Is the urinary iodine/Creatinine ratio applicable to assess individual iodine status in Chinese adults? Comparison of iodine estimates from 24-hour urine and timed-spot urine samples in different periods of the day

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Research

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Is the urinary iodine/Creatinine ratio applicable to assess individual iodine status in Chinese adults? Comparison of iodine estimates from 24-hour urine and timed-spot urine samples in different periods of the day

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Abstract  
Background: Urinary iodine concentration (UIC) is routinely used to evaluate the population iodine status while the uniform method for the individual level assessment is uncertain.  
Objectives: To explore the 24-hour urinary iodine excretion (UIE) in five different periods of the day and the corresponding prediction equations respect by the use of creatinine-corrected UIC.  
Methods: We collected 24-hour, spot and fasting urine in five periods of the day to estimate 24-hour UIE by the six different prediction equations. We compared the estimated creatinine-corrected UIC to the collected 24-hour UIE and identified the most suitable equations in each period of the day.  
Results: Among the six different prediction equations, the equation of Kawasaki T was the best to estimate the 24-hour UIE by fasting urine among Chinese adults. Among the five periods of time, the equation of Knudsen N was the best to estimate the 24-hour UIE except the morning period.  
Conclusion: Urinary iodine status at the individual level could be estimated by different creatinine-based equations at different periods of the day.  
Key words: Urinary iodine/Creatinine ratio, Individual iodine status, Urinary iodine excretion,
Timed-spot urine

Background

Iodine, as a key micronutrient for thyroid hormone synthesis [1], plays an essential role in metabolism. It is estimated that around 1.88 billion people globally are still at risk of insufficient iodine intake [2], and therefore iodine deficiency is regarded as a public health issue in both developing and developed countries [2-3], especially in Eastern Mediterranean, Asian, African and most of the Eastern European countries [2,4]. Due to the limited kinds of iodine rich food [5], WHO introduced a salt iodization program to improve iodine status at the populational level as a way of lowering the risk of iodine insufficiency [6]. China, as well as most other countries [7-8], has considerably improved this issue by the universal salt iodization program [9].

In order to identify people at risk of iodine deficiency, it is important to monitor iodine status at both population and the individual level [10-11]. The amount of iodine collected at the spot urine sample is usually presented as the urinary iodine concentration (UIC) or the urinary iodine/creatinine concentration ratio [12]. UIC can be used to evaluate the population iodine status [8,13], however, we still lack the uniform standard in the prediction of the individual iodine status. Some researchers used serum iodine [14], serum thyroglobulin [15] and 24-hour urine [16] to assess individual iodine status. This study compared the six prediction equations in five periods of the day to the real 24-hour urine iodine excretion (UIE), aiming for providing evidence of individual iodine status assessment.

Methods

Participants

126 healthy adults aged 18-to-59-year olds were recruited from May to September 2016 in Fujian Province. Data on age and sex was collected.

People included in this study were based on the following criteria: (1) non-pregnant and breastfeeding women; (2) no history of thyroid illnesses; (3) no recent (within 6 months) use of iodine contrast agents or amiodarone medications; (4) no severe infectious disorders, chronic diseases, renal or other systemic diseases; (5) long-term residency (Longer than six months) in Fujian Province. And the exclusion criteria were: (1) pregnant and lactating women; (2) recent (within 6 months) coronary angiography, endoscopic retrograde cholangiopancreatography (ERCP), and other use of iodinated contrast agent and amiodarone drugs; (3) having renal dysfunction and (or) other major diseases; (4) with mental diseases; (5)
having cognitive disorders; (6) not living in Fujian Province for six months.

