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ORIGINAL ARTICLE

Restricted intraindividual urinary iodine concentration variability in nonfasting subjects

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Objective: Individual urinary iodine concentration (UIC) reflects iodine intake over a short time prior to sampling. Since eating habits are relatively constant in single subjects, UIC should be relatively constant in a given individual. The aim of our study was to verify this hypothesis by assessing UIC in repeated single urine samples from a group of healthy subjects.
Design and Setting: A prospective sequential investigation was performed in 131 volunteer health workers or students recruited in our University hospital.
Interventions: Single urine samples were taken in a nonfasting state, between 0900 and 1100 hours. Group 1 was composed by 131 subjects who collected one urine sample. Group 2 was composed by 11 subjects of the group 1, who collected multiple repeated urine samples (as a whole 158 urine samples, mean 14 samples each). UIC mean $\pm$ s.d., median and coefficient of variation (CV%) was measured in both groups.
Results: Interindividual UIC variation was wide, UIC ranging from 21 to 382 μg/l, mean 136 $\pm$ 84 μg/l, median 124 μg/l, CV 62%. Also in the 11 subjects repeatedly sampling there were considerable differences among individual UIC average levels (ranging from 37 $\pm$ 15 to 221 $\pm$ 91 μg/l). However, in this second group, the intraindividual variation was considerably restricted (CV% 36).
Conclusions: The present study shows that in a nonfasting state in mid-morning UIC is more stable from day to day in a single subject, depending on his eating habits, than in various subjects. Thus, a single urine sample even in nonfasting state may give some rough information about the individual's iodine status.
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Keywords: thyroid; Italy; milk; diet; urinary iodine concentration; iodine supply

Introduction

A 24 h urinary iodine excretion (UIE) is the most reliable index of iodine availability in a stated population and is universally employed in epidemiological studies on goiter and iodine deficiency disorders, since it corresponds to 95% of the dietary iodine intake (Vought *et al*., 1963; Als *et al*., 2003b). However, as it is difficult to obtain 24-h urine samples, single urine samples taken in the morning are generally used, and the iodine contents are given as a concentration (urinary iodine concentration, UIC, μg/l) or as an iodine–creatinine (I/Cr) ratio. Gender, age, socio-cultural and dietary influences, drug interferences, geographical location and period of the year are the most important factors determining UIE and UIC (Als *et al*., 1994, 1995, 2000; Dunn, 1996; WHO, 1996; Rasmussen *et al*., 2000, 2002).

Iodine intake from foodstuff is efficiently absorbed by the gut (more than 90%) (Hurrell, 1997) and an individual's UIC reflects iodine intake shortly before sampling. (Rasmussen *et al*., 1999; Als *et al*., 2000; Andersen *et al*., 2001). Indeed, most iodine is excreted on the day it is ingested (Rasmussen *et al*., 1999), its peaks following the three main meals by 4–5 h. (Als *et al*., 2000; Andersen *et al*., 2001). Iodine contents in food vary considerably, being high in relatively few foods, such as sea fish, milk (Saxholt, 1996; Rasmussen *et al*., 1999; Als *et al*., 2003a; Girelli *et al*., 2004). Since eating habits are presumably relatively constant in a given subject, individual UIC variations from day to day are presumably limited as well, while there is a great variability in different subjects.

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Only few reports have investigated intraindividual UIC variations in the short term (Bourdoux, 1998; Rasmussen *et al*., 1999; Als *et al*., 2000, 2003a; Andersen *et al*., 2001). The aim of this study was to evaluate intra- and interindividual variations of UIC to ascertain whether the UIC measured in a single urine sample, taken in a nonfasting state, may give some information about the individual iodine status.

Materials and methods

UIC was measured in a single urine sample taken in the morning (between 0900 and 1100 hours). For clear reasons of convenience, urine samples were collected in nonfasting conditions, a few hours (about two) after Italian breakfast, usually consisting in coffee without or with a little milk, some biscuits and jam. No subject has taken a second breakfast before the urine collection. All subjects were healthy and motivated volunteers (students or people employed at our Institute). None had clinical goiter neither personal history of thyroid disorders. None had undergone examinations with iodized contrast media in the 6 months preceding the study. All participants were living in Padova or neighboring villages up to about 20 km away. Padova is in the Veneto region of northeast Italy, an iodine-sufficient area according to WHO, 1994, 2001 criteria, as found in previous studies (Frigato *et al*., 1996; Busnardo *et al*., 2003). In this region, the use of iodized salt is only volunteer and assumed by 30% of the population (Busnardo *et al*., 2003).

