. The ratios between potassium, phosphate, and iodine, one at a time in the numerator, and creatinine concentration in the denominator were calculated for the 24-h urine and the three urinary spot samples.

To transform the urinary concentrations of potassium, phosphate, and iodine in the urinary spots into 24-h urinary excretion estimates, the following equations were used: Remer [15] and Mage [6, 16] for potassium, phosphate, and iodine, Kawasaki [17, 18] for potassium, Robinson-Cohen [11] for phosphate, and Montenegro-Bethancourt [12] and Zimmermann [19] for iodine (see detailed equations in Online Appendix 1). To compare the estimated 24-h urinary excretions from the different equations and spots with the corresponding measured 24-h urinary excretions, several statistics were used: mean bias, i.e., mean difference between the estimated and measured 24 h with the micronutrient excretion; Pearson correlation coefficient between estimated and measured excretion; precision, i.e., proportion of children with a between estimated and measured excretion difference within ± 1 SD of the 24-h mean; misclassification, i.e., the proportion of children who were incorrectly classified to ≥ 3.5 g/day or < 3.5 g/day for potassium [20], ≥ 1.0 g/day or < 1.0 g/day for phosphate [21], and ≥ 120 µg/day or < 120 µg/day for iodine [19]. Moreover, scatter plots and

Bland–Altman diagrams [22, 23] were plotted for each spot and equation to allow visual comparisons.

Statistical analyses were conducted with R (version 3.3.1) and R Analytic Flow (version 3.0.6).

Results

Participants' characteristics

Among the 101 children recruited, 94 were able to collect a 24-h urine sample and were included in the analyses. There were 39 girls (41%). The children were on average 10.6 years of age (SD 2.9, range 6–16). They weighed 36.2 kg (SD 14.2, range 17.4–88.0) and were 142 cm (SD 17, range 113–186) tall.

Potassium

The mean concentrations of potassium were 54 g/L (SD 20) in the 24-h urine samples, 55 g/L (SD 38) in the evening spots, 41 g/L (SD 23) in the overnight spots, and 82 g/L (SD 46) in the morning spots. The potassium-to-creatinine ratios were higher in the 24-h urine than in the spot samples: 19.5 mmol/mmol (SD 114.1) in the 24-h urine samples; 6.8 mmol/mmol (SD 4.0) in the evening spots; 4.1 mmol/mmol (SD 2.2) in the overnight spots; and 9.7 mmol/mmol (SD 5.1) in the morning spots. The 24-h potassium urinary excretion measured in the 24-h urine samples was 1.76 g/24-h (SD 0.68; min 0.37; max 4.38). The distribution of this variable is shown in Online Appendix 2A.

Comparisons between the 24-h potassium excretion measured in the 24-h urine samples and estimated in the three urinary spot samples with the different equations are shown in Table 1. The smallest bias was with the Kawasaki equation and the overnight spot. The highest correlation was with the Mage equation and the evening spot. The highest precision was with the Kawasaki equation and the overnight spot. The lowest misclassification was with the Kawasaki equation and the overnight spot. The scatter plots are shown in Fig. 1 and the Bland–Altman plots in Online Appendix 3A. In the scatter plots, the Remer and the Mage equations with the morning spot show the best results. The Bland–Altman plots indicate that the difference between estimated and measured are the smallest with the overnight spots. Overall, the equation and spot combination that provided the best estimates was the Mage equation with the morning spot.

Phosphate

The mean concentrations of phosphate were in 24 g/L (SD 11) in the 24-h urine samples; 31 g/L (SD 17) in the evening spots; 36 g/L (SD 15) in the overnight spots; and 22 g/L (SD 12) in the morning spots. The phosphate-to-creatinine ratios were higher in the 24-h urine than in the spot samples: 7.0 mmol/mmol (SD 35.9) in the 24-h urine samples; 3.7 mmol/mmol (SD 1.3) in the evening spots; 3.5 mmol/mmol (SD 1.1) in the overnight spots; and 2.4 mmol/mmol (SD 1.0) in the morning spots. The total 24-h phosphate excretion in the 24-h urine samples was 0.61 g/24-h (SD 0.27; min 0.13; max 1.79). The distribution is shown in Online Appendix 2B.

