Age-adjusted reference ranges for creatinine clearance were determined in 279 women, ages 40–95 years, who were housed in a metabolic research unit and consumed a meat-free diet. Creatinine clearance, but not serum creatinine, declined with age by 0.63 mL/min per 1.73 m² per year. Serum and urine creatinine concentrations, used to calculate clearances, were analyzed by a kinetic Jaffé procedure. In a subset of 100 subjects, fasting serum creatinine values averaged 8.3 ± 5.2 (SD) µmol/L higher when measured by the kinetic Jaffé procedure than by an enzymatic method (creatinine PAP). The Cockcroft–Gault formula for estimating creatinine clearance from serum creatinine in women was validated, and the modification factor for the male equation was determined to be 0.84 (95% confidence interval 0.83–0.86) confirming the suggested 15% correction. A prediction formula derived from this population was similar in accuracy to the Cockcroft–Gault formula.

Indexing Terms: reference interval/sex- and age-related effects/ glomerular filtration rate

Glomerular filtration rate (GFR) has been described as the most important clinical renal function test to monitor with age (1). The gold standard for GFR has been inulin clearance, as inulin is neither secreted nor absorbed by the kidney tubules; however, it is an impractical test for routine use (2, 3). Creatinine clearance has been generally accepted as a clinically useful measure of GFR despite some limitations (4). Since creatinine is secreted by the proximal tubule, creatinine clearance can exceed inulin clearance by 10–30% when serum creatinine is measured by a “true” creatinine method that does not measure noncreatinine chromogens (1, 5). This error is potentially offset when serum creatinine is analyzed with a “total” chromogen method (1, 3). The accuracy of creatinine clearance is also influenced by the completeness of timed urine collections (6), the amount of meat in the diet and the method of cooking (7, 8), the within-subject and analytical variability (3), strenuous exercise, and stress (9). Trauma, severe infection, and the menstrual cycle, as reviewed by Heymsfield et al. (10), have been shown to affect urinary creatinine excretion and therefore potentially creatinine clearance.

Cross-sectional studies of GFR, by inulin clearance (11, 12) and creatinine clearance (13, 14), have shown a decline with age in adults. Longitudinal studies by Lindeman et al. in men (14), however, suggest that loss of renal function with age is not an inevitable process: In a group of 446 men from the Baltimore Longitudinal Study of Aging with five or more serial creatinine clearance determinations who were followed for up to 23 years, one-third of the subjects had no decrease in renal function, and a small number had increases. Creatinine clearance is proportional to body size, resulting in gender differences (5). Correction for lean body mass or body surface area may eliminate or reduce the gender variation (2, 5, 15). Consequently, age- and gender-adjusted reference ranges are warranted for creatinine clearance. Age-adjusted reference ranges for creatinine clearance have been established in several previous studies (13, 16–18). In general, reference values for women have not been determined directly, but have been calculated by adjusting male values.

Creatinine clearance can be calculated from serum creatinine by using previously established nomograms or formulas without collecting a timed urine specimen (19). Estimated clearances are most often computed to adjust the dosage regimen of drugs, which are primarily excreted by the kidney (19). Use of formula estimates eliminates the need for the often inaccurate urine collection, the cost of an additional laboratory test, and the wait for completion (usually 24 h) of the urine collection (20). The calculated creatinine clearance is frequently used in the clinical setting for elderly patients, who, as a group, may often suffer urinary incontinence, mental confusion, or forgetfulness. The most commonly used formula is the one developed by Cockcroft and Gault (21) because of its ease of use and its inclusion of age and weight as predictors of urinary creatinine excretion. This formula was developed from data for 249 men, ages 18–90 years, and a 15% reduction in the resulting creatinine clearance for women was proposed based on data from the literature (21). Validation of this correction factor for women has been limited and inconclusive (20, 22–26).

No studies of creatinine clearance in men or women have controlled for the effects of diet, and most have not included supervised urine collections. Creatinine clearance reference ranges have in general been only estimated in females; therefore, we determined reference ranges for creatinine clearance in 279 healthy women who resided in a metabolic research unit and consumed a meat-free diet for 3 days before and during the testing

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Received May 31, 1994; accepted September 6, 1994.

