Creatinine Clearance: Enzymatic vs Jaffé Determinations of Creatinine in Plasma and Urine

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We measured creatinine in plasma and urine samples from 17 normal subjects and 10 clinically impaired subjects by four different methods: two enzymatic—Ektachem iminohydrolase and Boehringer Mannheim amidohydrolase—and two Jaffé reaction based—Beckman Astra 8 and Technicon AutoAnalyzer I. Creatinine clearances, standardized for body surface area, were also calculated. In both groups of subjects plasma creatinine values were significantly (p <0.05) lower, by 3 to 4 mg/L, when measured enzymatically than when measured by the Jaffé reaction. Additionally, creatinine clearances were significantly (p <0.05) greater by at least 30 mL/min when calculated from enzymatically measured creatinine values vs Jaffé method values for creatinine. The benefits of lack of interference with enzymatically measured creatinine concentrations and clearances should be assessed in relation to the lack of agreement with long-established (Jaffé) methods for determining creatinine (and inulin) clearances.

The analytical method used most commonly in clinical laboratories to measure plasma and urine creatinine concentrations is based on the Jaffé reaction, which involves an alkaline solution of picric acid. Many modifications of the Jaffé method have been suggested for eliminating interference by noncreatinine substances in serum, but with incomplete success (1-3). Recently, two alternatives to the Jaffé reaction have been developed for measuring creatinine, both involving enzymes specific for creatinine. The method used in the Kodak Ektachem analyzer is based on the enzymatic hydrolysis of creatinine by creatinine iminohydrolase (creatinine deiminase; EC 3.5.4.21) to produce ammonia and N-methylhydantoin (4, 5). In the second, developed by Boehringer Mannheim, creatinine is converted to creatine by the enzyme creatinine amidohydrolase (creatininase; EC 3.5.2.10) (6, 7). Both methods are sensitive and specific for creatinine and are not affected by endogenous noncreatinine substances (ketoacids, cephalosporins, bilirubin, drugs) that interfere with the Jaffé reaction.

Here we have compared creatinine clearances derived from creatinine concentrations measured by two enzymatic and two Jaffé-based methods in subjects with normal renal function and in patients with renal impairment, looking for potential difficulties in the interpretation of normal ranges for creatinine clearances derived from data on plasma and urine creatinine concentrations measured by enzymatic methodologies.

Materials and Methods

Plasma was sampled either before, during, or immediately after collection of a 24-h urine specimen. The subject's height and weight were recorded.

Creatinine concentrations in plasma and urine were measured by four procedures. Enzymatic creatinine analyses were performed by the iminohydrolase procedure in the Ektachem 400 (Eastman Kodak Co., Rochester NY 14600) according to the manufacturer's specifications, with use of calibration materials and reagent slides provided (8) and by the amidohydrolase procedure in the Multistat III Centrifug-
gal Analyzer (Instrumentation Laboratory, Spokane, WA 99207) utilizing the enzymatic creatinine method of Boehringer Mannheim Diagnostics (BMD), which is a modification of the procedure of Jaynes et al. (7). Jaffé-based creatinine measurements were made in an Astra 8 Analyzer (Beckman Instruments, Inc., Brea, CA 92621) (9) and in a continuous-flow AutoAnalyzer I (Technicon Instruments Corp., Tarrytown, NY 10591) according to the manufacturer’s guidelines (10).

Day-to-day means (±SD, n = 30) and CVs for each method were as follows: Ektachem: 1.10 ± 1.0 mg/L, 7.3%; BMD: 14.5 ± 0.8 mg/L, 5.6%; Astra: 12.0 ± 0.6 mg/L, 5.1%; AutoAnalyzer I: 16.0 ± 0.7 mg/L, 4.3%.

Creatinine clearances were calculated by use of the standard formula, corrected for body surface areas, as follows: CC = Ucr · V · 1.73/(Scr · T · BSA), where CC is creatinine clearance (mL/min); Ucr, urinary creatinine (mg/dL); Scr, serum creatinine (mg/dL); V, volume (mL); T, time (min); 1.73, standard body surface area; BSA, individual body surface area extrapolated from weight and height from an established nomogram (11). By analysis of variance we identified differences in creatinine results as measured by the different methods.

Results

Table 1 shows the mean values for plasma and urinary creatinine concentrations and the calculated creatinine clearances for all four methods. For the 17 normal volunteers, both enzyme-determined plasma creatinine concentrations were significantly (p <0.05) lower than the Jaffé-determined creatinine concentrations. Enzymatic plasma creatinine concentrations ranged from 3 to 9 mg/L, Jaffé-determined creatinine from 6 to 12 mg/L. The 3 to 4 mg/L lower enzymatic results probably are ascribable to the lack of interference by endogenous substances that normally do interfere with Jaffé creatinine measurement. By all four methods, there was a wide range (660 to 2900 mg/L) of urinary creatinine concentrations. Values for iminohydrolase-derived creatinine clearance (155 ± 46 mL/min) and amidohydrolase derived creatinine clearance (154 ± 44 mL/min) were significantly (p <0.05) higher than clearances measured by either the Astra Jaffé (92 ± 27 mL/min) or the AutoAnalyzer Jaffé (112 ± 32 mL/min) procedures. In addition, the AutoAnalyzer/Jaffé creatinine clearance results significantly (p <0.055) exceeded the Astra/Jaffé results. The best correlation between clearances derived by use of the four different methods was between the two Jaffé methods: AutoAnalyzer vs Astra, r = 0.98. The iminohydrolase-derived clearances also correlated well with the AutoAnalyzer/Jaffé (r = 0.94) and the Astra/Jaffé (r = 0.91) derived clearances. The correlations were poorer between the amidohydrolase method vs iminohydrolase-derived clearances (r = 0.71) or the Astra/Jaffé (r = 0.83) and the AutoAnalyzer/Jaffé-derived clearances (r = 0.78). When we compared 10 patients with mildly increased plasma creatinine concentrations by the iminohydrolase and Astra/Jaffé methods, we saw significant (p <0.05) differences (Table 1): clearances determined by the enzymatic methodology were 1.32 times those by the colorimetric Jaffé method. However, results by the two methods correlated well (r = 0.98; iminohydrolase creatinine clearance = 1.64 (Astra creatinine clearance) – 18).

Discussion

Our preliminary results show (Table 1) that measured clearances were significantly greater for normal subjects when enzymatic determinations of creatinine were utilized than when established Jaffé methods were used. In addition, patients with mild renal impairment showed significantly greater clearance values when measured by enzymatic methods than by Jaffé methods. Data derived by the enzymatic methods in determining creatinine clearances would lead to misinterpretation of renal function if expressed in comparison to creatinine clearances derived from the Jaffé-based methods, and clinicians should be cognizant of such potential problems.

Creatinine has been measured by methods based on the Jaffé reaction for a century (1). The influence of an abrupt decline in glomerular filtration on creatinine balance and plasma creatinine concentrations have shown that substantial changes in creatinine clearance are not reflected in plasma creatinine concentrations (12). Thus, plasma creatinine assay may not be as useful in detecting a mild impairment of the glomerular filtration rate. If clinical laboratories utilize one of the enzymatic creatinine methodologies, an additional interpretation problem arises, as shown in Table 1, where a mean clearance of 91 mL/min by the enzymatic method would be falsely interpreted as being within normal limits based upon Jaffé creatinine or inulin clearance studies. The mean Jaffé creatinine clearance was 66 mL/min, indicating mild renal impairment.

The potential benefits of lack of interferences (4–7) with the enzymatic methodologies for creatinine measurements might be outweighed by the lack of agreement with established values for Jaffé-based creatinine and inulin clearance. Higher enzymatic-based calculations of creatinine clearance may be detecting greater tubular secretion of creatinine in both normal and impaired renal function.

Table 1. Creatinine Concentrations (Mean ± SD) In Plasma and Urine, and Creatinine Clearances in Normal Subjects and Renally Impaired Patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Enzymatic</th>
<th>Jaffé-based</th>
</tr>
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<tbody>
<tr>
<td>Normal subjects (n = 17)</td>
<td></td>
<td></td>
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<tr>
<td>Plasma creatinine, mg/L</td>
<td>7.1 ± 1.5</td>
<td>6.1 ± 1.5</td>
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<tr>
<td>Urinary creatinine, mg/L</td>
<td>1680 ± 650</td>
<td>1390 ± 530</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>155 ± 46</td>
<td>154 ± 44</td>
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<tr>
<td>Renal patients* (n = 10)</td>
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<tr>
<td>Serum creatinine, mg/L</td>
<td>14 ± 6</td>
<td>—</td>
</tr>
<tr>
<td>Urine creatinine, mg/L</td>
<td>680 ± 210</td>
<td>—</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>91 ± 43</td>
<td>—</td>
</tr>
</tbody>
</table>

*Significantly (p <0.05) different from Jaffé-based results.

*BMD and AutoAnalyzer assays not performed because of insufficient sample volume.
However, clinicians and laboratorians must be extremely careful in the interpretation of creatinine clearance measurements performed by the enzymatic creatinine methods. Plasma creatinine concentrations are precisely measured by all four methods, but the utility of enzymatically derived clearances needs further investigation in relationship to the established Jaffé methods currently used worldwide. Although our observations are not definitive, they reinforce the need to establish normal reference intervals for plasma creatinine and for creatinine clearance measurements.

We thank Professor Esther Freier, Department of Laboratory Medicine and Pathology, University of Minnesota, for performing creatinine measurements by the BMD method for this study.

References

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Corrections

p 1628, Fig. 6: Although faint, there is a band at the origin in lane g.

p 1656: The accompanying figure should be substituted for the figure 2 shown on page 1656, a figure that was to have been deleted. The figure legend is unchanged.

p 1705: The second paragraph in the left column should replace the third paragraph of the Discussion. Thus the authors find it "likely . . . that the principal alkali-labile oxalate precursors in urine are ascorbate and some of its metabolites . . . ."

p 1927: The name of the author of a book review, Robert Rej, was omitted.

p 2014: On line 7, the 95 percentile interval for plasma ammonia should be 16–53 μmol/L, as correctly stated in the Abstract.