**Urine samples collection**

Participants were given a uniform urine collection bag, a sterile plastic urine collection tube with a handle for dipping, and clear instructions for 24-hour urine collection. Participants were required to record the start and finish times. All the participants were informed about the collection methods and announcements. The urine was firstly collected after the first urination in the early morning; then it was collected till the first urination on the next morning, lasting for 24 hours. Each voiding time was recorded. There were five periods of the day for urine collection: morning (after discarding the first void -12:30), afternoon (12:31-17:30), evening (17:31-23:59), early morning (00:00-03:59), and fasting time (the first void collected the next morning after the longest duration of sleep) [17]. 24-hour urinary creatinine excretion [18] was used as quality control for 24-hour urine collection, and participants with urinary creatinine excretion of less than 75 per cent were eliminated across genders and ages [19].

**Laboratory Analysis**

All results were assayed in the Fujian Provincial Center for Disease Control and Prevention, which satisfied the requirements of the quality control of the National Iodine Deficiency Disorders Reference Laboratory in China. Urine iodine concentration was assayed by Arsenic-Cerium Catalytic Spectrophotometry for Urine Determination (WS/T107-2016) [20], and urinary creatinine concentration was assayed applying urinary creatinine alkaline picric acid spectrophotometry (WS/T97-1996) [21].

**Equations of the estimated and measured 24-hour urinary iodine excretion**

For spot urine and fasting urine in five periods of the day, the six prediction equations were employed to estimate 24-hour UIE in the same individual. The equations in Table 1 were used to estimate UIE.

**Table 1 Equations of the estimated and measured 24-hour urinary iodine excretion**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Abbreviation</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine: creatinine ratio (μg/g)</td>
<td>I/Cr</td>
<td>UIC (μg/L) / UCr (g/L)</td>
</tr>
<tr>
<td>Measured 24-hour urine iodine excretion (μg/d)</td>
<td>Measured 24h</td>
<td>24hUIC (μg/L) × 24h urine (L)</td>
</tr>
<tr>
<td>Estimated 24-hour urine iodine excretion (μg/d)</td>
<td>Estimated 24h</td>
<td>I/Cr (μg/g) × Pr24hCr (g/d)</td>
</tr>
<tr>
<td>Estimated 24-hour urine iodine excretion (μg/d)</td>
<td>Estimated 24h</td>
<td>Pr24hCr (g/d) = 24hUCr (g/L) × 24h urine (L)</td>
</tr>
<tr>
<td>Estimated 24-hour urine iodine excretion (μg/d)</td>
<td>Estimated 24h</td>
<td>I/Cr (μg/g) × Pr24hCr (g/d)</td>
</tr>
</tbody>
</table>
iodine excretion\(^2\) (\(\mu g/d\)) \quad \text{UIE}\(^2\) \quad \text{Pr24hCr (mg/d)} = [(-2.04 \times \text{age (year)}) + (14.89 \times \text{weight (kg)}) + (16.14 \times \text{height (cm)}) - 2244.45

Estimated 24-hour urine iodine excretion\(^2\) (\(\mu g/d\)) \quad \text{(Tanaka T, et al [22])}

Estimated 24-hour urine iodine excretion\(^3\) (\(\mu g/d\)) \quad \text{UIE}\(^3\) \quad \text{Males:}
\quad \text{Pr24hCr (mg/d)} = [-12.63 \times \text{age (year)}] + [15.12 \times \text{weight (kg)}] + [7.39 \times \text{height (cm)}] - 79.9
\quad \text{Females:}
\quad \text{Pr24hCr (mg/d)} = [-4.72 \times \text{age (year)}] + [8.58 \times \text{weight (kg)}] + [5.09 \times \text{height (cm)}] - 74.5

Estimated 24-hour urine iodine excretion\(^4\) (\(\mu g/d\)) \quad \text{UIE}\(^4\) \quad \text{Males:}
\quad \text{Pr24hCr (mg/d)} = 0.00179 \times [140 - \text{age (year)}] \times [\text{weight (kg)}^{1.5} \times \text{height (cm)}^{0.5}] \times [1 + 0.18 \times A \times [1.366 - 0.0159 \times \text{BMI} (\text{kg/m}^2)]].
\quad \text{Females:}
\quad \text{Pr24hCr (mg/d)} = 0.00163 \times [140 - \text{age (year)}] \times [\text{weight (kg)}^{1.5} \times \text{height (cm)}^{0.5}] \times [1 + 0.18 \times A \times [1.429 - 0.0198 \times \text{BMI} (\text{kg/m}^2)]]; \text{where A is African American or black race} = 1, \text{other race} = 0.

