Seasonal effects on urinary iodine concentrations in women of reproductive age: An observational study in Tanzania and South Africa

Leonie Arns-Glaser,[1] Lizelle Zandberg,[2] Vincent D Assey,[3,4] Jeannine Baumgartner,[1,2] Fatma Abdallah,[4,5] Valeria Galetti,[1] Omar Dary,[6] Michael B Zimmermann,[1,4] and Maria Andersson[4,7]

[1]Human Nutrition Laboratory, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland; [2]Centre of Excellence for Nutrition, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa; [3]Nutrition Services Section Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, Tanzania; [4]Iodine Global Network, Ottawa, Canada; [5]Tanzania Food and Nutrition Centre, Dar es Salaam, Tanzania; [6]Division of Nutrition and Environmental Health, Office of Maternal and Child Health and Nutrition, Bureau for Global Health, United States Agency for International Development, Washington, DC, USA; and [7]Nutrition Research Unit, Children's Research Centre, University Children's Hospital Zurich, Zürich, Switzerland

ABSTRACT

Background: Iodine intake in populations is usually assessed by measuring urinary iodine concentrations (UICs) in spot samples. Hot climate conditions may reduce urine volume, thus leading to overestimations of UIC and thereby masking inadequate iodine intake.

Objectives: We investigated the effects of season on UICs in 2 populations exposed to high-temperature climates.

Methods: In this observational study, we examined women (18–49 years) in Tanzania ($n_{cold} = 206$; $n_{hot} = 179$) and South Africa ($n_{cold} = 157$; $n_{hot} = 126$) during cold and hot seasons. From each woman in both seasons, we obtained two 24-hour urine collections and 2 spot urine samples, as well as salt, water, and cow's milk samples. We measured the urine volume, UIC, and urinary creatinine concentration (UCC). The 24-hour urinary iodine excretion (UIE) was calculated and used to estimate the iodine intake. We used linear mixed-effects models to test for differences between seasons.

Results: In Tanzanian women, we observed no seasonal effect on the urine volume, 24-hour UIE, 24-hour UIC, spot UIC, spot UIC:UCC ratio, or salt iodine concentration. In South African women, the median 24-hour urine volume was 1.40 L (IQR, 0.96–2.05 L) in the winter and 15% lower in the summer ($P < 0.001$). The median 24-hour UIE was 184 μg/day (IQR, 109–267 μg/day) in the winter and 34% lower in the summer ($P < 0.001$), indicating a lower iodine intake. As a result, UICs did not significantly differ between seasons in 24-hour collections and spot samples, whereas the spot UIC:UCC ratio differed by 21% ($P < 0.001$) and reflected the lower iodine intake. In both study populations, the within- and between-person variabilities in urine volume, 24-hour UICs, and spot UICs were higher than the variability between seasons.

Conclusions: Spot UIC may slightly overestimate the iodine intake in hot temperatures due to concentrated urine, and methods to correct for urine volume may be considered. Local seasonal differences in iodine intakes may also occur in some populations. This trial was registered at clinicaltrials.gov as NCT03215680. *Am J Clin Nutr* 2022;115:298–309.

Keywords: iodine, urinary iodine concentration, urine volume, season, climate, women, Tanzania, South Africa

Introduction

Iodine adequacy in populations is conventionally assessed in cross-sectional studies by measuring urinary iodine concentrations (UICs) in spot urine samples (1). The obtained median UIC is evaluated against recommended thresholds (1). The UIC is considered a reliable biomarker of iodine intake, as >90% of dietary iodine is excreted in the urine within 24 hours after consumption (2, 3). However, urine samples may vary in dilution depending on the individual hydration state at the time of collection, which in turn may affect the UIC (4, 5).

This work was supported by the US Agency for International Development (Washington, DC), the Nutrition Section of the United Nations International Children's Emergency Fund (New York, NY), the Iodine Global Network (Ottawa, Canada), the Swiss-African Research Cooperation (SARECO) of University Basel (Basel, Switzerland), and ETH Zurich (Zürich, Switzerland).

The funders had no influence on the implementation, analysis, or interpretation of the data. The views and statements in this article are the authors' own and do not necessarily represent the views or policies of the Iodine Global Network, US Agency for International Development, or US government.

Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to MA (e-mail: maria.andersson@kispi.uzh.ch).

Abbreviations used: GPAQ, global physical activity questionnaire; MET, metabolic equivalent; SIC, salt iodine concentration; UCC, urinary creatinine concentration; UCE, urinary creatinine excretion; UIC, urinary iodine concentration; UIE, urinary iodine excretion; UNaC, urinary sodium concentration; UNaE, urinary sodium excretion.

Received March 30, 2021. Accepted for publication September 24, 2021.

First published online October 28, 2021; doi: https://doi.org/10.1093/ajcn/nqab327.

Low urine volumes may overestimate the iodine intake and mask the risk of iodine deficiency, whereas large urine volumes may underestimate the iodine intake. Methods to correct for urine dilution in spot UICs have been suggested (6–8), but uncorrected spot UICs remain the most commonly used measure for population iodine intake (9).

Urine volumes vary between countries and cultures and are typically influenced by a combination of several different factors (10–14). Hot temperatures and physical activity may decrease urine volumes due to higher fluid losses via sweat, whereas large fluid intakes, cold temperatures, and hypoxia may increase the urine output (15, 16). Cross-sectional studies in adults observed lower urine volumes in Mediterranean populations compared to populations in central Europe with a more temperate climate (12, 16–18). This difference was primarily explained by lower fluid intakes in the Mediterranean populations, not due to temperature differences between countries. Although fluid intakes were higher during the summer in all countries (16, 17), there was no difference in urine volume between the winter and summer seasons, possibly due to higher fluid loss via sweat in the summer (16–19).

Seasonal variability in UIC is frequently reported (20–22) and has mainly been attributed to seasonal differences in the iodine intake. Cow's milk and dairy products are important dietary iodine sources in many countries, and the iodine concentration in cow's milk is typically higher during the winter, when cattle are fed fodder enriched with iodine (23). However, the effects of temperature differences between seasons on the hydration status and UIC remain unclear.

Our study objective was to investigate the seasonal influence on UICs in women exposed to moderate- to high-temperature climates. We enrolled women of reproductive age in Tanzania and South Africa and assessed participants in the cold and hot seasons. We evaluated seasonal differences in dietary iodine intakes by assessing the total urinary iodine excretion (UIE) over 24 hours. We measured the 24-hour urine volume and hypothesized that the daily urine volume decreases in the hot season, leading to more concentrated urine and a higher UIC, which overestimates the iodine intake.

Methods

Study design

We conducted an observational study in healthy, nonpregnant, and nonlactating women (18–49 years) at 2 study sites with known marked temperature differences between seasons. We obtained 2 spot urine samples (days 1 and 4) and two 24-hour urine collections (days 2 and 3) over 4 different days in both the cold and hot seasons. We measured UICs and urinary creatinine concentrations (UCCs) in all urine samples. In 24-hour urine collections, we measured urine volume and calculated the daily UIE to estimate the daily iodine intake.

We assumed iodized salt to be the primary dietary source of iodine in both populations, although drinking water and cow's milk may also contribute. All participants provided 1 household salt sample, 1 drinking water sample, and 1 cow's milk sample in each season for determination of the iodine concentrations and assessment of potential seasonal differences. We administered 3 questionnaires to obtain information on: *1*) general subject characteristics; *2*) dietary patterns and beverage consumption; and *3*) physical activity.

Study sites

The study was carried out in the division of Kilema North in the Moshi, Kilimanjaro region in Tanzania and Potchefstroom in South Africa, 2 areas with cold winters (night 0°C/day 15°C) and hot summers (night 15°C/day 30°C). We obtained the daily minimum and maximum temperatures over 24 hours. In Tanzania, we measured outdoor temperatures with a thermometer at the study center, and in South Africa, we obtained the information from the local weather station.