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period. Serum and urine creatinine concentrations were measured with a kinetic Jaffé procedure; however, a subset of serum specimens was analyzed with an enzymatic (creatinine PAP) true creatinine method for comparison. In addition, the validity of the correction factor for the Cockcroft–Gault formula for use in women was investigated and a new formula was derived and compared with measured clearances.

**Materials and Methods**

The 280 healthy ambulatory women who participated in this study were recruited from the Boston area and ranged in age from 40 to 95 years. The research protocol was approved by the Tufts University Human Investigation Review Committee and written informed consent was obtained from each subject. All subjects accepted into this study had normal results for physical examination and laboratory tests (standard clinical chemistry and hematologic profiles, and urinalysis). Women with evidence of renal, hepatic, coronary artery, or cerebral vascular disease, gout, urinary tract infections, congestive heart failure, diabetes mellitus, or uncontrolled hypertension (mean blood pressure [diastolic/1/3 (systolic–diastolic)] = 107 mmHg) were excluded. No subjects were currently being treated with diuretics, antihypertensives, steroid medications, vasodilators, or amphetamines or had used aminoglycoside antibiotics, phenacemide, cimetidine, or thyroxine within 1 month of the study. Subjects who smoked cigarettes were admitted to the study, but were asked to refrain from smoking immediately before and during the course of the study. No exercise was permitted prior to or during the study. Premenopausal women were admitted immediately after the cessation of their last menses.

Subjects spent 1.5 days and 2 nights in the Metabolic Research Unit at the USDA Human Nutrition Research Center on Aging at Tufts University. The first evening meal and the subsequent three meals were meat free and subjects were requested to eat no cooked meals 3 days prior to admission. Subjects were advised by Human Nutrition Research Center dieticians on alternative sources of dietary protein. Because of their diuretic effects, no tea or coffee was allowed immediately prior to or during the testing period. Caffeine-free soft drinks were allowed. For the subject to be fully hydrated, water was consumed (orally) at 10–15 mL/kg of body weight.

On the second day of the study, blood was drawn at 0800 after an overnight fast and again at 2000. A 24-h urine collection was started at 0800 and completed the next morning. Specimens were stored at −20°C until analysis. Serum and urine creatinines were analyzed by a kinetic modification of the Jaffé reaction with Roche reagents on the Cobas Fara centrifugal analyzer (Roche Instruments, Belleville, NJ). The morning and evening serum specimens from each subject were analyzed in the same run. Creatinine clearances were calculated by averaging the two serum creatinine values or by using only the fasting morning values; the results obtained were very similar. Because morning specimens are used most often clinically to calculate clearances, they are reported here. Correction for body surface area was made with the formula 1.73A, where log A = 0.425 log weight (kg) + 0.725 log height (cm) − 2.144 (27). A subset of the fasting serum specimens (n = 100) was analyzed for creatinine with an enzymatic method, the creatinine PAP kit (Boehringer Mannheim Diagnostics, Indianapolis, IN), adapted to the Cobas Fara.

A creatinine clearance of 138 mL/min per 1.73 m² from one subject of age 70 was determined to be an outlier in a normal probability plot and was rejected by Grubb’s test (28). This volunteer was excluded from analyses. Reference intervals were calculated as the mean ± 1.96 SD. Aging effects were assessed by linear regression analysis. Paired Student’s t-test and Pearson correlation coefficients were used to compare creatinine clearances with those corrected for body surface area, measured creatinine clearances with those estimated from serum creatinine, and serum creatinine values measured by a Jaffé method with those measured by an enzymatic method. Least-squares regression analysis through the origin was used to determine the factor necessary to correct the Cockcroft–Gault formula for men for use in women.

**Results**

Age-adjusted references ranges for fasting serum creatinine, 24-h urinary creatinine excretion, and creatinine clearance, both uncorrected and corrected for body surface area, were determined in the 279 healthy women (Table 1). Creatinine clearances were highly correlated with corrected clearances (r = 0.91, P < 0.0001), and mean (SD) corrected values were significantly higher [82.8 ± 15.6 mL/min per 1.73 m² vs 80.8 ± 16.6 mL/min, P < 0.001] than uncorrected values. Except for the group >80 years (six subjects), the ranges for corrected and uncorrected creatinine clearances were very similar. To convert serum creatinine values analyzed with enzymatic methods to comparable Jaffé values so that these reference ranges could be applied, we analyzed 100 fasting serum specimens by both methods (Fig. 1). There was a significant correlation (r = 0.80) between the kinetic Jaffé (y) and creatinine PAP (x) methods (P = 0.0001, y = 0.85x + 17.9 μmol/L), and the Jaffé results averaged 8.3 ± 5.2 (SD) μmol/L (0.94 ± 0.59 mg/L) higher (P = 0.0001) than the corresponding creatinine PAP values.

