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Urinary Iodine Concentration follows a Circadian Rhythm: A Study with 3023 Spot Urine Samples in Adults and Children*

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ABSTRACT

Our overall aim is to monitor iodine supply in a prospective study before and after the September 1998 increase of salt iodide content in Switzerland. Because iodide is supplied by alimentation, we moreover wondered whether urinary iodine concentration (UI) is governed by circadian rhythmicity.

Forty-two subjects (18 males and 24 females, including 13 children) collected 3023 urine spots between May 1996 and May 1998, at a rate of three to five samples per month, at any time of the day. The results show that circadian rhythmicity of UI in adults and children was found independent of the individual subject, age, gender, and season. Lowest UI levels were found between 8–11 h. A curve increasing progressively between 12 and 24 h was obtained. UI returned to base-line levels between 21 and 22 h in children only. UI peaks occurred 4–5 h after main meals; children's peaks occurred later than that of adults.

Although the existence of a circadian rhythm of UI is probably universal, its profile, however, depends on alimentation. Because nadir of UI is represented by morning spots, this might seem an appropriate collecting period. In view of the significant circadian rhythmicity of UI, studies with restriction of sampling time to morning hours, for example, cannot be directly compared with studies in which urine is sampled all over the day. (*J Clin Endocrinol Metab* **85:** 1367–1369, 2000)

URINARY IODINE CONCENTRATION (UI) is influenced by gender, age, socio-cultural and dietary factors, drug interferences, geographical location, and season. In Switzerland, severe iodine deficiency (ID) had progressively resolved with the introduction of iodized salt in 1922. Our ongoing prospective study is primarily intended for monitoring iodine supply before and after the increase of salt iodization from 15–20 ppm iodide (20–26 mg potassium iodide per kg salt) that took place in September 1998. As iodine is supplied by alimentation, we wondered whether UI is governed by a circadian rhythm. Could the known chronobiological rhythmicity of the thyroid parameters serum TSH and T_3 be paralleled by an as yet unknown circadian rhythmicity of UI (1)?

In the present study, our aim was to determine whether a relation between UI and the spot sampling hour exists in adults and children. Is the hour of spot urine sampling a significant factor to be considered in the design of epidemiologic studies and in the interpretation of own data on UI?

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Subjects and Methods

Subjects

This ongoing prospective study, approved by the Ethical Commission of the University Hospital of Bern, is patronized by the Swiss National Foundation for Scientific Research (number 32-49424.96). An informed consent was signed by all study subjects. The subjects in the present study were 42 healthy volunteers: 18 males and 24 females (aged 4–60 yr at the beginning of the study in May 1996), including 13 children (Table 1). Of a total of 50 subjects at the beginning of the study, 8 subjects aged 47–75 yr had been excluded because of: 1) long-lasting iodine exposure by oral ingestion of oligoelements (n = 4) or percutaneous application of betadine (n = 1); 2) lack of compliance (n = 2); and 3) incomplete age category with only one 66-yr-old subject left after exclusion of the other subjects more than 60 years of age.

Spot urine sampling

Between May 1996 and May 1998, a total of 3023 urine spots were collected at a rate of three to five samples per month, at any time of the day, as convenient. The urines were immediately frozen. The subjects recorded the sampling hour, but not the hour of meals. The main meals were breakfast, lunch, and supper; some subjects moreover took small meals in-between. Due to the long-time study design aimed primarily at monitoring a potential increase of UI in Switzerland, no special emphasis had been laid on sampling during late night. As a consequence, only few spot samples were collected between 1 and 5 h in the morning. Therefore, those hours were left out from statistical analysis.

Laboratory analyses

The laboratory analyses of all 3023 spots, primarily frozen at −30 C, were realized in a continuous run-through between May and September 1998 in the laboratory of the Swiss Federal Office of Public Health. Urinary iodine was measured according to a new method: inductively coupled plasma mass spectrometry (ICP-MS) (2). ICP-MS allows the

TABLE 1. Characteristics of adult and child subjects

Subject	Male (n = 17)					Female (n = 25)				
	Spots (n)	Spots/person (mean ± SD)	Age (yr) (mean ± SD)	Urinary iodine (μg/L)		Spots (n)	Spots/person (mean ± SD)	Age (yr) (mean ± SD)	Urinary iodine (μg/L)	
				(mean ± SD)	(median)				(mean ± SD)	(mean)
Adults	859	76 ± 22	47 ± 8	120 ± 61	110	1136	69 ± 27	41 ± 8	93 ± 60	82
Children	600	86 ± 12	8 ± 1.2	154 ± 73	147	428	72 ± 15	11 ± 3	127 ± 66	117
Total	1459	79 ± 19	31 ± 20	134 ± 68	125	1564	69 ± 24	33 ± 15	102 ± 64	93

direct determination of iodine in urine. The application of isotope dilution analysis by using the long-lived radioisotope of iodine I-129 as a spike offered possibilities for automatic and accurate measurement.