Estimated 24-hour urine iodine excretion\(^5\) (\(\mu g/d\)) \quad \text{UIE}\(^5\) \quad \text{Males:}
\quad \text{pr24hCr (g/d)} = 1.74 \text{g (25-49 years), 1.63 g (50-59 years)}
\quad \text{Females:}
\quad \text{pr24hCr (g/d)} = 1.23 \text{g (25-49 years), 1.15 g (50-59 years)}

Estimated 24-hour urine iodine excretion\(^6\) (\(\mu g/d\)) \quad \text{UIE}\(^6\) \quad \text{Males:}
\quad \text{iodine excretion (\(\mu g/d\)) = 0.9 \times \text{iodine intake (\(\mu g/d\))}
\quad \text{Females:}
\quad \text{iodine intake (\(\mu g/d\)) = Spot UIC (\(\mu g/L\))/ 0.92 \times (0.0009 L \cdot h^{-1} \cdot kg^{-1} \cdot 24h \cdot d^{-1}) \times \text{weight (kg)}}

I, Iodine; Cr, creatinine; UIC, urinary iodine concentration; UIE, urine iodine excretion; Pr24hCr, 24-hour creatinine prediction.

**Statistical analysis**

The analysis was conducted by SPSS software (SPSS 20.0; IBM Corp, Armonk, NY, USA). The normality of data was checked by the Kolmogorov-Smirnoff test. Age, height and weight were presented as means ± SD for normally distributed data, and 24-hour urine volume was presented as medians (95% CI). The difference between the estimated UIE and measured UIE (standard method) were examined by applying independent samples \(t\)-tests for normally distributed continuous variables, Mann-Whitney \(U\) tests for non-normally
distributed continuous variables. The correlations between the estimated UIE and measured UIE (standard method) were tested by Pearson correlation for normally distributed data and Spearman correlation for non-normally distributed data. The consistency between the estimated UIE and measured UIE (standard method) was tested by a Bland-Altman plot. \( P<0.05 \) was considered as significantly different.

Ethics approval was obtained from the Ethics Committee of Fujian Provincial Centers for Disease Control and Prevention (No. 2017002). Written informed consents were obtained from all the participants before their urine samples were collected.

**Results**

**Demographic characteristics of the participants**

A total of 126 healthy adults, of which 59 males and 67 females were enrolled in this study. Age, height, weight, and 24-hour urine volume were shown in Table 2.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female (n=67)</th>
<th>Male (n=59)</th>
<th>Total (n=126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), mean ± SD</td>
<td>40.1 ± 11.83</td>
<td>37.2 ± 10.45</td>
<td>38.7 ± 11.25</td>
</tr>
<tr>
<td>Height (m), mean ± SD</td>
<td>1.60 ± 0.04</td>
<td>1.69 ± 0.05</td>
<td>1.64 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg), mean ± SD</td>
<td>55.5 ± 7.47</td>
<td>67.3 ± 8.15</td>
<td>61.0 ± 9.74</td>
</tr>
<tr>
<td>24-hour urine volume (L), IQR</td>
<td>2.14 (1.54, 2.68)</td>
<td>2.09 (1.45, 2.59)</td>
<td>2.09 (1.47, 2.62)</td>
</tr>
</tbody>
</table>

SD, standard deviation; IQR, inter-quartile range.

**Comparison between the measured and the estimated 24-hour urinary iodine excretion in different periods of the day**

In this study, 121 spot urine samples were collected in the morning, 126 spot urine samples were collected in the afternoon, 125 spot urine samples were collected in the evening, and 48 spot urine samples were collected in the early morning, and 126 in the fasting time.

