RESEARCH ARTICLE

Validation of daily urinary creatinine excretion measurement by muscle-creatinine equivalence

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Background: Twenty-four-hour urinary creatinine excretion (24hUCrE) is strongly correlated with skeletal muscle mass (SMM). This study suggests how to exploit the power of the SMM-24hUCrE correlation to assess the accuracy of 24hUCrE measurement.

Methods: Four hundred and sixty-six men, a subgroup of participants in the 2002-2004 follow-up examination of the Olivetti Heart Study, performed a 24-h urine collection to measure 24hUCrE and underwent bioelectrical impedance analysis to evaluate SMM. Linear regression analysis between 24hUCrE and SMM was used to calculate the muscle-creatinine equivalence and to develop an equation to predict the 24hUCrE depending on SMM. The accuracy of the 24hUCrE measurement was assessed using the change in the SMM-24hUCrE correlation coefficient upon variation in the percentage deviation (%D) between the measured and predicted 24hUCrE.

Results: The calculated muscle-creatinine equivalence was 1 g of 24hUCrE = 22.73 kg of SMM. The %Ds and the corresponding SMM-24hUCrE correlation coefficients were as follows: %D = 3.0, $r = .997$; %D = 4.7, $r = .989$; %D = 8.1, $r = .963$; %D = 10.5, $r = .940$; %D = 12.6, $r = .909$; %D = 18.9, $r = .825$; %D = 25.8, $r = .707$; %D = 33.5, $r = .595$; %D = 41.4, $r = .453$.

Conclusion: The increase in %D corresponds to a reduced correlation between muscle mass and creatinine excretion, which indicated a poor performance in the measurement of the 24hUCrE. For studies on single individuals, where small variations in 24hUCrE could be significant, a %D up to 12.6% is suggested; on the other hand, a wider %D interval could be acceptable for population studies.

KEYWORDS

24-h urinary creatinine excretion, creatine, creatinine, phosphocreatine, skeletal muscle mass

1 | INTRODUCTION

The measurement of 24-h urinary creatinine excretion (24hUCrE) is usually used to estimate the creatinine clearance and a number of urinary analyte excretions (e.g. albumin, sodium, potassium, lithium, iodine) as analyte/creatinine ratio.^{1,2} Unfortunately, the measurement of 24hUCrE may be impaired by several factors³ such as normal daily variation, incomplete 24-h urine collection, exhausting

physical exercise, emotional stress, changes in the meat content of the diet, renal disease, and other acute conditions such as infection, fever, or trauma. Thus, there is often uncertainty about the validity of 24hUCrE measurement. To estimate the accuracy of the measurement of 24hUCrE, a few studies^{4,5} suggest assessing the strength of the correlation between skeletal muscle mass (SMM) and creatinine excretion. Indeed, the majority of creatine (together with phosphocreatine) is contained within the SMM in normal conditions so that,

FIGURE 1 Scatter plot of the SMM (kg) and measured 24hUCrE (M24hUCrE, g/24 h) of the whole study population. The equation of regression line was $RC24hUCrE = 0.044 \times SMM + 0.410$. The linear regression coefficient was $r = .37$ and $P < .0001$. (RC24hUCrE = recalculated 24hUCrE)

on a meat-free diet, the amount (g) of creatine (and phosphocreatine) per kg of muscle remains constant.³ Following the spontaneous conversion of creatine and phosphocreatine to creatinine at a constant daily rate, the latter is constantly eliminated by the kidney. As a consequence, the 24hUCrE is strongly correlated with the total SMM^{6,7} and the muscle-creatinine equivalence enables to predict the daily urinary creatinine excretion. The estimation of SMM can be currently performed with good accuracy by a quick and inexpensive method such as bioelectrical impedance analysis (BIA).⁸⁻¹⁰ A possible difference between the measured and predicted 24hUCrE values (i.e., the accuracy of 24hUCrE measurement) translates into a change in the SMM-24hUCrE correlation coefficient.

