Spot Urine Protein To Creatinine Ratio Corrected With Estimated Urine Creatinine Generalized From Cystatin C Is Invaluable For Estimating Daily Urinary Protein In Patients With Decreased Muscle Mass.

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Research article

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Abstract

Background: Spot urine protein to creatinine ratio (sUP/Cr) is used to evaluate proteinuria; however, conventional equations do not reflect 24-hour (24H) urine creatinine (24HUCr), especially in patients with decreased muscle mass. This study aimed to establish a methodology for estimating 24HUCr in patients with decreased muscle mass and to assess whether multiplying the sUP/Cr with estimated 24HUCr would improve the accuracy of 24HUP estimation.

Methods: Nine patients with decreased muscle mass and 14 patients with normal muscle mass were included. Serum samples were obtained during 24H urine collection. Serum creatinine (sCr) and cystatin C (sCysC) as well as urine creatinine and protein concentrations were measured. The correlation coefficient (R) between the estimated glomerular filtration rate (eGFR) calculated with sCysC (eGFRCysC) and 24H creatinine clearance was obtained. We defined the formula as eGFR = R × [(UCr × 24H urine volume × 1.73)] / [(sCr × 1440 × body surface (m^2)] and then e24HUCr (UCr × 24H urine volume) = [eGFR × sCr × 1440 × body surface (m^2)] / (1.73 × 100 × R).

Results: The new formula, i.e., e24HUCr = eGFRCysC × sCr × 1440 × BS/1.73 × 100 × 0.97, was used to estimate 24HUCr. The sUP/Cr corrected with e24HUCr from the new formula was closer to the actual 24HUP compared to that corrected with e24HUCr derived from existing formulas.

Conclusion: The sUP/Cr corrected with the estimated urine creatinine generalized from sCysC is useful for estimating 24HUP in patients with normal and decreased muscle mass.

Background

The degree of proteinuria reflects the severity and long-term prognosis of patients with kidney disease. Thus, determining the degree of proteinuria is essential in kidney disease treatment. Several methods are used to evaluate proteinuria levels. Measurement of protein levels in samples obtained during 24-hour (24H) urine collection has been used as the standard method of evaluating urine protein (UP) levels. However, urine collection is not easy in young children and patients who cannot urinate independently. Aside from the 24H urine collection, the spot urine protein to creatinine (sUP/Cr) ratio has become popular. However, inter-individual variability in urine creatinine measurement and gravity heavily affects the results [1–3]. In patients with decreased muscle mass, such as those with muscular dystrophy and severe physical and mental impairment, the simple sUP/Cr ratio frequently overestimates the 24H urine protein (24HUP) because of the low urine creatinine concentration, and the value may be even more than double [4]. These patients belong to a high-risk group of patients with renal dysfunctions, and accurate evaluation of proteinuria is very important for their management[5]. Yang et al. reported that multiplying the sUP/Cr ratio with the estimated 24H urine creatinine (e24HUCr) using the adult Cockcroft-Gault equation significantly improved the accuracy of the estimated 24HUP [6]. However, formulas used to calculate e24HUCr, such as the adult Cockcroft-Gault equation, only include patient’s age, height, and sex. Therefore, these formulas are not applicable to patients with decreased muscle mass. Thus, the primary aim of this study was to establish a methodology of estimating 24HUCr in such a patient group, and the secondary aim was to assess whether multiplying the sUP/Cr ratio with the e24HUCr derived from this study would improve the accuracy of 24HUP estimation.

Patients And Methods

Study population

The study subjects were selected from patients who were diagnosed with chronic kidney disease (CKD) by blood and/or urine examination, from June 2016 to June 2018, at the Faculty of Medicine, University of Yamanashi and the National Hospital Organization Kofu Hospital. For diagnosis, CKD was defined as persistent proteinuria or a decrease in the renal function.

