

Urinary Excretion of Sodium, Potassium, and Chloride, but Not Iodine, Varies by Timing of Collection in a 24-Hour Calibration Study^{1–4}

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Abstract

Because of the logistic complexity, excessive respondent burden, and high cost of conducting 24-h urine collections in a national survey, alternative strategies to monitor sodium intake at the population level need to be evaluated. We conducted a calibration study to assess the ability to characterize sodium intake from timed-spot urine samples calibrated to a 24-h urine collection. In this report, we described the overall design and basic results of the study. Adults aged 18–39 y were recruited to collect urine for a 24-h period, placing each void in a separate container. Four timed-spot specimens (morning, afternoon, evening, and overnight) and the 24-h collection were analyzed for sodium, potassium, chloride, creatinine, and iodine. Of 481 eligible persons, 407 (54% female, 48% black) completed a 24-h urine collection. A subsample ($n = 133$) collected a second 24-h urine 4–11 d later. Mean sodium excretion was 3.54 ± 1.51 g/d for males and 3.09 ± 1.26 g/d for females. Sensitivity analysis excluding those who did not meet the expected creatinine excretion criterion showed the same results. Day-to-day variability for sodium, potassium, chloride, and iodine was observed among those collecting two 24-h urine samples (CV = 16–29% for 24-h urine samples and 21–41% for timed-spot specimens). Among all race-gender groups, overnight specimens had larger volumes ($P < 0.01$) and lower sodium ($P < 0.01$ to $P = 0.26$), potassium ($P < 0.01$), and chloride ($P < 0.01$) concentrations compared with other timed-spot urine samples, although the differences were not always significant. Urine creatinine and iodine concentrations did not differ by the timing of collection. The observed day-to-day and diurnal variations in sodium excretion illustrate the importance of accounting for these factors when developing calibration equations from this study. *J. Nutr.* 143: 1276–1282, 2013.

Introduction

Evidence indicates excess dietary sodium intake is associated with increased blood pressure and subsequently increased risk of cardiovascular disease (1–5). To prevent cardiovascular diseases, the 2010 *Dietary Guidelines for Americans* recommend reducing sodium intake to <2.30 g/d and to 1.50 g/d for individuals ≥ 51 y and those of any age who are African American or have

hypertension, diabetes, or chronic kidney disease (6). Monitoring sodium intake in the U.S. population is imperative to track current and future national sodium reduction efforts (7). The Institute of Medicine and the Pan American Health Organization both recommend the measurement of 24-h urinary sodium excretion as the gold standard for assessment of sodium intake (7,8). To estimate the distribution of usual sodium intake within the U.S. population, e.g., the proportion of individuals who meet 2010 *Dietary Guidelines*, experts also recommend the collection of a second 24-h urine sample in a subsample to account for within-individual, day-to-day variability (9,10). However, to date, no 24-h urine samples have been collected in U.S. nationally representative surveys, mainly for logistical reasons and the concerns about excessive respondent burden and poor data quality.

The NHANES is the primary data system that provides objective information to monitor the health and nutritional status

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³ Supplemental Methods 1–3 and Supplemental Tables 1–5 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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in the U.S. population. The survey includes measures relevant to cardiovascular disease risk, such as medical history, blood pressure, blood lipids, medication use, dietary assessment, and linkage to U.S. mortality and morbidity data. Although timed-spot urine specimens are collected in the NHANES, they are not currently recommended to be used to assess and monitor sodium intake, largely because they do not provide good measures of an individual's sodium intake (7,8). However, there are studies suggesting that timed-spot urine samples may be a valid low-burden and low-cost alternative to the 24-h collection for estimating sodium intakes at the population level (11–14).

Diurnal variation in sodium excretion is well recognized (13–15). However, data comparing different procedures, such as the timing of partial urine collection, for estimating 24-h sodium excretion are limited. In addition, some studies suggest that there is a difference in diurnal patterns of sodium excretion between black and white persons, which may potentially relate to the racial differences in hypertension status (2,16). Very few studies evaluating the use of spot specimens in estimating 24-h sodium excretion have reported results for black persons (17–19).

