24-Hour Urine Samples Are More Reproducible Than Spot Urine Samples for Evaluation of Iodine Status in School-Age Children


Abstract

Background: Variation in different urinary measurements for evaluation of iodine status is of concern to clinicians and researchers.

Objective: This study evaluated variations between urine iodine concentration (UIC), spot and 24-h urine sample creatinine concentrations, and 24-h urine iodine excretion (24-h UIE) in repeated samples from school-age children.

Methods: Urine samples (24 h and morning spot) were collected on 2 occasions from 981 children in Ningjin and Lingxian counties, China. Samples from Ningjin were collected in October and November 2013, and samples from Lingxian were collected in April and May 2014. Morning spot urine iodine concentration (MUIC), morning spot urine creatinine, 24-h UIC, and 24-h urine creatinine were measured in all samples. The 24-h UIE was calculated by multiplying the 24-h UIC by the 24-h urine volume.

Results: In Ningjin County, the 24-h UIC and 24-h UIE did not differ between repeated collections (192 and 172 μg/L, respectively, for 24-h UIC (P = 0.08); 123 and 120 μg/L, respectively, for 24-h UIE (P = 0.56)), whereas the MUIC was lower in November 2013 than in October 2013 (170 and 190 μg/L, respectively; P = 0.034). In Lingxian County, no significant differences were observed in 24-h UIC between the repeated collections (230 and 218 μg/L, respectively; P = 0.79), whereas the 24-h UIE and MUIC were higher in the samples collected in May 2014 than in April 2014 (161 and 155 μg/L, respectively, for 24-h UIE (P = 0.002); 244 and 203 μg/L, respectively, for MUIC (P < 0.001)). When data from both counties were combined, no difference was observed between repeated 24+4 UIC (214 compared with 196 μg/L, P = 0.17) and 24+4 UIE (143 compared with 143 μg/d, P = 0.06), but MUICs were lower in the first collection than in the second collection (199 and 207 μg/L, respectively; P = 0.002). The k values were >0.4 for 24-h UIC and mean UIE, whereas relatively low k values were observed for MUIC and mean UIE.

Conclusion: The 24-h UIC was more accurate and reproducible than the MUIC in evaluating iodine status in a large-scale population study of school-age children. J Nutr doi: 10.3945/jn.115.215806.

Keywords: Urinary iodine concentration, urinary iodine excretion, urinary creatinine, urinary volume, children

Introduction

Iodine, an essential element for the synthesis of thyroid hormones, is primarily obtained through diet and iodized salt and is excreted in urine. Therefore, urinary iodine excretion is a good marker for recent dietary iodine intake. Urinary iodine measurements are performed either in spot or 24-h urine samples. Population iodine status is typically assessed by the median urinary iodine concentration (UIC); in μg/L from spot urine samples, as the sampling is convenient (1). However, day-to-day and within-day variation in UICs considerably affects the reliability of estimates of population and individual iodine status (2–4). It has been estimated that 500 spot urine samples are required to describe population iodine excretion with ±5% precision (5, 6), and >10 repeated spot samples from an individual are needed to reliably assess individual iodine intake (7). Although it is known to lead to overestimates of iodine deficiency, the proportion of individuals with UIC <100 μg/L is often used inappropriately to estimate the number of iodine-deficient individuals in a population (8, 9).

The 24-h urinary iodine excretion (UIE; in μg/d), calculated from the 24-h UIC and urine volume (U-vol) from a 24-h urine sample, is generally regarded as the reference standard (10, 11). However, 24-h urine samples are difficult to obtain, and there is considerable intra-individual variability of 24-h UIC values (12). An alternative method, estimating daily UIE by using the urinary
TABLE 1 Characteristics of participating children1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ningjin (n = 412)</th>
<th>Lingxian (n = 569)</th>
<th>P</th>
<th>Total (n = 981)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys, n (%)</td>
<td>183 (44.4)</td>
<td>258 (45.3)</td>
<td>0.77</td>
<td>441 (45.0)</td>
</tr>
<tr>
<td>Girls, n (%)</td>
<td>229 (55.6)</td>
<td>311 (54.7)</td>
<td>540</td>
<td>55.0</td>
</tr>
<tr>
<td>Age, y</td>
<td>8.9 ± 1.5</td>
<td>10.6 ± 1.2</td>
<td>&lt;0.001</td>
<td>9.9 ± 1.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>134.1 ± 9.4</td>
<td>140.5 ± 9.4</td>
<td>&lt;0.001</td>
<td>137.8 ± 10.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>31.1 ± 7.7</td>
<td>35.6 ± 9.3</td>
<td>&lt;0.001</td>
<td>33.7 ± 8.9</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs unless otherwise indicated.

iodine/creatinine ratio (I/Cr) from spot urine samples, can correct for differences in urine volume and sample dilution (13). However, the estimated UIE has been reported to be unreliable, particularly when protein intake and, consequently, creatinine excretion are low (14).

