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Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese schoolchildren

Tomiko Tsuji[1,2], Tsutomu Fukuwatari[1], Satoshi Sasaki[3] and Katsumi Shibata[1,*]
[1]Department of Food Science and Nutrition, School of Human Cultures, The University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522-8533, Japan: [2]Department of Health and Nutrition, School of Health and Human Life, Nagoya Bunri University, Aichi, Japan: [3]Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, Tokyo, Japan

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Abstract

Objective: To examine the association between 24 h urinary water-soluble vitamin levels and their intakes in free-living Japanese schoolchildren.
Design: All foods consumed for four consecutive days were recorded accurately by a weighed food record. A single 24 h urine sample was collected on the fourth day, and the urinary levels of water-soluble vitamins were measured.
Setting: An elementary school in Inazawa City, Japan.
Subjects: A total of 114 healthy, free-living, Japanese elementary-school children aged 10–12 years.
Results: The urinary level of each water-soluble vitamin was correlated positively to its mean intake in the past 2–4 d (vitamin B_1: $r = 0{\cdot}42$, $P < 0{\cdot}001$; vitamin B_2: $r = 0{\cdot}43$, $P < 0{\cdot}001$; vitamin B_6: $r = 0{\cdot}49$, $P < 0{\cdot}001$; niacin: $r = 0{\cdot}32$, $P < 0{\cdot}001$; niacin equivalents: $r = 0{\cdot}32$, $P < 0{\cdot}001$; pantothenic acid: $r = 0{\cdot}32$, $P < 0{\cdot}001$; folic acid: $r = 0{\cdot}27$, $P < 0{\cdot}01$; vitamin C: $r = 0{\cdot}39$, $P < 0.001$), except for vitamin B_{12} ($r = 0{\cdot}10$, $P =$ NS). Estimated mean intakes of water-soluble vitamins calculated using urinary levels and recovery rates were 97–102 % of their 3 d mean intake, except for vitamin B_{12} (79 %).
Conclusions: The results show that urinary levels of water-soluble vitamins, except for vitamin B_{12}, reflected their recent intakes in free-living Japanese schoolchildren and could be used as a potential biomarker to estimate mean vitamin intake.

Keywords
Urinary water-soluble vitamin
Biomarker
Free-living
Japanese schoolchildren

Since vitamin deficiencies cause various disorders in the growth of schoolchildren, a method to evaluate vitamin status easily and accurately is desired for early screening at a primary preventive stage. Methods using biomarkers for assessing vitamin intakes offer an effective approach to evaluate vitamin status in individuals. Many preceding studies have investigated urinary excretion as a biomarker for vitamin intake[1–3]. We have also reported recently that 24 h urinary levels of water-soluble vitamins correlate highly with their intakes for Japanese college students in a strictly controlled environment[4,5]. Performing a study under a free-living environment without any interventions is the next step to confirm the applicability of the biomarker method. In the present study, we examined the association between 24 h urinary excretion of water-soluble vitamins and their dietary intakes for free-living schoolchildren to confirm the validity of the findings obtained in the controlled environment.

To capture dietary intake and calculate nutrients under a free-living environment, we used a weighed food record for four consecutive days. Although a weighed food record can provide relatively precise information regarding dietary intake compared with other dietary assessment methods[6], it is difficult for schoolchildren to complete a weighed food record without support. Few studies have reported this kind of assessment for free-living schoolchildren[7], while many studies have reported using a 24 h recall[8], a dietary diary[9] or an FFQ[10]. To overcome the difficulty of using a weighed food record for schoolchildren, we formed a close and cooperative relationship not only with the children but also their parents and teachers in the target elementary school before starting the study, through supporting the prolonged dietary education programme provided by the school board.

Methods

Participants

A total of 132 healthy, free-living schoolchildren aged 10–12 years voluntarily participated in the present study.

*Corresponding author: Email kshibata@shc.usp.ac.jp

The purpose and protocol were explained to all participants, as well as their parents, before joining the study, and written informed consent was obtained from each parent because all participants were less than 20 years old. We excluded participants diagnosed with the common cold or influenza, and those who had taken multivitamin supplements at least once during the previous month. In addition, we excluded participants whose 24 h urine collection or dietary records were considered incomplete, with a collection time outside the range of 22–26 h, urine volume <250 ml, creatinine excretion in relation to body weight outside the range of 10·8–25·2 mg/kg[11,12] or extremely low or high energy intake (<2092 or >16 736 kJ/d)[13]. After these screenings, 114 schoolchildren (sixty-seven boys and forty-seven girls) were found to be eligible. The study was reviewed and approved by The Ethical Committee of The University of Shiga Prefecture.

Dietary records

This was a 4 d dietary assessment in which the participants were living freely and consuming their normal diet. The assessment was performed at one of the elementary schools in Inazawa City (population >130 000) in Aichi Prefecture, Japan, in June 2007 and June 2008. The first day (Monday) of the experimental period was defined as day 1, the second day as day 2, the third day as day 3, and the fourth day as day 4. All foods consumed during the 4 d period were recorded using a weighed food record[14]. A digital cooking scale (1 g unit; Tanita Inc., Tokyo, Japan), a set of dietary record forms, a dietary record manual and a disposable camera were distributed to the participants in advance. Upon entry in the dietary record, the status of food at oral intake was identified as 'raw', 'boiled', 'cooked', 'the presence of skin', 'a part of cooking ingredients' or 'with or without seasoning', and coded according to the fifth revised and enlarged edition of the *Standard Tables of Food Composition in Japan*[15]. The participants with support from their parents took photographs with the disposable camera of the dishes before and after eating. Several experienced dietitians used the photographs to check the records, asking participants or their parents to resolve any discrepancies or to give further information when needed. The food that remained after eating was measured with a digital scale and was deducted from the dietary record. For school meals, the registered dietitians completed the records on behalf of the participants. Nutrient and energy intakes were calculated using the SAS statistical software package version 6·12 (SAS Institute Inc., Cary, NC, USA), based on the current *Standard Tables of Food Composition in Japan*[15]. For vitamins, the intakes of eight water-soluble vitamins –vitamins B_1, B_2, B_6, B_{12}, niacin, pantothenic acid, folic acid and vitamin C – were calculated, except for biotin which is not designated in the current *Standard Tables of Food Composition in Japan*. Since niacin is synthesized from tryptophan, the amount of niacin equivalents was handled separately from niacin. Since 1 mg nicotinamide is synthesized from 60 mg tryptophan[16], niacin equivalents was calculated as the sum of niacin and 1/60 tryptophan intakes. For calculating mean vitamin intakes, the 2 d mean intake corresponds to average intakes on days 3 and 4. Similarly, the 3 d mean intake corresponds to average intakes on days 2–4, and the 4 d mean intake corresponds to average intakes on days 1–4.

24 h urine sampling

A single 24 h urine sample was collected on the fourth day to measure urinary levels of water-soluble vitamins and their metabolites. It was collected from the second passage of urine on the fourth day to the first passage on the fifth day. The participants were asked to record all the times of urination on the sheet. After the total urine sample was collected, the volume was measured. Aliquots of the urine were stabilized to avoid destruction of water-soluble vitamins and their metabolites, and then stored at −20°C until analysis.

Urinalysis

Urinary thiamine was determined by post-HPLC labelled fluorescence[17]. Urinary riboflavin was determined by HPLC[18]. Urinary vitamin B_6 metabolite, 4-pyridoxic acid, was determined by HPLC[19]. To measure urinary vitamin B_{12}, urine samples were added to 0·2-mM acetate buffer (pH 4·8), vitamin B_{12} was converted to cyanocobalamin by boiling for 30 min with 0·0006 % w/w potassium cyanide at acidic pH, and cyanocobalamin was determined by a microbioassay using *Lactobacillus leichmanii* ATCC 7830[20]. Urinary N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide[21] and N^1-methylnicotinamide[22] were determined by HPLC, and the sum of these compounds was determined as nicotinamide metabolites. Urinary pantothenic acid was determined by a microbioassay using *Lactobacillus plantarum* ATCC 8014[23]. Urinary folic acid was determined by a microbioassay using *Lactobacillus casei* ATCC 2733[24]. Urinary reduced and oxidized ascorbic acid and 2,3-diketogluconic acid were determined by HPLC[25].

Statistical analysis

To exclude extraordinarily abnormal urinary vitamin levels which might be caused by taking unexpected fortified foods, participants in the upper 5 % limit in terms of urinary excretion for each vitamin were removed from the 114 eligible participants, and a total of 108 samples were identified to be valid for data analysis for each water-soluble vitamin. Similar to a previous free-living study[2], males and females were not separated for analysis. The SPSS for Windows statistical software package version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Values are presented as means and standard deviations. Since measurements of urinary and dietary water-soluble vitamins were not distributed normally, the data were converted logarithmically. Pearson correlation

Table 1 Characteristics of the participants: 114 eligible Japanese elementary-school children aged 10–12 years

	Total (*n* 114)		Boys (*n* 67)		Girls (*n* 47)	
Variable	Mean	SD	Mean	SD	Mean	SD
Anthropometric variables						
Age (years)	10·8	0·7	10·7	0·7	11·0	0·7
Body height (cm)	144·0	7·7	142·2	7·7	146·5	7·0
Body weight (kg)	36·7	8·3	34·6	7·2	39·8	8·9
Rohrer index (kg/cm^3×10^7)	122·0	17·9	119·3	15·9	125·7	20·1
Obesity index (%)	−4·01	3·8	−6·5	13·3	0·4	13·8
Dietary intake†						
Total energy (kJ/d)	8489	1298	8665	1409	8238	1086
Protein (% of energy)	14·9	2·5	14·9	2·6	14·8	2·1
Fat (% of energy)	29·0	5·8	29·1	6·0	28·8	5·5
Carbohydrate (% of energy)	54·8	8·7	54·7	9·3	55·1	7·7
% Energy intake‡						
Breakfast	21·3		21·7		20·8	
Lunch	32·7		32·1		33·6	
Supper	31·1		31·4		30·8	
Snacks	14·8		14·8		14·9	

†Dietary intake assessed from the consecutive 4 d dietary records.
‡Average starting time of each meal: breakfast, 06.50 hours; lunch, 12.30 hours; supper, 18.40 hours.

coefficients were calculated to determine the association between urinary and dietary measurements, and between dietary and estimated water-soluble vitamin intakes. $P < 0{\cdot}05$ was considered statistically significant. An ANOVA random-effects model was used to quantify inter- and intra-individual CV (%CV), which was used to estimate variability in vitamin intake.

Results

The characteristics of the 114 eligible participants are presented in Table 1. Since each value was almost the same as those reported for children aged 10–11 years in the *Dietary Reference Intakes for Japanese* in 2005[13], the participants were considered as typical elementary-school children in Japan. During the experimental period, all participants were living freely. Inter- and intra-individual variations in dietary intake of water-soluble vitamins for the consecutive 4 d period are shown in Table 2. For intra-individual variations, %CV was 25–45 %, except for vitamin B_{12} and vitamin C. For inter-individual variations, vitamin B_1, vitamin B_{12}, folic acid and vitamin C exceeded 50 %.

The correlations between 24 h urinary excretion of water-soluble vitamins and their intakes are shown in Table 3. For all vitamins except for vitamin B_{12}, a significant positive correlation was found between urinary excretion and dietary intake on day 4. For all vitamins except for pantothenic acid, the correlations on day 4 were higher than those on other days.

To examine the influence of dietary intake during the past few days on 24 h urinary excretion, we calculated the correlations between 24 h urinary excretions and mean dietary intakes, which are shown in Table 4. For all vitamins except for B_{12}, niacin equivalents and folic acid, the correlations between the urinary excretion (column 2 in Table 3) and the 3 d mean intake (column 5 in Table 4) were higher than those based on daily intake shown in Table 3 (columns 6, 9, 12 and 15). Because the most significant correlations were found between the urinary excretion and the 3 d mean intake, recovery rates (column 11 in Table 4) were derived from the urinary excretions (column 2 in Table 3) and the 3 d mean intakes (column 5 in Table 4), which are also shown in Table 4. Estimated mean intakes of water-soluble vitamins (column 13 in Table 4) were calculated using these recovery rates and urinary excretions. Estimated mean intakes, except for vitamin B_{12}, niacin equivalents and folic acid, correlated with 3 d mean intakes and were 97–102 % of the 3 d mean intake, except for vitamin B_{12} (79 %).