We designed a calibration study to examine the utility of timed-spot urine specimens, as collected in NHANES, for estimating intake of sodium and related analytes that may be affected by sodium reduction in the food supply, including potassium, chloride, and iodine (7). The objectives of this paper are to describe the study methods, characteristics of the participants, and the distributions of the analytes by race, gender, and the timing of the urine specimen collection. In addition, within-individual, day-to-day correlations of each analyte were presented for 4 race-gender subgroups. We also provided data on the completeness of the 24-h urine collections, including an assessment using expected 24-h creatinine excretion criterion.

Methods

Participants. Participant recruitment procedures included announcements sent to employees of the National Center for Health Statistics and its contractor, Westat, and persons on a Westat database of people known to be interested in participating in human studies. Five hundred volunteers aged 18–39 y living in the Washington, DC metropolitan area were then invited to participate in the study during a phone contact at which a screener questionnaire was administered. Of these, 481 were scheduled for an initial visit to receive instructions on study protocol and 19 were not scheduled for the initial visit, because the sample target had been met. Recruitment of participants was stratified by gender and race to yield an equal number of men and women and ~50% black or African Americans within each gender. The screener questionnaire (Supplemental Methods 1) administered during recruitment was used to exclude pregnant women or those trying to become pregnant, persons who reported taking loop diuretics, those with self-reported chronic kidney disease, or those with reported new or modified hypertension treatment in the past 2 wk from participation. During recruitment, volunteers were also asked 3 screening questions (see Screener Questionnaire in Supplemental Methods 1) pertaining to high- or low-dietary sodium intake to provide a study sample with wide range of sodium intake. These questions were developed using 2003–2008 NHANES dietary behavior and food consumption data (20). The goal was to recruit at least 50 participants (25 men and 25 women) who were more likely to be at the high end and 50 (25 men and 25 women) more likely to be at the low end of gender-specific sodium intake distributions.

Study design. The study was conducted from June to August 2011 in Rockville, Maryland. Eligible participants were scheduled for an initial visit to have their weight and height measured and to receive detailed verbal and written instructions along with the collection kit for their 24-h urine collection. Each participant was randomly assigned to a weekday or weekend day to collect the 24-h urine sample. The target was to

collect 50–70% of the samples within each gender during Monday to Thursday. The date to start the 24-h urine collection was scheduled for each participant within the assigned weekday/weekend category. A reminder call was placed to the participant the day before their scheduled starting date. On the morning of the starting day, upon rising, participants were asked to discard the first void of the day and record the date and time as the starting point for the urine collection period. They were instructed to collect all subsequent urine voids over the next 24-h period, including the first void of the following day. They were told to collect each void using a separate container and to record the date and time of each void on the container label. There were no preservatives in the urine containers. Participants were instructed to store their urine samples in a refrigerator or in the provided insulated bag with icepacks. They were asked to return their urine samples the day they completed the collection. The length of collection, total urine volume, and the responses to 8 questions asked in person upon return of the specimens were used to assess the completeness of the collection (Supplemental Methods 2). If the urine sample was determined to be complete, a 24-h dietary recall interview was administered. The urine sample was considered incomplete or invalid if any of the following occurred: 1) the total 24-h urinary volume was <500 mL; 2) a female participant reported menstruation during the collection period; 3) the reported length of collection was <20 h; or 4) more than one void was reported as missed or urine was spilled or lost more than once during the collection. If the collection was incomplete or invalid, the participant was offered the option to redo the 24-h collection. If the participant was unable or unwilling to redo the collection, the existing 24-h sample was omitted from the analysis.

One-third of the participants with complete collections were invited to collect a second 24-h urine 4–11 d later, but not on the same day of the week as the first 24-h urine collection. A convenience sampling approach was used to yield an equal number of participants in each of the 4 race-gender categories for this second urine collection. The entire protocol was repeated, including the follow-up questions to assess completeness of the urine collection. A second 24-h dietary recall was administered to those with complete urine collections.

The study protocol was approved by the National Center for Health Statistics Ethics Review Board and written informed consent was obtained from all participants.

Urine processing and laboratory measurements. Once the urine samples were returned, each separate void was weighed and a 25-mL composite, 24-h urine sample was prepared by taking a proportional aliquot from each void as following: amount of aliquot from each void (mL) = [separate void volume (mL)/total volume from all voids (mL)] × 25. In addition, 4 timed-spot urine specimens were selected from each 24-h urine collection period: 1) morning sample, the first void occurring between 0830 and 1230 h; 2) afternoon sample, the first void occurring between 1231 and 1730 h; 3) evening sample, first void occurring between 1731 and 2359 h; and 4) overnight sample, the first void after the longest period of sleep and occurring between 0400 and 1200 h the next morning. The 4 timed-spot specimens correspond to the time of day when the NHANES timed urine samples are collected under the current survey protocol. A 1-mL (sodium, potassium, chloride, and creatinine) and 1.8-mL (iodine) aliquots were then taken from the composite 24-h urine sample and the 4 timed-spot urine specimens and vials were sent to the laboratories at CDC's National Center for Environmental Health for analysis. All vials were shipped frozen on dry ice within 7 d of collection. Urine specimens were analyzed for sodium, potassium, and chloride using ion-selective electrodes and the Cobas ion-selective electrode/Na⁺, K⁺, Cl⁻ assay on the Hitachi Modular P clinical analyzer (Roche Diagnostics). Urine creatinine was analyzed using the Roche Creatinine Plus enzymatic assay on the Hitachi Modular P clinical analyzer (21). Each analytical run (*n* ~60–70) contained 100 study samples and 2 levels of commercially prepared urine quality control (QC) materials (Cliniqa) measured in duplicate at the beginning and end of the run bracketing the study samples. The between-run measurement imprecision was: 0.8–0.9% for sodium (67 and 159 mmol/L), 1.7–3.4% for potassium (29 and 81 mmol/L), 1.0–1.1% for chloride (86 and 190 mmol/L), and 1.1–1.5% for creatinine (5.3 and 13.2 mmol/L). Urine iodine was measured using an Inductively Coupled-Plasma Dynamic-Reaction Cell Mass Spectrometer

ELAN DRC Plus (PerkinElmer Instruments) (22). The between-run (74 analytical runs; 20–50 study samples/run) measurement imprecision using 2 levels of in-house QC material was 1.9–2.7% (93 and 308 $\mu\text{g/L}$). QC samples contained within each analytical run for all analytes were evaluated for validity by use of a multi-rule QC program (23). Persons taking thyroid medications were excluded from iodine analysis.

Other measurements. Black or African American participants were identified by the question “Do you consider yourself to be black or African American?” during the screening. No information on other races or ethnicities was collected; participants of races other than black were grouped as “other races” in the analysis. Weight and height were measured using the standard NHANES protocol (24) and were used to calculate BMI as weight in kilograms divided by squared height in meters. Based on their BMI, participants were classified as normal weight ($18.5 \text{ kg/m}^2 \leq \text{BMI} < 25 \text{ kg/m}^2$), overweight ($25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$), or obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) (25). Because of limited sample size ($n = 5$), overweight participants ($\text{BMI} < 18.5 \text{ kg/m}^2$) were excluded from analyses using BMI categories. Dietary intake was obtained from 24-h dietary recall interviews administered in person with the standard NHANES protocol using the USDA’s Automated Multiple-Pass Method (26). Nutrient intakes were calculated from reported foods and beverages consumed by participants using USDA’s Food and Nutrient Database for Dietary Studies, version 5.0 (27), the same database used to report dietary data from the NHANES 2009–2010 cycle. This version of the Food and Nutrient Database for Dietary Studies is based on the National Nutrition Database for Standard Reference 24, which includes updated nutrient values for ~1500 foods, including top contributors to sodium intake (27). Same as the dietary data in NHANES 2009–2010, the sodium value of reported foods was not adjusted based on self-reported salt use in cooking or preparing foods in the household (28). We log-transformed sodium and potassium intake data to improve the distributions toward normality. Logarithmic means and 95% CIs were back-transformed to geometric means and 95% CIs on the original scale.

Statistical analysis. The amount of individual analytes in each urine specimen was calculated by multiplying the concentration of the analyte by the corresponding volume of the sample. The volume of the 24-h urine collection was adjusted for self-reported collection time as: (total volume collected/self-reported collection time) \times 24. Statistical tests of differences between subgroups were performed using *t* tests at the $P < 0.05$ level. Adjustments for multiple comparisons when the 4 time-spot specimens or the 3 BMI categories were compared were performed using the Bonferroni method (29). Within-person CVs were calculated between the d-1 and -2 collections as the square root of the within-person

variance divided by the mean of each analyte. We also calculated the ratio of within- to between-person variances to express the day-to-day variability.