Because of extremely high values between 2500–6000 μg I/L, 5 samples were excluded from the calculations, as also were 67 samples collected between 1 and 5 h in the morning. Results were expressed as I/volume (μg/L). The relation between daytime of spot urine sampling and UI was evaluated.

Results and Statistical Analysis

The obtained UI values are described by a right-skewed distribution. The application of the simple square root transformation $y_i = (x_i)^{0.5}$ yields values that follow approximately a normal distribution. To reveal the unbiased influence of daytime (sampling hour) on UI, the subject means $\bar{y}$ were subtracted from the respective y_i values for each subject to center the data around zero: $z_i = y_i - \bar{y}$. Accordingly, the transformed UI data (z_i) were evaluated by ANOVA. Thereby, the effects of season (summer, winter) and sampling hour were significant ($P < 0.001$), whereas the effects of individual subject, gender, and age were not ($P > 0.9$). A fully factorial ANOVA of the transformed UI data (z_i) with the factors sampling hour ($P < 0.001$) and season ($P < 0.001$) was next calculated and revealed that interaction between the two categorical variables did not occur ($P = 0.84$). Consequently, it was assumed that the factor sampling hour was not modified by seasonal variations.

Most urine spots were sampled in the morning (6–10 h, n = 191–390/h). Less specimens were obtained in the evening (19–23 h, n = 106–262/h) and between 11–18 h (n = 95–140/h); for obvious reasons, even less at 24 h (n = 20/h) and between 1 and 5 h (n = 2–11/h, n = 67 samples omitted in Fig. 1).

As can be seen in Fig. 1, a curve of UI increasing progressively between 12 and 24 h was obtained with significant differences between the mean values of the sampling hours ($P < 0.001$). Although the effect of age was not significant, the circadian rhythm is given separately for both children and adults to point out the differences. There was no return of UI to base-line levels between 12 and 24 h, except for children between 21 and 22 h. After midnight, however, the return took place progressively over about 12 h in adults and children (*i.e.* lowest levels were found in the morning, between 8–11 h). UI "peaks" appeared around 13–14 h, around 16–17 h, and most pronounced around 22–23 h. The child's first UI peak between 12–14 h was relatively higher than the corresponding adult peak; each child's peak was found delayed as compared to the corresponding adult's peak.

Discussion

The presented data of a large-scale study, designed primarily for the quantitative evaluation of iodine supply, demonstrate a circadian rhythm of UI in adults and children, independently of the individual subject, age, gender, and season. Contrary to a former study, in which complete urine spots over 24 h in 62 adults had failed to show consistent diurnal variations (3), a highly significant interaction between daytime and UI appeared in our longitudinal study over a 2-yr-period with as many as 3023 samples. The large number of spot urine samples in our study was obviously necessary to demonstrate this interrelationship.

Although the existence of a circadian rhythm of UI is most probably universal (nadir in the morning, followed by a progressive increase until about 24 h), its profile seems to depend on alimentary peculiarities. As UI peaks followed the three main meals by 4–5 h, the profile of the circadian rhythm suggests a short-timed relation to alimentary iodine intakes. Compared to an UI peak at about 20 h in 193 Romanian children (4), the third peak at 22–23 h in our study is possibly due to later intakes of qualitatively different suppers. In analogy, the different shape of the first peak in our children and adults, respectively, may be related to qualitatively different breakfasts. In this context, it has been shown that in Switzerland milk is an important provider of iodine, especially during the winter months, when cows are fed in the stables with industrially prepared animal foods (5). In our study, indeed, the reported milk intake at breakfast of participating children was high: 0.2–0.6 L. On the contrary, adults consumed much less milk at breakfast: 0–0.15 L. The abundant consumption of milk at breakfast by our child subjects should, thus, be considered an important provider of iodine, mirrored by the children's first peak of UI between 12 and 14 h.

In analogy to the circadian rhythmicity of UI described herein, other 24-h rhythmic profiles (*i.e.*, of serum TSH and T_3) have been described in young and elderly men, aged 20–27 and 67–84 yr, respectively (1). The chronobiological modulation of TSH and of T_3 is preserved in the elderly compared with young men, even if the amplitude of the overall normal TSH secretion over 24 h is decreased in the elderly. However, in contrast to our findings of a nadir of UI between 8 and 11 h both in adults and children, the nadir of serum TSH was found in the afternoon, both in young and elderly men. Moreover, the acrophases of UI between 13 and 14 h, 16 and 17 h, and 22 and 23 h anticipate (or follow!) the acrophase of serum TSH found between 0 and 8 h. Thus, thyroid economy underlies a chronobiological rhythmicity of several of its main parameters. To find out whether or not relations between circadian rhythmicity of UI and of plasma TSH or T_3 exist is beyond the scope of this study. Future studies should deal with this question.

As the significant interaction between daytime and UI had to, our knowledge, not been known as such up to now, it is

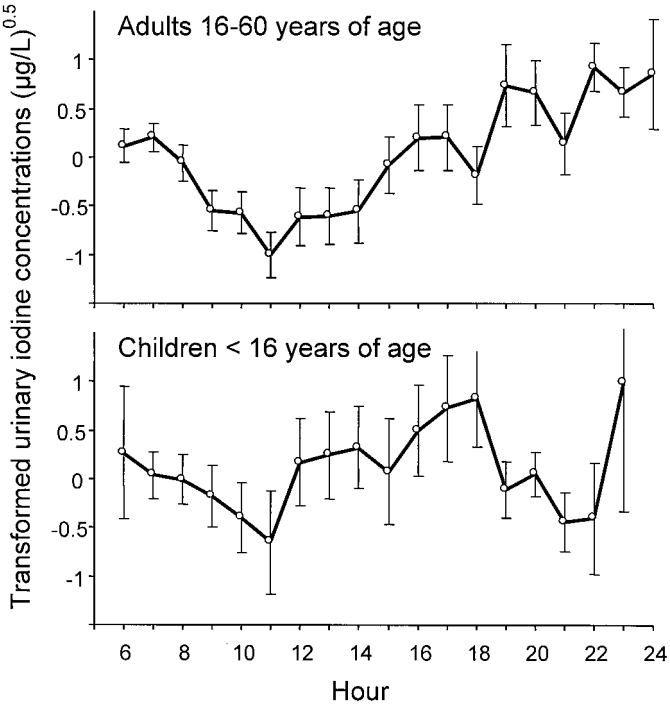

FIG. 1. Circadian rhythm of urinary iodine concentration (UI, in μg/L) in adults and children. The mean values of UI for each hour of the day from 6–24 h are represented as square root transformed values that were centered around zero for each individual subject (means with 95% CIs).

not astonishing that in virtually all reviews and studies, including ours, dealing on epidemiological or methodological aspects of iodine in urine, this point was not systematically addressed. On the contrary, spot urine sampling was often random [*i.e.* untimed (6–15); Brander, L., C. Als, H. Buess, F. Haldimann, M. Harder, W. Hanggi, U. Hermann, K. Lauber, U. Niederer, H. Odermatt, E. Schürch, T. Zürcher, U. Bürgi, and H. Gerber, submitted for publication]. The question remains open whether randomized sampling compared to early morning sampling leads to significantly different results of UI. However, only the integral of daily iodine excretion really counts. Indeed, complete urine collections of 24 h, albeit very difficult to obtain in field conditions, represent without doubt the most precise marker of daily iodine intake.

For a feasible and adequate assessment of a population's iodine status, however, the WHO and others (3) have recommended early morning, respectively, fasting urine samples. Our results show that spots taken in the morning or 8–12 h after the last meal represent the nadir of UI; hence, this might seem a sensitive collecting period. In view of the pronounced circadian rhythmicity of UI, studies with exclusive urine spot collections in the morning (*i.e.* in the nadir of UI), cannot be directly compared with studies in which urine spots are sampled all over the day (*i.e.* including the peaks that may not reflect real iodine intake). Our data are useful for standardization of the methodology of surveys assessing iodine intake of a population.

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