The relationship between creatinine clearance corrected for body surface area and age in this cohort of women is shown in Fig. 2. Creatinine clearance significantly declined at a rate of 0.63 mL/min per 1.73 m² per year (P < 0.0001) from ages 40 to 95. Unlike creatinine clearance, serum creatinine did not change with age in women between the fifth and tenth decades (Fig. 3).

Measured creatinine clearances (Ccr) were compared with clearances estimated from serum creatinine by using the formula of Cockcroft and Gault (21):

\[
Ccr = \frac{140 - \text{age (years)} \times \text{weight (kg)}}{7.2 \times \text{serum creatinine (mg/L)}}
\]
Table 1. Reference ranges for fasting serum creatinine, 24-h urinary creatinine excretion, and creatinine clearance, both uncorrected and corrected for body surface area.*

<table>
<thead>
<tr>
<th>Age range, years</th>
<th>Serum creatinine</th>
<th>Urine creatinine</th>
<th>Creatinine clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>mg/L</td>
<td>mmol/day</td>
</tr>
<tr>
<td>40–49</td>
<td>52–84</td>
<td>5.8–9.5</td>
<td>5.9–11.9</td>
</tr>
<tr>
<td></td>
<td>(68)</td>
<td>(7.7)</td>
<td>(8.9)</td>
</tr>
<tr>
<td>50–59</td>
<td>53–89</td>
<td>6.0–10.0</td>
<td>5.6–11.2</td>
</tr>
<tr>
<td></td>
<td>(71)</td>
<td>(8.0)</td>
<td>(8.4)</td>
</tr>
<tr>
<td>60–69</td>
<td>46–96</td>
<td>5.2–10.8</td>
<td>5.3–10.1</td>
</tr>
<tr>
<td></td>
<td>(71)</td>
<td>(8.0)</td>
<td>(7.7)</td>
</tr>
<tr>
<td>70–79</td>
<td>48–96</td>
<td>5.5–9.7</td>
<td>4.8–9.1</td>
</tr>
<tr>
<td></td>
<td>(67)</td>
<td>(7.6)</td>
<td>(6.9)</td>
</tr>
<tr>
<td>80+</td>
<td>44–90</td>
<td>5.0–10.2</td>
<td>4.1–7.2</td>
</tr>
<tr>
<td></td>
<td>(67)</td>
<td>(7.6)</td>
<td>(5.6)</td>
</tr>
</tbody>
</table>

*Means are shown in parentheses.

Fig. 1. Relation between a kinetic Jaffé method (y) and an enzymatic method (creatinine PAP, x) for measuring serum creatinine. A subset of 100 fasting serum specimens from 279 women was analyzed by both methods. The solid line represents the linear regression line (y = 0.86x + 17.9 μmol/L), and the dotted line, the line of identity.

Fig. 2. Relation between creatinine clearance corrected for body surface area (y) and age (x) in 279 healthy women, ages 40–95. The linear regression equation was y = −0.83x + 121 mL/min per 1.73 m².

and the suggested modification of Ccr = 15% for women (Fig. 4). The measured and estimated clearances were significantly correlated (r = 0.74, P <0.0001), and the standard error of the estimate decreased from 15.5 to 13.2 with the 15% adjustment. There was a significant mean difference of 12.8 ± 15.5 mL/min (P <0.001) between the estimated and measured clearances with the formula for men; however, that difference was eliminated (−1.2 ± 13.3, P = 0.13) when the 15% correction factor for women was applied. By using least-squares regression analysis through the origin, the correction factor for the Cockcroft–Gault (21) formula for men that was required to adjust the formula for application in women was determined to be 0.84 (95% confidence interval, 0.83–0.86). With the 15% correction factor, the formula estimate tended to slightly underestimate the true clearance for values <70 mL/min and slightly overestimate those clearances >70 mL/min. The mean absolute difference between the two was 10.7 ± 8.1 mL/min. Creatinine clearance estimates for 78% of the subjects were within 20% of the measured value, whereas 95% of subjects’ estimates were within 30% of measured values.

