British Journal of Nutrition (2015), **113**, 1433–1440
doi:10.1017/S0007114515000653

Misclassification of iodine intake level from morning spot urine samples with high iodine excretion among Inuit and non-Inuit in Greenland

Stig Andersen[1,2,3]*, Rasmus Waagepetersen[4] and Peter Laurberg[1,5]

[1]*Arctic Health Research Centre, Institute of Clinical Medicine, Aalborg University Hospital, Hobrovej 18-22, 9000 Aalborg, Denmark*
[2]*Department of Internal and Geriatric Medicine, Aalborg University Hospital, Aalborg, Denmark*
[3]*Department of Internal Medicine, Queen Ingrids Hospital, Nuuk, Greenland*
[4]*Department of Mathematical Sciences, Aalborg University, Aalborg, Denmark*
[5]*Department of Endocrinology, Aalborg University Hospital, Aalborg, Denmark*

(Submitted 27 September 2014 – Final revision received 28 January 2015 – Accepted 6 February 2015 – First published online 8 April 2015)

Abstract

Iodine nutrition is commonly assessed from iodine excretion in urine. A 24 h urine sample is ideal, but it is cumbersome and inconvenient. Hence, spot urine samples with creatinine to adjust for differences in void volume are widely used. Still, the importance of ethnicity and the timing of spot urine samples need to be settled. We, thus, collected 104 early morning spot urine samples and 24 h urine samples from Inuit and non-Inuit living in Greenland. Diet was assessed by a FFQ. Demographic data were collected from the national registry and by questionnaires. Iodine was measured using the Sandell–Kolthoff reaction, creatinine using the Jaffe method and *para*-amino benzoic acid by the HPLC method for the estimation of completeness of urine sampling and compensation of incomplete urine samples to 24 h excretion. A population-based recruitment was done from the capital city, a major town and a settlement (*n* 36/48/20). Participants were seventy-eight Inuit and twenty-six non-Inuit. The median 24 h iodine excretion was 138 (25th–75th percentile 89–225) μg/97 (25th–75th percentile 72–124) μg in Inuit/non-Inuit ($P=0{\cdot}030$), and 153 (25th–75th percentile 97–251) μg/102 (25th–75th percentile 73–138) μg ($P=0{\cdot}026$) when including compensated iodine excretion. Iodine excretion in 24 h urine samples increased with a rising intake of traditional Inuit foods ($P=0{\cdot}005$). Iodine excretion was lower in morning spot urine samples than in 24 h urine samples ($P<0{\cdot}001$). This difference was associated with iodine intake levels ($P<0{\cdot}001$), and was statistically significant when the iodine excretion level was above 150 μg/24 h. In conclusion, the iodine intake level was underestimated from morning spot urine samples if iodine excretion was above the recommended level.

Key words: Iodine excretion: 24 h urine: Spot urine: Creatinine adjustment: Urine collection timing: Ethnicity: Greenland Inuit

Iodine excretion is commonly assessed by the analysis of urine. A 24 h urine sample is ideal, but it is difficult to obtain complete and accurately timed collections, and compliance with 24 h urine sampling is often low[1,2]. Hence, spot urine samples are frequently used because they are simple to collect and pose minimal inconvenience to the subjects. However, concentrations in spot urine samples fluctuate with fluid intake, and creatinine adjustment is often used as an internal standard[3–8] to compute an estimated 24 h iodine excretion.

Marked variations in estimated 24 h iodine excretion have been described between days[3,9]. This was suggested to relate to variations in iodine content of the diet. Furthermore, the iodine content of spot urine samples may vary with the time of collection[10], and this was speculated to relate to timing of meals. This is in keeping with early studies of iodine metabolism in humans that showed a fast appearance of the ingested iodine in blood and a subsequent appearance in urine[11].

The traditional Inuit diet comprises fish and marine mammals that are rich in iodine[12]. The iodine nutrition survey of Greenland has shown that a high frequency of intake of the traditional Inuit diet is associated with a high iodine content of spot urine samples[13]. The iodine content of spot urine samples are used to calculate an estimated 24 h urinary iodine excretion (eUIE) by using ethno-specific creatinine excretions[14]. However, this needs to be validated.

The aim of the present study was to compare the 24 h urinary iodine excretion (UIE) with iodine excretion in morning spot

Abbreviations: eUIE, estimated 24 h urinary iodine excretion; PABA, *para*-aminobenzoic acid; UIC, urinary iodine concentration; UICC, ratio of urinary iodine concentration to creatinine concentration; UIE, urinary iodine excretion.

* **Corresponding author:** Dr S. Andersen, email stiga@dadlnet.dk

urine samples, and to asses the influence of ethnicity. We, thus, collected spot urine samples and 24 h urine samples among Inuit and non-Inuit living in Greenland. We included *para*-aminobenzoic acid (PABA) as a marker of the completeness of 24 h urine collections[15] and to allow us to compensate for incomplete 24 h urine collections[16] using the HPLC-method[17].

Methods

Populations

Participants were recruited from the general population in the capital city, Nuuk (n 36), in a major town in Greenland, Ilulissat (n 48), and in the settlement Saqqaq (n 20). The study population has been described in detail in the report on creatinine excretion in Inuit[14]. They were seventy-eight Inuit and twenty-six non-Inuit. The population in town and settlement was limited.

In Nuuk, we recruited a randomly selected subgroup of healthy participants of the population-based Greenland iodine nutrition survey[13]. In Ilulissat, participants in the survey of skeletal health[18] and seasonal variation[19] collected a 24 h urine sample in addition to a spot urine sample. These subjects were included stratified by age, sex, ethnicity and residence. None took diuretics, thyroxine, none were treated for hypertension, and none had renal disease. None of the participants were pregnant.

Inuit ethnicity was defined by having both parents born in Greenland and mixed ethnicity if one parent was born outside Greenland. Participants were divided into Inuit (both parents born in Greenland) and non-Inuit (at least one parent born outside Greenland) for some calculations. Participants with both parents born in Denmark were classified as Caucasians.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Ethics Committee for Medical Research in Greenland. Participants gave written informed consent before participation.

Procedures

In Nuuk, participants were visited at home. They were given careful written and verbal instructions for 24 h urine collection by one of the investigational doctors (S. A.). They were asked to empty the bladder completely and then the 24 h urine collection was started. The void sample just before the initiation of the 24 h sample was collected to represent the spot urine sample.

In Ilulissat, participants came to the hospital, and in Saqqaq, they came to the nursing station for instruction and initiation of urine collection. The void sample before the initiation of the 24 h sample was collected to represent the spot urine sample. They were given careful written and verbal instructions (S. A., P. L.). They took the first 80 mg of PABA, and were supplied with two additional doses of 80 mg to be taken with main meals.

All participants were given a bag large enough to contain two 2-litre collecting plastic containers with a handle, a jug that fitted under the toilet seat and a funnel.

Participants were visited in their homes for the final urine collection 24 h after the initiation of the urine collection. They were asked if the collection was complete, and if they had encountered any problems with the urine collection.

Participants were given no restrictions of diet or daily living.

Volumes were estimated by weight assuming a gravity of 1 g/ml, and 5 ml samples were stored at −20°C until analysis.

A physical examination was performed by the investigational doctors (S. A., P. L.). It included height without shoes and weight in indoor clothing.

Information regarding age and sex was obtained from the National Civil Registration System, while information regarding lifestyle and dietary habits was obtained by questionnaires. Participants were interviewed by a Greenlandic interpreter or by one of the investigational doctors completing a questionnaire in either Danish or Greenlandic by participant choice. Questions were asked as written in the questionnaires.

The intake of Inuit diet was recorded by an interview-based FFQ as described in detail previously[13,19]. It included seven traditional Inuit food items (seal, whale, wild fowl, fish, reindeer, musk ox and hare) and seven imported food items (pre-cooked meals, potatoes, vegetables, butter, cheese, egg and fresh fruit). These had been selected because they were typical to the diet in Greenland and they have been used previously. Frequencies were given in six categories from never to daily. Inuit food items scored positively and imported food items scored negatively. The sum of food frequency score for all food items consumed by each participant was calculated based on this recording, and participants were categorised into groups of intake of <40, 40–60 and >60 % traditional Inuit food item scores on a scale where 100 % was purely Inuit foods and 0 % was purely imported food. In addition, participants were asked how many days of the week the main meal was of Greenlandic food items and the number of days it was imported foods. This associated with the food frequency score[13,19]. Also, food frequency scores were validated by iodine as a biomarker of the intake of traditional Inuit foods[13]. Iodine was used as a biomarker of the intake of traditional Inuit foods as these are of marine origin and have been confirmed to be particularly rich in iodine[12].

Para-aminobenzoic acid

PABA (supplied by Unikem A/S) was used as a marker of completeness of urine collection. Its use is based on recovery in the urine of three 80 mg doses taken with meals[15,20]. It was used among sixty-five participants in Ilulissat and Saqqaq. The first dose of PABA was taken in the clinic at the start of urine collection, and the subsequent two doses were taken with main meals. Urine samples containing less than 78 % of PABA (187 mg/24 h) were designated incomplete as this was the level in single observations when 240 mg (three times 80 mg) of PABA were taken and the HPLC method was used for analysis[17].

One participant excreted only 32 % of the expected ingested dose and stated to have missed one of the three PABA doses. Two individuals had very low PABA recovery (3·9, 5·4 %) despite collection of expected urine volumes (2692, 1766 ml).

This suggested missing intake of PABA and they were excluded from the PABA compensations as recommended[16]. If PABA was below 187 mg/24 h (78 %), urine samples were considered incomplete. In such incomplete urine collections, iodine was compensated up to 93 % as recommended if the PABA excretion was above 120 mg/24 h[16,17].

Assays

Urine samples were analysed for PABA by the HPLC method as described in detail previously[17]. Urinary creatinine was measured by a kinetic Jaffé method[21]. Iodine was measured by the Sandell–Kolthoff reaction as described in detail previously[13,22].