Salt iodization is well established in both study locations and the iodine intake in the general population is considered adequate (24–28). Tanzania introduced mandatory salt iodization (using iodate) in 1995 at 20–80 mg/kg and revised the iodine concentration to 40–80 mg/kg in 2010 (24). The most recent national survey in 2015–2016 found a median salt iodine concentration (SIC) of 26 mg/kg and 61% of samples with an SIC >15 mg/kg, with large variations across the country (25). The nationwide median UIC in women of reproductive age was 180 μg/L (25). In the Kilimanjaro region, the median SIC was 37 mg/kg, 89% of the salt samples had an SIC >15 mg/kg, and the median UIC in women was 186 μg/L (25). South Africa introduced voluntary iodization of table salt in 1954 at 10–20 mg/kg. In 1995, mandatory table salt iodization (using iodate) was established at a level of 40–60 mg/kg salt, but the allowed range was broadened to 35–65 mg/kg in 2006 (26). Iodization of salt for food production or for animal consumption remains voluntary (26). A national study in 2005 reported 77% household coverage of salt, with an SIC >15 mg/kg (27) and a median UIC in women of reproductive age of 177 μg/L (26). A more recent national study carried out in 2015 reported a median UIC of 130 μg/L in adults (18–90 years) (8). A national demographic and health survey in 2016 reported an SIC >15 mg/kg in 89% of the household (28).

Subjects

Following a standard protocol for both study sites, participants were volunteers recruited as a convenience sample from the local communities. In Tanzania, subjects were typically farm workers, health workers, teachers, and students from a local boarding school. In South Africa, participants were recruited in central Potchefstroom and surrounding townships by local fieldworkers.

The inclusion criteria were: female, age between 18–49 years, nonpregnant and nonlactating, in generally good health as assessed by no reported chronic disease, nonsmoking, no known history of major medical illnesses or thyroid dysfunction, no use of X-ray/computed tomography contrast agents or iodine-containing medication within the last year, no use of iodine-containing disinfectants during the last 6 months, residence at the respective study site for at least a month, and consent to voluntarily participate in the study. In South Africa, women living with HIV were enrolled if they reported current antiretroviral treatment. Women who became pregnant or moved to a different area during the study were excluded from follow-up.

Sample size calculation

To our knowledge, no previous data are available reporting seasonal effects of temperature differences on UICs. Therefore, we applied sample size recommendations for cross-sectional UIC studies and aimed to enroll a convenience sample of 100 women at each study site location to determine the median UIC with 10% precision for each study site (29, 30). We assumed improved power thanks to the repeated sample collection. To account for an estimated drop-out rate of 30%, we aimed to recruit 150 women per study site.

Ethics

We obtained ethical permission from the Ethics Review Committee of ETH Zurich and locally from the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol IX/2503) and Health Research Ethics committee of the North-West University in South Africa (NWU-00,134–17-S1). Written informed consent was obtained from all participating women. All collected data were pseudonymized before analysis. The study was registered at clinicaltrials.gov as NCT03215680.

Study procedures

In Tanzania, the study was conducted in July 2017 (cold season) and October 2017 (hot season). In South Africa, the study times were July to August 2018 (cold season) and November 2018 (hot season). The study procedures were identical for both study sites and repeated during both seasons. The study period was 4 days for each season.

Anthropometrics.

Heights and weights were measured using standard anthropometric techniques on the first day of each study period (31).

Questionnaires.

The 3 questionnaires were completed by the field workers as an interview in the participant's language. The first questionnaire obtained information on socio-economic status, general health characteristics, knowledge and use of iodized salt, use of dietary supplements, and cigarette smoking and was administered once each season. The second was a dietary 24-hour recall questionnaire, completed at 3 different days in each season (days 1, 3, and 4). Women's fluid intake (beverages only) was assessed quantitatively. We obtained qualitative information of the intakes of selected foods, including iodine-containing foods, and used the data to assess differences in food habits between seasons. Once per season, women also completed a global physical activity questionnaire (GPAQ), which assessed physical activity via metabolic equivalents (METs) (32). We analyzed the GPAQ as described by Armstrong and Bull (32). The WHO recommendation on physical activity for health is to achieve at least 600 MET-minutes per week (33). An individual activity level <600 MET-minutes per week was categorized as sedentary.

Urine samples.

In both seasons, we obtained 2 spot urine samples (days 1 and 4) and two 24-hour urine collections (days 2 and 3) from each participant. The spot urine samples were collected at any time of the day (except the first morning void) on 2 nonconsecutive days: on day 1 before the first 24-hour urine collection and on day 4 after finishing the second 24-hour collection. Women were given a plastic cup and asked to provide ~20 mL of midstream urine. We provided 2 opaque 2.7-L collection containers with lids for each 24-hour collection and a 600-mL beaker to ease the urine collection. We stressed the importance of collecting all urine and avoiding spillage. The participants were instructed to store the container in a dark space and to return the container to the study center immediately after the 24-hour collection. We accepted collections stored up to 48 hours. The urine container was not cooled during the 24-hour collection, but measured variables are assumed to remain stable over the sample collection period (34). In Tanzania, the urine containers were brought by the women to the health center, and pick-up service was provided if needed. In South Africa, the containers were picked up from the participants by the fieldworkers or study assistants. The filled urine containers were weighed on return to the health center (Tanzania) or facilities of the North-West University (South Africa) with 5 g precision. Completeness of the 24-hour urine collection was assessed using the 24-hour urinary creatinine excretion (UCE) and urine volume. Incomplete 24-hour urine collection was defined as a 24-hour UCE < 0.57 g/d or as a UCE < 0.68 g/d and a urine volume < 1.0 L (35–37).

All urine samples were aliquoted into 2.0-mL Eppendorf tubes without filtration. In Tanzania, samples were transported in a cool box from the place of collection to the local storage freezer, transported frozen to the laboratory of the Tanzania Food and Nutrition Centre, and stored frozen at −20°C until analysis. In South Africa, samples were aliquoted and frozen at −20°C in the facilities of North-West University. One aliquot from each urine sample and each site was sent to ETH Zurich for the analysis of UCC.

Salt samples.

We asked all participants to provide a handful (50 g) of their regular household salt in a designated plastic container on day 4 in each study period for an assessment of the iodine concentration. The salt containers were stored tightly closed at room temperature until analysis.

Drinking water.

We asked women to collect 20 mL of household drinking and cooking water in provided plastic containers on study day 3 in each season. We prepared 2-mL aliquots of the water samples in Eppendorf tubes and stored them frozen until an analysis of the iodine concentration.

Cow's milk.

Women provided a 20-mL sample of cow's milk (fresh or powder) or drinking yogurt from a regularly consumed product identified in their FFQ on study day 3 each season in provided plastic containers. Milk samples were transported cold from the place of collection, separated into aliquots, frozen at −20°C,

and shipped frozen to ETH Zurich for an analysis of the iodine concentration.

Biochemical analysis

Urinary iodine and water iodine concentrations.

The iodine concentrations in urine and water were analyzed in duplicate at the Tanzania Food and Nutrition Centre (Dares Salaam, Tanzania) and North-West University (Potchefstrooom, South Africa) using the Pino-modification of the Sandell-Kolthoff method (38). The laboratory in Tanzania successfully participates in the Program to Ensure the Quality of Urinary Iodine Procedures (US CDC), including its quarterly external validation. The South African laboratory is not participating due to legal restrictions for sample imports. In Tanzania, the inter-assay variability results were 10% at 75 μg/L ($n = 81$), 8% at 133 μg/L ($n = 81$), and 6% at 228 μg/L ($n = 80$). The Tanzanian laboratory measured 2 external control urine samples provided by ETH Zurich: the inter-assay variability results of the low and high controls done at the South African laboratory were 8% at 98 μg/L ($n = 33$) and 6% at 290 μg/L ($n = 51$). Two further external control samples were measured by both laboratories to assess the analytical agreement in UICs between the study sites. The first urine sample was measured at 68 μg/L ($n = 13$; 13% inter-assay variability) in Tanzania and at 60 μg/L ($n = 6$; 4% inter-assay variability) in South Africa. The second urine sample was measured at 183 μg/L ($n = 17$; 4% inter-assay variability) in Tanzania and 181 μg/L ($n = 7$; 2% inter-assay variability) in South Africa. Urine samples were measured separately for each season.

Urinary creatinine concentration.

UCCs were measured in duplicate at ETH Zurich using the Jaffé reaction (39). In-house controls were used to ensure the quality of the UCC measurements. The between-assay variability results were 2.7% at 0.90–1.20 g/L ($n = 100$) and 2.5% at 1.91–2.18 g/L ($n = 100$). We assumed that UCCs remain stable under the described sample collection and storage conditions (34).

Urinary sodium concentration.

The urinary sodium concentration (UNaC) was measured in the South African 24-hour urine collections at Neuberg Global (Amanzimtoti, South Africa) using the indirect ion-selective electrode method (40).

Salt iodine concentration.