This study suggests how to exploit the value of the SMM-24hUCrE correlation coefficient to evaluate the accuracy of 24hUCrE measurement.

2 | MATERIALS AND METHODS

2.1 | Study description

The study population was composed of 466 men on their habitual diet, a subgroup of 994 men (age range 32-82 years) participating in the 2002-2004 follow-up examination of the Olivetti Heart Study (OHS). The OHS population was provided by the male workforce of the Olivetti factories of Pozzuoli (Naples) and Marcanise (Caserta). The study participants consented to undergo BIA and to perform a 24-h urine collection. A detailed description of the study procedures has been previously reported.¹¹

2.2 | Urinary creatinine excretion measurement

Participants were carefully instructed to perform a 24-h urine collection.¹² The day before the visit, they performed a urine collection

discarding the first void in the morning and then collecting all the urines until the following morning, including the first void. The volume of the 24-h collection was recorded, and a specimen was stored for the subsequent chemical analysis of creatinine. Urinary creatinine concentration was measured by an autoanalyzer (ABX Pentra 400; HORIBA ABX, Rome, Italy) using a picric acid colorimetric method with the kinetic reading of results.

2.3 | Bioelectrical impedance analysis and estimation of skeletal muscle mass

All the study participants were on their habitual diet and did not follow any physical exercise program. BIA measurements were carried out by the same operator in all patients using the same analyzer¹³ (BIA 101 model, Akern/RJL Systems, Florence, Italy). The measured resistance and reactance (in ohm) were used to assess total SMM. SMM by BIA was estimated using the Tengvall equation⁹ as applied for men, using the height (cm) and weight (kg) of the participants: $SMM \text{ (kg)} = -24.02 + (0.33 \times \text{height}) + (-0.031 \times \text{resistance}) + (0.083 \times \text{reactance}) + (0.046 \times \text{body weight})$.

2.4 | Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 13; SPSS, Inc., Chicago, IL, USA). Linear regression analysis was performed to compute the grams of creatinine production rate, predicted for kg of SMM. The study of the pattern of residuals allowed to recalculate the values of 24hUCrE according to the equation of the best fit line, using SMM as the independent variable (predictor, X-axis). Pearson's correlation analysis was used to estimate the correlation coefficient (r) between SMM and 24hUCrE. Two-sided P values $< .05$ were considered statistically significant.

3 | RESULTS

3.1 | Calculation of muscle-creatinine equivalence and prediction of daily urinary creatinine excretion

Figure 1 shows the regression line between the SMM (kg) and the measured 24hUCrE (M24hUCrE, g/24 h) of the whole study population. The linear regression coefficient was $r = .37$, $P < .0001$. The points on the graph of Figure 1 are represented by the equation:

$$M24hUCrE = 0.044 \times SMM + 0.410 + ER24hUCrE \quad (1)$$

where 0.044 is the slope, 0.410 is the constant (Y-intercept), and the term "ER24hUCrE" describes the residue between M24hUCrE and the recalculated 24hUCrE (RC24hUCrE) value according to the equation of the best fit line:

$$RC24hUCrE = 0.044 \times SMM + 0.410 \quad (2)$$

where the values of RC24hUCrE represent the points on the regression line recalculated by the equation (2). The reliability of the adopted model was evaluated by graphical and statistical studies of

the residues plotted against the independent variable values (SMM). The analysis of residues gave the following results:

- the mean of the residues was zero (Figure 2);
- the values were evenly distributed above and below a straight line that had slope = 0 and correlation coefficient = 0 (Figure 2);
- the value of residues do not depend on the variables considered (SMM or RC24hUCrE) (Durbin-Watson statistic tests = 1.84);
- the maximum and minimum critical values, in which the residues were permitted, were calculated as the average of 3 standard deviations (SD) of the residue values (SD = 0.31); the outliers were only 3 (Figure 2);
- the residual values were normally distributed (Figure 3, $P = .223$, One-Sample Kolmogorov-Smirnov test).