Group 1 was composed by 131 subjects (63 M, 68 F; aged between 18 and 56 years, median 33), who collected one urine sample. Group 2 was composed by 11 subjects of Group 1 (5 M, 6F; aged between 26 and 55 years, median 30), who collected multiple repeated urine samples every 2–3 days over a period of 3 months (range 6–30 samples per subject, mean 14 samples each, on the whole 158). Although most of these 11 volunteers were born in the Veneto region, three of them (IC, CC and RT) were born in central or southern Italy, but had lived in the northeast for at least 10 years. Another subject (DVD) was born in a Veneto mountain village (1000 m a.s.l.) and had also lived in the lowlands for at least 10 years. These 11 subjects filled in a simple questionnaire about their eating habits, particularly as regards milk intake, recorded as: no milk (0), occasional (1), one cup (200 ml) a day (2), and more than one cup a day (3); yogurt intake was recorded as: never (0), occasional (1), one or more pots a week (2); and the use of iodized salt as indicated (yes or no).

Urine samples were stored at $+4^{\circ}$C and assayed within 3 days from collection. UIC was measured in duplicate using the colorimetric ceric ion-arsenious acid method in a second-generation Technicon Auto-Analyzer (Brain Luebbe GmbH, Germany)(Garry *et al*., 1973). Intra- and interassay coefficients of variation were 2.3 and 4.6%, respectively. The UIC was expressed as μg/l.

Statistical methods

Data were analyzed using the STATISTICA version 6 by StatSoft Italy 2002 (www.statsoft.it). UIC mean$\pm$s.d., median and coefficient of variation (CV%) were assessed in order to evaluate UIC inter- and intravariability. In the 11 subjects who collected multiple urine samples, the individual UIC variability was expressed by the interval of values between the 25th and the 75th percentile (interquartile range). In this context, the 'outlier' values per subject were the values exceeding 1.5 times the UIC variability beyond the 75th percentile. The 'limit' values were the UIC values exceeding threefold the UIC variability.

The Mann–Whitney *U* Test was used to evaluate the differences in the UIC values between female and male subjects. A $P<0.05$ value was considered statistically significant.

Results

Interindividual variability

Four samples revealed UIC values above 400 μg/l, and were excluded, because indicative of iodine overload (Busnardo *et al*., 2003). UIC distribution was calculated in the remaining 116 samples, with the addition of the first sample of the 11 subjects who had multiple urine samples. Thus, interindividual UIC variability was calculated in a total of 127 urine samples, as it is shown, separately for male and female subjects, in Figure 1. UIC varied considerably in individual urine samples, from 21 to 382 μg/l, mean 136$\pm$84 μg/l, median 124 μg/l, CV 62%. UIC was below 50 μg/l in 12% of samples, below 100 μg/l in 41% of samples as a whole, between 100 and 200 μg/l in 38% and above 200 μg/l in 21%. Mean UIC was 128$\pm$75 μg/l in male subjects and 144$\pm$92 μg/l in female subjects, median was 110 and 136 μg/l, respectively: this difference was not statistically significant ($P=0.5$).

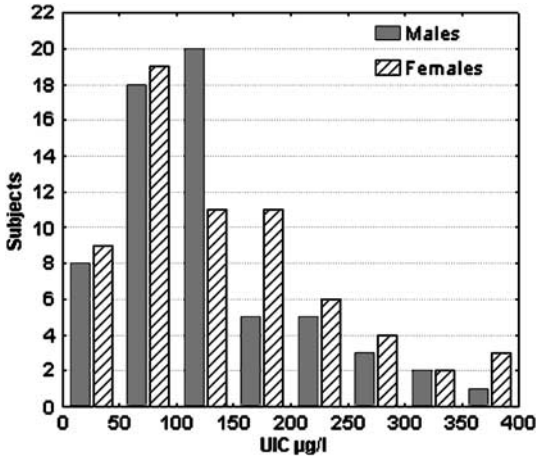

Figure 1 Urinary iodine concentration.