Table 2 Inter- and intra-individual variations in the dietary intake of water-soluble vitamins measured for the consecutive 4 d experimental period: eligible Japanese elementary-school children aged 10–12 years

	%CV (*n* 108)†	
Vitamin	Inter-individual variations	Intra-individual variations
Vitamin B_1	71·0	31·1
Vitamin B_2	28·8	29·5
Vitamin B_6	5·7	32·1
Vitamin B_{12}	166·8	95·0
Niacin	30·4	33·1
Niacin equivalents	8·8	25·2
Pantothenic acid	42·7	25·0
Folic acid	87·4	45·0
Vitamin C	62·2	65·5

†A total of 108 samples were valid for data analysis after removing the upper 5 % limit in terms of urinary excretion for each vitamin.

Discussion

In the present study we found a significant positive correlation between the urinary excretion and the dietary

Table 3 Measured values for 24 h urinary excretion collected on day 4 and daily vitamin intake for each water-soluble vitamin, and correlation between 24 h urinary excretion and daily vitamin intake (*n* 108), among eligible Japanese elementary-school children aged 10–12 years

	24 h urinary vitamin excretion†		Vitamin intake at day 4			Vitamin intake at day 3			Vitamin intake at day 2			Vitamin intake at day 1		
Vitamin	Mean	SD	Mean	SD	*r*‡	Mean	SD	*r*‡	Mean	SD	*r*‡	Mean	SD	*r*‡
Vitamin B_1 (μmol/d)	0·766	0·383	3·13	1·01	0·41***	2·90	0·85	0·25**	2·60	0·74	0·22*	2·75	0·92	0·07
Vitamin B_2 (μmol/d)	0·290	0·209	3·47	0·94	0·36***	3·75	1·13	0·36***	3·59	1·00	0·33***	3·60	1·17	0·23*
Vitamin B_6 (μmol/d)	2·36	0·92	5·93	1·86	0·42***	5·96	1·65	0·32***	5·97	1·69	0·36***	6·00	2·41	0·17
Vitamin B_{12} (nmol/d)	0·0256	0·0147	3·15	1·97	0·18	4·85	5·93	0·14	4·76	4·29	−0·02	4·64	3·37	0·11
Niacin (μmol/d)	–		97·0	32·3	0·28***	101·7	38·2	0·11	105·3	31·3	0·21*	101·4	32·5	0·23*
Niacin equivalents (μmol/d)	65·6	27·6	214	56	0·28**	218	56	0·23**	218	52	0·16	218	56	0·25**
Pantothenic acid (μmol/d)	11·6	5·5	27·6	6·9	0·23*	30·1	7·4	0·20*	27·0	6·3	0·31***	28·7	7·8	0·25**
Folic acid (nmol/d)	16·8	6·6	575	170	0·27**	615	423	0·12	491	123	0·18	532	164	0·24*
Vitamin C (μmol/d)	161	221	477	225	0·35***	448	313	0·23*	403	289	0·26**	445	328	0·18

†Urinary excretion for each vitamin corresponds to: thiamin for vitamin B_1; riboflavin for vitamin B_2; 4-pyridoxic acid for vitamin B_6; the sum of nicotinamide, N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide for niacin equivalents; the sum of reduced and oxidized ascorbic acid and 2,3-diketogluconic acid for vitamin C.
‡*r* indicates the correlation between urinary excretion and dietary intake of the vitamin; significance of the correlation: $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.