After exclusion of outliers, the new equation of the best fit line was:

$$\text{RC24hUCrE} = 0.044 \times \text{SMM} + 0.420 \quad (3)$$

As in the SMM-by-RC24hUCrE linear model the Y-intercept is the bias between the method that predicts 24hUCrE (i.e., by SMM) and the method that measures 24hUCrE (i.e., measurement of urinary creatinine by 24-h urine collection), then Y-intercept has only a functional role in the calculation of the Y values but it is biologically meaningless. Furthermore, the correction of the bias between the two methods allows the direct proportionality between the predictor (SMM) and the predicted (24hUCrE) as in the equation of the regression line:

$$\begin{aligned} \text{P24hUCrE} &= (\text{RC24hUCrE} - 0.420) = 0.044 \times \text{SMM} \\ \text{P24hUCrE} &= 0.044 \times \text{SMM} \end{aligned} \quad (4)$$

where the term “P24hUCrE” in the equation (4) represents the predicted value of 24hUCrE directly proportional to SMM, which becomes zero when SMM is equal to zero. In other words, the term on the left side of the equation (4) represents the portion of 24hUCrE arising exclusively from SMM, corrected by the regression errors

(ER24hUCrE) and by the bias of method (Y-intercept = 0.420) and may be indicated as P24hUCrE. The equation (4) represents, therefore, the muscle-creatinine equivalence and allows to predict the daily urinary creatinine excretion deriving exclusively from total skeletal muscle (0.044 g of theoretical P24hUCrE corresponds to 1 kg of SMM or 1 g of theoretical P24hUCrE corresponds to 22.73 kg of SMM).

3.2 | Assessment of the correlation coefficient between the SMM and the measured 24hUCrE upon variation in the measurement error

The percentage deviation (%D) from the measured and predicted value of 24hUCrE was calculated as the difference between the actual value and the one provided by the equivalence muscle-creatinine (calculated by the equation 4) divided by the latter and multiplied by one hundred:

$$\%D = (\text{M24hUCrE} - \text{P24hUCrE}) \times 100 / \text{P24hUCrE} \quad (5)$$

The %D between a single measured and predicted value of 24hUCrE does not give information about the power of the association in the SMM-M24hUCrE linear model. It is useful to calculate the coefficient of correlation (r) according to the %D in a large sample of measurements and then to compare the %D of a single measurement with those calculated in a population of measurements. In the SMM-M24hUCrE linear model, several r were calculated across the whole study population using ranges of calculated absolute errors (as the difference between the measured and predicted value). The clearly incomplete collections ($\text{M24hUCrE} - \text{P24hUCrE} \ll 0$) were excluded from the calculations ($n = 33$) because they distorted the relationship between %D and r . Table 1 shows the selected absolute error ranges, the corresponding r and the resulting accuracy of the measurements as the percentage of the determination coefficient (r^2). A %D lower or equal to 3% from the expected value corresponded to about 100% of the association between the muscle mass and the daily creatinine

FIGURE 2 Residues (ER24hUCrE, g/24 h) plotted against the independent variable values (SMM, kg). The values of regression errors were evenly distributed above and below the central dotted line with slope = 0 and correlation coefficient $r = 0$. The permitted range of the residues was calculated as the average of ± 3 standard deviations (SD) of the residue values (SD = 0.31), included between the dashed lines with dots. Black circles indicate the outliers

FIGURE 3 Frequency of the regression errors. The residual values were normally distributed ($P = .223$, One-Sample Kolmogorov-Smirnov test)

TABLE 1 Mean values of measured (M24hUCrE) and predicted (P24hUCrE) daily urinary creatinine excretion (g/24 h), percentage deviation (%D) between M24hUCrE and P24hUCrE, correlation coefficient (r) between skeletal muscle mass (kg) and M24hUCrE of several participants (cumulative n) and accuracy of the measurement calculated as percent of determination coefficient ($\%r^2$) according to the selected values of the absolute error (g/24 h) between the M24hUCrE and P24hUCrE