Comparisons between the 24-h phosphate excretion measured in the 24-h urine samples and estimated in the three urinary spot samples with the different equations are shown in Table 2, the scatter plots are shown in Fig. 2, and the Bland–Altman plots in Online Appendix 3B. The smallest bias was with the Mage equation and the evening spot. The highest correlation was with the Mage equation and the overnight spot. The highest precision and the

Table 1 Comparison between 24-h potassium urinary excretion measured in 24-h collections and estimated with equations

Equation	Urine sample	Bias (g/day)	Correlation	Precision (%)	Misclassification (%)
Remer	Evening spot	− 0.25	0.27	54	6
	Overnight spot	− 0.82	0.22	33	3
	Morning spot	0.39	0.24	53	9
Mage	Evening spot	− 0.41	0.29	52	5
	Overnight spot	− 0.90	0.18	26	3
	Morning spot	0.15	0.27	56	10
Kawasaki	Evening spot	0.39	0.10	57	6
	Overnight spot	− 0.08	0.07	67	2
	Morning spot	0.79	0.09	35	7

Bias: mean difference between estimated and measured 24-h potassium excretion; correlation: Pearson correlation between estimated and measured 24-h potassium excretion; precision: proportion of children with a difference between estimated and measured potassium excretion of less than 1 SD of the mean measured 24-h potassium excretion; misclassification: proportion of children misclassified to ≥ 3.5 g or < 3.5 g potassium excretion per day

