Can Overnight Urine Replace 24-Hour Urine Collection to Assess Salt Intake?

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SUMMARY Are overnight urine specimens adequate for characterizing the daily salt intake of individuals, i.e., can the overnight specimen replace the 24-hour specimen? Data from 142 male participants of an ongoing trial on the primary prevention of hypertension were used to examine this question with correlation analysis and quantile classification. Estimated correlation between the true mean 24-hour and the true mean overnight sodium excretion was 0.72. Furthermore, 67% of the individuals in the upper third of the distribution of true mean overnight urine sodium were also in the upper third of the distribution of true mean 24-hour sodium. Thus, these data are promising in regard to the use of overnight urine specimens for characterizing the salt intake of individuals.

The number of overnight urine collections required to estimate accurately the correlation between an individual's true mean overnight urine sodium and a variable of interest (e.g., blood pressure) was calculated. Given the observed intra- and inter-individual variation, the data indicate that 14 measurements are needed to limit the diminution of the correlation coefficient to 10%. (Hypertension 1: 529-536, 1979)

KEY WORDS • overnight urine sodium • 24-hour urine sodium • intra-individual variation • inter-individual variation • correlation • quantile classification

The question of the role of habitual high sodium intake as a conditioning factor for the mass occurrence of hypertension remains a major unsolved cardiovascular problem. In particular, it is unclear whether within a population, such as the U.S. population, there is a relationship between sodium intake of individuals and their risk of high blood pressure. Research efforts to test this hypothesis have been seriously hampered by uncertainty as to satisfactory methods for accurate assessment of the habitual salt intake of individuals, i.e., methods that validly distinguish one person from another within a more or less homogeneous population and hence permit classification of individuals based on this variable. Given the difficulties of estimating the salt consumption of individuals, the limited approaches to solving this key methodological problem in cardiovascular epidemiology have relied on urinary Na excretion. A recent study on sodium excretion in healthy American adults indicates that the intra-individual variation in the individual's daily output is about three times the inter-individual variation. This phenomenon can result in a serious underestimate of the true association between habitual salt intake and blood pressure within a population, unless several 24-hour urine specimens are collected. For example, 14 collections of 24-hour urine are necessary to limit the error in the correlation between the true mean 24-hour urine Na and a variable of interest to less than 10% under the cited circumstances. Their collection is a formidable task for epidemiological studies of free-living populations. The difficulties could be reduced if overnight urine specimens are sufficient, since they are easier to collect. However, it is not clear...
whether or not they adequately measure the daily salt intake of individuals, 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. This study systematically investigates these problems.

Materials and Methods

Data Sources

Data used in this study were collected as part of an ongoing trial on the primary prevention of hypertension involving 142 male business and administrative personnel at two work sites in Chicago, referred to here as Sample A and Sample B. A total of 2229 persons were participants in the periodic medical examination program of the two companies. Chart review at the company medical departments identified 312 individuals between 30 and 44 years of age who were free of medical illness and had a high-normal blood pressure (diastolic BP 80-84 mm Hg, relative weight 1.10-1.49 and/or pulse ≥ 80 beats per minute, or diastolic BP 85-89) or a borderline elevation of blood pressure (diastolic BP 90-94 mm Hg) at their last routine periodic examination. Relative weight is the ratio of observed weight to desired weight for height and sex, from the 1959 actuarial tables. A total of 167 volunteers were recruited, 25 of whom were women. Findings among the women were significantly different for every measure; since the numbers were too small to be considered separately, their data were excluded from this analysis.

Urine collection included four specimens, two weekday and two weekend, with each weekday and weekend pair about 3 months apart. Of these men, 142 collected a first specimen; 141 a second; 132 a third; 128 a fourth; 116 all four. Each 24-hour urine collection was divided into three time periods: morning, afternoon, and overnight.

Special attention was paid to the completeness of the 24-hour collections. In order to insure that 24-hour urine specimens were as complete as possible, the following protocol was employed.

Detailed instructions on the methods for urine collection during each period were enclosed in each urine collection kit. Each participant was also verbally instructed by the research staff until he fully understood the methods. The importance of the completeness of the 24-hour collection and of each separate period were strongly emphasized. Each participant was asked to record the starting time and the finishing time for each period. When the participant returned the 24-hour specimen, he was thoroughly questioned about its completeness.