Absolute error	M24hUCrE (mean)	P24hUCrE (mean)	%D (mean)	r	$\%r^2$	Cumulative n
<0.05	1.1607	1.1274	3.0	.997	100	15
<0.10	1.1697	1.1168	4.7	.989	98	27
<0.15	1.1693	1.0816	8.1	.963	93	50
<0.20	1.2053	1.0907	10.5	.940	88	75
<0.25	1.2285	1.0908	12.6	.909	83	94
<0.30	1.2779	1.1014	16.0	.867	75	130
<0.35	1.3116	1.1027	18.9	.825	68	167
<0.40	1.3310	1.0920	21.9	.775	60	205
<0.50	1.3774	1.0945	25.8	.707	50	260
<0.60	1.4308	1.0999	30.1	.655	43	316
<0.70	1.4684	1.1000	33.5	.595	35	359
<0.90	1.5107	1.1000	37.3	.518	27	401
<1.10	1.5343	1.1008	39.4	.489	24	417
<1.35	1.5578	1.1016	41.4	.453	21	430

n = number of participants included per range of selected absolute error.

excretion, indicating an extremely accurate creatinine excretion measurement. Increased values of %D corresponded to a reduced association between muscle mass and creatinine excretion and, consequently, to a poor performance in the evaluation of creatinine excretion. Figure 4 depicts the trend of r with the change in %D.

4 | DISCUSSION

Experimental studies on the equivalence between muscle mass and daily urinary creatinine excretion were originally performed to assess the total body muscle mass starting from the measurement of 24hUCrE.¹⁴ A thorough study indicated that an accurate urine collection, repeated at least three times, from subjects in normal clinical conditions and with an adequate meat-free diet, allowed to obtain a very accurate measurement of 24hUCrE from which it was possible to calculate with precision the corresponding total body muscle mass.⁷ In theory, the most specific method for the evaluation of the total body muscle mass should be just the daily urinary creatinine excretion measurement. Indeed, the precursors of creatinine (i.e., creatine and phosphocreatine) are present almost exclusively in the skeletal muscle (approximately 98%) and are steadily degraded and then eliminated as urinary creatinine at a constant rate.³ However, because of the restrictive dietary conditions to be applied to the subject, the long time required to perform various urine collections, and because of other unpredictable variables which may modify the 24hUCrE, it is preferred to use other methods. Among these, BIA seems to be an excellent method both in routine and in population studies because of its good accuracy, noninvasiveness, simplicity, and speed of execution (about 10 minutes), low cost of the measurement, as well as no dietary

FIGURE 4 Trend of the correlation coefficient between SMM (kg) and measured 24hUCrE (M24hUCrE, g/24 h) to change with the percentage deviation (%D) between the measured and predicted (P24hUCrE) values

restriction of the subjects.⁸⁻¹⁰ From these considerations, and as suggested by some studies, it could be possible to use the strength of the association between the body muscle mass and the daily urinary creatinine excretion even the other way,^{4,5} that is, to measure the skeletal muscle mass using a suitable and accurate method to evaluate the reliability of 24hUCrE measurement. To this end, it is required to calculate the actual value of the muscle-creatinine equivalence. Several investigators worked on the estimation of the equivalence between muscle mass and daily excretion of creatinine¹⁴ establishing