Variations in different urinary measurements for iodine status estimation are of concern to clinicians and researchers. The present study aimed to evaluate variations between 24-h UIE, UIC, and creatinine excretion in spot and 24-h urine samples and to determine the most feasible and robust method for the estimation of population iodine status by using urinary iodine as a biomarker.

Methods

Subjects. This study was conducted in Ningjin and Lingxian counties, Dezhou City, in the Shandong Province of China. The 2 counties are adjacent with similar climates, economic structures, culture, and dietary habits. Both regions have high levels of iodine in the drinking water, and therefore salt in the region is not iodized. Three elementary schools were randomly selected in each county. A total of 981 children aged 7–13 y were enrolled in these schools. Healthy children without any previous history of thyroid diseases and medication intake (including iodine supplements) were included. The Medical Ethics Committee of Tianjin Medical University approved the research protocols. Parents or caretakers of participating children provided written informed consent before the study.

Urine sample collections. Two 24-h urine samples were obtained from each participant within a 1-mo period. In Ningjin County, the first sample collection was performed in October 2013 and the repeat collection was performed in November 2013. In Lingxian County, the first collection was performed in October 2013 and the repeat collection was performed in November 2014. Children were instructed to void their bladders in the morning before the 24-h urine sample, and spot urine samples were taken from the first morning urine. Over the next 24 h, all urine, including the first morning void of the next day, was collected in polyethylene bottles. Teachers and parents were trained to assist children in completing the study. Children were instructed to inform teachers or investigators if they forgot or spilled urine samples, and in such cases they were asked to repeat the collection. Investigators asked children about missed urine samples during the 24-h collection. The 24-h samples reported to be complete or to have only a single missed void were considered acceptable, and the other samples were excluded. Urine volume was carefully measured, and 2 aliquots were taken from each sample. All urine samples were stored at −20°C until analysis.

Water sample collection. Children were given 10-mL polyethylene plastic bottles, and were instructed to rinse the bottle 3 times with drinking water before sample collection. A 10-mL household drinking water sample was obtained from each child, and stored at 4°C until analysis. Children whose families drink water from the same well in the village were required to submit 1 water sample.

Determination of UIC, water iodine concentration, and U-Cr. The determination of UIC, water iodine concentration, and U-Cr was done at the Key Laboratory of Hormone and Development (Ministry of Health, Metabolic Diseases Hospital and the Tianjin Institute of Endocrinology, Tianjin Medical University). Ammonium persulfate digestion with spectrophotometric detection of the Sandell-Kolthoff reaction was used for urinary iodine and water iodine measurement. Four levels of certified reference material-hyperboilized human urine (lot nos. GBW09108I, GBW09110n, GBW09111a, and GBW09112a; National Reference Laboratory for Iodine Deficiency Disorders, Beijing) with mean certified iodine concentrations of 68 µg/L (reference range: 59–77 µg/L), 195 µg/L (reference range: 185–205 µg/L), 558 µg/L (reference range: 541–575 µg/L), and 885 µg/L (reference range: 857–913 µg/L), respectively, were run with each batch of samples. U-Cr was measured by a national spectrophotometric standard method. With use of this method, the CV for urine creatinine concentration was 0.2%–3.2% in our laboratory.

Statistical analyses. All data analyses were carried out using SPSS 19.0 (IBM), Microsoft Excel (XP 2011; Microsoft), and Graph Prism (version 6.0c; Graph Pad Software Inc.). The 24-h UIE was calculated by multiplying the 24-h UIC by the U-vol. Proportions were compared using chi-square tests. Differences between means were analyzed using the t test for independent samples. The Wilcoxon matched-pairs Signed Rank test was used to assess the consistency between the 2 repeated urine collections. Comparisons between 24-h UIE and MUIC were assessed using the Wilcoxon Mann-Whitney U test. Children with UIC ≥90 µg/d (7- to 10 y old) or ≥110 µg/d (11- to 13 y old) were categorized as having sufficient iodine intake, as recommended by the 2013 Chinese DRIs (15). UIC was also used to define iodine status; children with UIC ≥100 µg/L were categorized as iodine sufficient. The Cohen’s k coefficient was