The 24-hour UIE of spot urine in the morning was estimated by Estimated 24h UIE\(^1\) and Estimated 24h UIE\(^5\), and there was a significant difference between measured and estimated UIE (\( P<0.001 \)). Similarly, there was no significant difference (\( P>0.05 \)) between measured and estimated 24-hour UIE of fasting urine and spot urine in the afternoon, evening, and early morning, respectively. Estimated 24-hour UIE\(^2\) and Estimated 24-hour UIE\(^3\) were used to estimate 24-hour UIE of spot urine in the morning, afternoon, and evening, and significant differences between measured and estimated results (\( P<0.01 \) for all) were found. Likewise,
the difference between measured and estimated 24-hour UIE of fasting urine and spot urine in
the early morning was non-significant ($P>0.05$). In all periods of time, the Estimated UIE was
significantly different from the measured ones ($P<0.05$ for all). The difference between
the measured iodine concentration and the estimated one was statistically significant ($P<0.001$
for all) by Estimated 24-hour UIE in the morning, afternoon, and nighttime. Similarly, the
estimated 24-hour UIE of fasting urine and spot urine in the early morning showed that the
measured and estimated results were not significantly different ($P>0.05$ for both). (Figure 1).

**Figure 1** Difference between measured and estimated urinary iodine excretion in different periods of the day.

UIE, urine iodine excretion; mUIC, median urinary iodine concentration;
*Estimated urinary iodine excretion was significantly different compared to the measured urinary iodine
excretion ($P<0.05$) by Spearman rank correlation analysis;
**Estimated urinary iodine excretion was significantly different compared to the measured urinary iodine
excretion ($P<0.01$) by Spearman rank correlation analysis;
***Estimated urinary iodine excretion was significantly different compared to the measured urinary iodine
excretion ($P<0.001$) by Spearman rank correlation analysis.

Correlation between the measured and estimated 24-hour urinary iodine excretion in the
different time periods of the day

The estimated UIE showed a significant linear correlation compared to the Measured
24-hour UIE urine samples collected in all periods of the day ($P<0.01$ for all). (Table 3)
Table 3 Correlation between the measured and estimated urinary iodine excretion in different periods of the day

<table>
<thead>
<tr>
<th></th>
<th>Measured 24h UIE VS Estimated 24h UIE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Afternoon</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Evening</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Night</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Fasting time</td>
<td>&lt;0.001***</td>
</tr>
</tbody>
</table>

UIE, urine iodine excretion;

* Estimated urinary iodine excretion was significantly different compared to measured urinary iodine excretion (P<0.01) by Spearman rank correlation analysis;

** Estimated urinary iodine excretion was significantly different compared to measured urinary iodine excretion (P<0.001) by Spearman rank correlation analysis.

Consistency between the measured and estimated 24-hour urinary iodine excretion

With the exception of the morning, there was no significant difference but a strong correlation between the measured and estimated 24-hour UIE in the different time periods.

For all prediction equations except Estimated 24-h UIE4, fasting urine might be a choice to measure 24-hour UIE.

Hereby, Bland-Altman plots were also designed to evaluate the consistency between the measured and the estimated 24-hour UIE. The bias of Estimated 24-hour UIE1 varied from -0.21 to 0.19 by urine sample collected time at the individual level. The high consistency occurred in the evening time period (bias= 0.07) and fasting time (bias= -0.09). (Figure 2)
Figure 2 Consistency between log-transformed Estimated 24-hour urinary iodine excretion and log-transformed Measured 24-hour urinary iodine excretion in different periods of the day. UIE, urine iodine excretion; The X-axis is the mean of log-transformed estimated 24-hour urinary iodine excretion and log-transformed measured 24-hour urinary iodine excretion; The Y-axis is the difference between log-transformed estimated 24-hour urinary iodine excretion and log-transformed measured 24-hour urine iodine excretion; The solid black line represents the bias, and the dashed line represents the 95% range of consistency for the mean relative difference; Upper limit: upper 95% limit of consistency; lower limit: lower 95% limit of consistency.

