Letter to the Editor

Suggested Sample Size of 24-hour Urine Collection in Assessing Iodine Status among Adult Males with Insufficient Iodine Intake*

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Iodine is an essential component of thyroid hormones' biosynthesis and is crucial for normal growth and healthy development[1]. If the amount of dietary iodine intake cannot match the long-term requirement, regardless of whether it is excessively low or high, it can cause various thyroid dysfunctions, including hyperthyroidism, hypothyroidism, and autoimmune thyroid diseases[2]. Currently, iodine deficiency remains a major public health issue in many parts of the world, though enormous progress has been made during the past decades.

There are so many indicators for assessing iodine status in a population, such as urinary iodine concentration (UIC), the goiter rate, thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), and thyroglobulin (Tg)[3]. The UIC is the most used indicator because approximately 90% of dietary iodine intake is excreted by the kidney. The World Health Organization (WHO) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) jointly recommend that spot UIC be used in population-based surveys[1]. However, the application of spot UIC is widely controversial because of the apparent fluctuations[4]. The indicator of 24-h urinary iodine is considered more reflective of current iodine status, although it varies somewhat since it mainly depends on daily iodine intake and water consumption. Variations in the 24-h urinary iodine can greatly affect the estimated sample size for assessing iodine status. The estimated sample size of 24-h urine collection is the primary question. Currently, it has been reported in populations with iodine repletion so far[5]. However, there is a dearth of data on sample size estimates in individuals with iodine deficiency for assessing iodine status. Therefore, the purpose of this study was to examine the variability of 24-h urinary iodine concentration (24-h UIC) and 24-h urinary iodine excretion (24-h UIE). Also, it was to obtain sample size estimates of 24-h urine collection for assessing iodine status in a population, or an individual, with insufficient iodine intake.

This study was a population-based iodine experiment conducted in 2018 during an iodine depletion period for 15 days. Adult males aged 19–22 years participated in this study. The subjects were recruited from Changzhi Medical College, located in the Shanxi province of China. Before starting the experiment, information was carefully collected by a questionnaire involving disease history and eating habits. All subjects were required to be healthy without metabolic diseases, normal thyroid and kidney function, no iodine-containing mineral intake, and no recent examination with contrast media. The diets were designed according to the eating habits and prepared with non-iodized salt. Daily dietary iodine intake was lower than the recommended iodine intake (120 μg/day), as suggested by the China Nutrition Society. All subjects were only allowed to eat designed diets and to avoid any other iodine intake. The study protocol was designed according to the guidelines laid down in the Declaration of Helsinki of the World Medical Association and approved by the Ethics Committee of the National Institute of Nutrition and Health of the Chinese Center for Disease Control and Prevention and registered at medresman.org (ChiCTR1800016184). Informed consent was signed and obtained from each subject after the nature of

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the study was explained. Trained workers performed the physical examination. Bodyweight was measured in kg to the nearest 0.1 kg, and height was measured in cm to the nearest 0.5 cm with a calibrated instrument. Body mass index (BMI) was calculated by dividing weight in kilograms (kg) by height in meters squared (m^2).

Participants were asked to collect a 24-hour urine specimen in a separate polyethylene container each day for 15 consecutive days. All 24-h urine specimens were required to be returned on schedule, and urine volumes (UVs) were measured by the corrected measuring instrument. The 24-h urine specimens were then divided into several vials and preserved at 4 °C for short-term storage and −20 °C for long-term storage until analysis. The UIC was determined by the inductively coupled plasma mass spectrometer (ICP-MS) using an Agilent 8800 ICP-MS system (Agilent Technologies Inc., CA, USA). The limit of quantification (LOQ) was 2 μg/L during the analysis. The certified urine reference materials GBW (E) 090016 and GBW (E) 090017 were employed to monitor the accuracy and precision. The recovery of the analytical method was between 90% and 95%, and the precision of intra-day and inter-day was 2%–3% and 3%−5%, respectively. The total amount of 24-h UIE was calculated by multiplying the 24-h UIC (μg/L) by the 24-h UV (Liter).