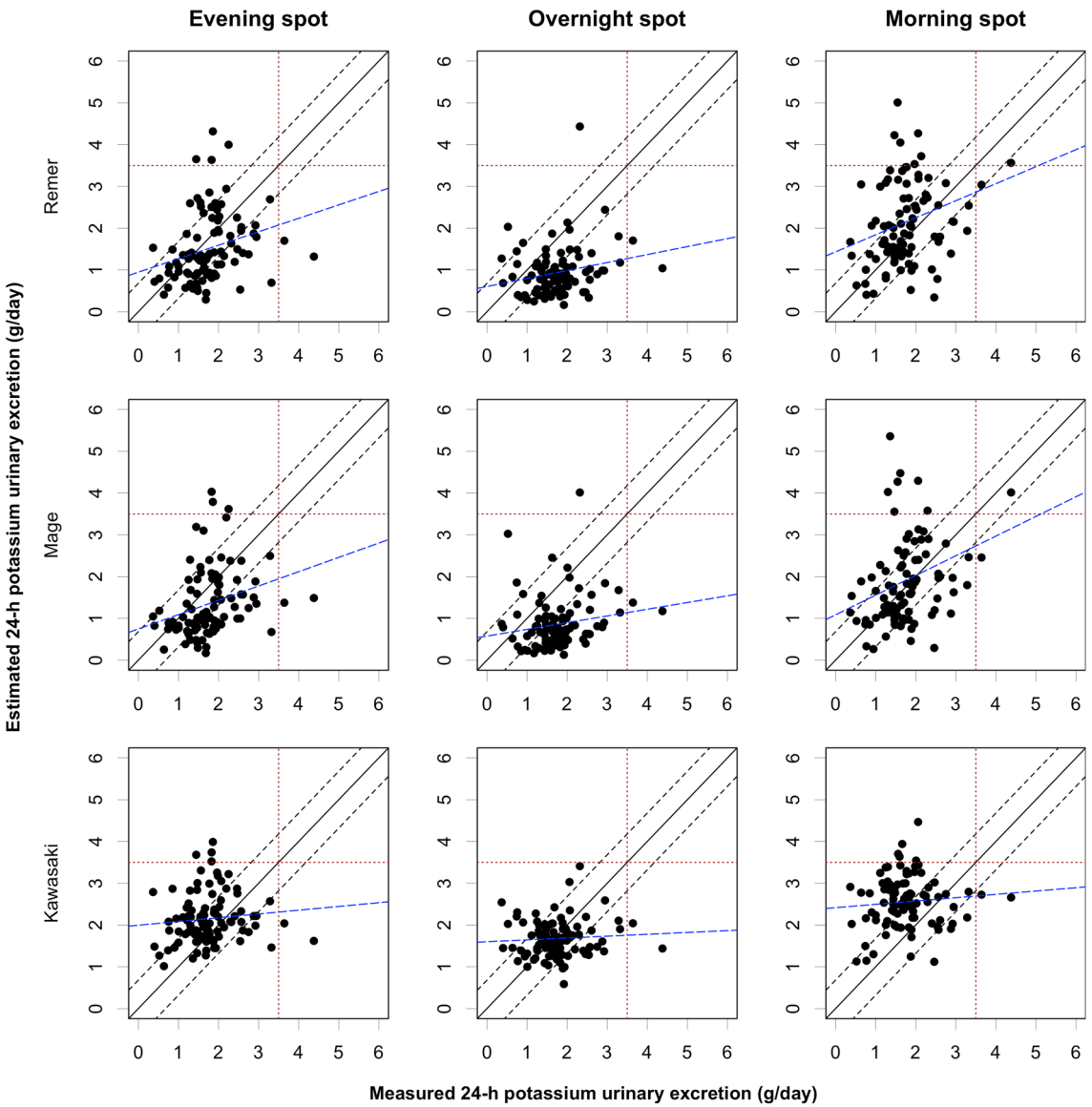

Fig. 1 Scatter plot of measured 24-h potassium excretion versus estimated 24-h potassium excretion from urine spot samples using different equations in g/day. Black continuous line: identity line, i.e., perfect correlation; black dashed lines: 1 SD difference between measured and estimated excretion; red dotted lines: threshold for high potassium intake; blue dashed line: linear regression

lowest misclassification were with the Remer equation and the overnight spot. In the scatter plots, the Remer equation with the overnight spot shows the best results. The scatter plots also show that the estimates with Robinson-Cohen 2 equation almost do not vary. Overall, the equation and spot combination that provided the best estimates was the Mage equation with the overnight spot.

Iodine

The mean concentrations of iodine were 115 µg/L (SD 53) in the 24-h urine samples, 155 µg/L (SD 91) in the evening spots, 150 µg/L (SD 72) in the overnight spots, and 124 µg/L (SD 58) in the morning spots. The iodine-to-creatinine ratios varied widely between the different urine samples: 265 mmol/mmol (SD 171) in the 24-h urine samples; 154 mmol/mmol (SD 125) in the evening spots; 894 mmol/mmol (SD 5) in the overnight spots; and 110 mmol/mmol (SD 5) in the morning spots. The total 24-h iodine excretion in the 24-h urine samples was 95 µg/24 h (SD 45; min 19; max 287). The distribution is shown in Online Appendix 2C.

The comparisons of the 24-h iodine excretion measured in the 24-h urine samples and estimated in the three urinary spot samples with the different equations are shown in Table 3, the scatter plots are shown in Fig. 3, and the

Table 2 Comparison between 24-h phosphate urinary excretion measured in 24-h collections and estimated with equations

Equation	Urine sample	Bias (g/day)	Correlation	Precision (%)	Misclassification (%)
Remer	Evening spot	0.07	0.30	66%	13
	Overnight spot	0.03	0.40	7	6
	Morning spot	− 0.16	0.32	62	7
Mage	Evening spot	0.02	0.38	72	16
	Overnight spot	− 0.03	0.54	72	9
	Morning spot	− 0.19	0.41	51	7
Robinson-Cohen 1	Evening spot	0.55	0.30	12	61
	Overnight spot	0.52	0.40	14	59
	Morning spot	0.34	0.35	37	37
Robinson-Cohen 2	Evening spot	0.05	− 0.03	68	7
	Overnight spot	0.03	− 0.01	67	7
	Morning spot	− 0.08	0.02	71	7