In Tanzania, the iodine concentration in salt was measured by iodometric titration (41), placing 5 g of salt in 25 mL of water. The CVs of repeated measures in different runs were 5% at 23.2 mg/kg ($n = 8$), 5% at 43.1 mg/kg ($n = 8$) and 2% at 76.4 mg/kg ($n = 8$). In South Africa, the SIC was measured using iCheck Iodine, solubilizing 10 g of salt. The CV between runs was 17% (BioAnalyt) (42). The salt was defined as noniodized when the iodine concentration was <5 mg/kg and was presented as the percentage <15 mg/kg to illustrate the distribution in SIC. The median concentration was calculated from salt samples with an SIC >5 mg/kg (43).

Cow's milk iodine concentration.

We analyzed the iodine concentrations in cow's milk and yogurt samples by multi-collector inductively coupled plasma mass spectrometry at ETH Zurich as previously described (44). We used whole-milk powder reference material (NIST SRM1549a whole milk powder; National Institute of Standards and Technology) as an external control. The average iodine concentration of the reference material was well within the certified acceptable range (3040–3640 μg/kg) for all measurements. The samples from the 2 sites were measured in 2 different batches. The mean ± SD iodine concentrations of the National Institute of Standards and Technology reference materials in the first ($n = 14$ runs; Tanzanian) and second ($n = 18$ runs; South Africa) batches were 3396 ± 33 μg/kg (CV, 1.0%) and 3110 ± 78 μg/kg (CV, 2.5%), respectively.

Statistical analysis

The primary outcome was UICs from spot urine samples. Secondary outcomes were the urine volume of the 24-hour collections, 24-hour UIC, 24-hour UIE, spot UCC, 24-hour UCC, 24-hour UCE, and the UIC:UCC ratio in spot samples, as well as the 24-hour UNaC (South Africa). We calculated the 24-hour UIE, 24-hour UCE, and 24-hour urinary sodium excretion (UNaE) by multiplying the 24-hour UIC, 24-hour UCC, and 24-hour UNaC with the urine volume of the 24-hour collections.

We used Excel 2010 (Microsoft) and R version 3.6.2 (45), using the packages "boot" (46, 47), "lattice" (48), "lmerTest" (49), "multcomp" (50), and "ggplot2" (51) for data processing and analysis.

Data for all enrolled subjects were included in the data analysis. We ran descriptive statistics for all variables by study site and sample time point. No outliers were removed from the data set. Incomplete 24-hour urine collections were excluded from the data analysis (**Supplemental Table 1**). The analysis was done using available data at each sampling time point, without imputing missing data. We assessed normality by visual inspection and by testing the distributions of continuous variables against a normal distribution using the Shapiro-Wilks test. We transformed skewed data before analysis [ln (x + 1)]. Normally distributed continuous data are presented as means (95% CIs), data that followed a normal distribution after log-transformation are presented as geometric means (95% CIs), and data that remained skewed after transformation are presented as medians (IQRs). Nonparametric 95% CIs around the median were obtained using the bootstrap technique ($n = 1000$). We tested differences between paired nonnormally distributed data using the Wilcoxon signed-rank test and between unpaired nonnormally distributed data using the Kruskal-Wallis test. Further, we used Fisher's exact test for count data for the analyses of contingency tables for water iodine concentration >15 μg/L, due to the small number of samples with high iodine concentrations. For all other analyses of contingency tables, we used Pearson's chi-square test for count data.

We assessed the effects of season on continuous variables (spot and 24-hour UICs, spot and 24-hour UCCs, 24-hour urine volume, 24-hour UIE, 24-hour UCE, and spot UIC:UCC ratio) using linear mixed-effects models and transformed data. We used the maximum likelihood procedure for the estimation

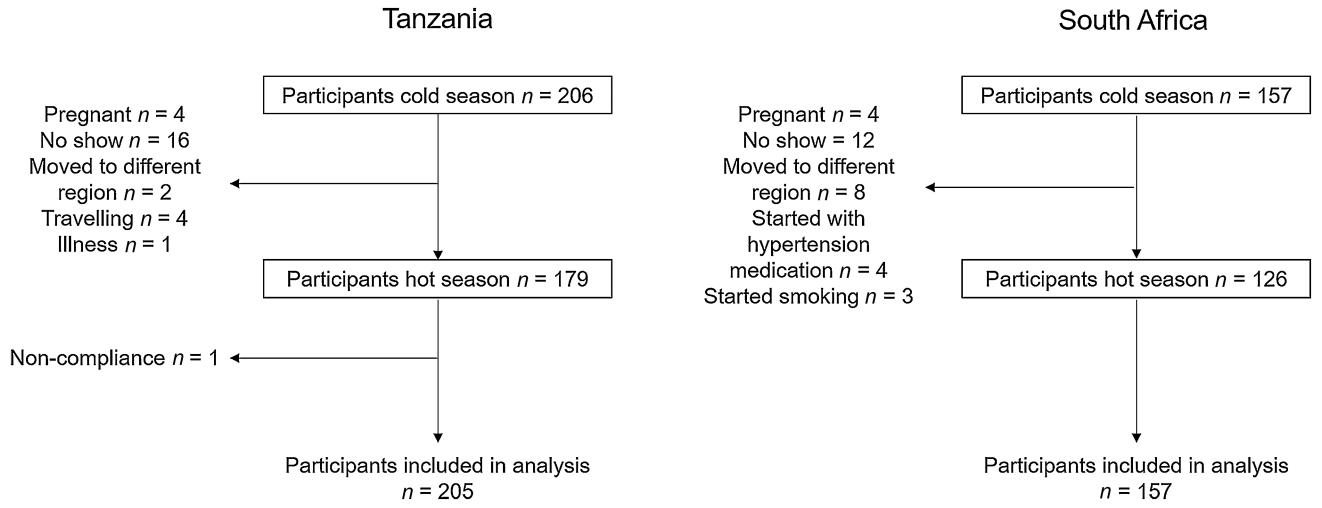

FIGURE 1 Study flow.

of variance. The analyses were conducted in 2 steps. First, we included data for both study sites. We defined site and season as fixed effects, with a site-season interaction term. We estimated the random effect of subject (1|subject) and random interaction of subject and season (1|subject:season). We assumed that the residuals from the random effects correspond to the within-person differences within season (day-to-day variability). Second, we conducted a site-specific analysis (same parameters as listed above, plus 24-hour UNaC and 24-hour UNaE for South African women). In this analysis, we defined season as a fixed effect and the random terms remained the same. The fixed effect assessed differences between the seasons. To quantify seasonal differences on the original scale, we pooled data for the 2 time points of each season, calculated the overall median per season, and obtained the difference between medians for cold and hot seasons. The P values for the fixed effects of the linear mixed-effects models were obtained by likelihood ratio tests. A P value < 0.05 was considered significant.

We calculated the relative variance ratio (VR; %) for the seasonal variability [$V_{Seasonal}$; i.e., seasonal variability for each person (fixed effect)], between-person variability [$V_{Between\text{-}person}$ (1|subject)], and within-person variability [$V_{Within\text{-}person}$ (1|subject:season)] from the linear mixed-effect model for each outcome parameter by study site using the following formulas:

$$VR_{Seasonal} = \frac{V_{Seasonal}}{V_{Seasonal} + V_{Between-person} + V_{Within-person}} \quad (1)$$

$$VR_{Between-person} = \frac{V_{Between-person}}{V_{Seasonal} + V_{Between-person} + V_{Within-person}} \quad (2)$$

$$VR_{Within-person} = \frac{V_{Within-person}}{V_{Seasonal} + V_{Between-person} + V_{Within-person}} \quad (3)$$

Results

We enrolled 206 women in Tanzania and 157 women in South Africa in the cold season (**Figure 1**). The response rates at follow-up in the hot season were 87% in Tanzania ($n = 179$) and 80% in South Africa ($n = 126$). Subject characteristics at baseline are outlined by study site in **Table 1**. We excluded 21% of the 24-hour urine collections from the data analysis due to incomplete sample collection: the compliance did not differ between the study sites (Supplemental Table 1).

Seasonal temperatures in the 2 locations during the study periods are presented in **Table 2**. The median (IQR) daily maximum temperatures during the cold and hot seasons were 21°C (20–22°C) and 38°C (34–41°C; $P < 0.001$) in Tanzania

TABLE 1 Baseline characteristics by study site

	Tanzania		South Africa	
	n	Value	*n*	Value
Age,[1] years	205	36 (20–44)	157	29 (25–36)
Height,[2] cm	205	159 (158–160)	157	159 (155–163)
Weight,[3,4] kg	203	61.9 (60.7–63.1)	157	72.2 (69.4–75.3)
BMI,[1] kg/m2	203	23.7 (21.3–27.9)	157	29.6 (23.8–34.2)
Completed secondary education, %	205	19.5	157	85.4

[1] Values are medians (IQR).
[2] Values are means and 95% CIs.
[3] Values are geometric means and 95% CIs obtained from log data.
[4] Body weight did not differ between seasons in Tanzania ($P = 0.07$) or South Africa ($P = 0.149$; Wilcoxon signed rank test).