In addition to the criteria mentioned in the study design section, we further assessed the completeness of urine collections using the ratio of observed:expected 24-h urine creatinine (Supplemental Methods 3).

The statistical analyses were conducted using SAS version 9.2 (SAS Institute).

Results

Of the 481 persons who were screened for eligibility and scheduled for an initial visit, 441 (92%) completed the appointment and received instructions and urine collection kits. A total of 407 (85%) persons returned a complete 24-h collection of urine specimens, 21 (4%) returned urine collections deemed as incomplete based on the length of collection, total urine volume, or self-reported information, and 13 (3%) did not return any urine. There were no gender, age, or race differences between participants who did not complete 24-h urine collections and those who completed a 24-h urine collection (Table 1). Of the participants with a complete 24-h urine collection, 133 (33%) completed a second 24-h urine collection 4–11 d later. Of those who completed 24-h urine collections, about one-half were female and one-half were black. Most of the participants who completed a 24-h urine collection also provided a complete 24-h dietary recall ($n = 403$, 99%, d 1; $n = 133$, 100%, d 2). Compared with those who only provided one 24-h urine collection, participants who completed a second 24-h urine collection were slightly older. However, gender, race, BMI, and self-reported dietary intakes of energy, sodium, and potassium did not differ between the 2 groups.

On average, each participant collected 7 voids/d (range: 3–13) (Table 2). The mean 24-h urine volume was 1397 mL/person for d 1 and 1505 mL for d 2. All but 2 of the 540 urine collections were collected for a period between 20 and 28 h. Both collections that took longer than 28 h (28.5 and 34.5 h) were d 1 collections. All analyses of 24-h excretions were standardized to 24 h of collection time.

Fewer urine specimens were collected during the 0830 to 1230 h time period compared with other urine collection time

TABLE 1 Characteristics of eligible study participants ($n = 481$)¹

	Participants who did not complete 24-h urine collection	Participants who completed at least one 24-h urine collection		
		Total	Participants who completed 2 d of collection	Participants who completed only 1 d of collection
Sample size, <i>n</i>	74	407	133	274
Female, <i>n</i> (%)	35 (47.3)	221 (54.3)	72 (54.1)	149 (54.4)
Age, <i>y</i>	25.8 \pm 5.1	27.0 \pm 6.0	28.1 \pm 6.0	26.4 \pm 5.9*
Race, <i>n</i> (%)				
Black	44 (59.5)	196 (48.2)	68 (51.1)	128 (46.7)
Other races	30 (40.5)	211 (51.8)	65 (48.9)	146 (53.3)
Self-reported hypertension diagnosis, <i>n</i> (%)	2 (2.7)	12 (3.0)	5 (3.8)	7 (2.6)
Thyroid medication users, <i>n</i> (%)	0 (0.0)	6 (1.5)	3 (2.3)	3 (1.1)
BMI, kg/m^2	N/A ²	27.8 \pm 6.6	27.7 \pm 6.7	27.9 \pm 6.6
d 1 dietary intake				
Energy, <i>kcal/d</i>	N/A ²	2544 \pm 1153	2497 \pm 1113	2567 \pm 1173
Sodium, <i>g/d</i>	N/A ²	3.79 (1.53, 9.41)	3.69 (1.45, 9.39)	3.84 (1.56, 9.47)
Potassium, <i>g/d</i>	N/A ²	2.65 (1.09, 6.41)	2.71 (1.09, 6.72)	2.62 (1.09, 6.30)

¹ Values are means \pm SDs or geometric means (95% CIs) unless otherwise noted. *Different from participants with 2 d of collection in a row, $P < 0.05$.

² Data were not available for participants who did not complete 24-h urine collections.