Iodine concentration in the 24 h urine samples (μg/l) was multiplied by urine volume collected to yield the UIE (μg/24 h). Iodine excretion in spot urine was expressed as concentration in spot urine samples (urinary iodine concentration; UIC, μg/l), as a ratio of μg iodine per g creatinine (I/creatinine) (UICC), and as an estimated 24 h UIE (eUIE) by stratifying I/creatinine ratio for age, sex and origin-specific creatinine excretions[14].

Urine sample volumes were standardised to a 24 h collection time by dividing the volume by the actual collection time and multiplying by 24. The actual urine collection times varied between 23·2 and 24 h except a single sample of 17 h.

Statistical analysis

Results are given as medians with 25th and 75th percentiles. UIE in groups were compared using non-parametric statistics: Wilcoxon signed-rank test for paired comparisons of UIE in the same individual, Mann–Whitney U test for unpaired comparison of two groups, Kruskal–Wallis test for comparing several groups and Kendall's τ for the relation between groups. Proportions were compared using χ^2 test. The difference between iodine in 24 h urine samples (UIE, μg/24 h) and in spot urine samples (UIC, μg/l) was computed (UIE − UIC), and the deviations from zero among the differences between 24 h urine sample iodine (UIE) and the spot urine iodine measurements (UIC) was tested using the one-sample Kolmogorov–Smirnov test. The different measures of iodine in urine were compared as they are all used to portray the iodine nutrition by the same unit (μg). Further, a linear regression provided an excellent fit of ln-transformed spot urine given ln-transformed 24 h urine. Based on this linear regression, optimal predictions and 95 % prediction intervals were obtained for ln spot urine. Finally, these predictions and prediction intervals were back transformed to obtain predictions and prediction intervals for the difference between spot urine and 24 h urine illustrated in Fig. 1. The correlations between differences in iodine excretion estimates and iodine level in 24 h urine samples were tested using Spearman's ρ. The distribution was positively skewed, and for multiple linear regression analysis UIE data were ln-transformed (one-sample Kolmogorov–Smirnov test for normal distribution before/after ln-transformation: P=0·002/0·93). Linear regression models were then used with ln-transformed UIE entered as dependent variable. Independent variables entered for adjusted comparisons were age, sex, origin, diet and weight. Random selection of participants in Nuuk was performed using MedStat (Astra). Data were processed and analysed using Corel Quattro Pro 8 (Corel Corporation) and the Statistical Package for the Social Sciences version 13.0 (SPSS, Inc.). A P value less than 0·05 was considered significant.

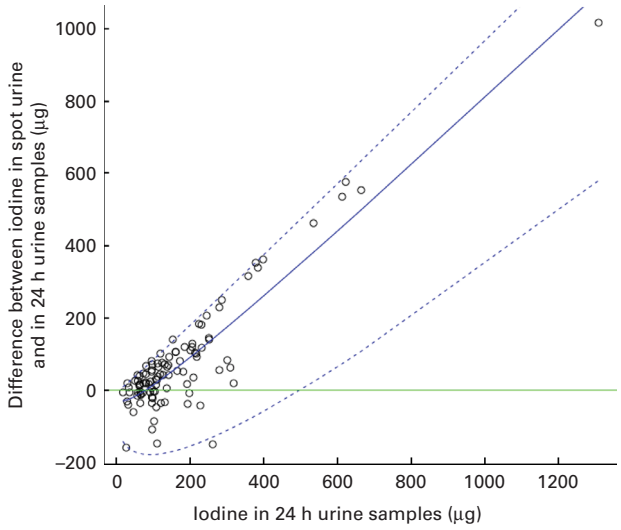

Fig. 1. Difference between iodine excretion in morning spot urine samples and 24 h urine samples plotted against 24 h urinary iodine excretion. The difference increased with rising 24 h iodine excretions. Negative values appeared when the 24 h urinary iodine excretion was below 250 μg, became frequent below 200 μg/24 h, and the association lost statistical significance in correlation analysis when the 24 h iodine excretion level was below 150 μg. Iodine concentration in the 24 h urine samples (UIC, μg/l) was multiplied by urine volume collected to yield the urinary iodine excretion (UIE, μg/24 h). Iodine excretion in spot urine was expressed as concentration in spot urine samples (UIC, μg/l). The solid line shows the optimal prediction of the difference between the iodine excretion in morning spot urine samples and 24 h urine samples given the iodine in the 24 h urine samples. The dashed blue lines show 95 % prediction intervals. The predictions and prediction intervals are based on a linear regression of ln-transformed iodine data. This explains the non-linear shape of the curves. A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn

Results

Table 1 lists participant characteristics. Participant inclusion in Ilulissat and Saqqaq was based on stratification according to age, sex and ethnicity. Non-Inuit were included in the town only. Non-Inuit were taller (P<0·001) and heavier (P=0·001) than Inuit, but Inuit and non-Inuit had similar BMI. More Inuit than non-Inuit were smokers (P=0·004), and Inuit had a more frequent intake of traditional Inuit food items (P<0·001; Table 1).