After the derivation of the Cockcroft and Gault formulation, a formula for estimating creatinine clearance from serum creatinine was derived from the 279 women in this study and determined to be:

\[
Ccr = \frac{185 - \text{age (years)} \times \text{weight (kg)}}{12.8 \times \text{serum creatinine (mg/L)}}
\]

or

\[
Ccr = \frac{185 - \text{age (years)} \times \text{weight (kg)}}{1.448 \times \text{serum creatinine (μmol/L)}}.
\]

Although not ideal, the formula was tested in the same population of women. There was a significant relation between the formula and measured values (r = 0.72, P
<0.0001, SEE = 12.6), as shown in Fig. 5. As with the Cockcroft-Gault formula for women, our formula tended to slightly underestimate the true clearance for lower clearances and slightly overestimate higher clearances, with a dividing point of 90 mL/min, there being a small but significant mean difference of 1.6 ± 13.1 (SD) mL/min (P = 0.04) between the estimated and measured clearances. Values estimated with this new formula (y) and the established Cockcroft-Gault formula (x) were in fact highly correlated (r = 0.98, P <0.0001, y = 0.91x + 9.9 mL/min).

**Discussion**

Reference ranges for creatinine clearance, both uncorrected and corrected for body surface area, were determined in 279 healthy women, ages 40 to 95 years. The women were housed in a metabolic research unit for the duration of the study, thus keeping variability in creatinine excretion due to incomplete urine collections to a minimum. In addition, subjects were not allowed to exercise prior to or during the study and premenopausal women started the study immediately after the cessation of their last menses, thereby eliminating potential variability from those factors (10). Coffee, tea, and drugs affecting GFR were also avoided. The effect of cooked meat on creatinine clearance was controlled in this study as well, with no intake of meat by the subjects during or 3 days prior to the study period. One hundred grams of meat contain 20–40 mg of creatinine and 350–500 mg of creatine, of which 18–65% will be converted to creatinine after cooking (3, 7). The diurnal variation of serum creatinine, which peaks in the afternoon (29), may be due to meat intake (7). Reports of changes in creatinine clearance in response to intakes of cooked meat have ranged from no effect (8, 30), attributed to the proportional increases in serum and urine creatinine (8), to increases of up to 50% (31, 32), with clearances 63% higher in meat eaters as compared with vegetarians (33). A daytime serum creatinine obtained after a cooked meat meal may underestimate creatinine.
clearance, whereas a cooked meat meal during the 24-h urine collection related to a fasting serum creatinine would overestimate it (8). Thus it has been suggested that cooked meats be substantially avoided during the clearance evaluation period (8, 31).

Serum and urine creatinines were measured in this study by the kinetic Jaffé method, which was the method most commonly used by laboratories participating in the College of American Pathologists’s chemistry survey in 1982–83 (34). Despite the elimination of interference of noncreatinine chromogens with enzymatic methods, use of these methods in the clinical laboratory has been limited because of the difficulty in adapting them to some automated instruments. A subset of samples from this study was analyzed with an enzymatic method (creatinine PAP) to determine whether there were differences in the methods and if so, to formulate an equation to convert the enzymatic results to a kinetic Jaffé result to utilize the reference ranges generated in this study. Lott and Hayton (19) have suggested, however, that correction for methodology is unnecessary. In our comparison, the modified Jaffé values were an average of 8.3 μmol/L higher than the enzymatic values and therefore the corresponding creatinine clearances would be lower. In previous comparisons between these methods, results obtained with the Jaffé method were similar (35, 36) or higher (37, 38) than corresponding creatinine PAP values. The kinetic modification of the Jaffé procedure may eliminate some, but not all, of the problems of interferences of noncreatinine chromogens in the Jaffé method and may explain the higher results here (4). An additional explanation may be a difference in calibration (35).

The relation between GFR and aging has been well defined (11–14). For creatinine clearance, the changes seen in our cross-sectional study of healthy women (0.63 mL/min per 1.73 m² and 0.72 mL/min per year) agree well with other studies of healthy women as well as men (16, 17, 39).