Table 4 Summary of values derived from measured values (daily vitamin intake and 24 h urinary excretion in Table 3), i.e. mean dietary intakes and their correlations with 24 h urinary excretion, recovery rates and estimated mean intakes (*n* 108), among eligible Japanese elementary-school children aged 10–12 years

	2 d mean vitamin intake† (day 3–day 4)			3 d mean vitamin intake (day 2–day 4)			4 d mean vitamin intake (day 1–day 4)			% Recovery‡		Estimated mean vitamin intake§			
Vitamin	Mean	SD	*r*‖	Mean	SD	*r*‖	Mean	SD	*r*‖	Mean	SD	Mean	SD	*r*¶	% Ratio††
Vitamin B_1 (μmol/d)	3·02	0·77	0·42***	2·88	0·63	0·42***	2·85	0·58	0·35***	27·6	12·2	2·83	1·42	0·37***	100
Vitamin B_2 (μmol/d)	3·61	0·85	0·41***	3·60	0·79	0·43***	3·60	0·78	0·42***	7·9	5·2	3·66	2·63	0·26**	102
Vitamin B_6 (μmol/d)	5·94	1·41	0·45***	5·95	1·29	0·49***	5·96	1·35	0·43***	39·8	14·0	5·90	2·30	0·41***	100
Vitamin B_{12} (nmol/d)	4·00	3·14	0·19*	4·25	2·55	0·10	4·35	2·10	0·10	0·7	0·6	3·72	2·14	0·06	79
Niacin (μmol/d)	99·4	26·0	0·24*	101·3	21·7	0·29**	101·4	20·4	0·32***	–		–		–	–
Niacin equivalents (μmol/d)	216	48	0·29**	217	43	0·29**	217	39	0·32***	30·7	12·6	215	91	0·20*	99
Pantothenic acid (μmol/d)	28·8	6·0	0·26**	28·2	5·6	0·32***	28·3	5·7	0·32***	41·4	19·5	28·1	13·3	0·27**	99
Folic acid (nmol/d)	595	236	0·23*	560	174	0·24*	553	147	0·27**	3·1	1·3	536	211	0·09	97
Vitamin C (μmol/d)	462	200	0·39***	442	183	0·39***	443	170	0·39***	36·4	50·3	447	613	0·39***	100

†Mean dietary intake was calculated using daily dietary intake (Table 3).
‡% Recovery rate was derived from 24 h urinary excretion (Table 3)/3 d mean intake ×100.
§Estimated mean intake was calculated using 24 h urinary excretion (Table 3) and recovery rate.
‖*r* indicates the correlation between 24 h urinary excretion (Table 3) and mean intake; significance of the correlation: $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.
¶*r* indicates the correlation between 3 d mean dietary intake and estimated intake; significance of the correlation: $^*P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$.
††% Ratio indicates the ratio between 3 d mean intake and mean estimated intake.

intake of seven water-soluble vitamins, except for vitamin B_{12}, in free-living Japanese schoolchildren aged 10–12 years. The correlation between the urinary excretion and the dietary intake on the same day as urine collection was highest, except for pantothenic acid, compared with the correlations on other days. Moreover, the correlations between the urinary excretion and the mean dietary intakes during the past 2–4 d showed higher correlations, except for vitamin B_{12} and folic acid, than those for daily intakes. These findings show that urinary levels of water-soluble vitamins are affected by not only their dietary intakes on the same day as urine collection, but also their intakes over the past few days.

The earlier intervention study showed extremely high positive correlations between urinary levels of water-soluble vitamins and their intakes[4]. In the earlier study, participants comprised college students and they consumed exactly the same defined diets, with or without synthesized water-soluble vitamin mixtures, for 4 weeks. In the present study, the dietary assessment for schoolchildren using a weighed food record was performed for four consecutive days without intervention. Assuming the dietary assessment protocol in the present study contributed best to reduce the errors in the dietary records, the similar results from the different groups and protocols indicate that the urinary levels of water-soluble vitamins are closely associated with vitamin intakes, and that this is true even for free-living schoolchildren.

Correlation coefficients between the urinary excretions and the 3 d mean intakes ranged from 0·24 to 0·49 with a mean of 0·36, except for vitamin B_{12}, which showed a lower level than reported in our earlier study[4]. The considerable inter- and intra-individual variability for vitamin intakes in a free-living environment might affect these modest correlations. In addition, several factors are also known to affect water-soluble vitamin metabolism. For example, carbohydrate and physical activity are known to affect vitamin B_1 metabolism[26–28], the bioavailability of pantothenic acid in food is half that of free pantothenic acid[29], and the single-nucleotide polymorphism of the methylenetetrahydrofolate reductase gene affects folic acid metabolism[30]. These factors might also affect the modest correlations.

