

1 INDIVIDUAL VARIATION IN URINARY SODIUM EXCRETION AMONG ADOLESCENT GIRLS ON A FIXED
2 INTAKE

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13
14
15 Abstract Word Count: 257

16 Overall Word Count: 4281

17 Number of Tables: 1

18 Number of Figures: 5

19
20 Running Title: Variation in Urinary Sodium Excretion

21
22 This work was presented at the AHA-EPI Life Style meeting in Baltimore, MD on March 4, 2015.

23
24 *Conflict of Interest and Funding Disclosure: None*

25
26 C.M.W., B.R.M., G.P.M., M.W., L.J.A. designed research; C.M.W., B.R.M., G.P.M., L.D.M., M.W., L.J.A.
27 conducted research; B.R.M., G.P.M., L.D.M. analyzed data; and C.M.W., B.R.M., G.P.M., L.D.M., M.W.,
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Abstract

Background: According to traditional understanding of sodium homeostasis, nearly all of daily sodium intake is excreted in urine, with intra-individual variability attributed to variability in dietary sodium intake and urine collection errors.

Objective: To analyze the variability of urinary sodium in excretion from a balance study with fixed sodium intakes.

Methods: Daily 24-h urine collections were assessed for sodium, potassium, and creatinine in 22 black and 13 white adolescent girls (11-15 y, BMI 15-29 kg/m²) in a randomized, crossover design with controlled diets containing either low (57 mmol/d) or high (167 mmol/d) sodium, each fed for three weeks.

Results: Coefficients of variation (CV) analysis indicated higher variation in urinary sodium excretion about the mean on low (vs high) sodium (40% vs 32%, $p=0.02$) and in black (vs white) girls (42% vs 30%, $p<0.001$). A mixed model showed no sodium intake x race interaction. Urinary sodium excretion was not correlated with urinary potassium or creatinine excretion. Excretion of 65 mmol/d (Adequate Intake) or less was documented on 16% on the days during the high sodium. Reliability of the mean of several urine sodium samples varied from 23% for one sample to 75% for ten samples for the high sodium diet.

64 Conclusions: The high intra-individual variability in urinary sodium excretion on a fixed diet highlights
65 the potential for substantial error in (a) using a single 24-hr urine collection to estimate an individual's
66 usual sodium intake and (b) relating sodium excretion from a single 24-hr collection with outcomes.
67 Further research is warranted to understand the causes of such variation.

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70 KEY WORDS: sodium; urinary excretion; potassium; adolescent; girls

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Introduction

High sodium intake has been related to several chronic diseases, including hypertension [1-3] and osteoporosis [4]. Hypertension is a major risk factor for the development of stroke, coronary heart disease, heart failure, and end-stage renal disease [5]. Hypertension is the most common cause of death worldwide [6] and is one of the most important risk factors for cardiovascular mortality [7]. Hypertension is the leading preventable cause of mortality after smoking [8]. High sodium intake is associated with hypertension through increases in plasma volume associated with increased cardiac output and stroke volume [9].

Determining relationships between dietary sodium and hypertension, or other chronic diseases, depends on accurate assessment of sodium intake. Urinary sodium excretion is considered to be superior to survey measures of dietary assessment or spot urine for measuring dietary sodium intake. The uncertainty in the amount of salt added by subjects during food preparation or at the table, and the variability of sodium in food formulations available in food composition databases, precludes accurate assessment of sodium intake using traditional diet assessment tools.

Use of daily urinary sodium as a biomarker of sodium intake assumes that urinary sodium reflects dietary intake. However, variation in daily sodium excretion has been reported to be high in adults [10] and children [11], although in these studies diets were not controlled so that the source of the variation, i.e. diet or excretion, could not be determined. It was assumed that the source was variation in sodium intake. However, a recent report from a Russian space flight simulation study in 4 cosmonauts documented unexpectedly high daily variation in urinary sodium excretion despite a fixed intake [12].

Their findings challenge the current practice of using a 24-h urine collection as an estimate of the previous day's sodium intake, although the sample size was small, the setting was unusual, and the participants were not representative of the general population. Racial differences in urinary sodium excretion on a fixed sodium diet (167 mmol/d) have been reported in adolescents, i.e. lower sodium excretion and higher sodium retention by 0.66 g/d in black compared to white girls in a controlled environment with excellent compliance [13]. Furthermore, blacks have greater whole body sodium content compared with white women, as determined by delayed Y-neutron activation analysis [14].