Bias: mean difference between estimated and measured 24-h phosphate excretion; correlation: Pearson correlation between estimated and measured 24-h phosphate excretion; precision: proportion of children with a difference between estimated and measured phosphate excretion of less than 1 SD of the mean measured 24-h phosphate excretion; misclassification: proportion of children misclassified to ≥ 1 g or < 1 g phosphate excretion per day

Bland–Altman plots in Online Appendix 3C. The smallest bias was with the Montenegro-Bethancourt equation and the overnight spot. The highest correlation was with the Remer equation and the overnight spot. The highest precision was with the Montenegro-Bethancourt equation and the overnight spot. The lowest misclassifications were with both the Remer and the Mage equation and the overnight spot. In the scatter plots, the Remer equation with the overnight spot shows the best results. The scatter plots appear similar between all the equations, except with the Zimmermann equation which shows an overestimation. Overall, the equation and spot combination that provided the best estimates was the Remer equation with the overnight spot.

Discussion

Summary of findings

Our study including 94 children between 6 and 16 years of age suggests that urinary spot samples could be an alternative to 24-h urine collections for the population biomonitoring of the intake of some micronutrients in children. For potassium, the best 24-h estimates were obtained with the Mage equation and morning spot (mean bias: 0.15 g, correlation: 0.27, precision: 56%, and misclassification: 10%). For phosphate, the best 24-h estimates were obtained with the Mage equation and overnight spot (mean bias: − 0.03 g, correlation: 0.54, precision: 72%, and misclassification: 9%). For iodine, the best 24-h estimates were obtained with the Remer equation and overnight spot (mean bias: − 8 µg, correlation: 0.58, precision: 86%, misclassification: 22%). This suggests that urinary spot samples could be an alternative to 24-h urine collections for the biomonitoring of iodine and phosphate intakes in children in the population. For potassium, the spot samples seemed to be less reliable.

Comparison with other studies

Potassium excretion in 24-h urine is considered as a biomarker of absolute intake and the recommended method to assessing daily potassium intake [24]. About 77% of potassium intake is excreted in urine and 18% in stool [24]. In a study including 1083 people aged 35–70 years [7], the Kawasaki formula provided the best agreement and least bias to estimate 24-h urinary potassium excretion from a morning spot urine.

Phosphate is not commonly assessed in urine to measure intake, unlike potassium and iodine. However, interest in phosphate is rising as its intake, hence its excretion, is believed to have increased with the rise of use of food additives [25]. Little literature exists on the subject. One study with 32 adults showed that phosphorus intake based on weighed dietary records correlates strongly with 24-h urine excretion [25].

Urinary excretion of iodine is considered to reflect a high portion of dietary intake, as > 90% is excreted in the urine within 24–48 h by adults [16] and is relatively constant over the time of the day [26], making urinary spots a very interesting alternative to 24-h urine collections. In the DONALD study, where 180 children collected a 24-h urine sample and, a few days later, a casual urine spot sample, and from which the Montenegro–Bethancourt equation was constructed, the correlation between the measured and estimated 24-h