Urine was analyzed for sodium by flame photometry and creatinine by the Jaffe method (Technicon SMA 12-60). For about 10% of the urine specimens, randomly selected, unidentified split samples were transmitted to the laboratory to assess reliability of the laboratory. Although extreme values were occasionally encountered, repeat measurements were within the range of laboratory error, hence no values were excluded from the statistical analyses. Laboratory and other data were collected on pre-coded forms, key punched, verified, edited for errors and stored on computer tape for statistical analysis.

Statistical Considerations and Methods

In order to determine whether or not overnight urine specimens can replace 24-hour specimens for measuring salt intake, it is necessary to compare the individual's true mean* 24-hour urine Na and his true mean* overnight urine Na for their consistency in characterizing the individual's daily salt intake. Unfortunately, due to the large intra-individual variation, the true mean values cannot be observed and therefore the problem cannot be solved directly.

Since the problem is seriously complicated by the large intra-individual variations, first consideration must be given theoretically to the statistical methods necessary for its solution. These are discussed below.

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, ..., 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:

\[ C_i = X + e_i \quad i=1, \ldots, n, \]
\[ D_i = Y + f_i \quad i=1, \ldots, n, \]

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 \( f_j \) can also be assumed to be independent. Preliminary analysis of our data indicated that \( e_i \) and \( f_i \) are dependent variables, i.e., if an individual's overnight urine Na is high (or low) on a given day, then this individual's daytime urine Na also tends to be high (or low) for the same day. Furthermore, \( e_i \) and \( f_i \) are also assumed to be independent of \( X \) and \( Y \).

Generally speaking, the degree of consistency of two variables can be represented by the conditional probability that the first variable is in a specific portion of its distribution given that the second variable is in the same portion (or a similar portion) of its distribution. An example serves to illustrate this idea. Suppose that one wishes to determine whether or not overnight urine Na is adequate for replacing 24-hour urine Na in detecting individuals with very high salt intake, e.g., those in the fifth (highest) quintile of the distribution of salt intake. To answer this question, one needs to calculate the conditional probability that

\*For the individual, the true mean of a variable (e.g., 24-hour urine Na) is defined as the real (actual) average about which he varies during a period, when a stable dietary pattern is maintained.
an individual's true mean 24-hour urine Na is in the fifth quintile of its distribution given that his true mean overnight urine Na is in the fifth quintile of its distribution. A very large value for this probability indicates that an individual with very high overnight urine Na excretion is also likely to have a very high 24-hour urine Na excretion, thus indicating that the overnight Na excretion can suitably replace the 24-hour value. If this probability is not sufficiently large, other conditional probabilities need to be examined, e.g., the conditional probability that an individual's true mean 24-hour urine Na is in the third tertile of its distribution given that his true mean overnight urine Na is in the fifth quintile of its distribution. A large value for this conditional probability may also reflect consistency of these two variables.

Under the assumption that X and 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

\[
P(Z_1 > Z_{\alpha}, Z_2 > Z_{\alpha}) = \frac{Pr(Z_1 > Z_{\alpha}, Z_2 > Z_{\alpha})}{Pr(Z_2 > Z_{\alpha})} \ldots (1)
\]

where \( Z_1 \) and \( Z_2 \) are two random variables having a joint bivariate normal distribution with \( \mu_1 = \mu_2 = 0 \), \( \sigma_1^2 = \sigma_2^2 = 1 \) and correlation coefficient \( \rho = \rho (X,Y) \), and \( Z_\alpha \) is the 100 \( \alpha \) percentile of the standard normal distribution.

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

\[
\rho (X,Y) = \rho (C_i, C_j + E_i) \sqrt{1 + \frac{\sigma_{x+y}^2}{\sigma_{x+y}^2}} \ldots (2)
\]