To add to the evidence of the relationship of sodium intake to urinary sodium excretion in an important age group where there is a dearth of information the aim of this study was to analyze individual variation in daily urinary sodium excretion in black and white adolescent girls on a fixed diet of low (57 mmol/d) and high (167 mmol/d) sodium intake, but within the normal range of female adolescents in the U.S. [15]. The former is similar to the recommended Adequate Intake (AI) of 65 mmol/d (1500 mg/d) sodium by the Institute of Medicine [16] and is lower than dietary levels analyzed for variation in urinary excretion on a fixed intake in adults [12]. This study was a secondary analysis of a metabolic balance study in which group means were previously reported [13]. We tested the hypotheses that 1) daily urinary excretion would be highly variable despite a fixed intake, and 2) the variation may be affected by level of sodium intake and race. Because the urinary sodium to potassium ratio may be a better predictor of hypertension than either cation alone [17], we also examined variability in potassium excretion and the urinary sodium to potassium ratio.

Methods

Study Participants

As previously reported [11], white and black adolescent girls aged 11 to 15 yr were recruited for a metabolic balance study ran as a summer research camp in West Lafayette, Indiana in the summer of 1999. Inclusion criteria were: being of black or white race determined from the participants' self report of their parents and grandparents; body mass index between the 15th and 85th percentile for age; and good general health determined from screening questionnaires, chemistry panel, and physical examinations. Black and white girls were matched, by group, for post menarcheal age, height, and weight. Protocols were approved by the Purdue University Use of Human Subjects Research Committee, and both participants and guardians gave informed consent.

Study Design

This study was a randomized order, cross-over trial of two 3 week periods using two diets which varied in dietary sodium content (measured at 57 mmol/d and 167 mmol/d), separated by a 2 week washout period. Participants were housed on the Purdue University campus for the two metabolic balance periods. The controlled diets consisted of 4-day menu cycles and varied only in sodium content provided through two servings of soup and two servings of an energy drink, Gatorade (The Quaker Oats Company, Barrington Illinois) daily with one serving given at each of lunch, afternoon snack, dinner, and evening snack. In both dietary sodium periods, dietary potassium was fixed at 56 mmol/d, calcium at 20 mmol/d, magnesium at 9.4 mmol/d, phosphorus at 36 mmol/d, protein at 70 g/d, fat at 73.6 g/d, and fiber at 10 g/d. Subjects could adjust energy intake only with provided sodium-free, sugar based products such as hard candy and frozen flavored ices. Compliance with diet was monitored by extensive

and continuous direct supervision of participants; at each eating occasion for the entire study. Compliance with diet was greater than 98% as determined by uneaten food at the end of the day. Body weight was measured by an electronic scale (Health O Meter) and height using a stadiometer. Information on general feeling of wellness and the menstrual cycle was collected nightly throughout the study.

Analysis

Complete 24-h urine collections were made from day 2 through 19 of each metabolic balance period in acid-washed containers. For each metabolic period, we included individuals with at least 17 collections in each period. Compliance with urine collections was monitored by supervision of all bathroom activities. Daily diet composites and urine samples were analyzed for sodium and potassium by atomic absorption spectrophotometry (5100 PC, Perkins-Elmer, Shelton, CT), and creatinine colorimetrically (Colas Miras Systems).

Statistical Analysis

Urinary sodium excretion was plotted versus day for each participant with means for each race and sodium intake combination. For a representative black girl and a representative white girl, plots versus day for each diet were made for urinary excretion of sodium, potassium, creatinine, and the sodium to potassium ratio. Variability of urinary sodium was quantified by the coefficient of variation (CV, standard deviation expressed as a percent of the mean), and the distributions for each race and diet combination were examined. CVs were analyzed in a mixed linear model that included terms for sodium

level, race, and the interaction. For each level of sodium intake, within-subject and between-subject components of variance were determined and these were used to compute intraclass correlations.

To assess the effects of hormonal patterns on variation in sodium excretion, each observation day was classified as premenarcheal, follicular (days 1 to 14 of the menstrual cycle) or luteal (days 15 or greater of the menstrual cycle). The resulting categorical variable was called phase. Girls who were premenarcheal and did not provide an initial date of menstrual flow were excluded from this analysis. The variable phase was added to a mixed linear model for the analysis of CVs that included terms for the study design. Furthermore, for girls who provided luteal and follicular data, we plotted urinary sodium excretion versus cycle day. Cycle day one was the day of initial menstrual flow. A smooth function was fit to explore this relationship.