The dietary habits of the schoolchildren who participated in this study were well disciplined. They had regular breakfast (before 07.00 hours), school lunch (around 12.30 hours) and supper (around 18.40 hours), with few snacks. The daily distributions of energy intakes were 21 % at breakfast, 33 % at lunch, 31 % at supper and 15 % for snacks, which is thought to be well balanced compared with that reported in a previous study: 24 % at breakfast, 30 % at lunch, 23 % at supper and 23 % for snacks[31]. Fifty-five per cent of energy intake was obtained from carbohydrates, 30 % from fats and 15 % from protein, which fits with the *Dietary Reference Intakes for Japanese*[13]. These data show that the participants had regular dietary habits with well-balanced nutrition.

In terms of the completeness of the dietary assessment in the present study, there are several limitations of using a weighed food record method. One of the limitations is the reliance on self-report. In the present study, to reduce errors associated with self-report, several dietitians reviewed the collated records along with the photos. Another limitation exists in the present food composition table in Japan. In a dietary assessment for free-living people, potential errors caused by the quality of the food composition table are inevitable, such as defects in food composition. For example, the composition of Japanese tea may vary depending on whether the extract of tea was made personally or whether it was a bottled tea beverage, because the present Japanese food composition table cannot differentiate such products. Such restrictions may lower the accuracy of the data obtained from a weighed food record. However, identifying the food status at oral intake and coding the intake according to the food composition table should contribute to increase the accuracy of the records.

In terms of completeness of 24 h urine collection, we used the INTERMAP criteria[11] as already described. Because the *p*-aminobenzoic acid (PABA) method requires intervention by taking PABA tablets orally and would be difficult for schoolchildren, we did not use that method to avoid any interventions. Because the participants in the present study were well motivated for the study, the proportion of them with incomplete urine samples was presumed to be small[32].

We have recently reported the intra-individual variations of urinary water-soluble vitamins in young Japanese, and our intervention study showed that the collection of 24 h urine samples for 1–5 d was required to estimate those values within 20 % of the true mean[33]. Indeed, correlation between the 30 d mean urinary thiamin excretion and 30 d mean thiamin intake was higher than that between daily excretion and daily intake[1]. In the present study, urinary water-soluble vitamins were measured based on a single 24 h urine sample. Thus the urinary vitamin contents have potential for data inaccuracy from variability, and the results should be interpreted cautiously. However, recent findings also suggest that using several days of 24 h urine sample would improve the relationships between urinary excretion and intake of water-soluble vitamins.

A significant correlation was not found between urinary vitamin B_{12} and dietary intake in this or a previous study[4]. This is consistent with studies showing that urinary vitamin B_{12} increased by only 1·5 to 2 times when 1 mg of vitamin B_{12}, which is 300 times higher than usual intake, was administered orally, and by 2–3 times when 0·45 mg was injected intramuscularly[34,35]. Foods including vitamin B_{12} were so limited that its intake showed an extremely high inter- and intra-individual variation in the present study.

Estimated mean intakes of water-soluble vitamins calculated using the urinary levels and recovery rates correlated

well with the 3 d mean intakes, except for vitamin B_{12} and folic acid, and the estimated mean intakes agreed exactly with the 3 d mean intakes. These findings suggest that urinary levels of water-soluble vitamins can be used as a biomarker to assess their estimated mean intakes. As training schoolchildren to collect urine samples is easier than completing weighed food records, a nutritional assessment for water-soluble vitamins using urine samples and recovery rates is expected to be one of the applications of the present study.

In conclusion, for free-living Japanese schoolchildren aged 10–12 years, we found that 24 h urinary levels of water-soluble vitamins, except for vitamin B_{12}, correlated with their recent intakes, and can be used as a biomarker to assess, compare and validate estimated mean intakes of water-soluble vitamins.

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