TABLE 2 Urine volume and creatinine, UIC, and UIE from 24-h or spot urine samples collected at different times from children living in Lingxian or Ningjin1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ningjin (n = 569)</th>
<th>Lingxian (n = 412)</th>
<th>P</th>
<th>Total (n = 981)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h U-vol, mL</td>
<td>640 (420–950)</td>
<td>700 (550–1150)</td>
<td>0.001</td>
<td>695 (450–1100)</td>
</tr>
<tr>
<td>24-h UIC, µg/L</td>
<td>192 (109–370)</td>
<td>218 (127–342)</td>
<td>0.79</td>
<td>205 (109–420)</td>
</tr>
<tr>
<td>24-h U-Cr, g/L</td>
<td>0.56 (0.41–0.77)</td>
<td>0.68 (0.48–0.93)</td>
<td>&lt;0.001</td>
<td>0.62 (0.48–0.92)</td>
</tr>
<tr>
<td>U/Cr ratio,1 µg/g</td>
<td>321 (187–571)</td>
<td>311 (201–573)</td>
<td>0.049</td>
<td>316 (187–571)</td>
</tr>
<tr>
<td>24-h UIC, µg/d</td>
<td>123 (66–232)</td>
<td>161 (85–265)</td>
<td>0.002</td>
<td>142 (66–232)</td>
</tr>
<tr>
<td>MUIC, µg/L</td>
<td>230 (149–375)</td>
<td>286 (121–266)</td>
<td>0.08</td>
<td>258 (149–375)</td>
</tr>
<tr>
<td>MU-Cr, g/L</td>
<td>190 (119–418)</td>
<td>230 (149–375)</td>
<td>0.034</td>
<td>210 (119–418)</td>
</tr>
<tr>
<td>U/Cr ratio,1 µg/g</td>
<td>0.64 (0.50–0.90)</td>
<td>0.83 (0.52–1.20)</td>
<td>&lt;0.001</td>
<td>0.76 (0.54–1.03)</td>
</tr>
</tbody>
</table>

1 Values are medians; ranges in parentheses. I/Cr, iodine/creatinine; MU-Cr, morning spot urine creatinine; MUIC, morning spot urine iodine concentration; UIC, urinary iodine concentration; UIC, urine iodine excretion; U-vol, urine volume; 24-h U-Cr, 24-h urine creatinine; 24-h UIE, 24-h urine iodine concentration; 24-h UIC, 24-h urinary iodine concentration; 24-h UIE, 24-h urine iodine excretion.

2 I/Cr ratio for 24-h urine samples.

3 I/Cr ratio for morning spot urine samples.
Table 3: Urine volume and creatinine, UIC, and UIE from 24-h or spot urine samples collected at different times from children1

<table>
<thead>
<tr>
<th>Variables</th>
<th>First collection</th>
<th>Second collection</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h U-vol, mL</td>
<td>873</td>
<td>783 (460–1000)</td>
<td>0.001</td>
</tr>
<tr>
<td>24-h UI, μg/L</td>
<td>783</td>
<td>213 (117–366)</td>
<td>0.17</td>
</tr>
<tr>
<td>24-h U-Cr, g/L</td>
<td>683</td>
<td>0.60 (0.43–0.83)</td>
<td>0.003</td>
</tr>
<tr>
<td>I/Cr ratio, μg/g</td>
<td>873</td>
<td>331 (211–577)</td>
<td>0.001</td>
</tr>
<tr>
<td>24-h UIE, μg/d</td>
<td>873</td>
<td>143 (75–246)</td>
<td>0.06</td>
</tr>
<tr>
<td>MUIC, μg/L</td>
<td>744</td>
<td>199 (112–373)</td>
<td>0.002</td>
</tr>
<tr>
<td>MU-Cr, g/L</td>
<td>744</td>
<td>0.69 (0.45–1.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I/Cr ratio, μg/g</td>
<td>744</td>
<td>278 (174–545)</td>
<td>0.426</td>
</tr>
</tbody>
</table>

1 Values are medians; ranges in parentheses. I/Cr, iodine/creatinine; MU-Cr, morning creatinine; UIC, urinary iodine concentration; UIE, urine iodine excretion; U-vol, urine volume; 24-h U-Cr, 24-h urine creatinine; 24-h UIC, 24-h urine iodine concentration; 24-h UIE, 24-h urine iodine excretion.

Results

Of the 981 children enrolled, 412 were from Ningjin County and 569 were from Lingxian County. Table 1 presents the basic anthropometric characteristics of all study participants. The majority of participants were girls. The children in Lingxian County were older than the children in Ningjin County (P < 0.001) and had greater heights and weights (P < 0.001).