In this study, the decreased muscle mass group comprised of patients with severe motor impairment and the inability of walking on their own. Nine patients with decreased muscle mass aged 6–64 years were included in this study. Of these patients, four were younger than 18 years of age. Patients with the following conditions were excluded: dehydration, fluid overload, estimated glomerular filtration rate (eGFR) calculated with serum cystatin C (eGFRCysC) > 180 mL/min/1.73 m^2, 24H creatinine clearance (24HCCr) > 216 mL/min/1.73 m^2, or eGFRCysC to 24HCCr ratio > 1.5 were excluded. For confirmation whether the e24HUCr formula from this study was applicable to normal muscle mass patients, 14 patients with normal muscle mass, aged 4–16 years were also investigated. The normal muscle mass group included patients who had a normal motor function.

Measurement of serum cystatin C and urine creatinine and calculations of eGFR and 24HCCr

Serum samples were obtained during 24H urine collection, and serum creatinine (sCr) and serum cystatin C (sCysC) were measured. The sCr level was determined by enzymatic methods, and sCysC was measured at the SRL Inc., Tokyo, Japan. Given that the use of eGFR with sCr was not suitable for patients with decreased muscle mass, eGFR values in this population were calculated using sCysC-based formulas shown in the upper row of Table 1 [7]. In patients with normal muscle mass, eGFR was calculated using sCr-based formulas shown in the lower part of Table 1 [8, 9].

24H urine samples were collected to obtain total volume, urine creatinine (UCr) concentration, and UP concentration. The spot urine sample was obtained from the first voiding urine after 24H urine collection, and spot UCr (sUCr) and spot UP (sUP) were then measured. In patients who cannot urinate independently, 24H urine specimen was collected using urethral catheterization.

The relationship between eGFRCysC and 24HCCr was plotted by scatter gram, and correlation coefficient (R) was obtained. Because eGFRCysC and 24HCCr have the following relationship, eGFR = R × 24HCCr, we defined this formula as follows: eGFR = R × [(UCr × 24H urine volume × 1.73)] / [(sCr × 1440 × body surface (m^2)]. UCr multiplied by 24H urine volume is equal to 24HUCr. Then, we redefined this formula as follows:

24HUCr (mg) = [eGFR × sCr × 1440 × body surface (m^2)] / (1.73 × 100 × R)

Correlation of 24HUCr obtained from 24H urine collection and e24HUCr obtained from various formulas.

We evaluated 24HUCr obtained from 24H urine collection (actual 24HUCr) and estimated 24HUCr obtained from various formulas (e24HUCr) in both patients with decreased muscle mass and normal muscle mass. The formulas used for e24HUCr are shown below:

Cockcroft-Gault formula: (24HUcr (mg)) = [28-(0.2 × age)] × weight (kg) × (0.85 if female) [10]

Ghazali-Barratt formula: (24HUcr (mg)) = [0.46(age) + 15.4] × weight (kg) [11]

New formula: (24HUCr(mg)) = [eGFR × sCr × 1440 × body surface (m^2)] / (1.73 × 100 × R)

Linear regression analysis was performed to evaluate relations between e24HUCr derived from the new formula and the actual 24HUCr. The Bland-Altman plot was used to determine the agreement between the actual 24HUCr and e24HUCr obtained from several estimation formulas.

Correlation of 24HUP and sUP/Cr ratio modified with e24HUCr.

The sUP/Cr ratio was corrected with e24HUCr derived from the Cockcroft-Gault formula, the Ghazali-Barratt formula, and the new formula developed in this study. We compared the sUP/Cr ratio modified with e24HUCr (modified 24HUP) and 24HUP with 24H urine collection (actual 24HUP). The Bland-Altman plot was used to determine the agreement between the modified 24HUP and the actual 24HUP.

All analysis was conducted using Microsoft Excel 2010 and the JMP8 tactical software (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statically significant.

Results

Patient characteristics

Nine patients with decreased muscular mass and 14 patients with normal muscular mass were included in this study. Patients' clinical characteristics including renal abnormality are shown in Table 2. Of the nine patients with decreased muscle mass, four were younger than 18 years of age.