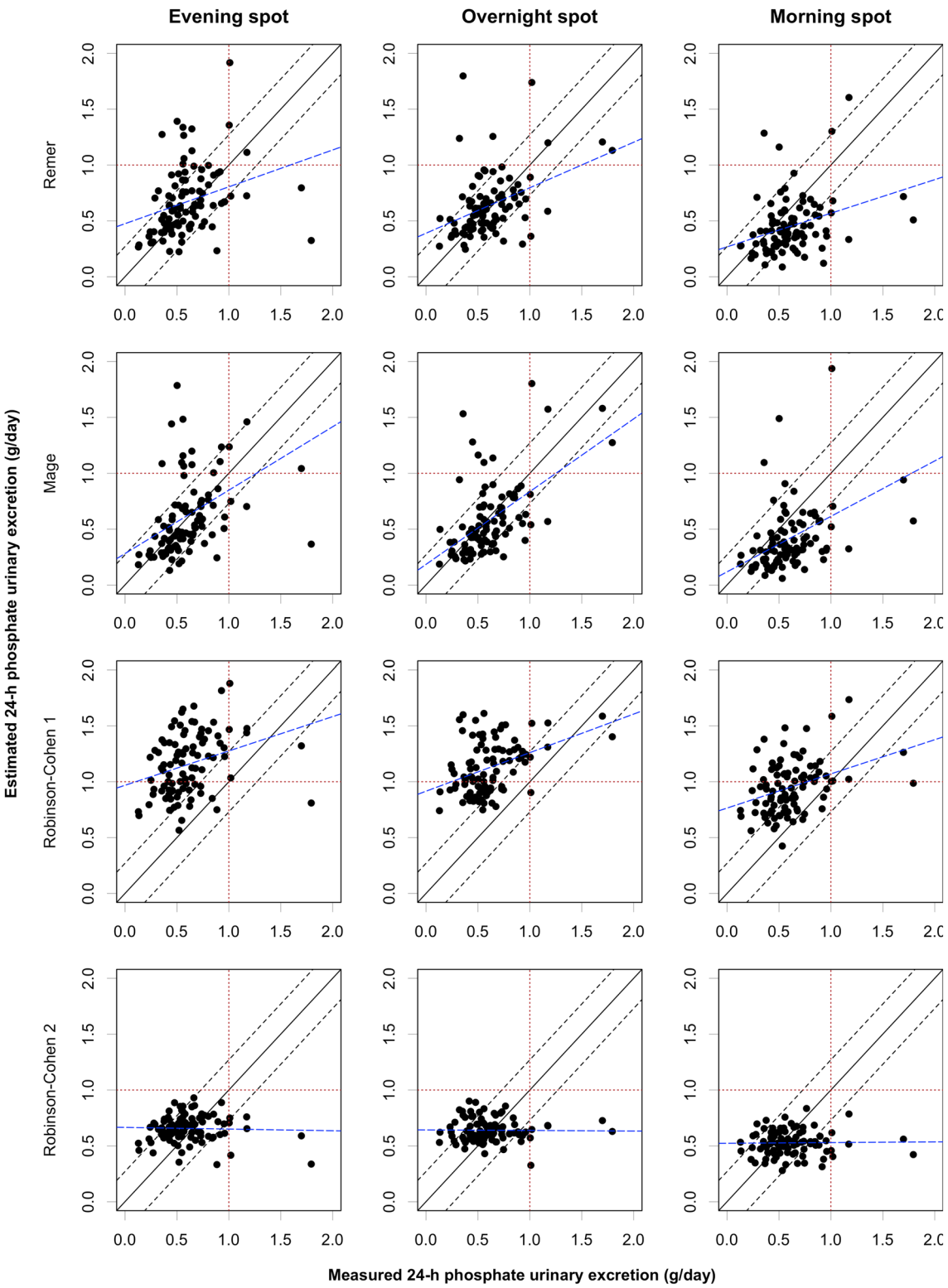

Fig. 2 Scatterplot of measured 24-h phosphate excretion versus estimated 24-h phosphate excretion from urine spot samples using different equations in g/day. Black continuous line: identity line, i.e., perfect correlation; black dashed lines: 1 SD difference between measured and estimated excretion;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

Table 3 Comparison between 24-h iodine urinary excretion measured in 24-h collections and estimated with equations

Equation	Urine sample	Bias (µg/day)	Correlation	Precision (%)	Misclassification (%)
Remer	Evening spot	20	0.44	80	25
	Overnight spot	− 8	0.58	86	22
	Morning spot	− 11	0.54	80	23
Mage	Evening spot	8	0.47	82	27
	Overnight spot	− 16	0.52	79	22
	Morning spot	− 18	0.49	72	24
Montenegro-Bethancourt	Evening spot	24	0.43	79	25
	Overnight spot	− 5	0.54	87	25
	Morning spot	− 8	0.51	80	27
Zimmermann	Evening spot	76	0.20	45	50
	Overnight spot	72	0.30	47	61
	Morning spot	43	0.21	57	51

Bias: mean difference between estimated and measured 24-h iodine excretion; correlation: Pearson correlation between estimated and measured 24-h iodine excretion; precision: proportion of children with a difference between estimated and measured iodine excretion of less than 1 SD of the mean measured 24-h iodine excretion; misclassification: proportion of children misclassified to ≥ 120 µg or < 120 µg iodine excretion per day

iodine excretion was moderate ($r = 0.41$–0.47) and similar to our study ($r = 0.43$–0.54). In a study of 400 adults, the 24-h iodine excretion estimated with the Mage equation and different spots was compared with a 24-h urine collection [27]; this study found mean biases similar to our study (− 9 to 16 µg/day; our study: − 18 to 8 µg/day). The equation by Zimmermann seemed not to provide satisfactory estimates, whatever the spot considered.

Strengths and limitations

The strengths of this study are that: (1) the 24-h urine collection was checked for completeness and corrected in case of incompleteness; (2) three different timed urinary spots were collected; (3) various equations were compared; (4) several statistics and plots were used to assess which equation and spot combination provided the best estimates.

The main limitation of this study was that only one 24-h urine sample was collected per child and therefore we could not measure the day-to-day variation in excretion. As a result, we could only assess whether urinary spots were useful to replace 24-h urine collections for group- or population-level estimates, not for individual-level estimates. In fact, for instance, up to ten urinary samples could be needed to accurately estimate the individual-level excretion of iodine [28]. Another limitation was that only three timed urinary spots were collected and no afternoon spot was collected. Finally, the equations used and compared were found through a non-systematic search of the literature and potentially more equations could have been identified through a systematic search. Some of these equations were developed in an adult population (Kawasaki and Robinson-Cohen), another in both adults and children (Mage), and the others in children (Montenegro-Bethancourt, Remer and Zimmermann).

Future research

It would be useful to replicate this study and to compare spots and equations in another sample of children to confirm which equation and spot are best to estimate 24-h urinary excretion of phosphate and iodine, and eventually potassium. To improve the reliability of urinary spots for potassium, it is possible that a combination of several spots would be more informative than a single spot. In addition, collecting multiple 24-h urine collections and multiple spots would allow the assessment of the validity of spots to estimate the average 24-h urinary excretion of micronutrients at the individual level. Moreover, other micronutrients could be measured in the urine and be used as biomarkers of intake. For example, urea in the urine could be measured as this could be a biomarker for protein intake [29]. Finally, it would be useful to conduct a study where the participants change their intake in micronutrients to assess whether these changes are measurable with the urinary spots and as a result could be used to assess the effectiveness of nutrition interventions.