It is useful to be able to determine creatinine clearance without the need for urine collections, which are inconvenient to obtain, often inaccurate, and prevent timely determinations of drug dosages. In addition, the within-subject variability in creatinine excretion has been reported to range from 3% to 14%, irrespective of collection and analytical errors (40). Therefore, a number of formulas and nomograms have been developed that are all minimally based on serum creatinine. The principle behind these estimates is that creatinine excretion is constant and equal to creatinine production, which is proportional to muscle mass and can be predicted from age and weight (3). Differences in body composition between men and women also influence these estimates. The Cockcroft–Gault formula, which was generated from data in men, is most commonly used because of its simplicity and inclusion of age and weight, which vary with creatinine clearance (19). The developers of this formula suggested a correction factor of 15% for women because of women’s lower muscle mass, which they determined from a review of available literature (21). Thus, the Cockcroft–Gault formula was evaluated in this cohort of women in relation to measured clearances and to the validity of the 15% correction factor.

The majority of studies evaluating the Cockcroft–Gault formula have looked at small numbers of older subjects, the population for which these formulas are potentially the most useful (22–25, 41, 42). However, two studies of hospitalized patients (20, 43) involved large numbers of subjects and found correlations of 0.93 (20) and 0.82 (43) between measured and predicted clearances for men and women. Cockcroft and Gault found a correlation of 0.83 in a group of male patients (21). In the elderly, the relation between measured and calculated creatinine clearance has been examined in free-living men and women [r = 0.79 (22); r = 0.81 (23)], male nursing home residents [r = 0.60 (41)], nursing home residents of both genders [r = 0.80 (25)], and free-living women, half of whom were in chronic renal failure [r = 0.74 (22)]. Two studies have compared the use of the Cockcroft–Gault formula in young and older subjects (24, 42). Goldberg and Finkelstein (42) compared older outpatients, r = 0.43, and inpatients, r = 0.74, with younger outpatients, r = 0.58, whereas Friedman et al. (24) looked at only ambulatory subjects (older, r = 0.73; younger, r = 0.37). Goldberg and Finkelstein (42) have suggested that the poorer correlations were due to inaccurate urine collections, and Sawyer et al. (43) have stated that the variability between the predicted and measured values is due to inaccuracy in the clearance, not the formula. The correlation between measured creatinine clearance and creatinine clearance estimated by the Cockcroft–Gault formula for women in this study was 0.74, similar to other studies of women and of both genders (21–25, 42). Results from previous studies regarding the accuracy of the formula have been mixed, claiming the formula was similar to measured values (20), underestimated (22–24), or overestimated at low values (42). The direction is obviously dependent upon the ages and disease states of the subjects studied. The formula estimates are unacceptable in situations of muscle wasting, obesity, or edema, and when serum creatinine is not in a steady state (3); however, there is no consensus of whether this formula is accurate enough for clinical use, especially in the elderly (42). Cockcroft and Gault have pointed out that the average prediction error of their formula is no greater than the variability in paired clearances, which they attribute to biological variation and errors in urine collection and analysis of creatinine (21).

The variability in some of the correlations between the measured and formula estimates may be due to the proposed correction factor for women. The correction factor has been described as both necessary (23) and unnecessary (20, 22, 24, 25), and several attempts have been made to determine the appropriate factor (26, 44). We determined here that the correction factor for women should be 0.84 (95% confidence interval, 0.83–0.86), which thus validates the assumption a 15% correction made by Cockcroft and Gault (21).

Additional
evidence for the appropriateness of the correction factor is the similarity between the published formula and the formula derived from the data presented here from 279 healthy women.

In summary, age-adjusted reference ranges for creatinine clearance were determined in healthy women over age 40 by controlling for factors, primarily diet, that affect the variability of creatinine clearance. A formula was generated to convert serum creatinine values measured with an enzymatic method (creatinine PAP) to the generally higher kinetic Jaffe values, to apply these ranges. There was a progressive decline of creatinine clearance with age in this cross-sectional study, as has been previously seen (1, 13, 16, 17, 39), and the Cockcroft–Gault formula with a correction factor of 15% for women was validated as an accurate estimate of creatinine clearance in healthy women.

Supported by the USDA Human Nutrition Research Center on Aging at Tufts University (Contract No. 53-3K06-5-10).

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