Table 1 Characteristics, dietary habits and urinary iodine concentration (UIC) variability in subjects with multiple urine samples

Subjects	*Gender M/F*	*Age years*	*Milk*[a]	*Iodized salt*[b]	*No.*[c]	*Range*[d] *(min–max)*	*Mean*[e]	*±s.d.*[f]	*Median*[g]	*CV%*[h]
IC	m	35	0	No	12	16–58	37	15	37	40
CC	f	28	0	No	18	12–285	61	61	46	42
RT	f	28	1	No	6	21–94	50	26	50	53
PM	m	46	1	No	20	45–158	79	27	78	35
SM	m	30	1	No	10	58–136	88	23	84	26
GE	f	30	2	No	12	49–148	95	28	90	30
DVD	m	28	1	No	30	45–221	98	35	96	36
MC	f	33	3	No	9	140–253	193	37	181	19
ZL	f	26	3	No	9	82–316	196	72	188	37
VAP	f	32	3	Yes	14	82–460	221	91	211	41
BB	m	55	3	No	18	106–317	219	55	219	26
Total	11				158					

[a]Milk consumption is stated as a score and iodized salt usage as yes/no (see Material and methods).
[b]Milk consumption is stated as a score and iodized salt usage as yes/no (see Material and methods).
[c]Number of collected samples for each subject.
[d]Range of UIC values.
[e]Mean UIC in μg/l.
[f]$\pm$s.d. of the mean.
[g]Median UIC in μg/l.
[h]Coefficient of variation (CV) of individual UIC as a percentage.
s.d. and CV% were weighted for sample number

Intraindividual variability

The characteristics and dietary habits recorded for the 11 volunteers providing multiple urine samples are given in Table 1. Mean UIC$\pm$s.d., median and CV% for each subject are also shown in Table 1. In the total of 158 urine samples, mean UIC value was $117\pm75\,\mu$g/l, median was $93\,\mu$g/l. In female subjects a total of 68 urine samples were collected: mean UIC value was $127\pm83\,\mu$g/l, median $108\,\mu$g/l; in male subjects a total of 90 urine samples were collected: mean UIC was $109\pm68\,\mu$g/l, median $87\,\mu$g/l. This difference was not statistically significant ($P=0.3$). The 11 sets of multiple samples revealed considerable differences in individual average levels: the individual average UIC (mean of the individual's 8–30 samples) varied from 37 ± 15 to $221\pm91\,\mu$g/l. The intraindividual variability in these 11 subjects was much smaller than the inter-individual one, being median CV 36 versus 63%. Three subjects had median levels $\leqslant 50\,\mu$g/l (IC, CC and RT): all three drank no milk and were born outside the Veneto region. All four subjects (BB, VAP, ZL and CM) with a high median UIC (219, 211, 188 and $181\,\mu$g/l, respectively) were milk drinkers, and one of them (VAP) used iodized salt too (Table 1 and Figure 2).

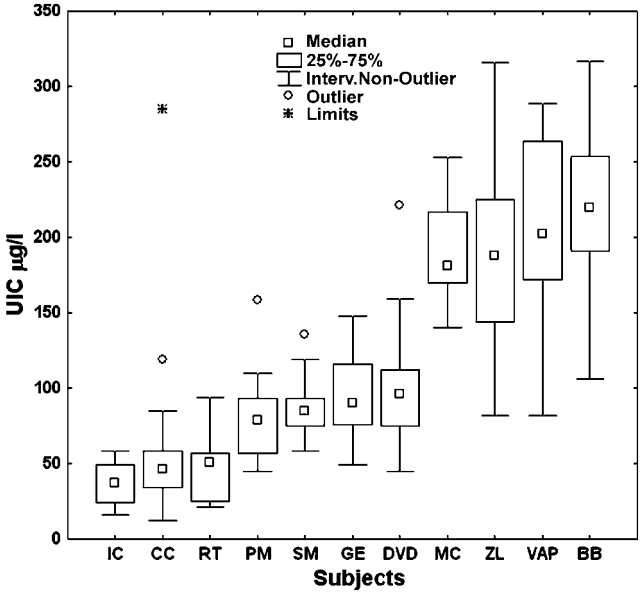

Figure 2 Median, 25–75% percentile, outliers and limits of UIC distribution in 11 subjects with repeated urine samples.

Discussion

In this study, UIC was expressed as a concentration, like in some reports (Andersen *et al*., 2001; Als *et al*., 2003a), instead of the iodine/creatinine ratio used by others (Rasmussen *et al*., 1999). According to some authors (Thomson *et al*., 1997; Bourdoux, 1998), the iodine/creatinine ratio is not suitable for evaluating daily iodine excretion and it is probably an additional source of error, increasing the scattering of urinary iodine excretion values. The gold standard, to reduce to the minimum the UIC variability, would have been to collect urines in fasting situation. We have decided to collect all urine samples after breakfast and not after lunch or supper in order to have a lesser variation in iodine concentration.