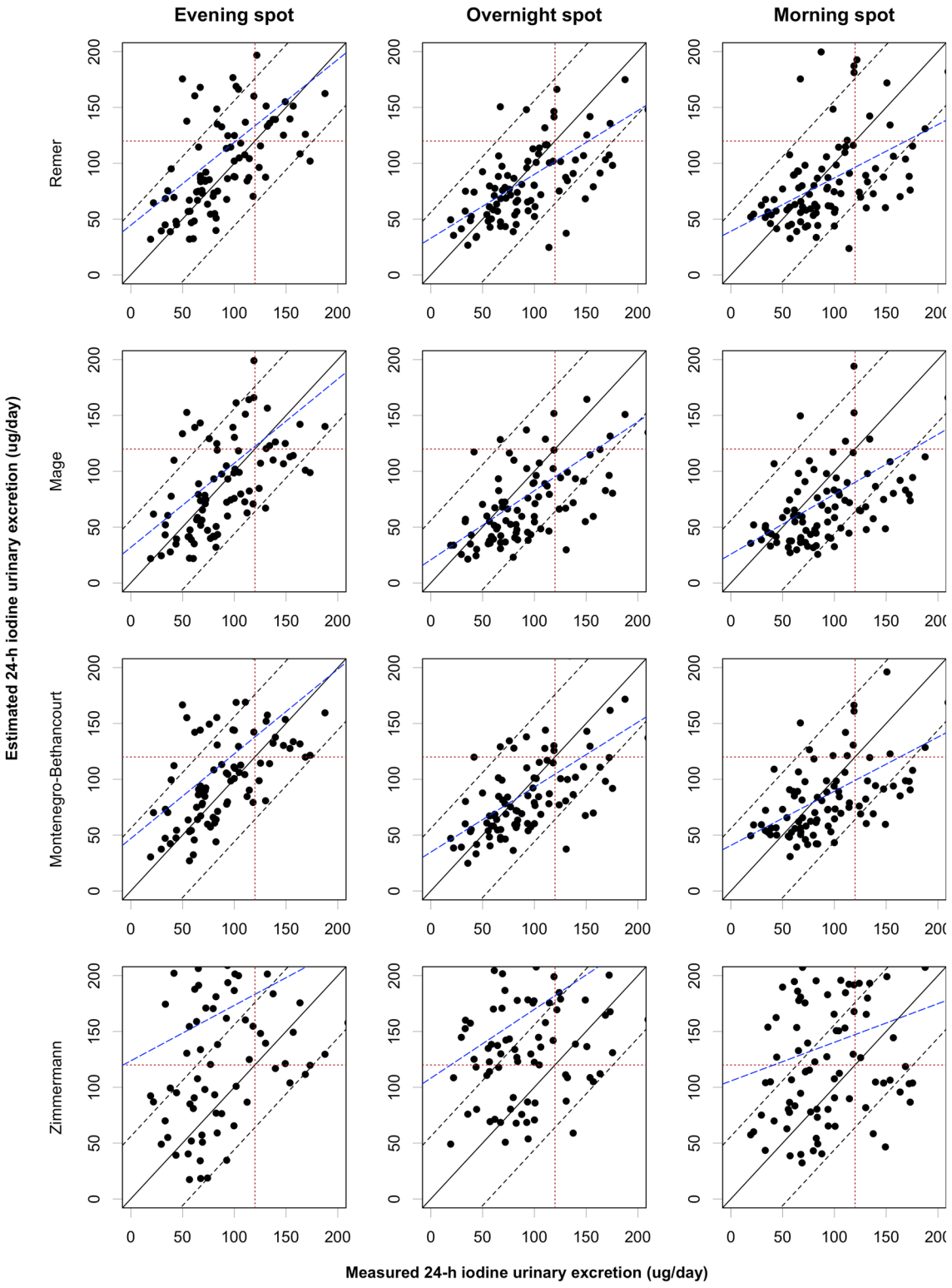

Fig. 3 Scatter plot of measured 24-h iodine excretion versus estimated 24-h iodine excretion from urine spot samples using different equations in µg/day. Black continuous line: identity line, i.e., perfect correlation; black dashed lines: 1 SD difference between measured and estimated excretion; red dotted lines: threshold for adequate iodine intake; blue dashed line: linear regression

Conclusions

Our findings suggest that urinary spot samples could be an alternative to 24-h urine collections for the population biomonitoring of iodine and phosphate intakes in children between 6 and 16 years of age if adequate timing and equations are used. In our study, the most reliable estimations were obtained with the Mage and Remer equations using the overnight spot sample. For potassium, spot samples collected in the evening, overnight, and morning appeared to be less reliable.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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