a huge range between 17 and 23 kg of muscle mass/1 g urinary creatinine/day (compared to our result 22.73 kg of muscle mass/1 g urinary creatinine/day). This wide range of muscle-creatinine equivalence suggested by previous studies was probably due to the selection of M24hUCrE as the independent variable (X-axis) in the SMM-by-M24hUCrE linear model. This choice results in a large variability of the coefficients in the regression equation as they depend on the accuracy of the 24hUCrE measurement method. Indeed, the ordinary least squares method used in the regression analysis is based on the assumption that the X variable (independent variable) is measured in a much more accurate way than the Y variable (dependent variable), so it can be considered error-free.¹⁵ For example, in the study by Wang et al⁷ the same raw data provide two different equations depending on which variable is selected as the independent one. If the independent variable was M24hUCrE, then the regression equation was $P24hUCrE = 0.052 \times SMM$, whereas if it was SMM, then the regression equation was $P24hUCrE = 0.044 \times SMM$. Although our study (which considered a cohort of middle-aged free-living men) differs greatly from that of Wang et al⁷ (a study specifically designed and based on a controlled and meat-free diet), also for the methods used in the measurement of SMM and M24hUCrE, the slopes of the two equations were the same when M24hUCrE was considered as dependent variable. This result confirms that the linear relationship between SMM and 24hUCrE, namely the ratio of the change in Y variable per unit change in X variable, is represented by the slope of the regression equation and that the Y-intercept (the constant in the regression equation) must be subtracted from the values of Y variable as it is the bias of the measurement method.

4.1 | Estimation of the correlation coefficient between skeletal muscle mass and the measured daily urinary creatinine excretion

The main purpose of this study was to assess the strength of the association between muscle mass and daily urinary creatinine excretion according to the deviation between the M24hUCrE and that provided by the equivalence muscle-creatinine. As the evaluation of the 24hUCrE measurement should refer to its correlation with the SMM, the correlation coefficient of the change with %D was evaluated in a large sample of measurements to obtain a tool that compared the %D of a single measurement with those calculated in a population of measurements. As previously reported^{3,6,7} and according to our data, the amount of creatinine excreted in the urine could be strongly affected by the diet (mainly meat diet or meat-free diet). In fact, our results indicated that the error in the urinary creatinine excretion measurement was unaltered by urine volume or serum creatinine concentration (data not shown). As the study participants were unaffected by overt renal disease or other acute situations such as infection, fever, or trauma, then the most plausible cause of error in the urinary creatinine measurement was an uncontrolled diet. The heterogeneity of the uncontrolled diet of the OHS participants allowed to acquire a wide range of deviations between the measured creatinine excretion and that predicted by

the evaluation of muscle mass. The correlation coefficients between SMM and M24hUCrE in different groups of study participants were calculated relying on gradual increases in the absolute error between the M24hUCrE and P24hUCrE. Figure 4 provides a useful tool for assessing the accuracy of the measurement of 24hUCrE according to the value of %D. In a study that requires the measurement of 24hUCrE, it is possible to establish in advance the desired degree of accuracy. As both the evaluation of muscle mass and the chemical measurement of the urinary creatinine are rapid and inexpensive, it is possible to perform them immediately and then quickly assess whether the measurements satisfy the requirements for the study. This would allow deciding in real time whether to repeat a urine collection or increase the number of study participants. Studies not specifically designed for the measurement of urinary creatinine excretion, as well as studies with a single urine collection and participants on an uncontrolled diet, could provide a small percentage of usable values. For studies on single individuals, where small variations in 24hUCrE could be significant, a value of %D up to 12.6% ($r = .91$, $\%r^2 = 83\%$) is suggested. Perhaps, a wider interval could be acceptable for population studies. However, the required degree of accuracy for the 24hUCrE measurement could depend on the type of study, the number of study participants, and other physiological and clinical factors. In any case, to validate the measurement of 24-h urinary creatinine excretion, it is necessary to measure the subject's muscle mass, to calculate the %D between the measured and predicted 24hUCrE, and to estimate r or $\%r^2$ from Table 1 or Figure 4.

In summary, the results of this study provide the value of the muscle-creatinine equivalence, a simple equation to predict the daily urinary creatinine excretion by measuring SMM and a method to assess the degree of accuracy in the measurement of 24hUCrE.

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