All data were analyzed using SAS software (version 9.3, SAS Institute) and Microsoft Excel (Version 2016, Microsoft). The variables were expressed as the mean, median, variance, and coefficients of variance (CV%). The sample size (*N*) of urine collection was estimated using the equation $N = (Z \times CV\%/D)^2$. The CV% was the square root of the variance divided by the mean as a percentage. The D was the precision of a set-point in the biochemical variables generally defined from ± 1% to ± 50%. The confidence interval (*CI*) used the Z value was 2.58 for 99%, 2.33 for 98%, 1.96 for 95%, 1.64 for 90%, 1.28 for 80%, 1.04 for 70%, 0.84 for 60%, and 0.67 for 50%. According to the suggestion by Fraser & Harris[6], the calculation using the Z statistic could underestimate the sample size up to 30% when compared with that by the t-statistic. The mean variances of intra-individual and inter-individual were relatively similar whether they were assessed as the mean variance among individuals or using ANOVA techniques. The CV% was used to estimate the sample size of 24-h urine collection for assessing iodine status with a specified precision range for intra-individual and inter-individual variations.

In this study, a total of 38 healthy adult males, aged 19.1 ± 0.6 years, were finally included, and BMI was 21.4 ± 1.2 Kg/m^2. The daily iodine intake for subjects was 107 ± 24 μg/day, whereas the 24-h urine volume (UV) was 2.1 ± 0.7 L and 24-h UIC was 77.6 ± 29.5 μg/L. The urinary creatinine concentration was 86.7 ± 13.4 μmol/L. The concentrations of thyroid hormones involving thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) were 2.4 ± 0.7 uIU/mL, 18.5 ± 2.1 pmol/L, and 5.6 ± 0.5 pmol/L, respectively.

Table 1 presents the intra-variations in the 24-h UIC and UIE for each subject with insufficient iodine intake. In total, 570 of 24-h urine specimens were collected during the 15 consecutive days. The mean 24-h UIC was 78 μg/L with the concentration from 45 μg/L to 170 μg/L, and the mean 24-h UIE was 119 μg/day with dietary iodine intake from 92 μg/day to 172 μg/day, respectively. The intra-individual CV% was 31.2% in the 24-h UIC and 21.5% in the 24-h UIE. The intra-variation in the 24-h UIC was slightly higher than that of the 24-h UIE.

As shown in Table 2, we calculated the inter-variations of 24-h UIC and 24-h UIE in a population in the 15 consecutive days. At the population level, the mean 24-h UIC and 24-h UIE were 78 μg/L with the concentrations ranged from 55 μg/L to 104 μg/L, and the mean 24-h UIE was 119 μg/day with the iodine intake from 92 μg/day to 152 μg/day, respectively. The population-based mean CV% was 45.9% in the 24-h UIC and 22.2% in the 24-h UIE. The inter-variation was higher in the 24-h UIC than that of the 24-h UIE.

Table 3 illustrates sample size estimates of the 24-h urine collection in assessing iodine status at the population and individual level. We observed that the estimates of a sample size could increase rapidly in association with an elevation in the precision range, regardless of whether it was in a population or an individual. Moreover, the sample size estimates of the 24-h urine collection were sharply reduced when the precision range was higher than 10%. In particular, based on the variability of 24-h UIC and 24-h UIE, the estimated sample size was distinctly larger in a population than that for an individual. Then, narrowing the precision range from 10% to 2%, the sample size of 24-h urine collection could increase from 105 to 2,630 in a population, or from 44 to 1,101 in an individual, according to the 24-h UIC. Similarly, the sample size could also increase from 25 to 621 in population, or from 20 to 499 in an individual, according to the 24-h UIE. In theory, the estimates of a sample size could be

changed accordingly by the requirement in a population or an individual, when a reliable assessment of iodine status could be provided.

At present, our study had estimated the sample size according to the variability of the 24-h urinary iodine concentrations (24-h UIC) and 24-h urinary

Table 1. Intra-individuals' variations in the 24-hour urinary iodine concentration (UIC) and excretion (UIE) at the individual level