TABLE 2 Characteristics of d 1 and 2 urine collections¹

	d 1 collection		
	Total (n = 407)	Participants who completed 2 d of collection (n = 133)	d 2 collection (n = 133)
Voids collected per person, n	6.9 (3–13)	7.3 (4–13)	7.0 (3–12)
Total urine volume, mL	1397 ± 654 (503–4534)	1558 ± 689 (503–4097)	1505 ± 771 (547–3746)
Total collection time, h	24.0 ± 1.1 (20.6–34.5)	24.0 ± 0.9 (20.8–28.5)	24.2 ± 1.0 (20.3–27.4)
Missed one void during collection	7 (1.7)	2 (1.5)	3 (2.3)
Total timed-spot specimens collected, n			
Morning	339 (83.3)	116 (87.2)	116 (87.2)
Afternoon	389 (95.6)	127 (95.5)	126 (94.7)
Evening	402 (98.8)	131 (98.5)	132 (99.3)
Overnight	406 (99.8)	132 (99.3)	131 (98.5)
Timed-spot specimens provided by participants, n			
2	6 (1.5)	2 (1.5)	2 (1.5)
3	80 (19.7)	22 (16.5)	23 (17.3)
4	321 (78.9)	109 (82.0)	108 (81.2)

¹ Values are means (range), means ± SDs (range), or n (%).

periods. After discarding the first void on the morning of the initial day of collection, 17% of the participants did not urinate again until the afternoon. Approximately 80% of the d 1 and 2 collections had a timed-spot urine specimen for all 4 urine collection periods.

Completeness of the urine collections. The completeness of the urine collections in the study was assessed by the length of collection, total urine volume, and the responses to a questionnaire similar to the one used in the International Cooperative Study on Salt, Other Factors, and Blood Pressure (INTERSALT) (31). Completion was defined as a set of urine samples collected through >20 h with total volume >500 mL and missing no more than one void during the collection. Only 7 (1.7%) participants on d 1 and 3 (2.3%) participants on d 2 with valid urine samples reported missing one void during their collections (Table 2). Exclusion of these data did not affect the distribution of estimates for any of the analytes reported and their data were included in this study.

We further verified the completeness of the 24-h urine collections using expected creatinine excretion (Supplemental Methods 3, Supplemental Tables 1–4). We conducted sensitivity analyses excluding individuals who did not meet the expected creatinine excretion criterion and found no effect on the reported mean urine volume or any of the analyte excretions (Supplemental Tables 2 and 3). Therefore, data from all 407 individuals are included in the analytic sample.

Urine analyte excretions measured in 24-h collections. The means and percentile distributions of total urine volume and 24-h urine excretion of the 5 analytes measured are presented in Table 3 for each race and gender group. Compared with other races, black males and females had significantly lower total urine volume and potassium, but higher creatinine excretion. Females had lower creatinine excretion than males, regardless of race. Compared with males, females also had significantly lower sodium, potassium, and chloride excretions among participants of other races, but not among blacks. The observed iodine excretion did not differ by gender or race.

Urine analyte concentrations measured in timed-spot urine specimens. Overnight specimens had a larger volume than other timed-spot urine samples across all race and gender

groups (Table 4). There were no volume differences among morning, afternoon, or evening specimens, except for non-black females' morning specimens, which had a lower volume than the other timed-spot urine samples for this group. Compared with other timed-spot urine samples, overnight specimens also had lower sodium, potassium, and chloride concentrations across all race and gender groups. There were generally no significant differences in creatinine or iodine concentrations among different timed-spot urine specimens.

Compared with males of other races, black males had a lower urine volume in their timed-spot specimens, although the difference was not significant in the morning specimens. Black females had a similar volume in their timed-spot urine samples compared with females of other races, except for afternoon specimens. Compared with persons of other races, blacks had higher urine sodium concentrations in their timed-spot urine samples, although the differences were not always significant. Black participants also had higher urine creatinine concentrations than participants of other races in morning, afternoon, and overnight specimens among males ($P = 0.003$ – 0.046) and in afternoon and evening specimens among females ($P < 0.001$).

In general, females had a lower urine creatinine concentration in their timed-spot urine samples than males. The observed gender differences were significant in the morning, afternoon, and overnight specimens for blacks and in the afternoon and evening specimens for participants of other races. Across all urine specimens, sodium, potassium, and chloride concentrations did not differ by gender. Iodine concentrations did not significantly differ by race or gender in any of the timed-spot urine specimens.

Within-person variance between the d 1 and 2 collections. Among the subset of individuals with a second 24-h urine sample, the within-person variances between the d 1 and 2 collections are shown in Table 5 by analyte, race, and the timing of urine collections. For 24-h sodium, chloride, and potassium excretions, the within-person, day-to-day variances were ~16–29% of the mean excretion values. The variances were larger for timed-spot urine samples, which ranged from 21 to 41% of the mean excretions in the spot specimens. The evening specimen showed generally larger within-person variance in sodium, chloride, and potassium excretions compared with other spot specimens.