Para-aminobenzoic acid check

Incomplete 24 h urine collection was suggested in twenty-six out of sixty-five (40 %) of the 24 h urine samples in Ilulissat that used the PABA check, as the excretion was below 187 mg/24 h. Of the 24 h urine samples collected, twelve (18 %) had a PABA excretion below 50 % of the expected; fourteen urine samples (22 %) could be compensated.

Table 1. Characteristics of participants in the study of iodine excretion in spot- and 24 h urine samples among Inuit and non-Inuit in Greenland*

(Number of participants, or median values and 25th–75th percentiles)

	Inuit† (*n*)	Non-Inuit‡ (*n*)	Mix§ (*n*)	All (*n*)	*P*‖
Number of participants	78	20	6	104	
Men	43	13	1	57	
Women	35	7	5	47	
Height (cm)					<0·001
Median	163	177	170	167	
25th–75th percentiles	158–179	172–182	163–173	160–174	
Weight (kg)					<0·001
Median	71·4	84·2	70·1	72·7	
25th–75th percentiles	63·1–76·9	71·9–93·6	61·7–80·2	65·6–82·5	
BMI (kg/m^2)					NS
Median	26·0	26·2	25·4	26·0	
25th–75th percentiles	23·1–29·8	23·8–29·6	21·2–28·0	23·3–29·6	
Age groups					0·033
30–39	24	10	5	39	
40–49	14	6	1	21	
50–59	27	3	0	30	
60–69	13	1	0	14	
Residence					<0·001
City	35	1	2	36	
Town	24	19	3	48	
Settlement	19	0	1	20	
Traditional Inuit diet intake					0·086
<40 %	39	16	5	7	
40–60 %	32	4	1	37	
>60 %	7	0	0	60	
Days/week¶					<0·001
0–2	20	19	4	43	
3–4	31	1	1	33	
5–7	25	0	1	26	
Smoker¶**					0·004
Never	18	11	0	29	
Past	9	3	0	12	
Present	49	5	6	60	

* The study population has been described in detail in the report on creatinine excretion[14].
† An individual whose both parents were born in Greenland.
‡ An individual whose both parents were born outside Greenland.
§ An individual who had one parent born in Greenland.
‖ Inuit *v.* non-Inuit.
¶ Information missing in two Inuit.
** Information missing in one non-Inuit.

The iodine excretions with the different PABA check levels are listed in Table 2. Differences between PABA groups were not statistically significant.

Iodine excretion

Table 2 shows the iodine excretion in 24 h urine samples (UIE) and in spot urine samples (UIC). UIE was slightly higher in Inuit than in Caucasians. Iodine excretion was higher when it was compensated for incomplete urine collection according to the PABA check (excretions <187 but >120 mg/24 h). Thus, median UIE level rose from 173 (25th–75th percentile 64–286) to 234 (25th–75th percentile 91–413) μg in Inuit that were compensated (*n* 11) and from 95, 80–245 μg to 139, 127–381 μg in the three non-Inuit that were compensated (Inuit/non-Inuit; *P*=0·003/NS). The UIE level was 153, 97–251 μg/24 h (*n* 69) in Inuit and 102, 73–138 μg/24 h (*n* 25) in non-Inuit when including both the complete and the compensated 24 h urine samples (data not shown).

Iodine excretion in 24 h urine samples increased with a rising number of days with intake of traditional Inuit foods (Table 2) in the direct comparison (101/139/193 μg/24 h; *P*=0·002) as well as in the adjusted comparison (*P*=0·005). Ethnicity did not influence UIE after adjustment for diet. Spot urine samples (UICC) were influenced by ethnicity (*P*=0·002) and diet (*P*=0·007) after correction for creatinine while not when comparing the crude iodine content (UIC) of spot urine samples in the multivariate analysis.

The 24 h UIE differed from crude iodine content in spot urine samples (UIE *v.* UIC; 119 μg/24 h *v.* 74 μg/l; *P*<0·001), iodine in spot urine samples after creatinine correction (UIE *v.* UICC; 119 μg/24 h *v.* 78 μg/g; *P*<0·001) and estimated 24 h UIE (UIE *v.* eUIE; 119 μg/24 h *v.* 100 μg/24 h; *P*<0·001; Table 2). This difference is detailed in Table 3, and it increased with rising iodine excretion levels.

Fig. 1 shows that the magnitude of the difference between iodine excretion in 24 h urine samples and spot urine samples (UIE − UIC) paralleled the iodine excretion levels as evaluated from 24 h urine samples (UIE) (Spearman's $\rho = 0{\cdot}7$; *P*<0·001). The difference decreased with lower UIE levels. Negative differences were seen in 7 % of samples above 200 μg/24 h, 20 % of samples between 150 and 200 μg/24 h,

Table 2. Iodine excretion in 24 h urine samples and in spot urine samples
(Median values and 25th–75th percentiles)