where \( \rho (C_i, C_j + E_i) \) denotes the correlation coefficient between the \( i \)th overnight urine Na and the \( j \)th 24-hour urine Na (\( \neq j \)), \( \sigma_{x+y}^2 \) and \( \sigma_{x+y}^2 \) are 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 \( \sigma_{x+y}^2 \) and \( \sigma_{x+y}^2 \) are the intra-individual variance (i.e., the variance of \( e_i \)) and the inter-individual variance (i.e., the variance of \( X + Y \)) of 24-hour Na, respectively. The parameters, \( \rho (C_i, C_j + E_i) \), \( \sigma_{x+y}^2 \) and \( \sigma_{x+y}^2 / \sigma_{x+y}^2 \) can be estimated by \( r(C_i, C_j + E_i) \), \( (1-r(C_i, C_j))/r(C_i, C_j) \) and \( (1-r(C_i+D_i, C_j+D_j))/r(C_i+D_i, C_j+D_j) \) (see Appendix A), where \( r(U,V) \) is the sample correlation coefficient between two variables, \( U \) and \( V \). The estimate \( r(X,Y) \) of \( \rho (X,Y) \) can then be obtained by substituting the three estimates in equation 2. Use of that value in equation 1 then allows calculation of approximate values for the desired conditional probabilities. The reasonableness of the assumption of bivariate normality is examined in Appendix C.

### Results

#### Concordance of the Two True Means

Group means and standard deviations of 24-hour, daytime and overnight urine sodium for each of the 4 days, and the averages of the four collections are presented in table 1. The mean 24-hour urine Na ranges from 156.1 mEq to 177.6 for Sample A, and from 174.2 mEq to 196.4 mEq for Sample B. The average value of the mean 24-hour urine Na excretion for each of the 4 days is significantly higher for Sample B than for Sample A. When the 24-hour Na is divided into daytime and overnight periods, the same pattern persists; however, the two average daytime sodium values are not significantly different. Based on these findings, it was decided to analyze the data both for the two groups separately and for the pooled group.

Urine creatinine was measured for each individual. The median coefficient of variation (CV) for creatinine is 0.116 for Sample A, 0.163 for Sample B and 0.142 for the pooled sample.

The weighted averages (weighted by sample size) of the six estimated correlation coefficients between two 24-hour, two daytime and two overnight urinary Na values are shown in table 2. Based on these correlation coefficients, the estimates of the ratio of intra-individual to inter-individual variances for 24-hour urine Na is 3.37 and 3.34 for Samples A and B, respectively (table 3). For overnight urine Na, the ratios are slightly different: 3.15 for Sample A, and 3.89 for Sample B. The corresponding ratios for the pooled sample are fairly consistent, 3.26 for 24-hour urine Na and 3.20 for overnight urine Na. The estimated correlation coefficients between the observed 24-hour urine Na for 1 day and the observed overnight urine Na for another day are 0.177, 0.135 and 0.171 for Sample A, Sample B and the pooled sample, respectively.

From these results, the correlation coefficient between the true mean 24-hour Na and the true mean overnight Na can be estimated. By applying the method previously described, the estimated correlation coefficient for Sample A is 0.752, for Sample B 0.622, and for the pooled sample 0.722.

The concordance of an individual's true mean 24-hour urine Na and his true mean overnight urine Na was measured by estimating appropriate conditional probabilities (table 4). 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 true mean overnight urine Na is in the same quintile of the distribution was estimated for both samples. The conditional probabilities are 0.60 for Sample A and 0.51 for Sample B. Both 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 for Sample A, 69% of the individuals in the fifth quintile of overnight urine Na are in the fourth quartile of the distribution of 24-hour urine Na; 79% in the upper third and 92% in the upper half of the distribution of 24-hour urine Na. For Sample B, 59%, 70%, and 85%, respectively, of the individuals in the fifth quintile of overnight urine Na were estimated to have their true mean 24-hour urine Na in the fourth quartile, third tertile and upper half, respectively, of the distribution. Furthermore, less than 3% of the 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 extremely high true mean overnight urine Na excretion are likely to have high true mean 24-hour urine Na excretion.

Similar conditional probabilities were also calculated for individuals with true mean overnight urine Na excretions in the third tertile of the distribution. For example, for Sample A, 69% of such individuals were estimated to be from the third tertile of overnight urine Na excretion. Therefore, almost all individuals with extremely high true mean overnight urine Na excretion are likely to have high true mean 24-hour urine Na excretion.

### Table 1. Mean and Standard Deviation for the Group Urinary Sodium Excretion (mEq): 24-hour, Daytime and Overnight

<table>
<thead>
<tr>
<th>Day</th>
<th>No.</th>
<th>24-hour values Mean</th>
<th>SD</th>
<th>Daytime Sodium Mean</th>
<th>SD</th>
<th>Overnight Sodium Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled</td>
<td>142</td>
<td>182.7</td>
<td>60.4</td>
<td>138.7</td>
<td>49.4</td>
<td>46.0</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td>165.1</td>
<td>70.0</td>
<td>115.7</td>
<td>55.3</td>
<td>49.3</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>132</td>
<td>184.0</td>
<td>59.3</td>
<td>138.9</td>
<td>48.5</td>
<td>45.1</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>175.4</td>
<td>73.4</td>
<td>118.6</td>
<td>53.7</td>
<td>56.8</td>
<td>36.6</td>
</tr>
<tr>
<td>Average</td>
<td>116</td>
<td>176.2</td>
<td>42.5</td>
<td>125.9</td>
<td>32.6</td>
<td>49.3</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Sample A

<table>
<thead>
<tr>
<th>No.</th>
<th>24-hour values Mean</th>
<th>SD</th>
<th>Daytime Sodium Mean</th>
<th>SD</th>
<th>Overnight Sodium Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>177.6</td>
<td>60.6</td>
<td>136.4</td>
<td>50.4</td>
<td>41.2</td>
<td>20.4</td>
</tr>
<tr>
<td>2</td>
<td>156.1</td>
<td>68.1</td>
<td>110.3</td>
<td>55.0</td>
<td>45.8</td>
<td>24.1</td>
</tr>
<tr>
<td>3</td>
<td>173.7</td>
<td>51.2</td>
<td>131.5</td>
<td>41.0</td>
<td>42.2</td>
<td>19.9</td>
</tr>
<tr>
<td>4</td>
<td>166.5</td>
<td>63.2</td>
<td>118.4</td>
<td>50.7</td>
<td>48.0</td>
<td>25.1</td>
</tr>
<tr>
<td>Average</td>
<td>166.6</td>
<td>39.2</td>
<td>122.8</td>
<td>30.0</td>
<td>43.8</td>
<td>15.3</td>
</tr>
</tbody>
</table>

Sample B

<table>
<thead>
<tr>
<th>No.</th>
<th>24-hour values Mean</th>
<th>SD</th>
<th>Daytime Sodium Mean</th>
<th>SD</th>
<th>Overnight Sodium Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>187.8</td>
<td>60.1</td>
<td>137.0</td>
<td>48.8</td>
<td>50.7</td>
<td>26.6</td>
</tr>
<tr>
<td>2</td>
<td>174.2</td>
<td>71.3</td>
<td>121.3</td>
<td>55.5</td>
<td>52.9</td>
<td>26.4</td>
</tr>
<tr>
<td>3</td>
<td>196.4</td>
<td>66.0</td>
<td>147.8</td>
<td>55.3</td>
<td>48.6</td>
<td>30.1</td>
</tr>
<tr>
<td>4</td>
<td>185.9</td>
<td>83.0</td>
<td>118.8</td>
<td>57.6</td>
<td>67.1</td>
<td>44.6</td>
</tr>
<tr>
<td>Average</td>
<td>185.6</td>
<td>43.8</td>
<td>130.9</td>
<td>34.7</td>
<td>54.6</td>
<td>10.4</td>
</tr>
</tbody>
</table>

### Table 2. Estimated Correlation Coefficients Between Two 24-hour, Two Daytime and Two Overnight Urine Sodium Excretions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pooled</th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td>0.24</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Daytime</td>
<td>0.22</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>Overnight</td>
<td>0.23</td>
<td>0.24</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*The weighted average (weighted by sample size) of the six estimated correlation coefficients, i.e., r_i1, r_i2, r_i3, r_i4, r_i5, r_i6, where r is the estimated correlation coefficient between Day i and Day j.

### Table 3. Intra-Individual and Inter-Individual Variations of 24-hour, Daytime and Overnight Urine Sodium Excretions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intra-individual SD</th>
<th>Inter-individual SD</th>
<th>Ratio of variances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled</td>
<td>24-hour</td>
<td>24.9</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>Daytime</td>
<td>24.9</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>Overnight</td>
<td>25.6</td>
<td>3.26</td>
</tr>
</tbody>
</table>

Sample A

| 24-hour     | 52.9                | 28.8                | 3.37               |
| Daytime     | 44.8                | 22.9                | 3.81               |
| Overnight   | 20.0                | 11.3                | 3.15               |

Sample B

| 24-hour     | 61.8                | 33.8                | 3.34               |
| Daytime     | 49.8                | 27.2                | 3.36               |
| Overnight   | 30.1                | 15.3                | 3.89               |
TABLE 4. Conditional Probabilities for Measuring the Consistency of an Individual's True Mean 24-hour and True Mean Overnight Urine Sodium Excretions

<table>
<thead>
<tr>
<th>Quantile for true mean overnight Na</th>
<th>Quantile for true mean 24-hour Na</th>
<th>Conditional probability*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>Fifth quintile</td>
<td>0.58</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>Fourth quartile</td>
<td>0.66</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>Third tertile</td>
<td>0.77</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>Upper half</td>
<td>0.91</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>Lower half</td>
<td>0.09</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>First tertile</td>
<td>0.03</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>First quartile</td>
<td>0.01</td>
</tr>
<tr>
<td>Fifth quintile</td>
<td>First quintile</td>
<td>0.01</td>
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<tr>
<td>Fourth quartile</td>
<td>Fourth quartile</td>
<td>0.62</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>Third tertile</td>
<td>0.73</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>Upper half</td>
<td>0.88</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>Lower half</td>
<td>0.12</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>First tertile</td>
<td>0.04</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>First quartile</td>
<td>0.02</td>
</tr>
<tr>
<td>Third tertile</td>
<td>Third tertile</td>
<td>0.67</td>
</tr>
<tr>
<td>Third tertile</td>
<td>Upper half</td>
<td>0.84</td>
</tr>
<tr>
<td>Third tertile</td>
<td>Lower half</td>
<td>0.16</td>
</tr>
<tr>
<td>Third tertile</td>
<td>First tertile</td>
<td>0.06</td>
</tr>
<tr>
<td>Upper half</td>
<td>Upper half</td>
<td>0.76</td>
</tr>
<tr>
<td>Upper half</td>
<td>Lower half</td>
<td>0.24</td>
</tr>
</tbody>
</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 distribution of the true mean 24-hour urine Na, 86% from the upper half and less than 5% from the first tertile. For Sample B, the corresponding percentages are 61%, 79% and 9%, respectively. Moreover, 77% (Sample A) and 71% (Sample B) of the 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 true mean 24-hour urine sodium.

If one interchanges the first quantile with the last quantile in table 4, the probabilities remain unchanged. For example, for Sample A, 0.60 is also the estimated conditional probability that an individual's true mean 24-hour urine Na is in the first quintile given that his true mean overnight urine Na is in the first quintile of its distribution. Therefore the results are also applicable for individuals with low salt intake.

These findings confirm that although the true mean overnight and the true mean 24-hour urine Na do not agree completely, they are sufficiently consistent to determine individuals with high versus low salt intake. Therefore, whether or not 24-hour urine specimens can be replaced by overnight measurement for salt assessment depends on the desired degree of accuracy.

Required Number of Overnight Specimens

Based on the ratio (3.15) of the intra-individual to the inter-individual variances estimated from Sample A, 13 measurements are necessary to reduce the resultant diminution of the correlation coefficient to 10% and 28 measurements are required to limit the attenuation to 5%. For Sample B, where the ratio is 3.89, the corresponding numbers of measurements required are 17 and 35, respectively. For the pooled sample, the numbers of measurements are 14 and 29, respectively.

The numbers of overnight specimens required for accurately classifying individuals into quantiles were also estimated from these samples. For Sample A, five specimens are necessary to limit the probability of misclassifying an individual in quintile 1 versus quintile 5 to 0.01 and nine specimens are needed to limit the probability of misclassifying an individual in tertile 1 versus tertile 3 to 0.01. For Sample B, the corresponding numbers of specimens are five and 12, respectively. For the pooled sample, the required numbers are five and 10, respectively.
Discussion

Data from epidemiological, clinical and animal experimental work clearly suggest a causal relationship between excessive salt consumption and hypertension. However, in studies of the relationship between sodium excretion and blood pressure for free-living individuals within a more or less homogeneous population either low order or zero correlations have been reported. One important factor possibly leading to these low correlations is the large intra-individual variation in daily salt output, a phenomenon that can result in serious underestimation of the true relationship. In order to minimize this problem, several daily urine specimens are required for each individual. The collection of these specimens is likely to be impractical in epidemiologic studies. This difficulty will be reduced if overnight urine samples can replace 24-hour specimens for assessing daily salt intake of individuals. Two studies have considered the possibility of the use of overnight urine specimens. Unfortunately, published results were based on the correlation coefficient of the observed 24-hour urine Na and the observed overnight urine Na, and therefore were seriously affected by the large intra-individual variations (24-hour and overnight). It was reported that overnight Na excretion correlates reasonably well with 24-hour Na excretion, but when overnight Na excretion was compared with 5-day Na excretion, the correlation became quite small. These seemingly inconsistent results can be explained by the presence of intra-individual variation (see Appendix B). When the overnight urine Na excretion is a part of the 24-hour measurement, the correlation coefficient is affected more by the dependency of the two intra-individual variations than by the attenuation caused by the presence of intra-individual variation. Thus, a fairly large value will be obtained. On the other hand, when a single overnight urine Na excretion is compared with a long-term Na excretion, the attenuation caused by the intra-individual variation is stronger than the influence of the dependence of the two intra-individual variations. In this instance, a smaller correlation coefficient is obtained.

The analysis in this study was based on the estimated correlation coefficient of the true mean 24-hour urine Na and the true mean overnight urine Na in order to eliminate the influence of intra-individual variation. The results indicate a moderate degree of consistency between these two variables. In a large epidemiologic study on the relationship between daily salt intake and blood pressure for individuals, the collection of a large number of 24-hour urine specimens is practically very difficult. In this case, the overnight urine Na output may be a reasonable alternative to the 24-hour specimen.

The number of overnight urine specimens required for estimating a correlation coefficient or for classifying individuals into quantiles depends on the desired degree of accuracy. For example, six measurements may be adequate for separating individuals with very high Na excretion from persons with very low Na excretion; however, nine to 12 measurements may be needed for separating individuals with moderately high or low Na excretion. It is suggested that at least a week of overnight urine specimens should be taken to avoid marked diminution of the correlation and the significant potential for misclassification due to the intra-individual variation of overnight Na excretion.

Finally, the data on the coefficient of variation for creatinine indicate that the intra-individual variation calculated from overnight urine specimens may be slightly overestimated. Previous studies have consistently reported a coefficient of variation in the range of 0.10, in contrast to the value 0.162 obtained for Sample B. (For Sample A, the value of 0.116 is consistent with previous results.) Several possibilities may explain this phenomenon. First, a longer series of examinations on a much smaller number of participants was generally utilized in these other studies, and the participants were often scientific personnel, the investigators themselves, or patients in a metabolic ward. Therefore a smaller variation could be expected. Second, the 3-month interval between the sets of collections in the present study could also have contributed to the increased variation observed here. Third, the time reported for beginning and ending of the collection period was not always 24 hours, but varied randomly depending on the day’s routine. The larger intra-individual variation may reduce the strength of the estimated correlation coefficient between the true mean 24-hour and the true mean overnight urine sodium values and therefore weaken the degree of the consistency of the two distributions. However, results obtained from Sample B are fairly consistent, although the correlation estimated from Sample B is slightly smaller than the value estimated from Sample A. Both results indicate a moderate degree of consistency between the true mean 24-hour and the true mean overnight urine sodium. Thus, any overestimate of the intra-individual variation for Sample B should not affect the conclusions of the present study. Because the analyses in this study are based on a sample of middle age men, strictly speaking the results of this study are generalizable only to this population. However, the major goal of the current study is not only to obtain the numerical results but also to select an appropriate statistical method for studying this problem given the phenomenon of intra-individual variation. When other populations are under consideration, the same method can be applied for studying the consistency between 24-hour and overnight sodium values.

Abnormal elevation of blood pressure represents a health hazard of enormous magnitude. The great majority of individuals diagnosed as hypertensive have only mild elevations of blood pressure, six out of seven falling in the range of 90–104 mm Hg diastolic. Actuarial data and prospective medical studies have shown that risk for adults is increased above a diastolic reading of 84 mm Hg. Despite the recent significant increase in proportion of hypertensive persons detected, treated and controlled compared to the recent past, pharmacological means are limited in
their ability to eliminate the risk of mass disease. Primary prevention represents the key long-term strategy. Unfortunately, the gaps in current understanding of the etiology and pathogenesis of hypertension limit ability to develop preventive strategies. With improved epidemiologic techniques it may be possible more definitively to resolve the important issue of the role of habitual salt intake in the etiology of hypertension, and thereby give a firmer foundation to primary preventive measures.

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Appendix A

Under the assumptions described in the text, the correlation coefficient between the $i^{th}$ observed overnight Na and the $j^{th}$ observed 24-hour Na is

$$\rho(C_1, C_j + D_1) = \rho(X + e_i, X + Y + e_j + f_j)$$

$$= \frac{\text{Cov}(X + e_i, X + Y + e_j + f_j)}{\sqrt{\text{Var}(X + e_i)} \sqrt{\text{Var}(X + Y + e_j + f_j)}}$$

$$= \frac{\text{Cov}(X, X + Y)}{\sqrt{\sigma_Y^2 + \sigma_{X,Y}} \sqrt{\sigma_Y^2 + \sigma_{X,Y}^2}}$$

$$= \rho(X, Y + \frac{1}{\sqrt{1 + \frac{\sigma_{X,Y}^2}{\sigma_Y^2}}} \frac{1}{\sqrt{1 + \frac{\sigma_{X,Y}^2}{\sigma_Y^2}}})$$

Therefore,

$$\rho(X, X + Y) = \rho(C_i, C_j + D_1) \sqrt{1 + \frac{\sigma_{X,Y}^2}{\sigma_Y^2}} \sqrt{1 + \frac{\sigma_{X,Y}^2}{\sigma_Y^2}}$$

(A1)
Under the same assumptions, the correlation coefficient between the \(i^{th}\) and the \(j^{th}\) overnight sodium values \(i,j\) is

\[
\rho_{(C_i, C_j)} = \frac{\text{Cov}(X+e_i, X+e_j)}{\sqrt{\text{Var}(X+e_i)} \sqrt{\text{Var}(X+e_j)}}
\]

or

\[
= \frac{\sigma^2_{x_{i,j}}}{\sigma^2_{x_i} + \sigma^2_{x_j}} = \frac{1}{1 + \frac{\sigma^2_{x_j}}{\sigma^2_{x_i}}}
\]

Therefore,

\[
\frac{\sigma^2_{x_{i,j}}}{\sigma^2_{x_i}} = \frac{1 - \rho_{(C_i, C_j)}}{\rho_{(C_i, C_j)}}.
\]

The variance ratio can then be estimated by substituting the sample correlation coefficient between the \(i^{th}\) overnight Na and the \(j^{th}\) overnight Na, \(\hat{r}_{(C_i, C_j)}\), for \(\rho_{(C_i, C_j)}\) in (A2). Similarly, for 24-hour Na, the variance ratio is

\[
\frac{\sigma^2_{x_{i,j}}}{\sigma^2_{x_i}} = \frac{1 - \hat{r}_{(C_i+D_i, C_j+D_j)}}{\hat{r}_{(C_i+D_i, C_j+D_j)}}.
\]

where \(\hat{r}_{(C_i+D_i, C_j+D_j)}\) is the correlation coefficient between the \(i^{th}\) and the \(j^{th}\) 24-hour sodium values. Again this ratio can be estimated by substituting the corresponding sample correlation coefficient for \(\hat{r}_{(C_i+D_i, C_j+D_j)}\) in (A3).

In this study, since four 24-hour urine specimens were collected for each individual, there are six sample correlation coefficients between the 24-hour urine sodium values of 2 different days; there are six sample correlation coefficients between the overnight urine sodium values of 2 different days, and there are 12 sample correlations between the 24-hour and the overnight sodium collected on 2 different days. The correlation coefficients in (A1), (A2) and (A3) were estimated by the averages of the corresponding sample correlation coefficients.

**Appendix B**

The correlation coefficient between the observed overnight and the observed 24-hour Na excretion is

\[
\hat{r}_{(C_i, C_i + D_i)} = \frac{\text{Cov}(X+e_i, X+e_i+e+f_i)}{\sqrt{\text{Var}(X+e_i+e+f_i)} \sqrt{\text{Var}(X+e_i+e+f_i)}}
\]

\[
= \frac{\text{Cov}(X, X+Y) + \text{Cov}(e_i, e_i+e+f_i)}{\sqrt{\sigma^2_{x_i} + \sigma^2_{x_i} + \sigma^2_{x_j} + \sigma^2_{x_j}}}.
\]