We also discovered that the bias of Estimated 24-h UIE varied from -0.16 to 0.19 in different periods of the day. As for point urine, the estimated UIE showed a high consistency when compared to the measured ones in the afternoon (bias = 0.09) and evening (bias = 0.05). (Figure 3)

Figure 3 Consistency between log-transformed Estimated 24-hour urinary iodine excretion and log-transformed Measured 24-hour urinary iodine excretion in different periods of the day. UIE, urinary iodine excretion.

In the prediction of fasting urinary iodine excretion, Estimated 24-hour UIE (bias=-0.02) showed the best consistency while Estimated 24-hour UIE (bias= 0.23) was the worst one, and Estimated 24-hour UIE (bias=-0.09), Estimated 24-hour UIE (bias=0.06), Estimated 24-hour UIE (bias=-0.10), and Estimated 24-hour UIE (bias=0.06) showed a relatively good consistency. (Figure 4)
Figure 4 Consistency of the six estimated 24-hour urinary iodine excretion equations and the measured 24-hour urinary iodine excretion in fasting urine.

UIE, urine iodine excretion.

Estimated 24h UIE for (A), Estimated 24h UIE for (B), Estimated 24h UIE for (C), Estimated 24h UIE for (D), Estimated 24h UIE for (E) and Estimated 24h UIE for (F);

Discussion

The findings of our study demonstrated that Estimated 24-h UIE might be a choice to estimate 24-hour UIE at all times except the morning. Fasting urine could be used to estimate 24-hour UIE in several prediction equations. With the exception of Estimated 24-hour UIE, the other prediction equations by fasting urine are feasible in the prediction of 24-hour UIE, especially Estimated 24-hour UIE.

Currently, there is no uniform method for individual iodine status assessment and each method has its limitations. The 24-hour UIE, computed by multiplying the 24-hour UIC and the 24-hour urine volume, is acknowledged as "gold standard" for individual iodine status assessment. Some researchers have proposed numerous equations to estimate individual 24-hour UIE given that it is not easy to collect 24-hour urine. Jakobsen J et al. verified the completeness of 24-hour urine collection by comparing the concentration of para-aminobenzoic acid (PABA) in urine to tablets, but it may be harmful to people’s health [26]. To perform the quality control of the completeness of 24-hour urine collection, we set a creatinine reference value at 75%, which ensured that the 24-hour urine volume for all volunteers was entirely collected. The equation concerning 24-hour urine volume seemed reliable due to its results, particularly the Measured 24-hour UIE. Once it was reliable, the
estimated equation which was compared to Measured 24-hour UIE could be used in the
evaluation of individual iodine status. Although it is easy for spot urine collection, there is
little evidence to support spot urine as a way to reflect iodine intake over the course of 24
hours[27]. As a consequence, some researchers have proposed a variety of prediction
equations to estimate 24-hour UIE[18, 22-25]. Some researches showed that multiplying I/Cr
by estimated 24-hour urine creatinine could be used to estimate 24-hour UIE while others
could not [28].

Moreover, UIE varies during the day, but it is controversial on the circadian pattern.
UIC which was at the lowest level in the morning showed an upward trend from the morning
to the evening; and spot urine in the afternoon can represent 24-hour UIE, according to Als C
and Vanacor R et al [29-30]. As for fasting urine, one article pointed out that the UIC in
fasting urine samples was 10% lower compared to non-fasting urine samples [31].
Nonetheless, some investigations reported that UIC in spot samples do not have a similar
tendency during the day [32]. Similarly, the results presented in Figure 1 do not have an
upward trend for all the participants, conversely, each participant had a different tendency.

According to our findings, the six prediction equations may have the applicability in the
assessment of individual iodine status. We still needed to employ 24-hour urine volumes for
calculation when using Estimated 24-hour UIE¹ albeit the most periods of the day (except the
morning) showed the good consistency. In addition, there was a large difference in 24-hour
urine volume between male and female [33-34]. Furthermore, there is currently no reference
range for urine volume by sex in China. Despite the fact that Estimated 24h UIE² and
Estimated 24h UIE³ were proposed on the Japanese population, which means there seems no
racial differences in the Chinese and Japanese population. And these two equations can be
used to estimate measured 24-hour UIE in the early morning and fasting time. Figure 1 also
showed that the 24-hour UIE in any period could not be predicted by Estimated 24-hour UIE⁴,
which implied that iodine excretion in various groups varied considerably depending on
ethnicity, social economic status, and dietary patterns [35]. Estimated 24-hour UIE⁵ was one
of the prediction equations that may be suggested to estimate UIE in all periods except the
morning. However, it was difficult to collect urine samples in the evening although it had the
highest consistency. Estimated 24-hour UIE⁶, as an equation for children UIE prediction,
showed the infeasibility among adults, which verified the premise that only for the children population.

Similarly, estimated 24-hour UIE in different periods of the day also showed the inconsistent results. The prediction equation except Estimated 24-hour UIE\(^4\) showed no significant difference between the early morning spot urine and fasting urine although they had a high correlation. It is not suggested to estimate by early morning spot urine since not all participants urinate during this period of the day, therefore, it is hard to assess iodine status for every person by collecting early morning spot urine. In addition, several researchers pointed that it was better to estimate 24-hour UIE by fasting urine compared to other spot urine [36-37]. The findings of this study also suggested that it was preferable to estimate 24-hour UIE by fasting urine samples in the most periods of the day. Also, The Bland-Altman diagram indicated that estimated 24-hour UIE\(^3\) was the best for fasting urine estimation.

There are some strengths and limitations in this study. One strength was the efficiency of creatinine-corrected UIC in the evaluation of individual iodine status among Chinese adults. Secondly, the quality of the 24-hour urine collection completeness was assured. Lastly, a number of prediction equations were employed to identify the optimum prediction equation for 24-hour UIE assessment. For the limitation, firstly, currently, there is no reference range of creatinine for the Chinese population by age groups, so Knudsen N's equation has its limited replication within the Chinese adults. Secondly, we had only 126 participants, which meant the sample size was not big enough. The external validity is uncertain since the participants were only recruited from Fujian Province instead of all the provinces in China. Lastly, the applicability for school-age children, pregnant women and lactating women was of uncertainty since adults were the target population in our study.

**Conclusion**

It is feasible to estimate 24-hour UIE of Chinese adults by the creatinine-corrected fasting urine iodine concentration, especially Kawasaki T's equation. 24-hour UIE for adults could be estimated by Knudsen N's equation except for the morning period. And the prediction equations could be suggested for the assessment of the individual iodine status among adults, but the feasibility for school-age children, pregnant women and lactating
women is unknown.

Abbreviations

I: Iodine; Cr: creatinine; UIC: urinary iodine concentration; UIE: urine iodine excretion; Pr24hCr: 24-hour creatinine prediction; SD: standard deviation; IQR: inter-quartile range; mUIC: standard median urinary iodine concentration.

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Authors’ contributions

Zhihui Chen was involved in the study's design and served as the corresponding author. This article was written by Zhuan Liu, who analyzed the data and wrote it. The paper was revised by Zhihui Chen, Zhuan Liu, and Yixuan Lin. Participants were recruited and urine samples were collected by Jiani Wu, Diquan Chen, Xiaoyan Wu, and Lan Ying. The laboratory analysis was carried out by Jiani Wu and Xiaoyan Wu. The published version of the work has been read and approved by all of the writers. Zhuan Liu, Yixuan Lin and Jiani Wu contributed equally to this article.

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Availability of data and materials

Please contact author (Zhihui Chen) for data or material requests.

Ethics approval and consent to participate

All the participants were recruited following the informed consent and ethical review.

Consent for publication

The authors consent to the publication of the data.

Competing interests

The authors declare that they have no competing interests.
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