TABLE 2 Temperature, salt, water, and milk iodine concentrations in the 2 study sites by season[1]

	Tanzania					South Africa				
	Cold Season		Hot Season			Cold Season		Hot Season		
	n	Value	*n*	Value	*P*	*n*	Value	*n*	Value	*P*
Daily minimum temperature,[2] °C	7	12 (12–13)	12	22 (20–25)	<0.001	23	4 (2–5)	26	15 (11–17)	<0.001
Daily maximum temperature,[2] °C	7	21 (20–22)	12	38 (34–41)	<0.001	23	24 (20–26)	26	32 (30–35)	<0.001
Salt iodine concentration,[3,4] mg/kg	149	34 (30–40)	128	37 (32–42)	0.337	150	21 (7–40)	126	13 (7–40)	0.150
Salt iodine concentration <5 mg/kg,[5] *n* (%)	149	0 (0)	128	0 (0)	1	153	3 (2)	126	2 (2)	1
Salt iodine concentration <15 mg/kg,[5] *n* (%)	149	14 (9)	128	12 (9)	1	153	67 (44)	126	66 (53)	0.185
Milk iodine concentration,[4] μg/kg	86	243 (160–346)	70	227 (146–322)	0.495	130	217 (147–287)	109	185 (90–279)	0.635
Water iodine concentration >15 μg/L,[5] *n* (%)	175	0 (0)	139	0 (0)	1	146	17 (12)	110	23 (21)	0.055
Fluid intake,[4] L/day	201	2.0 (1.4–2.3)	175	2.0 (1.5–2.5)	0.016	155	2.1 (1.7–2.6)	126	2.1 (1.6–2.6)	0.583
GPAQ score,[4] MET	205	1428 (7680–24,000)	176	7500 (3940–13,200)	<0.001[2]	157	540 (0–2592)	126	300 (80–1185)	0.761

[1] All values are medians (IQRs) unless otherwise noted. GPAQ, global physical activity questionnaire; MET, metabolic equivalent.
[2] The Kruskal-Wallis test was used to evaluate differences between seasons for each study site.
[3] Only samples with a salt iodine concentration >5 mg/kg were included.
[4] A Wilcoxon signed-rank test with continuity correction was used to evaluate differences between seasons for each study site.
[5] A Fisher's exact test for count data was used to evaluate differences between seasons for each study site.

and 24°C (20–26°C) and 32°C (30–35°C; $P < 0.001$) in South Africa, respectively (Table 2).

Iodized salt (≥5 mg/kg) was available in the households of almost all study participants (98%–100%; Table 2). The median SICs in salt samples collected during the cold season were 34 mg/kg (IQR, 30–40 mg/kg) in Tanzania and 21 mg/kg (IQR, 7–40 mg/kg) in South Africa, and we observed no statistically significant difference between the seasons ($P = 0.337$ and 0.150, respectively; Table 2). The median iodine concentrations in cow's milk were 243 μg/kg (IQR,160–346μg/kg) in Tanzania and 217 μg/kg (IQR, 147–287 μg/kg) in South Africa in the cold season, and did not differ compared to the hot season ($P = 0.495$ and 0.635, respectively; Table 2). The iodine concentrations in drinking water were low in Tanzania, and no sample had a concentration >15 μg/L ($P = 1.000$ between seasons; Table 2). In South Africa, 12% of the samples in the cold season and 21% in the hot season had iodine concentrations >15 μg/L ($P = 0.055$). The estimated median fluid intake in the cold season was 2.0 L (IQR, 1.4–2.3 L) in Tanzania and the intake was only 0.08 L higher in the hot season ($P = 0.016$); the median fluid intake was 2.1 L (IQR, 1.7–2.6 L) in South Africa, with no seasonal difference ($P = 0.583$). The qualitative analysis of the dietary questionnaire did not indicate any major differences in consumption of iodine-rich foods between seasons in either of the 2 sites (data not shown). Physical activity levels, assessed as GPAQ scores, are presented in Table 2. South African women had overall sedentary lifestyles, and their activity levels did not differ between seasons ($P = 0.761$). Participants in Tanzania were 3 times more active than those in South Africa, in both the cold ($P < 0.001$) and hot ($P < 0.001$) seasons. During the hot season, most of the Tanzanian women were engaged in farming and were 5 times more active than during the cold season ($P < 0.001$).

We assessed the iodine intakes in the 2 study populations by measuring the UIEs over 24 hours. In the first linear mixed-effects model analysis including both study sites in 1 model, season ($P < 0.001$), site ($P = 0.004$), and the season-by-site interaction ($P < 0.001$) were significant predictors of UIE. The post hoc test revealed a lower 24-hour UIE in the summer than in the winter in South Africa ($P < 0.001$), whereas no seasonal difference in 24-hour UIEs was observed in Tanzania ($P = 0.878$). This finding suggested seasonal differences in iodine intake in South Africa, but not in Tanzania. To allow for evaluation of the potential effects of seasonal differences in urine volume on UICs at a stable iodine intake, as observed in Tanzania, all further data analyses were conducted separately for each site.

In Tanzanian women, the median 24-hour urine volume in the cold season was 1.63 L (IQR, 1.21–2.10 L), obtained by pooling samples 1 and 2, and the volume did not differ compared to the hot season ($P = 0.518$; **Table 3**; **Figure 2**). Further, we observed no seasonal differences in the 24-hour UIE ($P = 0.468$), spot UIC ($P = 0.204$), or 24-hour UIC ($P = 0.339$; Table 3; Figure 2). The total 24-hour UCE and the UCCs in spot samples and 24-hour collections did not differ between seasons ($P = 0.361$, $P = 0.340$, and $P = 0.798$, respectively). There was no difference in the UIC:UCC ratios of the spot samples between the winter and summer ($P = 0.123$), in concordance with the 24-hour UIE data. For all parameters, we observed higher variability within and between individuals than between season (**Figure 3**).

TABLE 3 Urinary parameters in women from the 2 study sites by season[1]

	Cold season				Hot season				
	Sample 1		Sample 2		Sample 3		Sample 4		
	n	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	*n*	Median (IQR)	*P*
Tanzania									
Urine volume, L	164	1.69 (1.24–2.11)	167	1.57 (1.21–2.09)	129	1.61 (1.20–2.16)	129	1.63 (1.31–2.12)	0.518
Iodine									
Spot UIC, μg/L	202	146 (70–281)	195	134 (62–213)	172	184 (86–296)	171	139 (56–266)	0.204
24-h UIC, μg/L	162	112 (74–178)	166	122 (78–189)	128	118 (78–184)	127	111 (69–194)	0.339
24-h UIE, μg/day	162	195 (118–274)	164	193 (118–257)	128	197 (124–301)	126	203 (116–288)	0.468
Creatinine									
Spot UCC, g/L	204	0.92 (0.45–1.46)	199	0.87 (0.45–1.36)	175	1.03 (0.545–1.67)	170	0.86 (0.45–1.35)	0.340
24-h UCC, g/L	164	0.59 (0.46–0.84)	167	0.63 (0.47–0.82)	129	0.61 (0.46–0.80)	129	0.58 (0.44–0.76)	0.798
24-h UCE, g/day	164	1.01 (0.85–1.19)	167	0.98 (0.82–1.16)	129	0.98 (0.81–1.20)	129	0.94 (0.78–1.12)	0.361
Hydration correction									
Spot UIC:UCC, μg/g	201	177 (110–267)	195	152 (99–240)	171	177 (113–241)	169	176 (113–271)	0.123
South Africa									
Urine volume, L	130	1.43 (1.00–2.09)	128	1.40 (0.95–1.97)	96	1.13 (0.89–1.72)	94	1.24 (0.79–1.62)	<0.001
Iodine									
Spot UIC, μg/L	157	131 (65–225)	154	150 (72–298)	129	147 (93–230)	129	118 (63–199)	0.729
24-h UIC, μg/L	129	116 (66–237)	128	140 (78–247)	96	115 (70–169)	94	125 (72–167)	0.084
24-h UIE, μg/day	129	175 (101–245)	128	188 (115–281)	96	123 (94–179)	94	121 (86–185)	<0.001
Creatinine									
Spot UCC, g/L	157	1.05 (0.58–1.65)	154	0.95 (0.59–1.42)	129	1.31 (0.83–1.99)	125	1.04 (0.67–1.47)	0.004
24-h UCC, g/L	130	1.03 (0.52–1.09)	128	0.74 (0.53–1.11)	96	0.84 (0.59–1.29)	94	0.82 (0.53–1.37)	<0.001
24-h UCE, g/day	130	1.05 (0.86–1.24)	128	1.02 (0.82–1.08)	96	1.02 (0.84–1.24)	94	0.98 (0.81–1.22)	0.313
Hydration correction									
UIC:UCC spot, μg/g	157	127 (79–180)	154	160 (108–251)	128	108 (79–158)	125	117 (86–157)	<0.001
Sodium									
24-h UNaC, mmol/L	128	98 (66–129)	126	94 (63–142)	94	110 (68–147)	94	115 (74–173)	0.002
24-h UNaE, mmol/day	128	120 (95–175)	126	123 (94–178)	94	127 (84–166)	94	119 (93–157)	0.059

[1]Values are medians (IQRs). Linear mixed-effects models were used to estimate differences between season in each study population, using season as a fixed effect, the random effect of subject, and random interaction between subject and season. spot UCC, urinary creatinine concentration of the spot urine sample; spot UIC, urinary iodine concentration of the spot urine samples; spot UIC:UCC, urinary iodine to creatinine ratio in spot samples; 24-h UCC, urinary creatinine concentration of the 24-hour urine collections; 24-h UCE, urinary creatinine excretion over 24 hours; 24-h UIC, urinary iodine concentration of the 24-hour urine collection; 24-h UIE, urinary iodine excretion over 24 hours; 24-h UNaC, urinary sodium concentration of the 24-hour urine collection; 24-h UNaE, urinary sodium excretion over 24 hours.

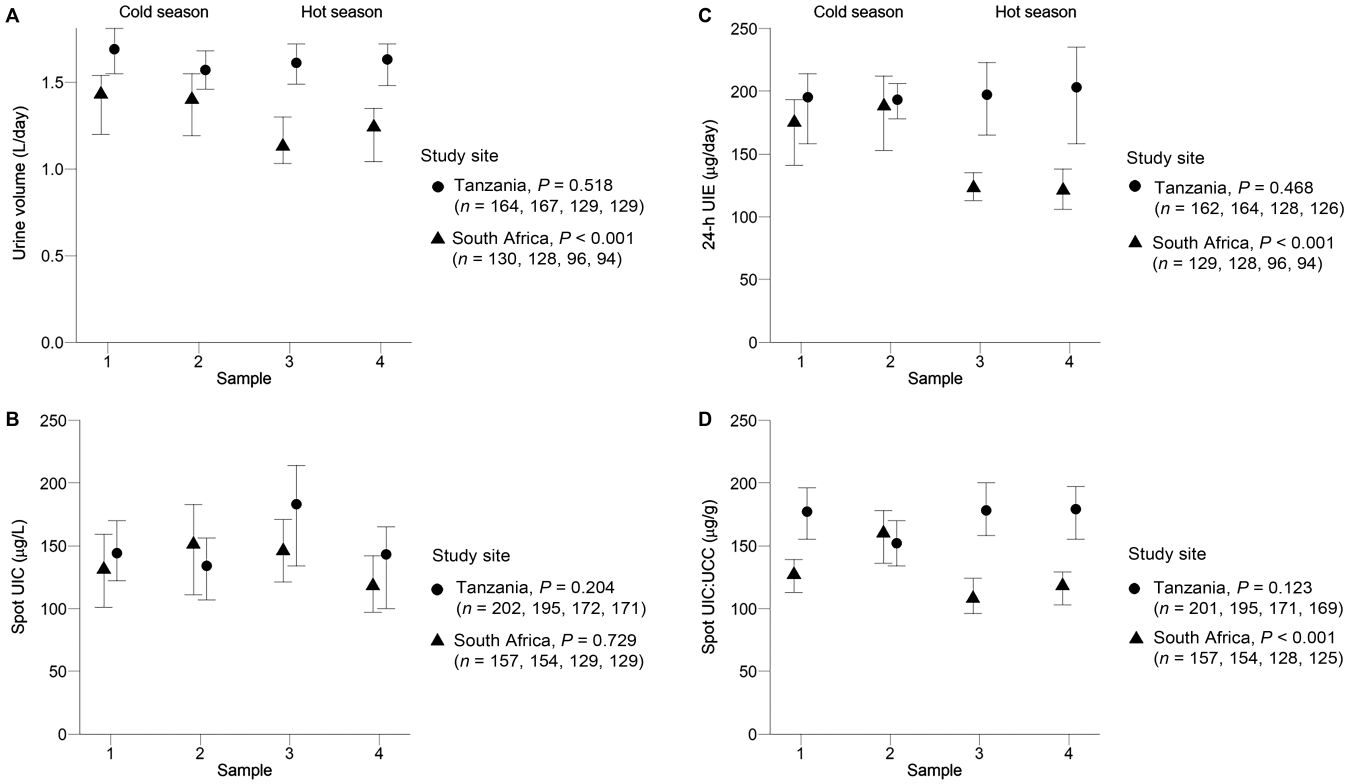

FIGURE 2 (A) Urine volume from 24-hour collections, (B) spot UIC, (C) 24-hour UIE, and (D) spot UIC:UCC ratio in Tanzanian and South African women at 2 repeated sampling occasions during the cold as well as the hot seasons. Data are presented as medians with 95% bootstrapped CIs. The *P* values indicate differences between seasons, evaluated using site-specific linear mixed-effects models with season as the fixed effect plus subject and the interaction between subject and season as random effects. Instances of *n* indicate the number of subjects at each sample occasion (samples 1–4). Subjects with incomplete 24-hour urine collections were excluded from the data analysis. spot UIC, urinary iodine concentration in spot urine samples; spot UIC:UCC, urinary iodine concentration to urinary creatinine concentration ratio in spot urine samples; 24-h UIE, urinary iodine excretion over 24 hours.

South African women had a pooled median 24-hour urine volume of 1.40 L (IQR, 0.96–2.05 L) in the cold season, and the urine volume was 0.22 L (15%) lower in the hot season ($P < 0.001$; Figure 2). However, the relative within- and between-person variabilities in urine volume were high (21% and 64%, respectively), and season explained only 15% of the total variability (Figure 3). UICs did not differ between seasons for spot ($P = 0.729$) or 24-hour collections ($P = 0.084$; Table 3; Figure 2). We observed a significant effect of season on the spot UCC ($P = 0.004$) and 24-hour UCC ($P < 0.001$), but no significant difference in the total 24-hour UCE ($P = 0.313$). The pooled median 24-hour UIEs were 184 μg/day (IQR, 109–267 μg/day) in the cold season and 122 μg/day (IQR, 90–182 μg/day; 34% lower) in the hot season ($P < 0.001$; Table 3; Figure 2). This seasonal difference in iodine intake in the South African women was not captured by the spot UIC, but when accounting for hydration by measuring UCC, the seasonal difference in iodine intake was picked up in the spot UIC:UCC ratio ($P < 0.001$). Season explained 47% of the total variability in the 24-hour UIE (Figure 3). For all other parameters, the within- and between-person variability were higher than the seasonal variability. To better understand differences in iodine intake between seasons in South Africa, we measured UNaC in the 24-hour urine collections. We observed significantly higher UNaC in the summer compared to the winter ($P = 0.002$), but the UNaE did not differ between the seasons ($P = 0.059$).

Discussion

This observational study in Tanzanian and South African women exposed to high seasonal temperature differences found no difference in median UICs in the hot and cold seasons. Tanzanian women had similar iodine intake and urine volume in both seasons, and the spot UIC was unaffected by the hot climate in the summer. However, in South African women, we observed a 34% lower iodine intake in combination with a 15% lower urine volume in the summer compared to the winter. The lower iodine intake in the summer season was not reflected in the spot UIC, as the urine was more concentrated and masked the lower intake. At a constant iodine intake, we estimate that a 0.22 L lower urine volume would have led to a 31 µg/L higher 24-hour UIC in the summer than in the winter. We demonstrate that seasonal effects on spot UIC may depend on several factors, such as the iodine intake, urinary dilution, and a combination of both.