Variation in repeated 24-h urine and morning spot urine samples. During the first sample collection in Ningjin County in October 2013, 412 children provided a total of 398 (96.7%) 24-h urine samples and 275 (66.7%) morning spot urine samples. The median 24-h UI and MUIC were 192 and 190 μg/L, respectively (P = 0.33). Repeat samples were collected in Ningjin County in November 2013, at which time 399 (96.8%) children provided 24-h urine samples and 375 (91.0%) provided spot urinary samples. The median 24-h UI and MUIC were 172 and 170 μg/L, respectively (P = 0.32). As illustrated in Table 2, there were no significant differences in 24-h U-vol (P = 0.07), 24-h UI (P = 0.08), or 24-h UIE (P = 0.56) between the October and November collections. The 24-h urinary creatinine concentration (24-h U-Cr) (P = 0.014) and urinary creatinine concentration in morning spot urine samples (MU-Cr) (P < 0.001) were slightly higher in November than in October; however, the MUIC was higher in October than in November (P = 0.034).

During the first sample collection in Lingxian County in April 2014, 569 children provided 524 (92.1%) 24-h urine samples and 518 (91.0%) morning spot urine samples. The median 24-h UI and MUIC were 230 and 203 μg/L, respectively (P = 0.09). Repeat samples were obtained in Lingxian County during May 2014, when 521 (91.6%) 24-h urine samples and 529 (93.0%) spot urine samples were collected. The median 24-h UI and MUIC were 218 and 244 μg/L, respectively (P < 0.001). As reported in Table 2, no significant differences were observed between 24-h UI (P = 0.79) and 24-h U-Cr (P = 0.08) in April compared with May, whereas the 24-h U-vol (P = 0.001), 24-h UIE (P = 0.002), MUIC (P < 0.001), and MU-Cr (P = 0.001) were all higher in May than in April.

When data from both counties were combined, there were 873 paired 24-h urine samples and 744 paired morning spot urine samples. No significant differences were observed in the 24-h UI and 24-h UIE between the first and second collections, but the 24-h U-vol, 24-h U-Cr, MUIC, and MU-Cr values all differed (P < 0.05) (Table 3). The medians for all of the 24-h urine samples and morning spot urine samples were 203 and 204 μg/L, respectively (P = 0.55).

Variations in UIE, UIC, U-vol, and U-Cr across 4 time points. Comparisons of the different urinary iodine parameters were conducted across all 4 collections. To exclude the effects of age, comparisons were conducted only in the 9- and 10-y-old children. There were no significant differences in 24-h UI, UIE, MU-Cr, and 24-h U-Cr; but the 24-h U-vol (P = 0.006) and MUIC (P = 0.044) differed across collections.

Individual iodine status categorized by 24-h UI, MUIC, and UIE. The median of the mean 24-h UIE values was 152 μg/dL (range: 96–266). Based on median UIE, 24.3% of the children were defined as iodine insufficient. Iodine status categorized by 24-h UI and MUIC from the first collection and by mean UIE are shown in Figure 1A. The χ2 value was 0.451 (P < 0.001) for 24-h UI and mean UIE, demonstrating good consistency between the mean UIE and 24-h UIE values. The χ2 value was 0.378 (P < 0.001) for the MUIC and mean UIE, demonstrating fair consistency. Significant differences were observed in the distributions of the 24-h UI and MUIC (P < 0.001), but the...
samples were different in both regions as well ($P < 0.001$) (Figure 2). The sensitivity and specificity of the first 24-h UIC were 51.4% and 90.7%, respectively. For MUIC in the first collection, the sensitivity and specificity were 48.6% and 87.8%, respectively. The results for the second collection were similar. The sensitivity and specificity were 48.6% and 93.4%. The sensitivity and specificity of the second MUIC were 39.6% and 88.8%.

As shown in Table 2, the values of median I/Cr ratio for morning spot urine samples differed significantly on repeated samplings in Ningjin County ($P < 0.001$). In Lingxian County, the median I/Cr ratio in May 2014 was relatively higher than April 2014 ($P < 0.001$). The median I/Cr ratios for 24-h urine samples were different in both regions as well ($P < 0.001$). When data from both counties were combined, no significant difference was observed between I/Cr ratios for morning spot urine samples ($P = 0.43$), whereas the I/Cr ratios for 24-h urine samples were different ($P = 0.001$) (Table 3).

**Drinking water iodine and 24-h UIE.** A total of 524 drinking water samples were collected from children. The median water iodine concentration was 112 μg/L (range: 37–169 μg/L). The children’s 24-h UIE was positively correlated with the drinking water iodine concentration ($r = 0.50, P < 0.001$) (Figure 3).