A key issue in sodium estimation is how many repeat 24h samples are required before a reliable estimate is obtained. The reliability of a mean value computed from a sequence of n daily urine samples per girl, r^* , was estimated using the Spearman–Brown formula [18],

$$r^* = nr / (1 + (n - 1)r)$$

where r = intraclass correlation. This formula was used to assess the effect of varying numbers of urine samples for each participant, together with associated 95% confidence intervals. We pre-specified that a reliability of 75% ($r^* = 0.75$) would be acceptable [18].

Results

Twenty-two black and thirteen white adolescent girls were studied. Of the 27 subjects on the low sodium diet and 25 subjects on the high sodium diet who met the inclusion criteria, we had complete

24-h urine collections for 485 out of 486 possible low sodium collections and 446 out of 450 possible high sodium collections. At baseline, whites were slightly older; no other statistically significant differences between races were found (Table 1).

The mean and individual urinary sodium and dietary sodium at the two studied intakes levels (57 and 167 mmol/d) are shown in Fig. 1. The mean urinary sodium excretion is below dietary intake on both low and high sodium diets and there is a large variation in urinary sodium, despite the fixed intakes. The distribution of CVs of urinary sodium excretion (Fig. 2) also indicates the large variation and the effect of race and level of sodium intake. Variation in urinary sodium (expressed as CV) about the mean on low compared with high sodium intakes was 40% vs 32%, $P<0.002$, and for black compared with white girls, was 42% vs 30%, $P<0.001$. There was no significant diet x race interaction ($P=0.3$). When urinary sodium excretion was corrected for creatinine excretion, the CV increased (47% vs 36%, $P<0.001$). On the low sodium diet, between-person variance in urinary sodium and within-person variance were 47.8 and 231.4, respectively. For the high sodium diet, the corresponding values were 466.9 and 1565.4 mmol/d, respectively. The intraclass correlations (95% CI) were 0.17 (0.09, 0.31) for low sodium and 0.23 (0.13, 0.39) for high sodium. Estimated reliability increases substantially when number of samples increases (Fig. 3). To reach a goal of 0.75 reliability [18], 10 to 15 samples would be required, depending on the sodium intake.

With respect to menstrual status, no differences in CVs due to phase were found: (n, Mean CV, standard error) premenarcheal 11, 35.8%, 3.5%; luteal 14, 36.5%, 3.6.7%; and follicular 14, 32.2%, 3.4%; $P=0.63$.

In plots of urinary sodium excretion versus cycle day for high and low sodium intake, smooth fits to the data did not suggest any consistent relationship between sodium excretion and cycle day (data not

shown). The variation of daily urinary sodium excretion on a fixed intake of 167 mmol/d was large (Fig. 4) and 85% of the samples were below the sodium intake of 167 mmol/d. Individuals on the high sodium diet had 16% of urinary excretion values <65 nmol/L (Adequate Intake, AI) compared with 95% on the low sodium diet (57 mmol/d).

Variance for urinary potassium and creatinine was also high. For urinary potassium, the CV was 35% on the high sodium diet and 41% on the low sodium diet for black girls; the CV was 31% and 33% for high and low sodium diets, respectively, for white girls. For urinary creatinine, the CV was 41% on the high sodium diet and 46% on the low sodium diet for black girls and 34% and 44% for the high and low diets, respectively, for black girls. There were no differences in variation due to race or dietary sodium. Urinary sodium was not correlated with urinary potassium or creatinine. Daily urinary excretion of sodium, potassium, and creatinine on high and low sodium for one representative black girl and one representative white girl are shown in Fig. 5.

When expressed as Na:K ratios, the CV on low sodium diet was also greater than the corresponding CV on the high sodium diet. For urinary Na:K, the CV was 44% and 30% for low and high sodium diets, respectively ($p=0.004$). For Na:Cr, the CV was 53% and 42% for the low and high sodium diets, respectively ($p=0.03$). There were no racial differences.

Discussion

Daily urinary sodium excretion in adolescent black and white girls was highly variable (30-42% CV), despite a fixed sodium intake. The variance in urinary sodium excretion was greater in black than white

girls and greater on lower than higher sodium intakes. None of these findings are explained by noncompliance or laboratory errors. In fact, the CVs for sodium to potassium and creatinine ratios, where any under-collection errors would be eliminated, were also high. Furthermore, urinary sodium was unrelated to excretion of potassium or creatinine. This rules out systematic under-collection errors where all measured components would track together. Intraclass correlations were weak and we found that at least 10 repeated 24-h samples were required on a high sodium intake to reach a desirable level of 75% reliability in estimation of individual levels of sodium excretion. Correction for the skewness of urinary sodium using the square root, as suggested by Cook et al. [19], did not change the results appreciably. Based on a study of children on self-selected diets, Liu et al [11] recommended 8 24-h urine samples. In adults aged 30 to 44y, Liu et al., [10] recommended 14 collections for estimating a correlation with blood pressure. On controlled diets in adult men, 7 24-h urine collections were necessary to detect a 3g salt difference with 92% accuracy [20].

The large variation in urinary sodium excretion on fixed sodium diets in adolescents is consistent with observations in 4 adult men participating in balance studies as part of a space flight simulation of 105 or 205 days [10]. In these long term balance studies, Rakova et al. [12] were able to observe infradian rhythm periods of about monthly and longer for sodium excretion and retention that were positively correlated with urinary aldosterone excretion and inversely correlated with urinary cortisol, but were independent of changes in body water or blood pressure. As salt intake was altered between 6, 9 and 12g/d in step changes, body weight, and extracellular water, aldosterone changed as predicted within the first week of adaptation. Our study in adolescents confirms the patterns observed in adults on similarly high sodium intakes (155 vs. 167 mmol/d). Uniquely, we studied low sodium intakes (57 mmol/d). On the low sodium intake the absence of any values for urinary sodium excretion over 129

mmol shows that the kidney is more efficient at conserving sodium than on high intakes, consistent with observed elevated plasma aldosterone levels and plasma renin activity on the lower sodium intake [13]. This is likely a physiological response to defending sodium homeostasis for vascular stability.

In adults, nearly all of dietary sodium is recovered in the urine. In contrast, dietary sodium is retained in adolescents and substantially less is excreted in urine. Specifically, we previously reported the low dietary sodium recovery in urine for adolescents (65 and 83% in white girls and 58% and 63% in black girls on low and high salt intakes, respectively) in the same subjects studied here [13].

The precautions taken to ensure compliance including extensive and continuous direct supervision of the participants for both diet and collection of excreta would suggest that the discrepancy between sodium intake and excretion was not due to noncompliance. This is supported by there being no significant correlation among excreted nutrients. We speculated that in adolescence, during this relatively short period of bone mass accretion, sodium is transferred to growing bone along with minerals needed for bone mineralization [13]. If poor compliance explained the low recovery of sodium intake in the urine, we would conclude that compliance in black girls was poorer than for white girls. However, the greater sodium, calcium, potassium, and magnesium retention on the same dietary intakes in black girls compared to white girls is consistent with the known greater bone accretion in black females [21-23].

An important consequence of large variation in urinary sodium excretion, even on fixed intakes, is the misclassification of individuals in observational epidemiological studies relating estimated sodium intakes to blood pressure, cardiovascular, and other diseases. We found that misclassification was

extreme on high sodium intakes. On 167 mmol/d of sodium, 16% of the samples were below the targeted intake of 65 mmol/d compared to 95% on the lower sodium intake of 57 mmol/d. Falsely classifying high sodium consumers as low sodium consumers would attenuate diet-disease associations, thereby reducing the ability to determine real relationships. Most epidemiological studies have used only one 24h urine collection, per person, to determine sodium intake. However, our data suggest that 10 urine collections are needed on high sodium intakes and 15 on low sodium intakes before reasonably robust estimates are obtained.

Strengths of this study include availability of complete daily urinary excretion for 3 week periods in an understudied group, i.e. adolescent girls, from two races on two levels of sodium, who were healthy with a normal range of BMI. Other strengths are the controlled feeding protocol with 2 levels of sodium intake and quality control efforts to ensure complete diet consumption and sample collections as well as the inclusion of confidence intervals and regions for our estimates of reliability and the number of samples required for a given reliability. Other environmental factors were also controlled as the volunteers lived in the same living quarters and had the same activity schedule. Limitations of this study are the small sample size, lack of sufficiently long periods to explore infradian patterns and the lack of daily measures of potential regulators of sodium excretion.

Together with results from Rakova et al., [12], our results challenge the notion that a single 24-h urine sodium excretion value is the gold standard estimate of sodium intake for either research or clinical monitoring of sodium intake for individuals. Requiring 10 to 15 24-h samples to achieve reliable estimates of sodium intake is impractical. Variation in self-reported sodium intake is at least as variable and likely less accurate because sodium in foods and sodium added at the table is difficult to assess. In 6

302 day diet records collected before the metabolic balance study began, variation in sodium intake was
303 42% CV in these adolescent girls. Given these considerations, controlled feeding studies are necessary
304 in order to be confident of knowing actual sodium intake.

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306 Nevertheless, single urinary sodium collections collected in a large population can be expected to
307 adequately describe the mean of that population's typical sodium intake.

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309 Acknowledgements: None

310 Source of Funding: None

311 Conflict of Interest: None

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