Correlations of eGFR and 24HCCr

We examined the correlations between eGFRCysC and 24HCCr in patients with decreased muscle mass. Scatter plots are shown in Fig. 1, and the following equation was derived: eGFRCysC = 0.84 × 24HCCr + 16.12 with a significant positive correlation coefficient of 0.95 ($P < 0.01$). The regression equation passing through the origin is eGFRCysC = 0.97 × 24HCCr. From the abovementioned results, the new formula to calculate e24HUCr was defined as follows:

24HUCr (mg/day) = [eGFR × sCr × 1440 × body surface (m^2)] / (1.73 × 100 × 0.97)

Correlations of the measured value of the actual 24HUCr and e24HUCr with eGFR (new formula)

Scatter plots of e24HUCr obtained from the new formula and the actual 24HUCr in patients with decreased muscle mass are shown in Fig. 2. The regression equation was e24HUCr = 0.99 × actual 24HUCr − 19.14 and that through the origin was e24HUCr = 0.92 × actual 24HUCr. A significant positive correlation was observed in patients with decreased muscle mass with a correlation coefficient of 0.87 (Fig. A, $P < 0.01$) Figures 3 and 4 show the Bland-Altman plot of the difference versus the average of both the actual 24HUCr and e24HUCr from each estimation equation. In patients with decreased muscle mass, the average bias of e24HUCr calculated from the new formula was − 20.8 mg/day [95% confidence interval (CI) -113.3 to 71.7 mg/day] (Fig. 3A). On the contrary, the average biases of e24HUCr obtained from the Cockcroft-Gault formula and Ghazail-Barrant formula were − 308.6 mg/day (95% CI -450.1 to -137.1) and − 316.8 mg/day (95% CI -66.1 to -33.5), respectively (Fig. 3B and C). In patients with normal muscle mass, the average bias between e24HUP obtained from the new formula and the actual 24HUCr was − 39.7 mg/day (95% CI -19.9 to 99.5) (Fig. 4A). The average biases of e24HUCr obtained from the Cockcroft-Gault formula and Ghazail-Barrant formula were − 200.4 mg/day (95% CI -257.0 to -143.8) and − 84.3 mg/day (95% CI -138.0 to -30.6), respectively (Fig. 4B and C).

24HUP modified by e24HUCr and actual 24HUCr in decreased muscle mass patients.

The clinical data of sUP/Cr ratio modified by e24HUCr generated from various formulas (modified 24HUP) and actual 24HUP are presented in Table 3. Although all patients with decreased muscle mass showed an abnormal range of sUP/Cr ratio, three had a normal level of actual 24HUP from the 24H urine collection (patients 3, 4, and 9). Patients 6, 7, and 8 showed high sUP/Cr ratio of > 1 g; however, the actual 24HUP was < 0.5 g per day. The Bland-Altman analysis to measure the validity of each coefficient is presented in Fig. 5. In patients with decreased muscle mass, the average biases between the actual 24HUP and modified 24HUP converted by the new formula and the actual 24HUCr were 74.5 mg/day (95% CI -114.1 to 263.1) and 80.5 mg/day (95% CI -119.3 to 280.2), respectively (Fig. 5A and B). Meanwhile, the average biases between the actual 24HUP and modified 24HUP converted by the Cockcroft-Gault formula and the Ghazail-Barrant formula were − 131 mg/day (95% CI -435.2 to 172.5) and − 92.2 mg/day (95% CI -335.4 to 151.0), respectively (Fig. 5C and D).

24HUP modified with e24HUCr and actual 24HUCr in patients with normal muscle mass.

The clinical data of the modified 24HUP and actual 24HUP are presented in Table 4. In most patients with normal muscle mass, the sUP/Cr ratio reflected the actual 24HUP. However, as the sUP/Cr ratio increased, the discrepancy between the sUP/Cr ratio and the actual 24HUP also increased. This tendency was evident in patients 6, 7, 9, and 12. By using the sUP/Cr ratio modified by various e24HUCr values, the sUP/Cr ratio reflected the actual 24HUP more accurately in these patients. The Bland-Altman analysis to measure the validity of each coefficient is presented in Fig. 6. In patients with normal muscle mass, the average bias of the actual 24HUP and modified 24HUP derived from the new formula was − 31.1 mg/day (95% CI -298.6 to -236.0) (Fig. 6A). The average biases of the actual 24HUP and modified 24HUP derived from the Cockcroft-Gault formula, Ghazail-Barrant formula, and the actual 24HUCr were − 438.5 mg/day (95% CI -1021.8 to 144.9), -220.7 mg/day (95% CI -581.6 to 140.2), and − 107.6 mg/day (95% CI -408.0 to 192.0), respectively (Fig. 6B-D).

Discussion

In this study, we investigated whether the new formula developed by us is capable of accurately estimating 24HUCr using sCysC. This formula can be used to estimate 24HUCr not only in patients with decreased muscle mass, but also in those with normal muscle mass. Moreover, we determined whether sUP/Cr ratio correction by using e24HUCr values derived from this new formula would be useful for estimating 24HUP. We observed that the actual and modified 24HUP, determined using the new formula-derived e24HUCr, were more accurate, especially in patients with decreased muscle mass.

Patients with severe physical and intellectual disabilities often have CKD due to urinary tract infections, congenital abnormalities of the urinary tract, hypo-reflex shock, and renal toxic drugs[5]. Furthermore, muscular dystrophy patients, due to their therapeutic medications, are more likely to develop proteinuria. The degree of proteinuria is an important indicator for the severity of renal injury and prognosis of renal function in these patients. However, there are several problems in proteinuria evaluation. Firstly, the simple sUP/Cr ratio that is widely used to evaluate proteinuria frequently overestimates 24HUP because of the low urine creatinine extraction in those patients[4, 12]. Secondly, some of these patients need the invasive 24-hour urethral catheterization for 24-hour urine collection for the assessment of 24HUP. Yang et al. reported that multiplying the sUP/Cr ratio with e24HUCr value derived from the adult Cockcroft-Gault formula significantly improved the accuracy of the estimated 24HUP in children [6]. Their method was also expected to be useful for decreased muscle mass patients. However, the existing 24HUCr-predicting formulas, including the adult Cockcroft-Gault formula, based only on height and weight, then these formulas are not appropriate for patients with non-standard physiques. Therefore, we developed a new formula in this study, which can be applied to patients with normal as well as decreased muscle mass. Compared to the existing formulas that only required weight and age, this new estimation formula requires a blood examination; however, frequent blood sampling is not needed unless there is a sudden change in the kidney function or the nutritional condition. By using this method, the invasive 24-hour urinary catheterization can be avoided, which is of great benefit to these patients.

While this study only targeted physically and mentally handicapped patients, this method is applicable for other patients, such as the elderly patients and patients on cancer treatment, whose nutritional status has been reduced. The 24HUCr estimated by this new method can be applied to the estimation of not only 24HUP but also of daily Na excretion in various patients. Furthermore, since urinary Cr excretion is also an indicator of nutritional status, estimation of 24HUCr using sCysC can also serve as an indicator for the same in patients with various wasting diseases.

This study has certain limitations. It included a relatively small number of patients with heavy proteinuria and severe renal dysfunction. Furthermore, recently, it has been noted that sCysC may fluctuate because of various factors. Thus, further analysis with a larger number of subjects is needed.

Conclusion

The new formula for assessing urinary creatinine excretion using eGFR with sCysC was suitable for estimating 24HUCr in patients with various conditions. Correction of the sUP/Cr ratio using this formula-derived 24UCr is useful for estimating the 24HUP.

List Of Abbreviations

Spot urine protein to creatinine ratio (sUP/Cr), 24-hour (24H) 24-hour (24H) , 24-hour urine creatinine (24HUCr), Serum creatinine (sCr) cystatin C (sCysC), correlation coefficient (R), estimated glomerular filtration rate (eGFR), eGFR calculated with sCysC (eGFRCysC), urine protein (UP), 24H urine protein (24HUP)chronic kidney disease (CKD), urine creatinine (UCr), spot UCr (sUCr), spot UP (sUP) obtained from 24H urine collection (actual 24HUCr), estimated 24HUCr obtained from various formulas (e24HUCr), the sUP/Cr ratio modified with e24HUCr (modified 24HUP), 24HUP with 24H urine collection (actual 24HUP)

Declarations

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Authors' contributions: MG: Conceptualization; Methodology; Data curation; Formal analysis; Investigation; Writing - original draft. HK: Data curation; Formal analysis; Investigation. A K: Data curation; Investigation; E S: Data curation; Investigation. All authors read and approved the final manuscript.

Ethics Approval and Consent to participate

This study was approved by the Institutional Review Board of Yamanashi University (IRB approval number 2139). Need for consent was waived by Yamanashi University IRB committee.

Consent for publication: Not applicable.

Competing interests: Not applicable.

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Tables

Tables 1-4 are available in the Supplemental Files section.

Figures

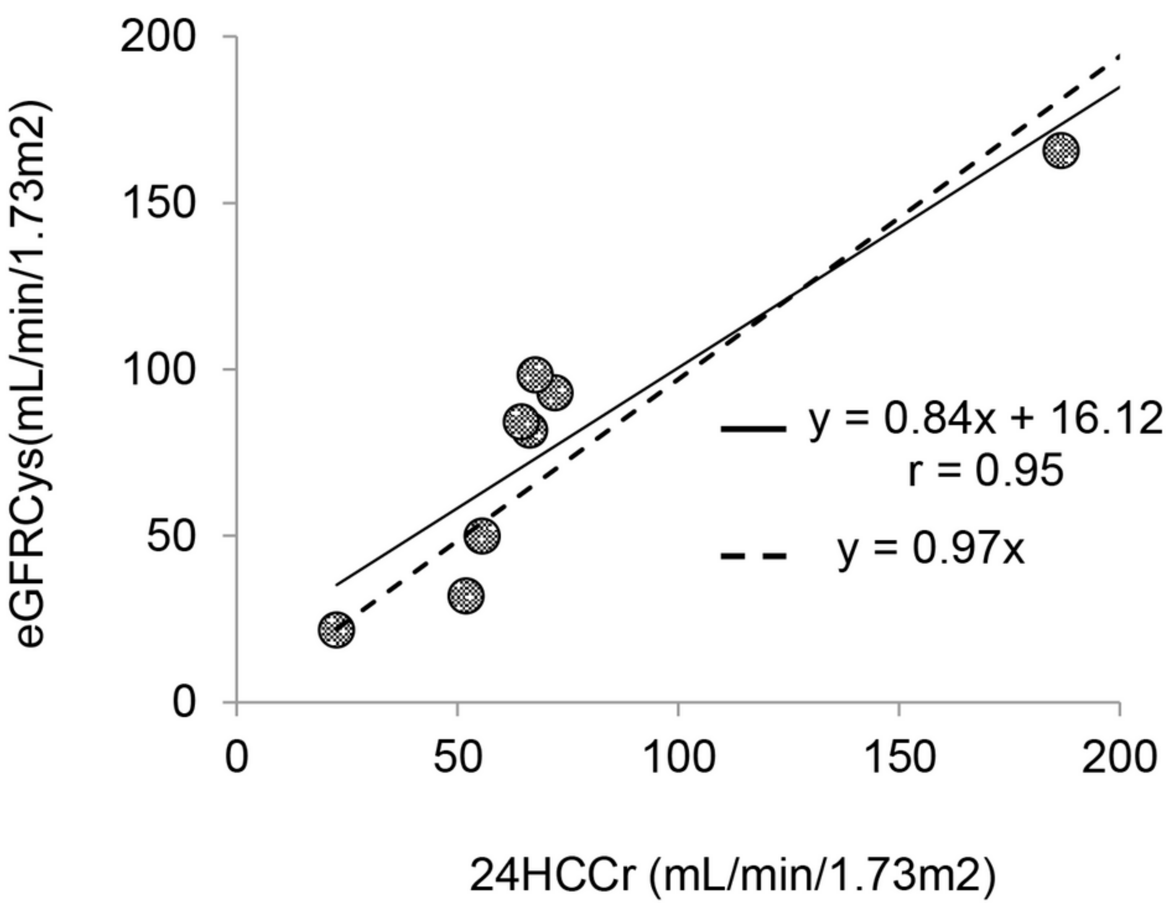

Fig1

Figure 1

Correlation of eGFRCysC and 24HCCr in decreased muscle mass patients

Dotted line is the regression equations passing through the origin

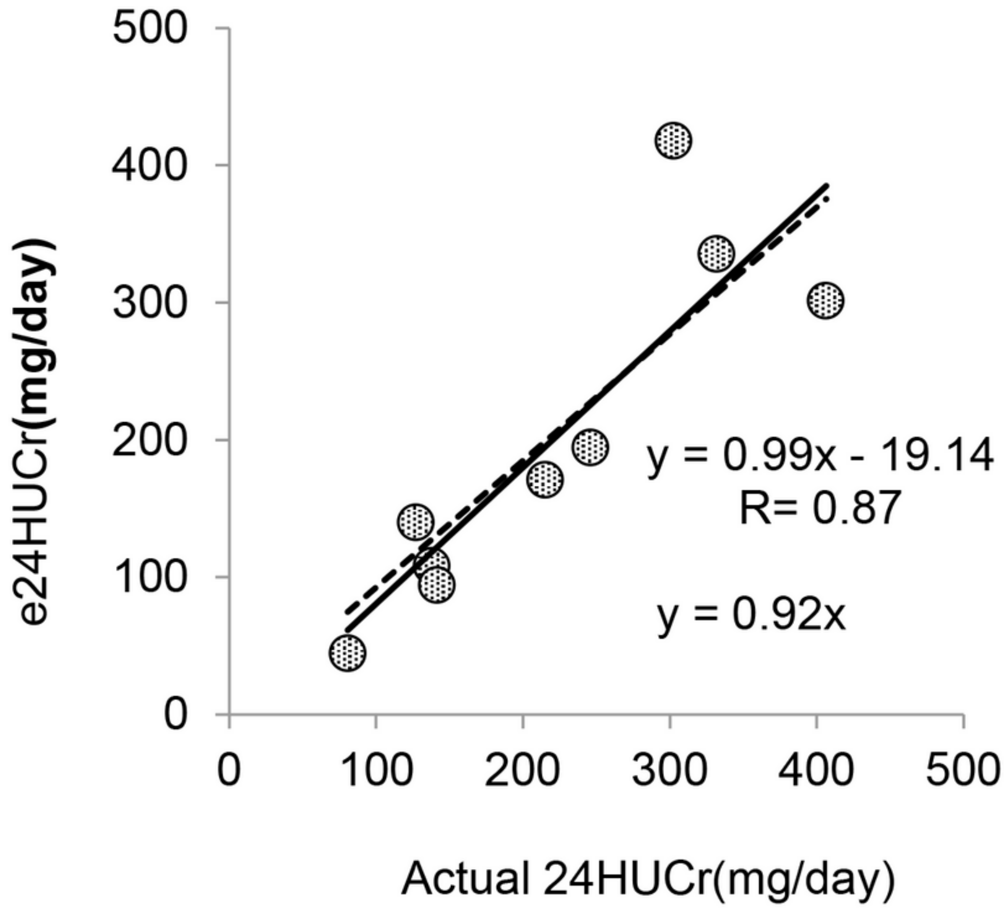

Fig2

Figure 2

Scatter plot between estimated 24HUCr and actual 24HUCr in muscle loss patients

Dotted line is the regression equations passing through the origin.