		24 h Urine samples		Spot urine samples					
		UIE* (μg)		UIC† (μg/l)		UICC‡ (μg/g)		eUIE§ (μg/24 h)	
	n	Median	25th–75th percentiles	Median	25th–75th percentiles	Median	25th–75th percentiles	Median	25th–75th percentiles
All participants	104	119·1	80–213	74·0	45–114	77·9	56–125	100·2	64–136
PABA excretion (mg/24 h)									
>187	39	118·9	91–206	76·0	49–111	62·3	42–106	96·1	60–126
120–186	14	141·8	64–255	74·5	37–132	73·8	43–119	104·9	70–149
<120	12	98·2	65–123	69·5	44–102	74·9	55–109	86·7	73–134
Nuuk‖	39	141·0	80–251	75·0	40–130	108·7	74–165	100·3	64–180
Inuit	78	138·0	89–225	75·0	44–120	95·1	63–140	101·1	69–139
Mix	6	84·0	51–153	49·0	20–165	55·1	31–113	NA	NA¶
Caucasians	20	104·9	80–133	72·5	50–92	58·2	40–74	92·3	56–116
Men									
Inuit	43	136·8	92–227	78·5	44–117	92·4	58–119	127·0	75–158
Caucasians	13	107·9	76–172	78·0	54–91	51·6	36–72	96·1	66–108
Women									
Inuit	35	141·0	65–214	70·0	43–130	108·7	69–143	83·0	58–124
Caucasians	7	102·0	80–119	71·0	38–105	62·0	40–114	62·6	45–157
Traditional Inuit diet									
<40 %									
Inuit	39	139·2	64–218	69·5	42–110	89·9	65–139	92·7	61–136
Caucasians	16	104·9	82–118	72·5	55–92	58·2	40–98	98·3	56–142
40–60 %									
Inuit	32	129·1	93–212	80·5	41–126	91·1	57–121	110·2	68–130
Caucasians	4	150·9	52–241	63·5	41–98	53·1	35–68	74·0	56–106
>60 %									
Inuit	7	227·5	97–301	130·0	45–218	251·0	120–256	204·4	100–340
Caucasians	0	NA	NA	NA	NA	NA	NA	NA	NA
Days/week**									
0–2									
Inuit	20	100·9	60–150	55·0	32–125	78·3	57–147	88·7	59–187
Caucasians	19	102·0	80–119	74·0	54–93	57·0	40–74	88·5	55–118
3–4									
Inuit	31	139·2	92–224	68·0	40–110	86·5	57–115	83·9	59–128
Caucasians	1	NA	NA	NA	NA	NA	NA	NA	NA
5–7									
Inuit	25	193·4	100–347	91·0	65–211	110·6	83–171	123·6	89–154
Caucasians	0	NA	NA	NA	NA	NA	NA	NA	NA

UIE, urinary iodine excretion; UIC, urinary iodine concentration; UICC, ratio of urinary iodine concentration to creatinine concentration; eUIE, estimated 24 h urinary iodine excretion; PABA, *para*-aminobenzoic acid; NA, not applicable.
* 24 h UIE: concentrations multiplied by 24 h urine volume.
† Crude iodine content of spot urine samples.
‡ Iodine per creatinine in spot urine samples.
§ Estimated from age, sex and ethno-specific creatinine excretions[14] in spot urine samples.
‖ Sampling in Nuuk did not include the PABA check.
¶ Uncertain creatinine excretion for mixed origin.
** Missing information in two Inuit participants.

British Journal of Nutrition

Table 3. Differences between 24 h urine samples and spot urine estimates of iodine excretion (Median values and 25th–75th percentiles)

	Inuit		Non-Inuit		All				
	Median	25th–75th percentiles	Median	25th–75th percentiles	Median	25th–75th percentiles	*n*	*P**	*P*†	*P*‡
Spot urine iodine concentration (μg/l)	45·1	−3·1–109·0	18·9	−13·0–72·0	42·4	−4·0–101·4	103	<0·001	0·83	<0·001
By 24 h urine iodine (μg)										
<100	15·8	−14·8–40·4	−6·7	−15·6–18·9	10·7	−13·0–27·1	40	<0·001		
100–150	35·1	−3·5–72·5	44·9	−46·4–66·7	37·7	−3·6–71·0	23			
150–200	55·4	5·0–113·0	107·0	NA	68·6	11·0–110·3	10			
>200	143·8	90·7–355·6	181·6	119·5–207·0	143·8	90·7–342·5	30			
Spot urine iodine/creatinine (μg/g)	40·4	−10·0–107·0	55·9	11·0–65·0	41·5	−5·5–99·3	103	0·010	0·52	<0·001
By 24 h urine iodine (μg)										
<100	−3·4	−81·5–20·8	27·9	−3·9–47·5	4·1	−24·8–37·5	40	<0·001		
100–150	35·8	−9·9–66·1	56·9	−2·4–62·0	38·2	−2·4–62·9	23			
150–200	90·0	12·4–101·6	102·0	NA	94·6	27·0–102·3	10			
>200	151·4	99·4–295·8	160·0	80·7–200·8	151·4	95·8–287·5	30			
Estimated 24 h iodine excretion (μg/24 h)§	26·4	−21·0–111·0	12·4	−19·0–43·0	19·2	−21·3–93·8	97	0·005	0·49	0·003
By 24 h urine iodine (μg)										
<100	−6·8	−66·6–13·3	6·4	−15·9–17·5	−4·1	−54·1–13·1	35	<0·001		
100–150	10·6	−20·5–53·5	17·8	−39·0–22·4	13·4	−26·4–48·9	23			
150–200	88·7	−5·2–108·8	98·4	NA	90·3	34·6–105·2	10			
>200	139·3	85·7–325·9	118·6	−22·3–156·5	137·1	83·8–296·5	29			

