Sex-Related Normal Intervals for an Index of Urinary Creatine Excretion

To the Editor:

The urinary excretion of creatine is increased in many muscle disorders (1) and has been used in the diagnosis and management of patients with muscle diseases (2). However, there are problems with both the assay and its interpretation. Apart from the difficulties of obtaining a reliable 24-h urine specimen, creatine converts spontaneously to creatinine on standing (3, 4). Creatine excretion is affected by diet (5) and by physical activity (6). These factors may account for the widely differing normal reference intervals published for urinary excretion of creatine (4, 6-9).

Determination of the ratio of creatine to creatinine in a single unimixed sample of urine may be a useful index of creatine excretion (9). Reference values have been quoted for this, but they vary: for example, values of <6% (2) and <10% (9) have been given for urine of persons over 15 years of age. Because of a local clinical demand for this test in the diagnosis of myalgic encephalomyelitis, we have measured the values in healthy individuals of both sexes. The values we find for women are at variance with the quoted ranges.

Our subjects were either ostensibly healthy laboratory workers or healthy volunteers participating in a reference-range study of calcium metabolism. Their ages ranged from 17 to 60 years. Untimed samples of urine were frozen within 1 h of voiding and stored frozen until assayed. We used the method of Clarke (10) to convert creatine to creatinine, which we measured by continuous-flow analysis (Technicon SMA 6/60) based on the Jaffé reaction (11).

Of 56 men, 55 gave values ≤10%; the remaining value was 12%. However, values for 15 of 51 women were >10%; the distribution is shown in the Figure. In this group of women we could find no age-related effect on the value (r = 0.17). Menstrual history was available from 28 women, but there was no association between the urinary creatine coefficient and the number of days since the onset of the last menstrual period. One woman was postmenopausal, and her value was 17%. Only three of the women were taking oral contraceptives, and their values were all ≤11%.

These data suggest that in most normal men the urinary creatine coefficient in a single urine sample is <10%, but values up to 45% must be expected for some women.

Among 41 women with myalgic encephalomyelitis no value exceeding 40% was found, and 24 had values <10%. Among 18 males with this disorder there were three results >10%: one 12%, one 16%, and one 54%. Thus measurement of the urinary creatine coefficient appears to be of little value in diagnosis of myalgic encephalomyelitis and most of the previously reported apparently increased values may in fact have been within normal limits (12).

We thank Mr. A. W. Musk and colleagues for the urinary creatine assays.

References
11. Technical publication no. THO-0160-60.
14. C. S. Goodwin J. R. L. Masarei

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Addendum to a Paper on Method-Comparison Analysis

To the Editor:

Having read the paper of Cornbleet and Gochman (Clin Chem 25:432–438, 1979), I would like to stress a point. The equation given for Deming bx in page 433,

$$b_x = U + \sqrt{U^2 + (1/\lambda)},$$

where $b_x$ is slope of the orthogonal regression line, is not entirely correct and results in meaningful slopes only when the coefficient of correlation (r) is positive. This results from the fact that $b_x$ is the solution of a quadratic equation (for simplicity omitting the λ):$\frac{b^2}{2} + \frac{Q_x}{Q_y} (Q_x - Q_y) - Q_x = 0$ where $Q_x = \Sigma (x_i - \bar{x})_i (y_i - \bar{y})_i$ and $Q_y = \Sigma (x_i - \bar{x})^2_i$. So in general two solutions can be found:

$$U + \sqrt{U^2 + 1} \quad \text{and} \quad U - \sqrt{U^2 + 1}.$$ The former results in an absolute minimum for the sum of the orthogonal distances, and the latter in a relative minimum, when the coefficient of correlation is positive. However, when r is
The HPLC analysis for theophylline metabolites was performed by a modification of the Mikeskie-Rodes procedure (1). The mobile phase was a 1:0.6:98.4 (by vol) mixture of tetrahydrofuran, methanol, and sodium acetate buffer (10 mmol/L, pH 5.0). We found high concentrations of the urinary metabolite, 1,3-dimethyluric acid (1,3-DMU) in both patients’ samples: 22 mg/L in patient A and 33 mg/L in patient B. This is the first reported finding of 1,3-DMU in human serum. We observed only trace amounts of the two other theophylline metabolites found in urine, 1-methyluric acid (1-MU) and 3-methylxanthine (3-MX), in the serum of these patients: <3 mg/L and <2 mg/L, respectively. The presence of 3-MX has been reported in some serum samples (2). We detected a trace of caffeine (2.4 mg/L) in the serum of Patient A, none in Patient B.

Patient A was a 74-year-old man with multiple medical problems, including chronic obstructive pulmonary disease, congestive heart failure, adult-onset diabetes, and substantial renal dysfunction (creatinine, 60 mg/L; urea N, 11.6 g/L).

