Variation in Urinary Creatinine Excretion and Its Relationship to Measurement of Urinary 17-Hydroxycorticosteroids

Philip E. Cryer and Jonas Sode

Urinary creatinine excretion, measured in 96 24-h urine specimens collected from a single subject, was less variable (CV, 9.3%) than daily urine volume (CV, 20.7%). However, a small variation in measured creatinine excretion did exist, since the observed CV of 9.3% was not explicable either on the basis of analytical error (CV for replicate determinations, 1.1%) or of collection error. Thus, 24-h urinary creatinine excretion is not constant. On the other hand, demonstrated variation in creatinine excretion was small, and urinary 17-hydroxycorticosteroids could be validly expressed in mg/g of creatinine. In a given individual, biologic variation in 24-h creatinine excretion is negligible as compared with variation in 17-hydroxycorticosteroid excretion.

Additional Keyphrase diagnostic aid to distinguishing obesity and Cushing's syndrome

Measurement of urinary creatinine is widely used as a gross index to the completeness of 24-h urine collections. In contrast, the use of urinary creatinine as a basis of expression for the excretion of other compounds is less well established and has been frequently criticized (1–5). Two examples of the latter application are: (a) measured urinary creatinine is used as a basis for correcting to 24-h the results of a determination performed on an incomplete 24-h urine collection (6), which assumes that daily and diurnal creatinine excretion is constant; and (b) complete 24-h urinary 17-oHcs\(^1\) is often expressed as mg/g of urinary creatinine.

The latter expression is of potential clinical usefulness in diagnosing Cushing's syndrome. It is known that 24-h urinary 17-oHcs excretion is greater in obese subjects than in persons of normal weight (7), and that 24-h urinary 17-oHcs may, therefore, exceed "normal limits" in common obesity as well as in Cushing's syndrome (8). Since most patients with clinically suspected Cushing's syndrome are obese, this enhanced 17-oHcs excretion complicates the biochemical distinction between common obesity and Cushing's syndrome. The findings of Streiten and coworkers (8) indicate that the distinction between obese normal individuals and patients with Cushing's syndrome is clearer if the 24-h 17-oHcs excretion is expressed as mg/g of urinary creatinine. This use of the 24-h 17-oHcs/creatinine ratio assumes that, in a given individual, any variation in 24-h creatinine excretion is negligibly small as compared with the variation in 24-h 17-oHcs excretion.

Three major variables are involved in measuring the urinary excretion rate of creatinine:

\(^1\) Abbreviations used: 17-oHcs, 17-hydroxy cortisol steroids; cv, coefficient of variation.

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analytical error, collection error, and biologic variation (including variation in the formation, transport, and excretion of creatinine). The variability of replicate determinations of creatinine in urine is small; cv's are about 1% (1, 3, 9). Measurement of biologic variation in creatinine excretion has been hampered primarily by the possibility of unrecognized collection error. Investigators have attempted to obviate this problem by using as subjects either patients hospitalized on a metabolic ward (3, 9) or well-informed, motivated personnel (1), and by the measurement of radioisotope excretion (during protein turnover studies) as an independent indicator of the adequacy of urine collection (2).

We have measured creatinine and 17-oHcs in nearly 100 24-h urines collected over a six-month period by a single subject. Only specimens for which no major error was made in collection were saved. We confirm that a small variation exists in creatinine excretion (2-6, 9), but we also find that 17-oHcs excretion may validly be expressed in mg/g of urinary creatinine (8) when 24-h urine collections are complete.

Methods

All urines were collected from 7 a.m. to 7 a.m. daily, Monday through Friday, for six months (September through February) from one subject, a healthy 30-year-old male physician who was ambulatory (though generally sedentary) and on an unrestricted diet during the collections. The subject's weight remained close to 90 kg during this time. The studies were begun after a period of adjustment to the routine of daily urine collections.

When a collection error was made, the collection for that day was discontinued and the specimen discarded. No collection of less than 23 h or more than 25 h was included in this study; variation of more than 15 min from 24 h was recognized on only six occasions. Urine aliquots were frozen soon after each day’s collection was completed.

Urinary creatinine was measured by the alkaline picrate (Jaffe) reaction (10). Though this reaction is widely used, and it is accepted clinical parlance to refer to the measured alkaline picrate positive material as “creatinine,” it is acknowledged that the Jaffe reaction is not specific for creatinine (11). Urinary 17-oHcs were measured in duplicate by the method of Silber and Porter (12), involving hydrolysis with β-glucuronidase and dichloromethane extraction.

Standard statistical methods were used (13). Probability values were determined by a t test and correlation by a least squares analysis.

Results

Results of creatinine determinations and volume measurements are shown in Table 1. Variation in

<table>
<thead>
<tr>
<th>Day of week</th>
<th>N</th>
<th>Urine creatinine</th>
<th>Urine volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean SD CV, %</td>
<td>Mean SD CV, %</td>
</tr>
<tr>
<td>Mon. 17</td>
<td>17</td>
<td>2.32 0.219 9.4</td>
<td>2050 480 23.3</td>
</tr>
<tr>
<td>Tues. 20</td>
<td>20</td>
<td>2.26 0.201 8.9</td>
<td>1540 310 20.1</td>
</tr>
<tr>
<td>Wed. 20</td>
<td>20</td>
<td>2.27 0.177 7.8</td>
<td>1680 300 17.9</td>
</tr>
<tr>
<td>Thur. 20</td>
<td>20</td>
<td>2.18 0.262 12.0</td>
<td>1800 290 16.4</td>
</tr>
<tr>
<td>Fri. 19</td>
<td>19</td>
<td>2.26 0.195 8.6</td>
<td>1830 440 24.0</td>
</tr>
</tbody>
</table>

Av CV = 9.3% Av CV = 20.7%

Table 2. Mean CV for Urinary Creatinine Excretion, from the Literature and Present Report

<table>
<thead>
<tr>
<th>Ref. no.</th>
<th>Subjects</th>
<th>Observations per subject</th>
<th>Range of mean creatinine, g/24 h</th>
<th>CV, % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>12</td>
<td>15-27</td>
<td>1.48-1.96</td>
<td>5.9 ± 1.3</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>11-23</td>
<td>1.29-1.83</td>
<td>9.2 ± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>10-58</td>
<td>0.91-2.11</td>
<td>10.0 ± 2.1</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
<td>96¹</td>
<td>2.18-2.32</td>
<td>9.3 ± 1.6</td>
</tr>
</tbody>
</table>

* Divided into five groups (by day of the week) of 17-20 observations each.

Table 3. Urinary 17-Hydroxycorticosteroids in 93 24-h Urines from One Subject

<table>
<thead>
<tr>
<th>Day of week</th>
<th>N</th>
<th>Urine 17-hydroxycorticosteroids</th>
<th>Urine creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean SD CV, %</td>
<td>Mean SD CV, %</td>
</tr>
<tr>
<td>Mon. 17</td>
<td>17</td>
<td>7.74 1.18 15.3 3.35 0.55</td>
<td>16.4</td>
</tr>
<tr>
<td>Tues. 19a</td>
<td>19</td>
<td>6.74 0.96 14.3 3.02 0.52</td>
<td>17.2</td>
</tr>
<tr>
<td>Wed. 19a</td>
<td>19</td>
<td>5.91 1.13 19.1 2.65 0.59</td>
<td>22.2</td>
</tr>
<tr>
<td>Thur. 19a</td>
<td>19</td>
<td>6.28 1.01 16.1 2.94 0.58</td>
<td>19.7</td>
</tr>
<tr>
<td>Fri. 19</td>
<td>19</td>
<td>6.88 1.30 19.1 3.07 0.68</td>
<td>22.2</td>
</tr>
</tbody>
</table>

Av CV = 16.9% Av CV = 19.5%

* 17-Hydroxycorticosteroids were not determined on three urine specimens on which creatinine measurements were performed.

creatinine excretion from day to day is indicated by the average cv, 9.3%. cv's for each day of the week ranged from 7.8 to 12.0%. The cv for duplicate determinations on five specimens performed in replicate on five separate occasions was 1.1%, indicating that analytical error was too small to account for the observed variability in urinary creatinine excretion. We compare our findings with data selected from the literature in Table 2.

As would be anticipated, the average cv for the urine volume measurements was 20.7%, significantly (p < 0.001) greater than that for the creatinine determinations.
Urinary 17-oHcs were measured in 93 of these same 24-h specimens (Table 3). The average cv for the 17-oHcs in mg/24 h was 16.9%; expressed as mg/g of urinary creatinine, the average cv was 19.5%—not significantly different. Indeed, the two modes of expression correlated highly (Figure 1).

Discussion

Since, in the present series, analytical error was small and collection error was minimized, the observed variation in measured urinary creatinine suggests that some biologic variation in creatinine excretion does occur and indicates that the urinary excretion of creatinine, as measured in the clinical setting, is not constant. Our findings agree closely with other studies in which collection error has been minimized (Table 2). Although the observed variation in measured 24-h urinary creatinine excretion is small, it may not accurately reflect true biologic variation in creatinine excretion, since (a) the alkaline picrate (Jaffe) reaction is not specific for creatinine, and (b) the observed discrepancy between the variation in measured urinary creatinine and the analytical error in the urinary creatinine determination is theoretically explicable on the basis of unrecognized collection error.

Because creatinine excretion apparently varies, the use of urinary creatinine as a correction factor for incomplete urine collections is not absolutely valid, although such use would give results close to the true value, since the variation is small. A more compelling criticism of the use of this correction for incomplete collection in measuring glucocorticoid excretion is that creatinine excretion does not parallel the marked diurnal variation in 17-oHcs excretion (14). However, use of the measured urinary creatinine to correct for an incomplete collection may yield a clinically useful, though admittedly approximate, 17-oHcs value under conditions in which the diurnal variation is eliminated—e.g., during dexamethasone suppression tests or ACTH infusions.

Since variation in creatinine excretion is small, 17-oHcs excretion, measured on complete 24-h collections, can appropriately be expressed per unit of urinary creatinine as well as per 24 h. Measurement of 17-oHcs on 93 of the same complete 24-h urine specimens used for creatinine determinations yielded two findings. First, urinary 17-oHcs are equally well expressed in mg/g of urinary creatinine or mg/24 h (Table 3). Second, the two modes of expression correlate highly (Figure 1). Therefore, the error introduced by variation in creatinine excretion was negligible in these measurements of 17-oHcs excretion.

In summary, a small variation in creatinine excretion does occur and, in a strict sense, the use of measured urinary creatinine to correct for incomplete 24-h urine collections is not valid. When 24-h urine collections are complete, on the other hand, the error introduced by variation in creatinine excretion (and analytical error in creatinine determination) is so small that 17-oHcs excretion can be expressed in mg/g of urinary creatinine without appreciable error. The latter finding supports the assumption, inherent in this expression, that, in a given individual, variation in creatinine excretion is so much smaller than variation in 17-oHcs excretion that it can be safely ignored.

These findings support the usefulness of measured urinary creatinine, not only as a gross index to the completeness of a 24-h urine collection, but also as an approximate mathematical correction factor for 17-oHcs determined on an incomplete collection when diurnal variation has been eliminated and as the basis of the 17-oHcs/creatinine ratio determined on complete 24-h urines to
facilitate the distinction between patients with common obesity and those with obesity due to Cushing's syndrome.

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References