NA, not applicable.
* *P* value for deviation from zero among Inuit was tested using the one-sample Kolmogorov–Smirnov test.
† *P* value for deviation from zero among non-Inuit was tested using the one-sample Kolmogorov–Smirnov test.
‡ *P* value for deviation from zero among all participants was tested using the one-sample Kolmogorov–Smirnov test.
§ Excluding 6 with mixed origin as creatinine adjustment was uncertain.

29 % of samples between 100 and 150 μg/24 h, 37 % of samples between 50 and 100 μg/24 h, and in 75 % of samples below 50 μg/24 h. Spot urine iodine (UIC) was markedly lower than 24 h iodine excretion (UIE) when the 24 h iodine excretion (UIE) was above 150 μg. The correlation between 24 h iodine excretion (UIE) and the difference between spot urine and 24 h urine iodine excretion (UIE − UIC) was non-significant when the 24 h UIE was below 150 μg.

Misclassification of iodine excretion occurred in 11 % when the 24 h UIE was below 150 μg, and misclassification occurred in 72 % when UIE was above 150 μg ($P=0{\cdot}03$). These numbers were similar for spot urine iodine content (UIC), iodine:creatinine ratio (UICC) and estimated 24 h iodine excretion (eUIE).

Discussion

We reported a systematic collection of 24 h urine samples among Inuit and non-Inuit in Greenland. The use of the PABA check provided a reliable new insight into the importance of Inuit ethnicity for iodine excretion. Furthermore, measurement of iodine in 24 h urine samples and the use of the validated urinary creatinine excretion[14] data indicated that spot urine samples should not be collected as morning urine samples among populations with an iodine intake above the level recommended by the WHO as misclassification of iodine excretion was frequent with high iodine intake levels.

It is cumbersome to collect 24 h urine samples and the PABA check suggested that 40 % of the collections in the present study were incomplete. This number is similar to previous findings[16], and it illustrates the consequences of the difficulties in obtaining complete and accurately timed urine collections. Also, it emphasises the low compliance with 24 h urine sampling. Thus, spot urine samples are a mainstay in everyday clinic and in population studies unless a check for completeness of 24 h sampling, such as the PABA check, is used to validate and possibly compensate the findings.

We included the PABA check and measured PABA using the HPLC method[17]. This method eliminates the risk of interference from aromatic amines from drugs such as sulphonamides and paracetamol that are co-determined when using the colorimetric method[15,17]. This contributed to the validity of our findings.

Creatinine concentration in casual urine sample is often used to standardise substance excretion in urine. The WHO recommendations on the assessment of iodine nutrition are based on iodine content of spot urine samples as correction for creatinine excretion is considered unreliable when protein intake is low[23]. Protein depletion accounts for populations in third world countries, but is rare in populations elsewhere. Correction for creatinine reduced variation in iodine excretion by 40 %[3], reduced the number of samples need by 20 %[6], and increased the reliability of studies of iodine nutrition[6] as confirmed in other populations[24] and after iodine fortification[25]. Thus, stratification according to factors important to creatinine such as age and sex has improved the validity of this adjustment[7], and adjusting for creatinine excretion is commonly adopted[26,27].

We recently showed that ethnicity influenced creatinine excretion by approximately 20 %[14]. Similarly, ethnicity has been reported to influence creatinine excretion among other groups. Thus, Blacks had a 5 % higher creatinine excretion per kilo body weight than Whites[28] as was found among other people of African descent[29], Pacific Islanders[30] and other populations in South East Asia[31]. Thus, the creatinine adjustments among populations in Greenland should be stratified for ethnic origin in addition to age and sex to enhance the reliability of the correction.

The traditional Inuit hunter diet is rich in iodine[12]. This caused a high-normal iodine intake among populations in Greenland mainly living on the traditional diet[13]. It was reported as estimated 24 h UIE because correction for creatinine excretion reduces variation in iodine[3] and hence increases the reliability of the estimated excretion[6,24,25]. The iodine nutrition survey in Greenland found a higher iodine intake level than what was suggested from the spot urine samples in the present study, but rather in resonance with the 24 h UIE. The cause for this discrepancy is described by the plot of the difference in iodine between spot urine and 24 h urine against the 24 h UIE. The increasing discrepancy with rising iodine intake levels are probably due to the fact that spot urine samples were collected in the morning hours in the present study. These urine samples thus reflect the iodine intake during late evening and night as iodine is excreted within a few hours after ingestion[9,11]. This extends the findings of a recent investigation that found a limited influence of time of spot urine samples[32] into higher iodine intake levels and explains the lower iodine excretion in the morning urine also demonstrated earlier[10]. Hence, morning urine samples do not include the excretion of iodine from the main meals, which in Greenland may be very rich in iodine[12]. The higher iodine excretion in the iodine nutrition survey in Greenland is most probably due to the fact that these spot urine samples were collected through all day and early evening and hence included iodine excreted after the main meals. The findings are likely to be similar when the excessive iodine intake is from drinking-water even though it may be consumed more evenly over the day as it is readily absorbed[8,11].