No.	24-h UIC (μg/L)			24-h UIE (μg/Day)		
	Mean	Variance	CV%	Mean	Variance	CV%
1	67	237	23.0	125	578	19.3
2	93	1,375	39.9	121	498	18.4
3	142	1,244	24.9	138	1,078	23.9
4	67	537	34.5	117	913	25.9
5	46	112	22.9	116	485	18.9
6	72	675	36.0	94	544	24.7
7	77	389	25.7	161	3,601	37.2
8	52	161	24.5	105	258	15.3
9	89	686	29.6	137	422	15.0
10	80	633	31.6	127	230	11.9
11	52	103	19.3	109	390	18.2
12	111	864	26.4	115	274	14.4
13	43	233	35.9	131	898	22.9
14	47	117	22.7	97	445	21.7
15	124	1,465	30.9	125	263	13.0
16	170	1,765	24.8	125	702	21.2
17	99	734	27.4	124	669	20.9
18	63	523	36.2	153	1,450	24.9
19	49	268	33.2	98	782	28.6
20	79	769	35.0	106	781	26.4
21	52	310	33.7	118	324	15.2
22	77	1,199	45.2	95	334	19.1
23	65	723	41.4	98	407	20.7
24	59	215	24.7	104	333	17.5
25	85	1,483	45.2	104	480	21.1
26	54	115	19.7	92	634	27.3
27	45	238	34.2	105	573	22.8
28	136	1,690	30.2	101	676	25.7
29	95	699	27.8	119	1,114	28.0
30	56	157	22.4	117	508	19.3
31	76	622	32.6	122	483	18.0
32	65	186	21.1	126	515	18.0
33	81	1,091	40.6	135	1,186	25.5
34	81	586	29.9	112	143	10.7
35	42	62	18.6	116	162	10.9
36	60	227	25.3	137	546	17.0
37	108	2,669	47.9	172	940	17.8
38	88	488	25.1	106	1,001	29.7
Median	74	562	29.7	117	530	20.0
Mean	78	675[†]	31.2[§]	119	674[†]	21.5[§]

Note. UIC, urinary iodine concentration; UIE, urinary iodine excretion; CV%, coefficients of variance; [†]Calculated using the mean variance in individuals and using ANOVA techniques gave similar results; [§]Calculated as $[(\Sigma CV\%^2_{1-38})/38]^{1/2}$.

Table 2. Inter-individuals' variations in the 24-hour urinary iodine concentration (UIC) and excretion (UIE) at the population level

Days	24-h UIC (μg/L)			24-h UIE (μg/Day)		
	Mean	Variance	CV%	Mean	Variance	CV%
1	62	686	42.4	101	778	27.6
2	65	658	39.6	99	635	25.4
3	69	1,214	50.2	104	588	23.2
4	59	960	52.6	92	275	18.1
5	55	549	42.8	99	467	21.9
6	72	1,405	51.8	116	547	20.2
7	85	1,515	45.7	117	679	22.3
8	78	1,252	45.1	118	477	18.6
9	82	1,140	41.4	131	974	23.9
10	78	1,094	42.5	112	590	21.7
11	101	2,567	50.4	141	597	17.4
12	78	1,270	45.8	140	1,258	25.3
13	95	1,800	44.5	152	734	17.8
14	104	2,374	46.8	136	987	23.2
15	81	1,317	44.6	121	813	23.5
Median	78	1,252	45.1	117	635	22.3
Mean	78	1,521[†]	45.9[§]	119	693[†]	22.2[§]

Note. UIC, urinary iodine concentration; UIE, urinary iodine excretion; CV%, coefficients of variance; [†]Calculated using the mean variance in individuals and using ANOVA techniques gave similar results; [§]Calculated as $[(\Sigma CV\%^2_{1-38})/38]^{1/2}$.

Table 3. Sample size estimates of the 24-hour urine collection with a specified precision range (D) at the population and individual level

Precision range (D)[*]	24-h UIC (μg/L)				24-h UIE (μg/day)			
	In population[‡]	In an individual [§]			In population[‡]	In an individual [§]		
		Median variance	Lowest variation	Highest variation		Median variance	Lowest variation	Highest variation
± 1%	10,522	4,405	1,728	11,458	2,484	1,998	572	6,911
± 2%	2,630	1,101	432	2,865	621	499	143	1,728
± 5%	421	176	69	458	99	80	23	276
± 10%	105	44	17	115	25	20	6	69
± 20%	26	11	4	29	6	5	1	17
± 30%	12	5	2	13	3	2	1	8
± 40%	7	3	1	7	2	1	1	4
± 50%	4	2	1	5	1	1	1	3