The correlation coefficient between the first overnight Na excretion and the average value of the five 24-hour Na excretions is

\[
\hat{r}_{(C_i, \sum_{i=1}^{5} (C_i+D_i)/5)} = \frac{\text{Cov}(X+e_i, X+Y+\frac{1}{5} \sum_{i=1}^{5} (e_i+f_i))}{\sqrt{\text{Var}(X+e_i) \sqrt{\text{Var}(X+Y+\frac{1}{5} \sum_{i=1}^{5} (e_i+f_i))}}}
\]

\[
= \frac{\text{Cov}(X, X+Y)}{\sqrt{\sigma^2_{x_i} + \frac{\sigma^2_{x_j}}{\sigma^2_{x_i}}} \sqrt{\frac{1}{5} \sigma^2_{x_j} + \frac{\sigma^2_{x_j}}{\sigma^2_{x_i}}}} + \frac{\text{Cov}(e_i, e_i+f_i)}{\sqrt{\frac{1}{5} \sigma^2_{x_i} + \frac{\sigma^2_{x_j}}{\sigma^2_{x_i}}} \sqrt{\frac{1}{5} \sigma^2_{x_j} + \frac{\sigma^2_{x_j}}{\sigma^2_{x_i}}}}.
\]

In general, the second term in (B1) is much larger than the second term in (B2). Therefore the correlation coefficient \(\rho_{(X+e_i, X+Y+\frac{1}{5} \sum_{i=1}^{5} (e_i+f_i))}\) is larger than \(\rho_{(X+e_i, X+Y+\frac{1}{5} \sum_{i=1}^{5} (e_i+f_i))}\). For example, using the values estimated from the pooled sample (i.e., \(\rho(X, X+Y) = 0.722, \sigma^2_{x_i}/\sigma^2_{x_i} = 3.26, \sigma^2_{x_j}/\sigma^2_{x_i} = 3.20\) and \(\rho(e_i, e_i+f_i) = 0.636)\) the variance ratio is

\[
= \frac{1}{\sqrt{1+3.26}} = \frac{1}{\sqrt{1+3.20}} = \frac{1}{5} \times 0.636 \times \frac{1}{\sqrt{1+3.26}} = \frac{1}{5} \times \frac{1}{\sqrt{1+3.20}} = 0.35
\]

However, the estimated \(\rho_{(X+e_i, X+Y+e_i+f_i)}\) is 0.66.

**Appendix C**

Since the proposed model is based on the assumption that the joint distribution between the true mean overnight urine Na (X) and the true mean 24-hour urine Na (X+Y) is bivariate normal, this assumption needs to be examined. Unfortunately, the variables X and Y can not be observed and therefore no direct method can be employed to test this hypothesis. Instead, we tested the following hypotheses:

(a) The average 24-hour Na \(\frac{1}{4} \sum_{i=1}^{4} (C_i + D_i)/4\) and the average overnight Na \(\frac{1}{4} \sum_{i=1}^{4} C_i/4\) have normal distributions.

(b) The joint distribution of the average 24-hour Na and the average overnight Na is bivariate normal.

The hypotheses in (a) were tested by the Kolmogorov-Smirnov Test. For in order to test (b), the pooled sample was divided into 12 categories based on the boundaries, \(-\infty, 145, 170, and \infty\) for the average 24-hour urine Na and the boundaries, \(-\infty, 40, 55, and \infty\) for the average overnight urine Na. The Chi-square test was applied to test the goodness of fit for the bivariate normal distribution. Although both (a) and (b) are not directly based on the true means X and Y, the results of the tests can still be considered as a good indication of the validity of the bivariate normality assumption for the joint distribution of X and Y.

Table 5 provides the values for the K-S statistics for the hypotheses in (a). None of the hypotheses was rejected at the 0.05 level. For the hypotheses in (b), the value of the Chi-square statistic is 10.2, which is not significant at the 0.05 level (\(\chi^2_{(0.05)} = 12.6\)). These results indicate that the assumption of bivariate normality for the joint distribution of the individual's true mean 24-hour and true mean overnight urine sodium excretions is probably reasonable.

**Table 5. Values of Kolmogorov-Smirnov Statistics for Testing the Hypotheses of Normality of the Daytime and Overnight Urine Sodium Excretions**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pooled</th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour</td>
<td>0.738</td>
<td>0.748</td>
<td>0.852</td>
</tr>
<tr>
<td>Overnight</td>
<td>0.702</td>
<td>0.587</td>
<td>0.800</td>
</tr>
</tbody>
</table>

*\(D \times n\). The 0.05 critical point for this statistic is 0.888.
Can overnight urine replace 24-hour urine collection to assess salt intake?

K Liu, A R Dyer, R S Cooper, R Stamler and J Stamler

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