The iodine excretion level about which the iodine intake estimation method was no longer markedly influenced by sampling hour was 150 μg/d. This supports the reliability of the use of spot urine samples in studies of iodine deficiency while studies suggesting excessive iodine intake estimated from morning spot urine samples should be interpreted more cautiously as the true iodine intake level may be even higher than estimated from morning urine samples. Conversely, it may be suggested that if iodine excess is in focus, spot urine samples should not be collected during early morning hours.

Recent years have seen a focus on the importance of monitoring iodine intake levels in populations after iodine fortification[33], in populations that are iodine replete[34] and in populations with excessive iodine intake[35]. This is emphasised by the rising awareness of the possible adverse health consequences of an iodine intake at[36] or above[37,38] the recommended level. Our data imply that the use of morning spot urine samples may reduce the reliability of the estimated iodine intake levels in such populations as we found misclassification of iodine nutrition markedly more frequent at the higher iodine excretion level compared with the lower iodine excretion level. This finding was validated by the PABA check, which was measured using the more cumbersome HPLC method that reduced the risk of interference in the analysis.

In conclusion, early morning spot urine samples provide a useful classification of iodine nutrition when iodine deficiency is in focus. If iodine excretion is above the recommended level, then the iodine intake level is probably to be underestimated if spot urine samples are collected during early morning hours.

Acknowledgements

The authors thank Anne Marie Christensen at the hospital in Ilulissat for assisting in careful collection of 24 h urine samples in Ilulissat.

The present study was financially supported by grants from the Greenland Government and the Karen Elise Jensen foundation. The funders had no role in the design and analysis of the study or in the writing of the article.

The authors' contributions are as follows: S. A. involved in the conception of idea, study design, raising of funds, data collection, analysis and interpretation of the data, and writing of the manuscript; R. W. involved in the analysis and interpretation of the data, and reviewing of the manuscript; P. L. involved in the study design, collection of data, raising of funds, interpretation of the data and reviewing of the manuscript. All authors had full access to all of the study data, and took responsibility for the integrity of the data and the accuracy of the data analysis.

There are no conflicts of interest.

References

1. Garde AH, Hansen AM, Kristiansen J, *et al.* (2004) Comparison of uncertainties related to standardization of urine samples with volume and creatinine concentration. *Ann Occup Hyg* **33**, 171–179.
2. Bingham SA & Cummings JH (1985) The use of creatinine output as a check on the completeness of 24-hour urine collections. *Hum Nutr Clin Nutr* **39**, 343–353.
3. Andersen S, Pedersen KM, Pedersen IB, *et al.* (2001) Variations in urinary iodine excretion and thyroid function. A 1-year study in healthy men. *Eur J Endocrinol* **144**, 461–465.
4. Haddow JE, McClain MR, Palomaki GE, *et al.* (2007) Urine iodine measurements, creatinine adjustment, and thyroid deficiency in an adult United States population. *J Clin Endocrinol Metab* **92**, 1019–1022.
5. Ohira S-I, Kirk AB, Dyke JV, *et al.* (2008) Creatinine adjustment of spot urine samples and 24 h excretion of iodine selenium, perchlorate, and thiocyanate. *Environ Sci Technol* **42**, 9419–9423.
6. Andersen S, Karmisholt J, Pedersen KM, *et al.* (2008) Reliability of studies of iodine intake and recommendations for number of samples in groups and in individuals. *Br J Nutr* **99**, 813–818.
7. Vejbjerg P, Knudsen N, Perrild H, *et al.* (2009) Estimation of iodine intake from various urinary iodine measurements in population studies. *Thyroid* **19**, 1281–1286.