TABLE 3 Distributions of total urine volume and 24-h excretion of sodium, potassium, chloride, creatinine, and iodine in the d 1 collection^{1,2}

	Means ± SDs	Percentile		
		25th	50th	75th
Total volume, mL				
Black male	1272 ± 532	884	1152	1574
Black female	1252 ± 589	799	1163	1533
Other male	1599 ± 814*	969	1288	2052
Other female	1458 ± 569*	987	1346	1836
Sodium, g				
Black male	3.52 ± 1.61	2.28	3.34	4.61
Black female	3.18 ± 1.32	2.35	2.91	3.95
Other male	3.56 ± 1.43	2.66	3.20	4.09
Other female	3.01 ± 1.21 [†]	2.12	2.87	3.74
Potassium, g				
Black male	1.89 ± 0.77	1.34	1.79	2.21
Black female	1.70 ± 0.61	1.3	1.66	2.04
Other male	2.35 ± 1.05*	1.72	2.04	2.79
Other female	1.99 ± 0.68* [†]	1.52	1.88	2.43
Chloride, g				
Black male	5.07 ± 2.27	3.39	4.89	6.42
Black female	4.65 ± 2.02	3.35	4.25	5.43
Other male	5.25 ± 2.18	3.88	5.02	5.99
Other female	4.42 ± 1.76 [†]	3.17	4.32	5.32
Creatinine, g				
Black male	2.04 ± 0.62	1.66	2.07	2.42
Black female	1.47 ± 0.36 [†]	1.23	1.47	1.67
Other male	1.82 ± 0.47*	1.49	1.80	2.05
Other female	1.23 ± 0.32* [†]	0.99	1.24	1.37
Iodine, µg				
Black male	252 ± 226	109	171	293
Black female	223 ± 245	103	140	237
Other male	308 ± 292	139	225	374
Other female	259 ± 265	133	185	310

¹ The number of observations for total volume and sodium, potassium, chloride, and creatinine excretion are: black male (*n* = 89), black female (*n* = 107), male of other races (*n* = 97), and female of other races (*n* = 114). *Within an analyte, different from blacks within the same gender, *P* < 0.05. [†]Within an analyte, different from males within the same race category, *P* < 0.05.

² The number of observations for iodine excretion were: black male (*n* = 89), black female (*n* = 106), male of other races (*n* = 96), and female of other races (*n* = 109).

Across all urine specimens, the within-person variance in sodium excretion was similar in magnitude to the between-person variance, except for overnight specimens among persons of other races. For chloride and potassium, the within-person variances were relatively smaller than the between-person variances among persons of other races, but not among blacks.

The within-person variance for 24-h creatinine excretion was only 9% of the mean creatinine excretion and 10–20% of the between-person variance, although the variability of spot specimens was similar to that observed for other analytes. For iodine, the within-person variances were ~17–33% of the mean iodine excretion and were generally much less than the between-person variances.

Discussion

To our knowledge, this is the first study designed to assess the ability to characterize sodium intake from timed-spot urine samples calibrated to a 24-h urine collection with a sizable U.S. sample of black and other race adults aged 18–39 y. In this group

of healthy, young adult volunteers residing in the Washington, DC metropolitan area in 2011, 85% collected a 24-h urine sample considered complete for analysis; one-third (*n* = 133) of these completed a second 24-h urine collection 4–11 d later. Data from this study will provide valuable information in developing a monitoring system for sodium intake at the population level.

The mean urine volume in our study was comparable with that from participants in the Coronary Artery Disease in Young Adults study (C.M. Loria and K. Liu, unpublished data) and INTERSALT participants in Jackson, Mississippi (i.e., 1220 mL for black participants and 1630 mL for white participants) but higher than mean volumes for INTERSALT participants in Goodman, Mississippi (i.e., 850 mL for black participants and 1240 for white participants) (2). Moreover, our mean 24-h urine sodium excretions were similar for females and somewhat lower for males compared with those in a recent meta-analysis of 38 studies dated from 1957 to 2003 (3084 mg for females and

TABLE 4 Mean urine volume and concentration of sodium, potassium, chloride, creatinine, and iodine in timed-spot urine specimens in the d 1 collection by race and gender^{1,2}