Note. UIC, urinary iodine concentration; UIE, urinary iodine excretion; CV%, coefficients of variance; [*]Calculated with a *CI* of 95% (Z = 1.96); [†]Calculated from $N = (Z \times CV\%/D)^2$, where Z = 1.96 for 95% *CI* and D is the precision range; [‡]Number of individuals needed to produce one urine sample was calculated based on the variation in the population; [§]Variation differs between individuals. The sample size needed in an individual is given for individuals with the median, lowest, and highest variation.

iodine excretion (24-h UIE) derived from a group of adult males with insufficient iodine intake. Therein, with the 95% *CI* and ± 5% D, we suggested that sample size of the 24-h urine collection should require 421 in a population and 176 in an individual by the 24-h UIC, or 99 in a population and 80 in an individual by the 24-h UIE for assessing iodine status in population with insufficient iodine intake. However, the 24-h urine collection sample size could be selected accordingly and considered acceptable at the population or individual level.

A slight flaw remains in that great variability exists in urinary iodine within the same day and from day-to-day. As presented in our study, the higher intra-individual and inter-individual CV% was observed: 31.2% and 45.9% for the 24-h UIC and 21.5% and 22.2% for the 24-h UIE. A previous study showed a higher inter-individual CV% with 55% in the 24-h UIC and 41% in the 24-h UIE. The variation was much larger in the 24-h UIC than the 24-h UIE, whereas a smaller variation was seen in the intra-individual than the inter-individual. Similar findings were also reported in a study with the intra-individual and inter-individual CV% of 48%, 56.4% for spot UIC, 40%, and 49.9% for the estimated 24-h UIE[7]. However, a contrasting result from a pilot study in adult women indicated that the intra-individual and inter-individual CV% were 55% and 32% for the 24-h UIC, 48%, and 24% for the 24-h UIE[5]. This difference could be due to the 24-h UIE with the dilution effect considered urine volume, and subjects were provided the same diet, both of which reduce variation. In our study, sample size estimates of the 24-h urine collection were calculated using the reported equation[6]. As two key parameters, *CI* and D could affect the estimates of sample size. When broadening the D, we observed that it could induce a steep decrease in sample size estimates, whereas adjusting the *CI* had a smaller impact on sample size. As a rule of thumb, the estimated sample size of the 24-h urine collection could be obtained by combining the 95% *CI* and ± 5% D. In our study, the 24-h UIC was more easily influenced than the 24-h UIE. The resulting larger variation in the 24-h UIC could indicate more urine collection in a population or an individual. Regardless of whether it was short-term and long-term, urinary iodine variation was associated with many factors, including diet, sex, age, season, and circadian rhythms[8]. Of these, diet is a major determinant of urinary iodine. Some natural iodine content and its inconsistent bioavailability in consumed foods could increase the uncertainty of urinary iodine excretion[9]. Stored iodine in the human body also affects the variation of inter-individual and intra-individual, which might partly affect iodine utilization and absorption[10]. As presented in our study, there was much lower urinary iodine variability in individuals with insufficient iodine intake. A possible reason could be attributed to the activation and excretion of stored iodine. Thus, this rationale may indicate that the 24-h urine collection is lower depending on the variability of the 24-h UIE for assessing iodine status in individuals with insufficient iodine intake.

To our knowledge, several limitations should be noted. First, the diets designed and provided during the study period had low iodine content, which possibly reduced the unexpected variation. Also, subjects were of the same gender and had similar ages that might result in a limited 24-h urinary iodine variation. Thus, the sample size estimates of urine collection could be reduced. Second, our study was performed on Chinese adult males. The sample size estimates were much larger than the current 38 subjects. If more subjects were included, it would generate a relatively low variation and lower sample size estimation. The generalization of our results to the general population was fairly circumscribed. Third, a large amount of iodine could be stored in the thyroid gland, whereas the 24-h urinary iodine in subjects could not be affected by short-term low iodine intake. The variability of the 24-h UIC and 24-h UIE remains to be verified in a long-term study.

In conclusion, the 24-h UIC variability was higher than that of the 24-h UIE and verified in a population compared with an individual. With the 95% *CI* and the ± 5% D, the 24-h urine collection sample size could be adequate when 421 in a population and 176 in an individual by the 24-h UIC, or 99 in a population and 80 in an individual by the 24-h UIE. This data should establish sampling guidelines for understanding the variation when assessing iodine status in individuals with insufficient iodine intake.

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