8. Andersen S, Pedersen KM, Iversen F, *et al.* (2008) Naturally occurring iodine in humic substances in drinking water in Denmark is bioavailable and determines population iodine intake. *Br J Nutr* **99**, 319–325.
9. Rasmussen LB, Ovesen L & Christiansen E (1999) Day-to-day and within-day variation in urinary iodine excretion. *Eur J Clin Nutr* **53**, 401–409.
10. Als C, Helbling A, Peter K, *et al.* (2000) Urinary iodine concentrations follows a circadian rhythm: a study with 3023 spot urine samples in adults and children. *J Clin Endocrinol Metab* **85**, 1367–1369.
11. Keating FR & Albert A (1949) The metabolism of iodine in man as disclosed with the use of radioiodine. *Rec Prog Horm Res* **4**, 429–481.
12. Andersen S, Hvingel B & Laurberg P (2002) Iodine content of traditional Greenlandic food items and tap water in East and West Greenland. *Int J Circumpolar Health* **61**, 332–340.
13. Andersen S, Hvingel B, Kleinschmidt K, *et al.* (2005) Changes in iodine excretion in 50–69-y-old denizens of an Arctic society in transition and iodine as a biomarker of the frequency of consumption of traditional Inuit foods. *Am J Clin Nutr* **81**, 656–663.
14. Andersen S, Dehnfeld M & Laurberg P (2015) Ethnicity is important for creatinine excretion among Inuit and Caucasians in Greenland. *Scand J Clin Lab Invest* **75**, 44–50.
15. Bingham S & Cummings JH (1983) The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clin Sci* **64**, 629–635.
16. Johansson G, Bingham S & Vahter M (1999) A method to compensate for incomplete 24-hour urine collections in nutritional epidemiology studies. *Publ Health Nutr* **2**, 587–591.
17. Jakobsen J, Ovesen L, Fagt S, *et al.* (1997) *Para*-aminobenzoic acid used as a marker for completeness of 24 hour urine: assessment of control limits for a specific HPLC method. *Eur J Clin Nutr* **51**, 514–519.
18. Andersen S, Boeskov E & Laurberg P (2005) Ethnic differences in bone mineral density between Inuit and Caucasians in North Greenland are caused by differences in body size. *J Clin Densitom* **4**, 409–414.
19. Andersen S, Jakobsen A & Laurberg P (2013) Vitamin D status in North Greenland is influenced by diet and season: indicators of dermal 25-hydroxy vitamin D production north of the Arctic Circle. *Br J Nutr* **110**, 50–57.
20. Williams DRR & Bingham SA (1986) Sodium and potassium intakes in a representative population sample: estimation from 24 h urine collections known to be complete in a Cambridgeshire village. *Br J Nutr* **55**, 13–22.
21. Bartels H, Bohmer M & Heierli C (1972) Serum creatinine determination without protein precipitation. *Clin Chim Acta* **37**, 193–197.
22. Laurberg P (1987) Thyroxin and 3,5,3′-triiodothyronine content of thyroglobulin in thyroid needle aspirates in hyperthyroidism and hypothyroidism. *J Clin Endocrinol Metab* **64**, 969–974.
23. World Health Organization (2007) *Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination: A Guide for Programme Managers*, 3rd ed. Geneva: WHO (WHO/UNICEF/ICCIDD).
24. König F, Andersson M, Hotz K, *et al.* (2011) Ten repeated collections for urinary iodine from spot samples or 24 hour samples are needed to reliably estimate individual iodine status in women. *J Nutr* **141**, 2049–2054.
25. Karmisholt J, Laurberg P & Andersen S (2014) Recommended number of participants in nutrition studies are similar before and after an iodine fortification programme. *Eur J Clin Nutr* **53**, 487–492.
26. Kesteloot H & Joossens JV (1996) On the determinants of the creatinine clearance: a population study. *J Hum Hypertens* **10**, 245–249.
27. Kampmann J, Siersbæk-Nielsen K, Kristensen M, *et al.* (1974) Rapid evaluation of creatinine clearance. *Acta Med Scand* **196**, 517–520.
28. James GD, Sealey JE, Alderman M, *et al.* (1988) A longitudinal study of urinary creatinine and cretinine clearance in normal subjects. *Am J Hypertens* **1**, 124–131.
29. Taylor EN & Curhan GC (2007) Differences in 24-hour urine composition between black and white women. *J Am Soc Nephrol* **18**, 654–659.
30. Eisner BH, Porten SP, Bechias SK, *et al.* (2010) The role of race in determining 24-hour urine composition in white and Asian/Pacific Islander stone formers. *J Urol* **183**, 1407–1411.
31. Mente A, Honey RJD, McLaughlin JR, *et al.* (2007) Ethnic differences in relative risk of idiopathic calcium nephrolithiasis in North America. *J Urol* **178**, 1992–1997.
32. Perrine CG, Cogswell ME, Swanson CA, *et al.* (2014) Comparison of population iodine estimates from 24-hour urine and timed-spot urine samples. *Thyroid* **24**, 748–757.
33. Laurberg P, Jørgensen T, Perrild H, *et al.* (2006) The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. *Eur J Endocrinol* **155**, 219–228.
34. Andersen S, Iversen F, Terpling S, *et al.* (2012) Iodine deficiency influences thyroid autoimmunity in old age – a comparative population-based study. *Maturitas* **71**, 39–43.
35. Teng W, Shan Z, Guan H, *et al.* (2006) Effect of iodine intake on thyroid diseases in China. *N Engl J Med* **354**, 2783–2793.
36. Andersen S, Iversen F, Terpling S, *et al.* (2009) More hypothyroidism and less hyperthyroidism with sufficient iodine nutrition compared to mild iodine deficiency – a comparative population-based study of older people. *Maturitas* **64**, 126–131.
37. Lv S, Xu D, Wang Y, *et al.* (2014) Goitre prevalence and epidemiological features in children living in areas with mildly excessive iodine in drinking-water. *Br J Nutr* **111**, 86–92.
38. Teng X, Shan Z, Chen Y, *et al.* (2011) More than adequate iodine intake may increase subclinical hypothyroidism and autoimmune thyroiditis: a cross-sectional study based on two Chinese communities with different iodine intake levels. *Eur J Endocrinol* **146**, 943–950.