Patient B, a 47-year-old man in renal failure, was hemodialyzed several times during his hospitalization for the treatment of severe hypotension and sepsis; when the above theophylline specimen was analyzed, his serum creatinine concentration was 93 mg/L. Both patients were receiving many other medications in addition to theophylline.

To determine if there might be an effect of theophylline metabolites on these assay systems, we prepared two sets of samples by supplementing the Syva-emtr no. 0 and no. 4 calibrators (respectively containing 0 and 20 mg of theophylline per liter) with theophylline metabolites (Adams Chemical Co., Round Lake, IL 60073). These samples contained either 1-MU (13 mg/L), 3-MX (37 mg/L), or 1,3-DMU (26 mg/L), and a final theophylline concentration of either 0 mg/L (no. 0 calibrator) or 13.3 mg/L (no. 4 calibrator).

The Ames TDA assay showed considerable cross-reactivity with the metabolite 1,3-DMU; the no. 0 calibrator supplemented with 1,3-DMU gave an apparent theophylline value of 5.5 mg/L (actual concn, 0 mg/L), while the similarly supplemented no. 4 calibrator gave an apparent value of 19.8 mg/L (actual concn, 13.3 mg/L). Ames notes this cross-reactivity in their publication (3) and in their TDA-Theophylline package insert, stating that this interference can be disregarded because 1,3-DMU has not been found in the serum of patients taking theophylline. Our data show that this assumption is not valid, at least for some uremic patients. Thus, the presence of 1,3-DMU in the serum of patients A and B explains the falsely increased theophylline values that we observed with the TDA system.

The Abbott TDx assay showed only trace cross-reactivity with 1,3-DMU; the no. 0 and no. 4 calibrators supplemented with 1,3-DMU gave apparent theophylline values of 1.0 mg/L (actual concn, 0 mg/L) and 14.2 mg/L (actual concn, 13.5 mg/L), respectively. This small amount of interference has been noted by Abbott in the TDx operator’s manual but is not sufficient to explain the discrepancies that we observed. Possibly there is a substance besides theophylline or its three metabolites that crosses the TDx assay.

We conclude that theophylline values for samples from uremic patients must be interpreted with caution when determined with the TDx or TDA systems. In this study, HPLC and emtr (lot M01) appear to give comparable and accurate results. But there may be significant lot-to-lot variation in antibody quality, so each new lot of reagents from any manufacturer should be checked for accuracy against a reference method such as HPLC, with use of sera from uremic patients.

In 1980, several groups (4–6) noted falsely increased values for phenytoin when sera from some renal-disease patients were immunoassayed, an increase possibly ascribable to accumulation of the phenytoin metabolite, 5-(p-hydroxyphenyl)-5-phenylhydantoin glucuronide (7). Accumulation of drug metabolites (and, possibly, other substances) in uremic patients that could cross react with therapeutic drug immunoassays may be a general and unappreciated occurrence; it is therefore the responsibility of manufacturers and users of drug immunoassay systems to validate such assays for use in this patient population.

[Note added during revision:] Subsequent to the submission of this Letter, another report of discrepant theophylline values from a uremic patient has appeared (8). These authors also note that serum theophylline values obtained with fluorescence polarization.

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Table 1: Serum theophylline, mg/L

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>emtr lot M01</th>
<th>TDX lot 49-5554q</th>
<th>TDA lot 1072</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>24.4</td>
<td>22.0 (−10)²</td>
<td>28.2 (16)</td>
<td>32.6 (34)</td>
</tr>
<tr>
<td>Patient B</td>
<td>16.7</td>
<td>16.6 (1)</td>
<td>22.0 (32)</td>
<td>23.1 (38)</td>
</tr>
</tbody>
</table>

*Percent difference from HPLC is shown in parentheses.

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Fig. 1. Orthogonal regression line, calculated with theoretical data points

A using the equation of Cornbleet et al.: \(y = 0.9912x - 0.1954;\) B, using the corrected equation: \(y = -1.0086x + 7.805.\) Line A is perpendicular to line B.

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Increase in Apparent Theophylline Concentration in the Serum of Two Uremic Patients as Measured by Some Immunoassay Methods (Caused by 1,3-Dimethyluric Acid?)

To the Editor:

Recently we found discrepancies in theophylline concentrations when eight samples of serum from two uremic patients were analyzed by "high-performance" liquid chromatography (HPLC) (1) and three commercially available immunoassay methods ("emtr," Syva Co., Palo Alto, CA 94303; "TDx," Abbott Labs., North Chicago, IL 60064; "TDA," Ames Div., Miles Labs., Elkhart, IN 46615). Note the significantly higher theophylline values in the TDA and TDx assays as compared with the HPLC and emtr methods, as shown in the following two representative examples: