Variability in 24-Hour Urine Sodium Excretion in Children

KIANG LIU, PH.D., RICHARD COOPER, M.D., IVAN SOLTERO, M.D., AND JEREMIAH STAMLER, M.D.

SUMMARY How many 24-hour urine sodium measurements are adequate for characterizing a child's salt intake? Can overnight urine specimens accurately replace 24-hour collections for salt assessment? A sample of 73 6th-8th grade children was taken from two parochial schools in Chicago to investigate systematically these questions. Seven consecutive 24-hour-urine specimens were collected from each child. The estimated ratio of intra- to inter-individual variances was 1.94 for 24-hour-urine sodium. Based on this value, eight 24-hour specimens are necessary to limit to 10% the diminution of the estimated correlation coefficient between 24-hour-urine sodium and blood pressure. Six measurements are required to reduce to 0.01 the probability of misclassifying a child in tertile 1 versus tertile 3.

The overnight specimens show a moderate consistency with the 24-hour collections in detecting children with high or low salt intake. For example 92% and 85% of children in the fifth quintile and the third tertile respectively of the true mean overnight sodium have their true mean 24-hour Na in the upper half of the distribution. These results suggest that in a large scale epidemiologic study, overnight specimens may be reasonable alternatives when 24-hour-urine sodium is practically very difficult to collect. (Hypertension 1: 631–636, 1979)

KEY WORDS • sodium excretion • children • urine sample

A causal relationship between excess sodium intake and high blood pressure has been inferred from the findings of epidemiological, clinical and animal experimental studies. However, for individuals within a more or less homogeneous population, this relationship has not been consistently demonstrated. The clarification of this problem in children is of special importance, since a positive result would lend support to nutritional approaches for the early prevention of the mass occurrence of hypertension.

In order to study this matter accurately, an appropriate method is required for characterizing each child's mean urinary Na output, as an index of Na intake. A recent study by our group on sodium excretion in healthy American adults indicated that the intra-individual variation in the individual's daily output is about three times the inter-individual variation. This phenomenon can result in serious underestimation of the true association between habitual sodium intake and blood pressure within a population unless several 24-hour urine specimens are collected. For example, in the adult population studied, fourteen 24-hour urine samples are needed to limit to less than 10% the error in the correlation between true mean 24-hour urine Na and blood pressure. Therefore, as a first step in studying this relationship in children, it was deemed necessary to examine the ratio of intra- to inter-individual variation in 24-hour Na excretion, and to assess the number of urine specimens required for accurately characterizing each child's sodium intake.

Collection of large numbers of 24-hour urines per person, adult or child, is very difficult practically. This difficulty can be reduced if overnight urine collection can replace 24-hour specimens for Na assessment. For the middle-aged men previously studied, overnight
urine sodium was shown to be reasonably consistent with 24-hour Na, and therefore usable for detecting individuals with high versus low sodium intake. The present study assessed whether this consistency between 24-hour and overnight urine Na is present in children, and, if so, how many overnight specimens are sufficient, given intra-individual variation, to reduce to a desired degree the resultant weakening of the estimated correlation.

Methods

The present study was designed as an extension of a large-scale blood pressure survey among children in two parochial schools in Chicago (unpublished data, S. Levinson et al.). With the cooperation of science teachers, it was presented as a special project to the science classes of 6th–8th grades of the two schools and the children were asked, with the consent of their parents, to volunteer. Information on the relationship between nutrition and cardiovascular disease was presented in general terms, with salt intake mentioned as one of several dietary factors. Students were requested not to alter usual eating patterns. Of 108 students in three target classrooms, 78 (72.2%) volunteered, and 73 (67.6%) completed 7-day specimens.

Groups of 6–10 children were carefully instructed on the procedure for collection of 24-hour urine output. They were asked to divide each 24-hour sample into an overnight portion, defined as urine voided after emptying the bladder at bedtime, including urine voided upon arising, and a daytime portion, i.e., urine voided during the day, including urine voided just prior to retiring. Carrying cases and plastic bottles were provided for each student on a daily basis. When a day’s sample was missing, an additional one was collected in the following week; although this was not necessarily on the same day of the week, repeat weekend samples were transmitted blind to the laboratory and creatinine. For 20 specimens, randomly selected, split samples were discarded at the time of analysis as presumed inadequate collections.

Height and weight were measured in light street clothes with shoes. Urine samples were analyzed by automated laboratory methods for sodium, potassium and creatinine. For 20 specimens, randomly selected, split samples were transmitted blind to the laboratory to assess reliability. The technical error* was 5% of the mean for sodium.

Results

Group Mean 24-Hour and Overnight Na Excretion

Group means and standard deviations of 24-hour daytime and overnight urine sodium samples for each of the 7 days and the average of the seven collections are presented in table 1. The mean 24-hour urine Na ranges from 135.9 to 167.6 mEq for boys, and from 109.9 to 136.6 mEq for girls. The average value of the mean 24-hour urine sodium excretion for each of the 7 days is significantly higher for boys than for girls. For overnight urine sodium, the means range from 58.1 to 84.5 mEq for boys, and from 42.8 to 58.0 for girls. The average value of the mean overnight urine Na excretion for each of the 7 days is also significantly higher for boys than girls.

Since the large sex-related difference in urine Na excretion could seriously affect estimation of inter-individual variation, it had to be controlled in the analysis. For this purpose, a regression analysis was done, with average 24-hour urine Na the dependent variable, and sex, race, age, weight, height, heart rate as independent variables, to identify variables significantly related to 24-hour urine Na. Sex was the only significant variable (table 2). Because of the small sample sizes for the two sex groups (31 boys and 42 girls), it was decided to analyze the overall pooled data, controlling for sex (see Appendix), in addition to accomplishing separate analyses for boys and girls.

Intra- and Inter-Individual Variances in Na Excretion

The estimated partial correlation coefficient between any two 24-hour urine Na measurements, controlling for sex, is 0.340. Based on this value, the ratio of intra- to inter-individual variances for 24-hour urine Na is estimated to be 1.94 (table 3). For boys and girls, the estimated coefficients between two 24-hour urine Na's are 0.32 and 0.37, and the corresponding ratios of intra- to inter-individual variances are 2.20 and 1.68, respectively.

Required Number of 24-Hour Specimens

The number of 24-hour urine specimens required for estimating correlation coefficient or for quantile classification depends on the desired degree of accuracy. For example, based on the ratio (2.20) of intra- to inter-individual variances for boys, nine specimens are necessary, if the diminution of the correlation coefficient is to be reduced to 10%, and 20 specimens to limit it to 5% (table 4). For girls, where the ratio is 1.68, the corresponding numbers of specimens required are seven and 16, respectively. For the pooled sample, the numbers of specimens are eight and 18, respectively.

The numbers of 24-hour specimens required for classifying individuals accurately into quantiles were

*The technical error was calculated by the formula

$$\sqrt{\frac{\sum_{i=1}^{n} d_i^2}{2n}}$$

where $d_i$ is the difference between the two values of the $i^{th}$ specimen.
TABLE 1.  Mean and Standard Deviation for the Group 24-Hour and Overnight Urinary Sodium Excretion (mEq)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Standard Error</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (lbs)</td>
<td>0.3732</td>
<td>0.2576</td>
<td>0.152</td>
</tr>
<tr>
<td>Height (inch)</td>
<td>1.4309</td>
<td>1.3056</td>
<td>0.309</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>0.6871</td>
<td>0.5270</td>
<td>0.197</td>
</tr>
<tr>
<td>Sex*</td>
<td>-30.7415</td>
<td>9.9380</td>
<td>0.003</td>
</tr>
<tr>
<td>Age</td>
<td>4.6999</td>
<td>7.7860</td>
<td>0.548</td>
</tr>
<tr>
<td>Race†</td>
<td>0.9918</td>
<td>14.6618</td>
<td>0.835</td>
</tr>
<tr>
<td>Constant</td>
<td>-71.3760</td>
<td>113.7023</td>
<td>0.532</td>
</tr>
</tbody>
</table>

*1-male 2-female.
†1-white 2-black.

TABLE 2.  Regression Equation of Average 24-Hour Urine Sodium on Weight, Height, Heart Rate, Sex, Age and Race

also estimated from these samples. For boys, three specimens are necessary to limit the probability to 0.01 of misclassifying an individual in quintile 1 versus quintile 5, and seven specimens to limit the probability to 0.01 of misclassifying an individual in tertile 1 versus tertile 3. For girls, the corresponding numbers are three and five, respectively, and for the pooled sample, the required numbers are three and six, respectively.

Concordance of the True Means for the 24-Hour and Overnight Specimens

Several parameters need to be estimated before comparing true mean 24-hour and true mean overnight Na. Based on the methods given in the Appendix, the ratio of intra- to inter-individual variances for overnight urine Na is estimated to be 2.60 for boys, 2.38 for girls, and 2.50 for the pooled sample (table 3). The estimated correlation coefficients between observed 24-hour urine Na for 1 day and observed overnight urine Na for another day are 0.24 and 0.21 for boys and girls, respectively. For the pooled sample, the partial correlation coefficient controlling for sex is estimated to be 0.23. From these results, the correlation coefficient between true mean 24-hour and
overnight urine Na values can be estimated; the values are 0.83 for boys, 0.64 for girls, and 0.73 for the pooled samples (see Appendix for method).

The concordance of an individual's true mean 24-hour urine Na and his/her true mean overnight urine Na was measured by estimating appropriate conditional probabilities (table 5). Thus, to examine whether or not overnight urine measurements can accurately detect individuals with very high or very low salt intake, the conditional probability that an individual's true mean 24-hour urine Na is in the fifth (first) quintile of the distribution given that his/her true mean overnight urine Na is in the same quintile of the distribution, was estimated to be 0.67 for boys, 0.52 for girls, and 0.59 for the pooled sample. All estimated conditional probabilities indicate that these two variables are not completely consistent for detecting individuals with very high or very low salt intake. Therefore, further probabilities were computed. It was estimated that 76% of boys in the fifth quintile of overnight urine Na are in the fourth quartile of the distribution of 24-hour urine Na; 86% in the third tertile and 96% in the upper half of the distribution of 24-hour urine Na. For girls, 60%, 71% and 86%, respectively, of the individuals in the fifth quintile of overnight urine Na were estimated to have their true mean 24-hour urine in the fourth quartile, third tertile and upper half, respectively, of the distribution. These conditional probabilities were also estimated based on the pooled sample, controlling for sex; the corresponding values are 0.68, 0.78 and 0.92, respectively. Furthermore, less than 2% of individuals in the fifth quintile of overnight urine Na were estimated to be in the first tertile of the distribution of 24-hour urine Na.

These findings show that almost all individuals with high true mean overnight urine Na excretion are likely to have high true mean 24-hour urine Na excretion.

To assess whether overnight measurements are consistent with 24-hour urine Na for detecting individuals with moderately high salt intake, similar conditional probabilities were calculated for individuals with true mean overnight urine Na excretion in the third tertile of the distribution. For example, 74% of such boys were estimated to be from the third tertile of the distribution of the true mean 24-hour urine Na, 91% from the upper half and less than 2% from the first tertile. For girls, the corresponding percentages are 62%, 85% and 5% respectively. Moreover, 81% (boys), 72% (girls) and 76% (pooled sample) of individuals with their true mean overnight urine Na in the upper half of the distribution remain in the upper half of the distribution of the 24-hour urine Na. These results indicate that those who have moderately high 24-hour urine Na excrections are likely to have high overnight Na and vice versa.

<table>
<thead>
<tr>
<th>Table 5. Conditional Probabilities for Measuring the Consistency of an Individual's True Mean 24-Hour and True Mean Overnight Urine Sodium Excretions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantile for true mean overnight Na</td>
</tr>
<tr>
<td>Fifth quintile</td>
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<td>Third tertile</td>
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<tr>
<td>Upper half</td>
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<td>Upper half</td>
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</table>

*The conditional probability that an individual's true mean 24-hour urine Na is in the quantile indicated in Column 2 given that his true mean overnight urine Na is in the quantile indicated in Column 1.
The conditional probabilities listed in table 5 remain the same when one interchanges the first quantile with the last quantile. For example, for the pooled sample, 0.59 is also the estimated conditional probability that an individual's true mean 24-hour \( Na \) is in the first quintile given that his/her true mean overnight urine \( Na \) is in the first quintile of the distribution. Therefore, the results are also applicable for individuals with low salt intake.

**Required Number of Overnight Specimens**

Based on the ratio (2.60) of intra- to inter-individual variances estimated for boys, 11 measurements are necessary to reduce to 10% diminution of the correlation coefficient, and 24 measurements to limit this to 5%. For girls, the corresponding numbers of measurements required are 10 and 22, respectively, and for the pooled sample the numbers are 11 and 23, respectively.

For quantile classification, the required numbers are comparable for all three samples: four measurements to limit to 0.01 the probability of misclassifying an individual in quintile 1 versus quintile 5, and seven to eight measurements to limit the probability of misclassifying an individual in tertile 1 versus tertile 3 (table 4).

**Discussion**

The relationship between blood pressure and habitual salt intake has been explored in many studies. Results for free-living individuals within a more or less homogeneous population are still inconclusive. One reason for failing to resolve this matter is that salt intake of individuals has not been properly characterized due to inadequate numbers of urine \( Na \) measurements. The problem can be largely solved by assessing the number of measurements required for accurately estimating correlation coefficient and for quantile classification. For example, based on a previous study of adult men, 10 measurements of 24-hour urine are necessary to distinguish highest from lowest tertiles with a 0.01 probability of misclassification.

The data on seven consecutive 24-hour urine \( Na \) collections for children provide an opportunity to examine further these questions. Compared with adults, children were more stable in daily \( Na \) excretion. For example, estimated ratio of intra- to inter-individual variance is 1.98 for children compared with 3.20 for adult men. Nevertheless, a full week of 24-hour urine specimens is still needed to limit resultant diminution of correlation to less than 10%. This finding provides a possible explanation for the reported lack of association between daily \( Na \) output and blood pressure of individuals obtained in most previous studies.

In large-scale epidemiological studies, collection of seven 24-hour urine specimens is very difficult practically. Therefore, it is relevant to assess whether overnight urine collections can replace 24-hour urine specimens for assessment of salt intake. Similar to the previous finding for adult men, the data from the present study indicate a moderate consistency between these two variables (24-hour and overnight \( Na \) values) in detecting individuals with high or low \( Na \) intake. Thus, whether overnight urine samples can replace 24-hour samples depends on the specific purpose and the desired degree of accuracy of the study. For example, for a large scale study (e.g., sample size in the hundreds), where the collection of so many 24-hour urine specimens is often impossible, overnight specimens may be reasonable alternatives. However, for a study involving less than 100 individuals, 24-hour specimens may be worth collecting, since they provide more accurate results. When one decides to use overnight samples, at least 1 week of urine specimens should be taken to avoid diminution of the correlation and significant misclassification due to intra-individual variation.

**Acknowledgments**

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**References**

Appendix

Let $X$ denote the true mean overnight urine Na for an individual and let $Y$ denote the true mean daytime urine Na. For $i=1, \ldots, n$, let $C_i$ and $D_i$ be the observed overnight and daytime values on the $i$th day, respectively. Due to intra-individual variation, the observed values are given by:

$$C_i = X + e_i \quad i = 1, \ldots, n,$$

and

$$D_i = Y + f_i \quad i = 1, \ldots, n,$$

where $e_i$ and $f_i$ are the departures of the observed values on the $i$th day from the true mean overnight value $X$ and true mean daytime value $Y$. It is reasonable to assume that $e_i$, $i = 1, \ldots, n$, are independent identically distributed (i.i.d.) random variables and that $f_i$, $i = 1, \ldots, n$, are i.i.d. random variables. For $i \neq j$, $e_i$ and $f_i$ can also be assumed to be independent. Furthermore, $e_i$ and $f_i$ are also assumed to be independent of $X$ and $Y$.

Let $\sigma^2_{X}$ and $\sigma^2_{Y}$ be the intra-individual variance (i.e., the variance of $e_i$) and the inter-individual variance (i.e., the variance of $X$) of overnight Na, respectively, and $\sigma^2_{X+Y}$ and $\sigma^2_{X+Y}$ be the intra-individual variance (i.e., the variance of $e_i+f_i$) and the inter-individual variance (i.e., the variance of $X+Y$) of 24-hour Na, respectively. The ratio of the intra- to inter-individual variances for 24-hour urine Na can be given by the formula:

$$\frac{\sigma^2_{X+Y}}{\sigma^2_{X}} = \frac{1 - \rho(C_i+D_i, C_j+D_j)}{\rho(C_i+D_i, C_j+D_j)}$$}

(1)

where $\rho(C_i+D_i, C_j+D_j)$ is the correlation coefficient between two 24-hour urine Na. In the analysis, the ratio $\sigma^2_{X+Y}/\sigma^2_{X}$ was estimated by substituting in Equation 1 the average value of 21 sample correlation coefficients between two 24-hour Na measurements. For the pooled sample, the ratio was estimated by substituting in Equation 1 the average value of 21 partial correlation coefficients between two 24-hour Na measurements controlling for sex.

The number of 24-hour specimens required to limit to less than 100(1-P) percent the diminution of correlation coefficient was estimated by:

$$n = \frac{\left(\frac{P^2}{1-P^2}\right)}{\frac{\sigma^2_{X+Y}}{\sigma^2_{X}}}$$

For quantile classification, the number of 24-hour Na measurements required to limit to 0.01, the probability of misclassifying an individual in the lowest 100$\alpha$ percent of the distribution into the highest 100$\alpha$ percent (or vice versa) was estimated by selecting the number $n$ so that the conditional probability

$$P(Z_1 > Z_{1-a} | Z_1 < Z_a)$$

is less than 0.01, where $Z_1$ and $Z_2$ are two standard normal random variables having a joint bivariate normal distribution with correlation coefficient

$$\sqrt{\frac{1}{1 + \frac{\sigma^2_{X}}{\sigma^2_{X+Y}}}}$$

and $Z_1$ is the 100$\alpha$ percentile of the standard normal distribution. For overnight Na, the corresponding ratio or the required number of measurements was estimated in a similar manner as the 24-hour Na.

Under the assumption that $X$ and $X+Y$ have a bivariate normal distribution, the conditional probability that an individual's true mean 24-hour Na is above the 100$\alpha$, percentile of its distribution given that the true mean overnight Na is above the 100$\alpha$, percentile of its distribution can be expressed as,

$$P(Z_1 > Z_{1-a} | Z_1 > Z_{a1}, Z_2 > Z_{a2}) = \frac{Pr(Z_1 > Z_{a1}, Z_2 > Z_{a2})}{Pr(Z_2 > Z_{a2})}$$

(3)

where $Z_1$ and $Z_2$ are two random variables having a joint bivariate normal distribution with $\mu_1 = \mu_2 = 0$, $\sigma^2_1 = 1$ and correlation coefficient $\rho = \rho(X,X+Y)$, and $Z_0$ is the 100$\alpha$ percentile of the standard normal distribution.

In order to calculate the required conditional probabilities, it is necessary to estimate $\rho(X,X+Y)$. Since the true mean overnight Na and the true mean 24-hour Na are not observable, $\rho(X,X+Y)$ cannot be estimated directly. Based on the assumptions stated above, it can be shown that

$$\rho(X, X+Y) = \frac{\rho(C_i, C_j+D_j)}{\sqrt{\frac{\sigma^2_{X}}{\sigma^2_{X+Y}}}}$$

(4)

where $\rho(C_i, C_j+D_j)$ denotes the correlation between the $i$th overnight urine Na and the $j$th 24-hour urine Na (if $j \neq i$). In the analysis, the parameter $\rho(C_i, C_j+D_j)$ was estimated by the average value of the 42 sample correlation coefficients between the 24-hour Na measurement in 1 day and the overnight Na measurement in another day. Again, when the pooled sample was under consideration, partial correlation coefficients controlling for sex were used to estimate the parameters.

The details of these formulas were published elsewhere.\textsuperscript{14-18}
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