Only 41% of the participants met the recommended daily total water intake of 2.2 L (52), but we observed no major seasonal differences in the self reported fluid intake. The median urine volumes in Tanzanian and South African women were within the broad range previously observed in South Africa (8, 53) and

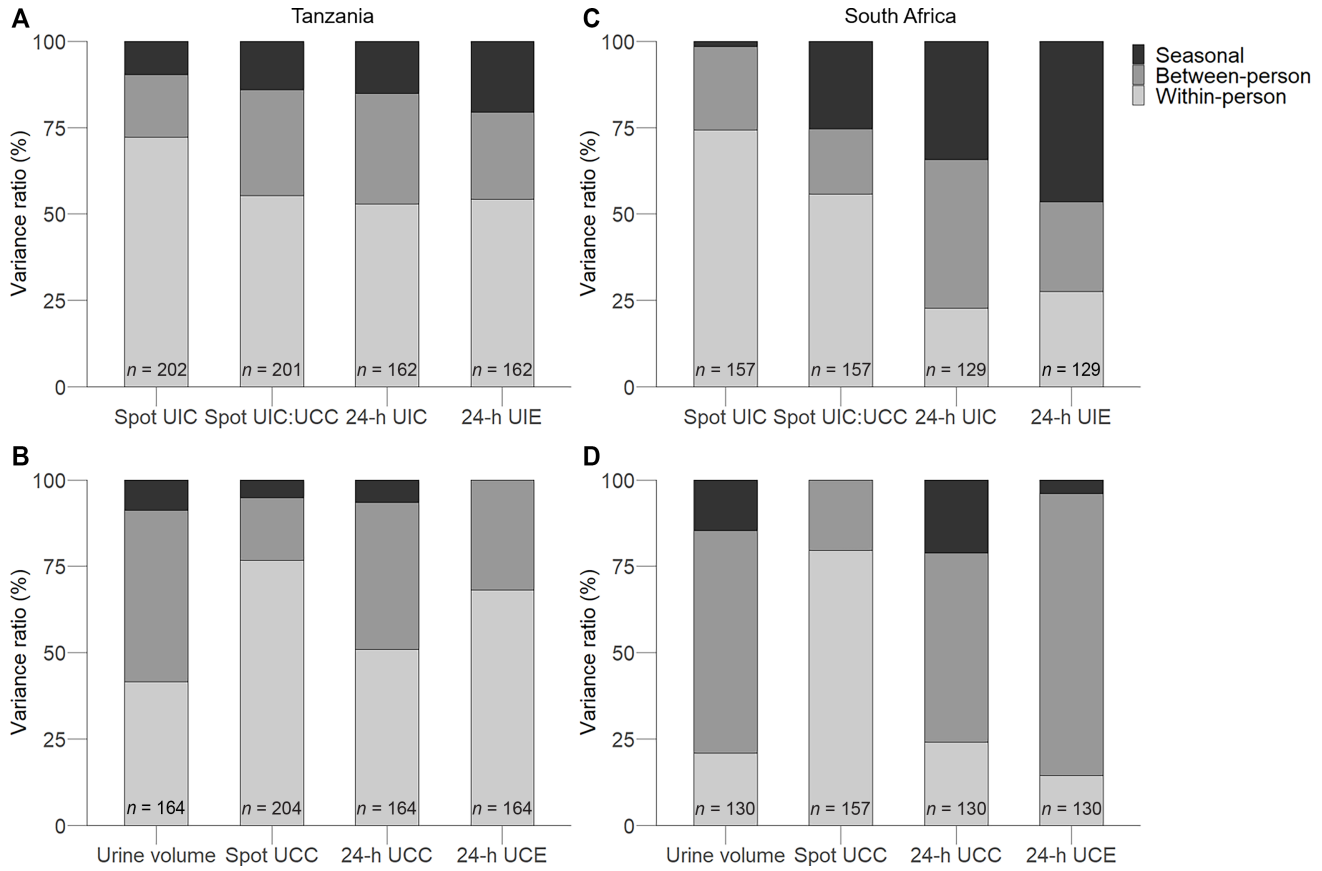

FIGURE 3 Relative variance ratio (%) of seasonal variability (fixed effect), between-person variability (random effect; 1|subject), and within-person variability (random effect; 1|subject:season) obtained from the linear mixed-effects models for each parameter by study site. spot UCC, urinary creatinine concentration from the spot urine sample; spot UIC, urinary iodine concentration from the spot urine sample; spot UIC:UCC, urinary iodine to urinary creatinine ratio in spot urine samples; 24-h UCC, urinary creatinine concentration of the 24-hour urine collection; 24-hour UCE, urinary creatinine excretion over 24 hours; 24-h UIC, urinary iodine concentration of the 24-hour urine collection; 24-h UIE: urinary iodine excretion over 24-hours.

southern Europe (17, 18), but lower than those reported in central Europe (17, 37) and the United States. (54). The seasonality in urine volume observed in South African women affected all urinary biomarkers, including UCC and UNaC. The overall median daily urine volume fluctuated only marginally from day to day in the study population (Table 3), in agreement with other studies (12). However, our study design using repeated measures allowed quantification of the sources of variability. We show that the within-person (21%–42%) and between-person (50%–65%) variabilities made up the largest proportions of the variance in daily urine volume, whereas season explained only 9%–15% of the variability. The data suggest that individual day-to-day differences in daily urine volume, as well as variability between subjects and differences between populations, are larger than the seasonal difference within a population. Consequently, cross-sectional studies aiming to detect small seasonal differences in urinary biomarkers require a large sample size or repeated sampling to account for the variability.

Our study was conducted in 2 countries with mandatory salt iodization (25, 55, 56), and we enrolled women with apparently adequate iodine intakes, assessed by a median spot UIC >100 μg/L in the winter season (1). However, in South African women the UIE was 62 μg/day lower in the summer compared to the winter. The lower iodine intake was not captured by the spot UIC alone due to more concentrated urine, but when accounting for hydration by measuring the UCC, the seasonal difference was picked-up in the spot UIC:UCC ratio (21% lower in the hot compared to the cold season). The UCC may improve the assessment of iodine nutrition from spot urine samples (6–8), and we demonstrate that an additional measurement of UCC may be particularly valuable in hot climates. However, criteria for adequate iodine nutrition has not been defined for the UIC:UCC ratio. The iodine intake may be estimated from this ratio, but population-specific reference ranges for UCC are required (57, 58).

To our knowledge, this is the first study to document seasonal differences in iodine intake in an African population. There may be several reasons for the lower iodine intake in the summer compared to the winter season observed in South African women. The salt intake agrees with previous studies (59, 60) and did not differ between seasons. However, we found a tendency toward a lower iodine concentration in salt in the summer compared to the winter (13 compared with 21 mg/kg), even if this trend was not statistically significant. Iodine losses in salt may be higher under hot and humid climate conditions (61, 62), although iodate, as used in South Africa, is more stable than iodide (63). Poor packaging and impurities increase iodine losses during storage. The iodine concentration in salt was highly variable, suggesting

heterogeneity in the level of fortification. We recognize that the variability in part may be due to the small amount of salt (10 g) used in the analysis and the fact that the iCheck method is unreliable at <10 mg/kg. Previous studies conducted in Europe and China reported lower iodine intakes in the summer as being associated with lower iodine contents in cow's milk when cows are grass-fed (20–22, 64). To our knowledge, our study is the first to report iodine concentrations in cow's milk in an African context. The concentrations were within the ranges reported in Europe and North America (23), implying that iodine/iodized salt are used for fodder and/or salt stones. However, unlike data reported in Europe (23, 64), we observed no seasonal difference in the milk iodine concentration. The daily milk consumption was low overall, and cow's milk is not the main dietary source of iodine in these 2 study populations. The qualitative analysis of the dietary questionnaire did not indicate any major differences in intakes of iodine-rich foods between seasons. It is possible that the overall food and energy intakes differed between seasons, but this was not assessed.

Our data indicate substantial within-and between-person variabilities in iodine intake (UIE) and iodine status measures (UICs in spot and 24-hour collections and UIC:UCC ratios from spot samples) in African women (Figure 3), in agreement with previous studies in European adults (21, 29, 30) and Chinese women (65). The relative within-person variance in UIC was higher in spot samples than in 24-hour collections. Although UIC is influenced by variability in hydration status, the major variability likely derives from the iodine intake.

The strengths of our study include 2 different study cohorts, follow-up of women in 2 seasons, and high response rates (80%–87%). We compared UICs in both spot and 24-hour urine collections and collected 2 repeat urine samples in 2 seasons, thereby accounting for within-person and between-person variances. We estimated the iodine intake based on the 24-hour urine excretion to assess differences in iodine intake between seasons. We measured the iodine concentrations in household salt, cow's milk, and drinking water to assess seasonal differences in major dietary iodine sources, as well as calculated the UNaC in South African women to assess salt intake. Although we administered a food questionnaire to help explain possible reasons for seasonal differences in iodine intake, it was not exhaustive or quantitative, and we cannot rule out differences in dietary patterns between seasons. A limitation to the study is incomplete 24-hour urine sampling (21%) and the subsequent data exclusion, but the targeted sample size of 100 was achieved at all time points except for the summer collection in South Africa. We did not record whether participants shared a household and could not account for this in the data analysis, but we expect minimal bias considering the overall high between- and within-subject variabilities in the reported outcome parameters (Figure 3).

In conclusion, our study findings suggest that using the spot UIC as a biomarker of iodine intake in populations exposed to hot temperatures can be biased, as concentrated urine may mask inadequate iodine intake. The risk for misclassification of iodine status due to a lower urine volume is mainly relevant in populations with borderline deficient iodine intakes. In such cases, the spot UIC:UCC ratio may be considered to correct for dilution, although no threshold for this ratio has been defined. Our study is the first to report a lower iodine intake in the summer season in African women, and seasonal differences in iodine intake also affect UICs. However, we show that the between- and within-person variabilities in urine volume, iodine intake, and UIC are larger than the seasonal variabilities. If seasonal differences in dietary iodine intake are likely, data collection in UIC studies may be spread throughout the year or be conducted during the season with the expected lowest iodine intake. Understanding the reasons for seasonal changes in iodine intake is important to prevent iodine deficiency, particularly in populations with incomplete coverage of iodized salt.

We thank the participating women from the Kilimanjaro region (Tanzania) and Potchefstroom (South Africa) and the fieldworkers in both study sites. We thank Peter Kaswahili, Michael Maganga, and Maria Ngilisho from the Tanzanian Food and Nutrition Centre (Dar es Salaam, Tanzania); Estelle Strydom from North-West University (Potchefstrooom, South Africa); and Sarah Bürki, Marco Germann, and Nicole Verhagen from ETH Zurich (Zürich, Switzerland) for assistance with the laboratory analysis. We acknowledge advice on statistical analysis from Statistical Consulting, ETH Zurich (Zürich, Switzerland).

The authors' contributions were as follows – MA, LA-G, MBZ, OD, JB, VG: conceived the study; MA, LA-G, MBZ: designed the study; LA-G, JB, VA, LZ, FA: conducted the fieldwork; LA-G, LZ: conducted the laboratory analysis; LA-G, MA, VG: conducted the statistical analysis; LA-G, MA: wrote the manuscript; MA: had primary responsibility for the final content; and all authors: edited the manuscript and read and approved the final manuscript. MBZ, VDA, FA, and MA are Iodine Global Network members. OD is an employee of the US Agency for International Development. All other authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application.

References

1. WHO, UNICEF, International Council for the Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. 3rd edition. Geneva, Switzerland: WHO; 2007.
2. Jahreis G, Hausmann W, Kiessling G, Franke K, Leiterer M. Bioavailability of iodine from normal diets rich in dairy products: Results of balance studies in women. Exp Clin Endocrinol Diabetes 2001;109:163–7.
3. van der Reijden OL, Galetti V, Burki S, Zeder C, Krzystek A, Haldimann M, Berard J, Zimmermann MB, Herter-Aeberli I. Iodine bioavailability from cow milk: A randomized, crossover balance study in healthy iodine-replete adults. Am J Clin Nutr 2019;110:102–10.
4. Johner SA, Thamm M, Schmitz R, Remer T. Examination of iodine status in the German population: An example for methodological pitfalls of the current approach of iodine status assessment. Eur J Nutr 2016;55:1275–82.
5. Pearce EN, Caldwell KL. Urinary iodine, thyroid function, and thyroglobulin as biomarkers of iodine status. Am J Clin Nutr 2016;104:898S–901S.
6. Johner SA, Boeing H, Thamm M, Remer T. Urinary 24-hour creatinine excretion in adults and its use as a simple tool for the estimation of daily urinary analyte excretion from analyte/creatinine ratios in populations. Eur J Clin Nutr 2015;69:1336–43.
7. Perrine CG, Cogswell ME, Swanson CA, Sullivan KM, Chen TC, Carriquiry AL, Dodd KW, Caldwell KL, Wang CY. Comparison of population iodine estimates from 24-hour urine and timed-spot urine samples. Thyroid 2014;24:748–57.
8. Charlton KE, Ware LJ, Baumgartner J, Cockeran M, Schutte AE, Naidoo N, Kowal P. Iodine status assessment in South African adults according to spot urinary iodine concentrations, prediction equations, and measured 24-hour iodine excretion. Nutrients 2018;10:736–50.

9. Zimmermann MB, Andersson M. Global perspectives in endocrinology: Coverage of iodized salt programs and iodine status in 2020. Eur J Endocrinol 2021;185(1):R13–21.
10. Manz F, Johner SA, Wentz A, Boeing H, Remer T. Water balance throughout the adult life span in a German population. Br J Nutr 2012;107:1673–81.
11. Zhang JF, Zhang N, Wang Y, Liang SX, Liu SF, Du SM, Xu YF, He HR, Cai H, Ma GS. Drinking patterns and hydration biomarkers among young adults with different levels of habitual total drinking fluids intake in Baoding, Hebei Province, China: A cross-sectional study. BMC Public Health 2020;20:468–79.
12. Braun H, von Andrian-Werburg J, Malisova O, Athanasatou A, Kapsokefalou M, Ortega JF, Mora-Rodriguez R, Thevis M. Differing water intake and hydration status in three European countries: A day-to-day analysis. Nutrients 2019;11:773–86.
13. Perrier E, Vergne S, Klein A, Poupin M, Rondeau P, Le Bellego L, Armstrong LE, Lang F, Stookey J, Tack I. Hydration biomarkers in free-living adults with different levels of habitual fluid consumption. Br J Nutr 2013;109:1678–87.
14. Swanson ZS, Pontzer H. Water turnover among human populations: Effects of environment and lifestyle. Am J Hum Biol 2020;32(1):e23365–78.
15. Agostoni C, Bresson JL, Fairweather-Tait S, Flynn A, Golly I, Korhonen H, Lagiou P, Lovik M, Marchelli R, Martin A, et al. Scientific opinion on dietary reference values for water. EFSA J 2010;8:1459–507.
16. Mora-Rodriguez R, Ortega JF, Fernandez-Elias VE, Kapsokefalou M, Malisova O, Athanasatou A, Husemann M, Domnik K, Braun H. Influence of physical activity and ambient temperature on hydration: The European Hydration Research Study (EHRS). Nutrients 2016;8:252–65.
17. Malisova O, Athanasatou A, Pepa A, Husemann M, Domnik K, Braun H, Mora-Rodriguez R, Ortega JF, Fernandez-Elias VE, Kapsokefalou M. Water intake and hydration indices in healthy European adults: The European Hydration Research Study (EHRS). Nutrients 2016;8:204–16.
18. Malisova O, Bountziouka V, Panagiotakos D, Zampelas A, Kapsokefalou M. Evaluation of seasonality on total water intake, water loss and water balance in the general population in Greece. J Hum Nutr Diet 2013;26:90–6.
19. Athanasatou A, Malisova O, Kandyliari A, Kapsokefalou M. Water intake in a sample of Greek adults evaluated with the water balance questionnaire (WBQ) and a seven-day diary. Nutrients 2016;8:559–75.
20. Moreno-Reyes R, Carpentier YA, Macours P, Gulbis B, Corvilain B, Glinoer D, Goldman S. Seasons but not ethnicity influence urinary iodine concentrations in Belgian adults. Eur J Nutr 2011;50:285–90.
21. Als C, Haldimann M, Bürgi E, Donati F, Gerber H, Zimmerli B. Swiss pilot study of individual seasonal fluctuations of urinary iodine concentration over two years: Is age-dependency linked to the major source of dietary iodine? Eur J Clin Nutr 2003;57:636–46.
22. Ji X, Liu P, Sun Z, Su X, Wang W, Gao Y, Sun D. Intra-individual variation in urinary iodine concentration: Effect of statistical correction on population distribution using seasonal three-consecutive-day spot urine in children. BMJ Open 2016;6:e010217–26.
23. van der Reijden OL, Zimmermann MB, Galetti V. Iodine in dairy milk: Sources, concentrations and importance to human health. Best Pract Res Clin Endocrinol 2017;31:385–95.
24. Republic of Tanzania. Salt acts: The mining act 1979: The mining (salt production and iodation) regulations 1994 and the food (control of quality) act 1978. Regulations made under section 16 (1) and (2). The food (iodated salt) regulation. Salt acts 1994. Government Gazette. Dar es Salaam, Tanzania; 1994.
25. Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), and ICF. Tanzania demographic and health survey and malaria indicator survey 2015-2016. Dar es Salaam, Tanzania: MoHCDGEC, MoH, NBS, OCGS, and ICF; 2016.
26. Jooste PZM. Progress towards eliminating iodine deficiency in South Africa. S Afr J Clin Nutr 2008;1:8–14.
27. Office E. National Food Consumption Survey-Fortification Baseline (NFCS-FB-I): South Africa, 2005. S Afr J Clin Nutr 2008; 21:245–300.
28. National Department of Health (NDoH), Statistics South Africa (Stats SA), South African Medical Research Council (SAMRC), and ICF. South Africa Demographic and Health Survey 2016. Pretoria, South Africa: NDoH, Stats SA, SAMRC, and ICF; 2019.
29. Karmisholt J, Laurberg P, Andersen S. Recommended number of participants in iodine nutrition studies is similar before and after an iodine fortification programme. Eur J Nutr 2014;53:487–92.
30. König F, Andersson M, Hotz K, Aeberli I, Zimmermann MB. Ten repeat collections for urinary iodine from spot samples or 24-hour samples are needed to reliably estimate individual iodine status in women. J Nutr 2011;141:2049–54.
31. WHO. Physical status: The use and interpretation of anthropometry. Report of a WHO expert committee. Geneva, Switzerland: WHO; 1995.
32. Armstrong T, Bull F. Development of the World Health Organization global physical activity questionnaire (GPAQ). J Public Health 2006;14:66–70.
33. WHO. Recommended population levels of physical activity for health. WHO 1st ed. In: Global recommendations on physical activity for health. Geneva, Switzerland: WHO; 2010;15–34.
34. Spierto FW, Hannon WH, Gunter EW, Smith SJ. Stability of urine creatinine. Clin Chim Acta 1997;264:227–32.
35. Reinivuo H, Valsta LM, Laatikainen T, Tuomilehto J, Pietinen P. Sodium in the Finnish diet: II trends in dietary sodium intake and comparison between intake and 24-hour excretion of sodium. Eur J Clin Nutr 2006;60:1160–7.
36. Murakami K, Sasaki S, Takahashi Y, Uenishi K, Watanabe T, Kohri T, Yamasaki M, Watanabe R, Baba K, Shibata K, et al. Sensitivity and specificity of published strategies using urinary creatinine to identify incomplete 24-hour urine collection. Nutrition 2008;24:16–22.
37. Haldimann M, Bochud M, Burnier M, Paccaud F, Dudler V. Prevalence of iodine inadequacy in Switzerland assessed by the estimated average requirement cut-point method in relation to the impact of iodized salt. Public Health Nutr 2015;18:1333–42.
38. Pino S, Fang SL, Braverman LE. Ammonium persulfate: A safe alternative oxidizing reagent for measuring urinary iodine. Clin Chem 1996;42:239–43.
39. Butler AR. The Jaffé reaction. Identification of the coloured species. Clin Chim Acta 1975;59:227–32.
40. Annino JS. Determination of sodium in urine by specific ion electrode. Clin Chem 1967;13:227–32.
41. Association of Official Analytical Chemists. Official methods of analysis. Gaithersburg, MD: Association of Official Analytical Chemists; 1984.
42. Rohner F, Garrett GS, Laillou A, Frey SK, Mothes R, Schweigert FJ, Locatelli-Rossi L. Validation of a user-friendly and rapid method for quantifying iodine content of salt. Food Nutr Bull 2012;33:S330–5.
43. United Nations Children's Emergency Fund. Guidance on the monitoring of salt iodization programmes and determination of population iodine status [Internet]. New York, NY: United Nations Children's Emergency Fund; 2018 . Available from: https://sites.unicef.org/nutrition/files/Monitoring-of-Salt-Iodization.pdf.
44. Dold S, Baumgartner J, Zeder C, Krzystek A, Osei J, Haldimann M, Zimmermann MB, Andersson M. Optimization of a new mass spectrometry method for measurement of breast milk iodine concentrations and an assessment of the effect of analytic method and timing of within-feed sample collection on breast milk iodine concentrations. Thyroid 2016;26:287–95.
45. R Core Team [Internet]. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org.
46. Canty A, Ripley B. Bootstrap R (S-plus) functions. R package. 1.3-24; 2019. Available from: https://CRAN.R-project.org/package=boot.
47. Davison A, Hinkley DV. Bootstrap methods and their applications. Cambridge, UK: Cambridge University Press; 1997.
48. Sarkar D. Lattice: Multivariate data visualization with R. New York, NY: Springer; 2008.
49. Kuznetsova A, Brockhoff PB, Christensen RHB. LmerTest package: Tests in linear mixed effects models. J Stat Softw 2017;82:1–26.
50. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. Biom J 2008;50:346–63.

51. Ggplot2 Wickham H. Elegant graphics for data analysis. New York, NY: Springer; 2016.
52. WHO. Water requirements, impining factors and recommended intakes. WHO 1st ed. In: Nutrients in drinking water. Geneva, Switzerland: World Health Organization; 2005;25–40.
53. Charlton K, Ware LJ, Baumgartner J, Cockeran M, Schutte AE, Naidoo N, Kowal P. How will South Africa's mandatory salt reduction policy affect its salt iodisation programme? A cross-sectional analysis from the WHO-SAGE wave 2 salt and tobacco study. BMJ Open 2018;8:e020404–13.
54. Terry AL, Cogswell ME, Wang C-Y, Chen T-C, Loria CM, Wright JD, Zhang X, Lacher DA, Merritt RK, Bowman BA. Feasibility of collecting 24-hour urine to monitor sodium intake in the national health and nutrition examination survey. Am J Clin Nutr 2016;104:480–8.
55. Smuts C, Baumgartner J. Are we neglecting iodine nutrition in South Africa? S Afr J Clin Nutr 2019;32:3–4.
56. Department of Health South Africa. Foodstuff cosmetics and disinfectants act, 1972 (act no. 54 of 1972) In: Government gazette 3753; 1972.
57. Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Andersen S, Rasmussen LB, Ovesen L, Jørgensen T. Estimation of iodine intake from various urinary iodine measurements in population studies. Thyroid 2009;19:1281–6.
58. Zimmermann MB, Andersson M. Assessment of iodine nutrition in populations: Past, present, and future. Nutr Rev 2012;70:553–70.
59. Eksteen G, Mungal-Singh V. Salt intake in South Africa: A current perspective. J Endocrinol Metab Diabetes S Afr 2015;20(1):9–13.
60. Menyanu E, Corso B, Minicuci N, Rocco I, Zandberg L, Baumgartner J, Russell J, Naidoo N, Biritwum R, Schutte AE, et al. Salt-reduction strategies may compromise salt iodization programs: Learnings from South Africa and Ghana. Nutrition 2021;84:111065.
61. Diosady LL, Alberti J, Mannar MGV, Stone TG. Stability of iodine in iodized salt used for correction of iodine-deficiency disorders. Food Nutr Bull 1997;18:1–9.
62. Shawel D, Hagos S, Lachat CK, Kimanya ME, Kolsteren P. Post-production losses in iodine concentration of salt hamper the control of iodine deficiency disorders: A case study in Northern Ethiopia. J Health Popul Nutr 2010;28:238–44.
63. Mannar MV, Dunn JT. Choice and dosage of iodine compound for salt iodization. 1st ed. In: Salt iodization for the elimination of iodine deficiency. Ottawa, Canada: International Council for Control of Iodine Deficiency Disorders; 1995. 19–25.
64. van der Reijden OL, Galetti V, Hulmann M, Krzystek A, Haldimann M, Schlegel P, Manzocchi E, Berard J, Kreuzer M, Zimmermann MB, et al. The main determinants of iodine in cows' milk in Switzerland are farm type, season and teat dipping. Br J Nutr 2018;119:559–69.
65. Chen W, Gao S, Guo WX, Tan L, Pan ZY, Dong SY, Jin Y, Zhang Y, Zhang WQ, Shen J. Intra-individual and inter-individual variations in iodine intake and excretion in adult women: Implications for sampling. Br J Nutr 2020;123:987–93.