	Timing of spot urine collection			
	Morning	Afternoon	Evening	Overnight
Total volume, mL				
Black male	184 ± 111 ^a	173 ± 81 ^a	184 ± 81 ^a	257 ± 123 ^b
Black female	168 ± 108 ^a	178 ± 91 ^a	182 ± 104 ^a	271 ± 178 ^b
Other male	206 ± 148 ^a	233 ± 139 ^{a*}	212 ± 100 ^{a*}	321 ± 163 ^{b*}
Other female	159 ± 111 ^{a†}	220 ± 125 ^{b*}	199 ± 114 ^b	288 ± 134 ^c
Sodium, mmol/L				
Black male	141 ± 62 ^a	146 ± 69 ^a	139 ± 74 ^{a,b}	121 ± 69 ^b
Black female	145 ± 64 ^a	148 ± 72 ^a	136 ± 75 ^{a,b}	121 ± 54 ^b
Other male	123 ± 61 ^a	117 ± 64 ^{a,b*}	129 ± 66 ^a	102 ± 57 ^{b*}
Other female	118 ± 58 ^{a*}	104 ± 62 ^{a,b*}	115 ± 63 ^{a*}	97 ± 52 ^{b*}
Potassium, mmol/L				
Black male	58 ± 35 ^a	58 ± 35 ^a	45 ± 31 ^b	35 ± 23 ^c
Black female	55 ± 31 ^{a,b}	56 ± 33 ^a	48 ± 32 ^b	32 ± 18 ^c
Other male	64 ± 35 ^a	59 ± 33 ^a	56 ± 35 ^{a*}	36 ± 26 ^b
Other female	56 ± 33 ^a	54 ± 38 ^a	55 ± 35 ^a	30 ± 19 ^b
Chloride, mmol/L				
Black male	150 ± 71 ^{a,b}	152 ± 76 ^a	131 ± 70 ^b	105 ± 62 ^c
Black female	150 ± 65 ^{a,b}	155 ± 72 ^a	133 ± 74 ^b	105 ± 51 ^c
Other male	144 ± 72 ^a	132 ± 73 ^{a,b}	130 ± 67 ^b	85 ± 55 ^{c*}
Other female	128 ± 66 ^{a*}	121 ± 67 ^{a*}	118 ± 66 ^a	77 ± 45 ^{b*}
Creatinine, mmol/L				
Black male	17 ± 9	18 ± 9	17 ± 10	18 ± 9
Black female	14 ± 8 [†]	14 ± 7 [†]	15 ± 9	14 ± 7 [†]
Other male	15 ± 9*	14 ± 8*	15 ± 9	15 ± 8 [‡]
Other female	12 ± 8 ^a	9 ± 6 ^{b*†}	10 ± 6 ^{a*†}	12 ± 7 ^{a†}
Iodine, µg/L				
Black male	198 ± 160	224 ± 292	201 ± 205	238 ± 296
Black female	196 ± 185	216 ± 284	206 ± 301	200 ± 219
Other male	242 ± 272	217 ± 227	227 ± 208	261 ± 325
Other female	205 ± 160	177 ± 157	229 ± 227	242 ± 346

¹ Values are means ± SDs. Within an analyte, labeled means in a row without a common letter differ, *P* < 0.05 (with Bonferroni adjustment). *Within an analyte, different from blacks within the same gender using the same timed-spot specimens, *P* < 0.05. [†]Within an analyte, different from males within the same race category using the same timed-spot specimens, *P* < 0.05.

² Timing of specimens: morning (the second void upon rising in the morning), 0830 to 1230 h; afternoon, 1231 to 1730 h; evening, 1731 to 2359 h; overnight (the first void after the longest period of sleep), 0400–1200 h the next morning. See **Supplemental Table 5** for number of observations in each cell.