Even if this is only indicative since populations are different, we would like to point out that the mean and median UICs recorded in this study are only slightly lower than those observed in a previous study performed on 1800 schoolchildren in the Veneto region (Busnardo *et al*., 2003) where the mean and median values were 148 ± 110 and 128 μg/l, respectively. In our region, iodine content of cow's milk is about 250 μg/l and milk is an important source of iodine in children (Girelli *et al*., 2004). Adults drink less milk: in a recent Swiss study, the UIC was 144 μg/l in school children and 96 μg/l in their mothers (Als *et al*., 2003a).

Both in the 11 subjects that sampled between 6 and 30 times and in the 116 that sampled only once, mean UIC values were higher in female than in male subjects, even though the difference was not significant. These data are different from those of our previous study on children (Busnardo *et al*., 2003) and from other studies (Hollowell *et al*., 1998; Als *et al*., 2000; Guttikonda *et al*., 2002; Als *et al*., 2003a). In all these studies male subjects show higher UIC than female subjects. We have no explanation for this difference, which appears to be the result of different dietary habits.

The results of the present study show a great variation in UIC values from one person to another (interindividual variation): in our population of 127 subjects the range was 21–382 μg/l, while in the 11 subjects repeatedly sampling the range was 12–317 μg/l, with a difference in the mean UIC value from a subject to another up to sixfold. This large interindividual variation is probably mainly due to different personal eating habits, particularly as regards the different intake of sea fish, milk and dairy products, and the use of iodized salt. However, different diets alone are probably not the only reason. Our observation that the three people with the lowest UICs were all born in distinctly iodine-deficient regions would suggest that other factors may play a part in determining individual UICs. Further studies are needed to investigate this issue.

On the contrary, the intraindividual variation is rather restricted, being the median intraindividual CV 36% as opposed to the CV 62% in the whole population. In the group of 11 subjects who sampled 6–30 times it was very infrequent to find an extreme or outlier outcome in each subject. (five out 157) (Figure 2).

Very few studies have evaluated short-term UIC variability in single individuals.

Bourdoux (1998) found little UIC variations in single samples collected from the same subject on 18 occasions over a period of 27 days, CV being 20.1%. Rasmussen *et al*. (1999) measured 24-h UIE in 10 adults for four consecutive days and they concluded that 'iodine excretion in one urine sample is insufficient to determine iodine status in an individual because of the great variation from one day to another'. However, only six of their subjects collected urine samples for all 4 days, one subject had three urine samples, two subjects had two urine samples and one subject only one sample: from their results it appears that most subjects showed little day to day variation in UIE and only few subjects showed a greater variation, up to threefold from one day to another. Thus, their results are not very different from ours, which were obtained from a greater number of urine samples (mean 14 urine samples for each subject) and their conclusion does not seem completely justified. Another Danish study (Andersen *et al*., 2001) assessed the UIC of 15 healthy adults in single urine samples collected monthly for a year. The mean annual UIC (mean of 12 samples) was 57 μg/l, ranging from 29 to 81 μg/l, while in all 180 samples UIC varied from 10 to 260 μg/l. The variation around the mean UIC was 2.4 times greater when it was calculated for all 180 individual samples (CV% 57.3) than when it was calculated for the 15 subjects' annual average values (CV% 23.6). They concluded that iodine levels measured in single urine samples from a group of subjects vary far more than the average iodine levels in a given individual over 1 year. The interindividual and the intraindividual variations reported in that study are very similar to and even smaller than those found in our study (CV 57 versus 62%, and 24 versus 36%, respectively).

The results of the present study are very similar to the data reported by Bourdoux (1998) and by Andersen *et al*. (2001), all obtained collecting single urine samples like in this study, and even to the data reported by Rasmussen *et al*. (1999), obtained by 24 h urine samples. It is also important to realize that in our study intraindividual UIC variations were measured in single urine samples collected a few hours after Italian breakfast. Our data show that the intraindividual UIC variability in nonfasting subjects is rather limited in comparison with the interindividual one. Presumably intraindividual UIC variability would have been even more restricted in fasting subjects.

A single urine sample is clearly not enough to evaluate whether an individual has iodine deficiency and to what extent. However, our data show that, even in a nonfasting state, a single urine sample may give some rough information about individual iodine status.

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