**Discussion**

As a group, the school-aged children surveyed in the present study were iodine sufficient by all of the urinary iodine parameters assessed, likely due to the high drinking water iodine content in the region. We found that the majority of the 7- to 13- y-old children studied were able to comply with instructions and provide complete 24-h urine samples. The 24-h UIC was more consistent on repeated sampling than the MUIC. These results demonstrate that 24-h urine iodine concentrations are not unduly cumbersome to collect, even in relatively young children, and can provide a robust measure for the assessment of population iodine status.

A strength of the study was the collection of repeated samples 1 mo apart, which allowed for assessment of reproducibility of results over time. The major limitation of the study was the reliance on self-report regarding completeness of the 24-h urine collections. Para-aminobenzoic acid administration is sometimes used as a marker for the completeness of 24-h urine samples (16), but this was not used in the present study.

Although spot urine samples are simpler to obtain than 24-h urine collections, the drawbacks of MUIC as a marker for population iodine status are well known. The UIC assessed in spot urine samples may be affected by factors such as hydration status (17, 18) and daily variation in iodine intake (7). It has been reported that the distribution of UIC measured in single spot urine samples is much broader than the distribution of iodine excretion in 24-h urine samples (19). This interindividual variation in MUIC may result in dispersion that would lead to incorrect estimations of population iodine status (7, 20), although large-scale population samples will improve precision (5, 6). In the present study, MUIC values differed significantly on repeated sampling, although the MUICs from both collections indicated adequate iodine status according to WHO/Iodine Global Network criteria. Previous studies have demonstrated seasonal fluctuations in UIC (21–23). In the present study, we found that the MUIC, but not the 24-h UIC, varied across different months (April 2014, May 2014, October 2013, and November 2013), which might relate to ambient temperature and hydration status. This issue is of particular concern in regions where drinking water is a significant source of ingested iodine (24). Optimally, to evaluate either individual level or population iodine intakes, multiple 24-h urine collections are preferable.

In addition, an overestimation of iodine deficiency would result from the use of the proportion of UIC <100 μg/L for spot urine samples in defining iodine insufficiency in population (8). With the use of the 24-h UIE as the standard reference for evaluating iodine status, 24.3% children had insufficient iodine intake in this study. However, iodine excretion in urine is probably lower than actual diet iodine intake (25, 26). A favorable consistency was found in distribution between 24-h UIC and 24-h UIE ($α > 0.4$) compared with MUIC. The sensitivity and specificity were better for 24-h UIC than for MUIC. It indicated that 24-h UIC is more appropriate for categorizing population iodine status than MUIC.

![Figure 2](image-url)  
**FIGURE 2** Distributions of 24-h UIC and MUIC in Chinese children in the first (A) and second (B) collections ($n = 793–922$). MUIC, morning spot urine iodine concentration; UIC, urinary iodine concentration; 24-h UIC, 24-h urinary iodine concentration.

![Figure 3](image-url)  
**FIGURE 3** Association between the water iodine concentration and UIE in Chinese children ($n = 524$). UIE, urine iodine excretion.
As a product of muscle metabolism, creatinine is excreted at a relatively constant rate throughout the day. Therefore, correction for urinary creatinine concentrations has been used in some studies to minimize the variation of UIC due to hydration status. The I/Cr ratio may be used to estimate 24-h UIE (10, 27), although many studies have reported that the I/Cr ratio is not a suitable proxy (28–30). Various factors affect creatinine excretion including strenuous exercise, emotional stress, dietary intake of creatine and creatinine, infection, fever, and trauma (31). In our study, similar to previous results (32), the U-Cr as well as the I/Cr in both spot and 24-h urine samples differed significantly between the 2 sample collections. Because there is no established reference creatinine excretion for 7- to 13-y-old children in China, the estimated 24-h UIE calculated through I/Cr ratios multiplying by reference creatinine excretion in children was not adopted in this study, which would be more accurate according to the study of Perrine et al. (27).

To the best of our knowledge, this is the first large-scale epidemiologic study to include repeated 24-h urine collections to compare different urinary iodine measurements. Although they are readily obtained, MUIC values are not consistent over time even in large-scale population studies. A single 24-h UIC is a relatively reliable and reproducible alternative for the assessment of population iodine status.

Acknowledgments
WC and YW conducted the research, analyzed the data, and wrote the paper; LL completed the laboratory analysis; LT, JS, XG, and WW conducted the research and data collection; ENP interpreted the results and edited the manuscript; JB and WJ provided the field survey for this study and coordinated the study; and WZ designed the research. All authors read and approved the final manuscript.

References