TABLE 5 Within-person, day-to-day CVs and the ratio of within- to between-person variance of sodium, potassium, chloride, creatinine, and iodine excretions in 24-h urine samples and timed-spot urine specimens^{1,2}

	Within-person CV, %					Ratio of within- to between-person variance				
	24 h	Morning	Afternoon	Evening	Overnight	24 h	Morning	Afternoon	Evening	Overnight
Sodium										
Black	23	29	31	39	31	0.9	0.8	0.6	0.9	1.1
Other races	19	38	30	39	21	0.9	0.7	0.8	1.2	0.3
Potassium										
Black	17	25	27	40	26	0.6	0.9	0.5	1.0	1.0
Other races	17	26	23	31	22	0.4	0.6	0.3	0.7	0.3
Chloride										
Black	29	24	38	41	27	1.2	0.4	1.3	1.2	0.6
Other races	16	34	25	33	24	0.5	0.6	0.6	0.8	0.3
Creatinine										
Black	9	23	31	35	27	0.2	0.5	0.7	0.9	1.2
Other races	9	24	25	29	17	0.1	0.7	0.7	1.2	0.4
Iodine										
Black	25	28	34	33	25	0.4	0.3	0.9	0.6	0.3
Other races	17	28	28	28	23	0.1	0.3	0.3	0.3	0.2

¹ Within-person CVs were calculated as the square root of the within-person variance divided by the mean of each analyte.

² Only included individuals with 2 d urine collections. See Supplemental Table 5 for number of observations in each cell.

3911 mg for males) (31). However, the study populations in most studies in the meta-analysis differed from ours on a number of factors: age, presence of hypertension, geographic region, and year of study.

It appears that the sodium intakes estimated from dietary recalls were somewhat higher than the excretion values measured from the 24-h urine collections in the study (Supplemental Table 2), which may raise questions about the completeness of the 24-h urine collections and the validity of the 24-h dietary recall in assessing the sodium consumption. The completeness of the 24-h urine samples in this study was determined during data collection by the length of collection, total urine volume, and the adherence of the protocol and was further verified by sensitivity analysis using expected creatinine criterion. Exclusion of participants with incomplete urine samples as identified by expected creatinine criterion did not eliminate the observed discrepancy. Studies comparing sodium intake based on dietary and urinary data are inconsistent, with some studies suggesting that food records or 24-h dietary recalls underestimate 24-h urinary sodium excretion (32–34), whereas others suggest 24-h sodium excretion underestimates dietary intake by 10 to 23% (19,35). The direction of differences in mean sodium intake based on dietary and urine data also varied by country in the International Study on Macronutrients and Blood Pressure (36).

This study was designed to provide an adequate sample size to develop a calibration equation specific for blacks. In the study, we observed that black individuals generally had lower urine volumes and higher urine sodium concentrations (mmol/L) than other races in 24-h urine and timed spot specimens. These observations were similar to the findings reported from previous studies (2,16,31), although one clinical trial among participants consuming a fixed sodium diet suggested no differences in 24-h sodium excretion by race (19).

The diurnal patterns observed in our study show that, in general, the overnight urine samples had the largest volume and lowest sodium, potassium, and chloride concentrations compared with urine samples collected during the other times of the day. In contrast, urine creatinine and iodine concentrations stayed relatively consistent throughout the 24-h period across all race

and gender groups. There was substantial day-to-day variability observed between the 2 urinary sodium, potassium, chloride, and iodine excretions 4–11 d apart across 24-h and timed-spot urine specimens. Compared with the between-person variance, the within-person, day-to-day variance was similar in sodium excretions but much less in iodine excretions.

The study sample was comprised of adults aged 18–39 y. Less than 1% were taking blood pressure-lowering medication. Therefore, our results may not apply to older adults, particularly to populations with higher rates of chronic disease. We did not measure blood pressure in this study and by design had a small sample size ($n = 12$) who reported having hypertension, precluding analyses stratified by hypertension status. However, exclusion of the few participants with self-reported hypertension or taking an antihypertensive medication did not affect the direction, magnitude, or significance of the results (analyses not shown).

A national surveillance system that is sensitive for monitoring U.S. efforts to reduce sodium intake is critical to evaluating such efforts. Due to logistic issues and limited resources, 24-h urine collection has not been included as part of the national surveys and thus sodium intake has been estimated using 24-h dietary recalls. This calibration study, which examines the associations between timed-spot and 24-h urine analytes in healthy young adults, is the first step toward determining if sodium intake can be measured from biomarkers already collected in the NHANES. This is the first report in a series. In this report, we described the design and basic characteristics of the study. Subsequent reports will focus on assessing the validity of published estimation equations for 24-h sodium and related nutrient excretions using partial samples collected at different times throughout the day and developing new calibration equations to estimate population-level, 24-h sodium and iodine excretion from multiple timed-spot urine samples.

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