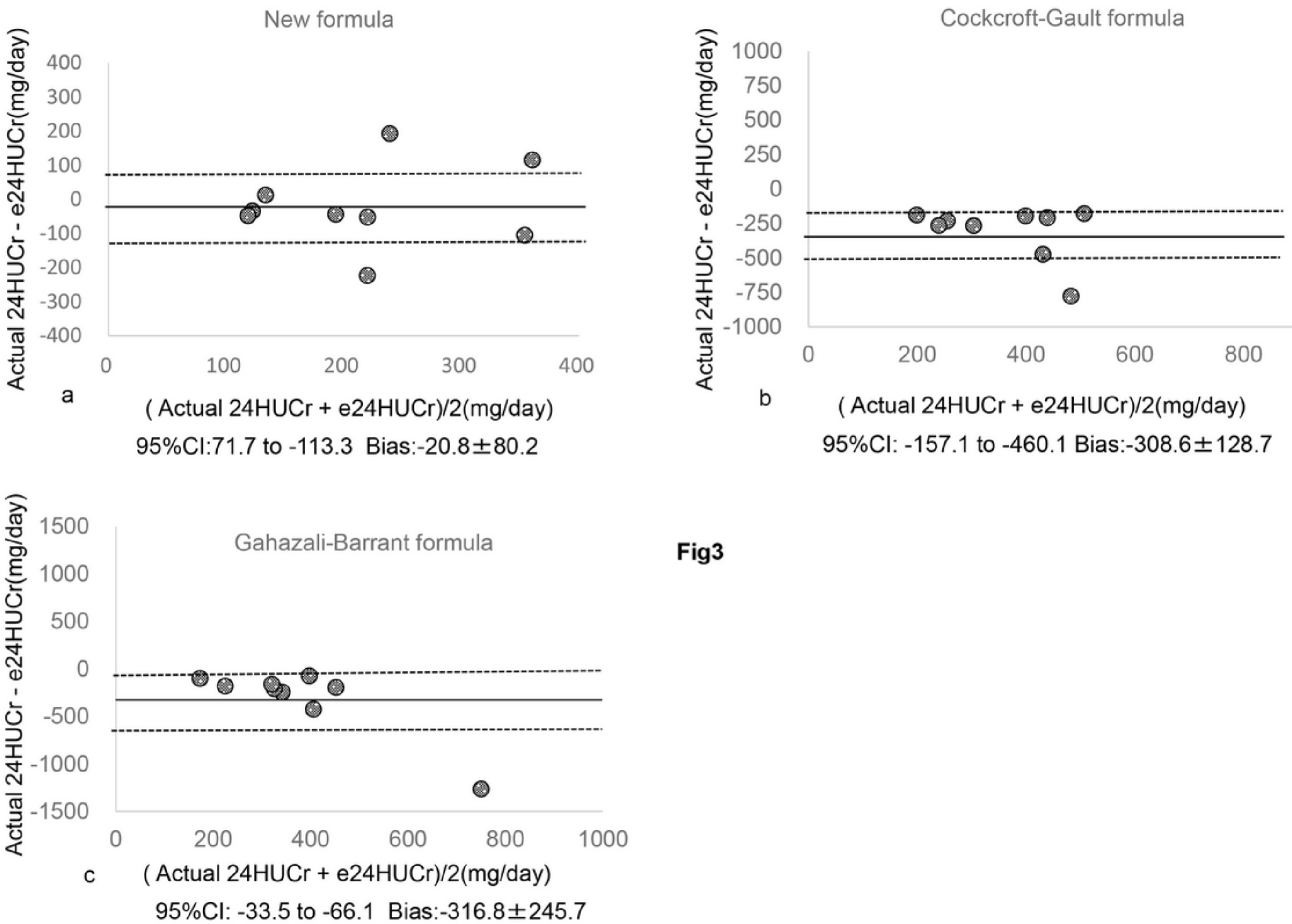

Figure 3

Brand-Altman plot. Difference versus average actual 24HUCr extraction and estimated 24HUCr form each three formulas in Decreased muscle mass subjects.

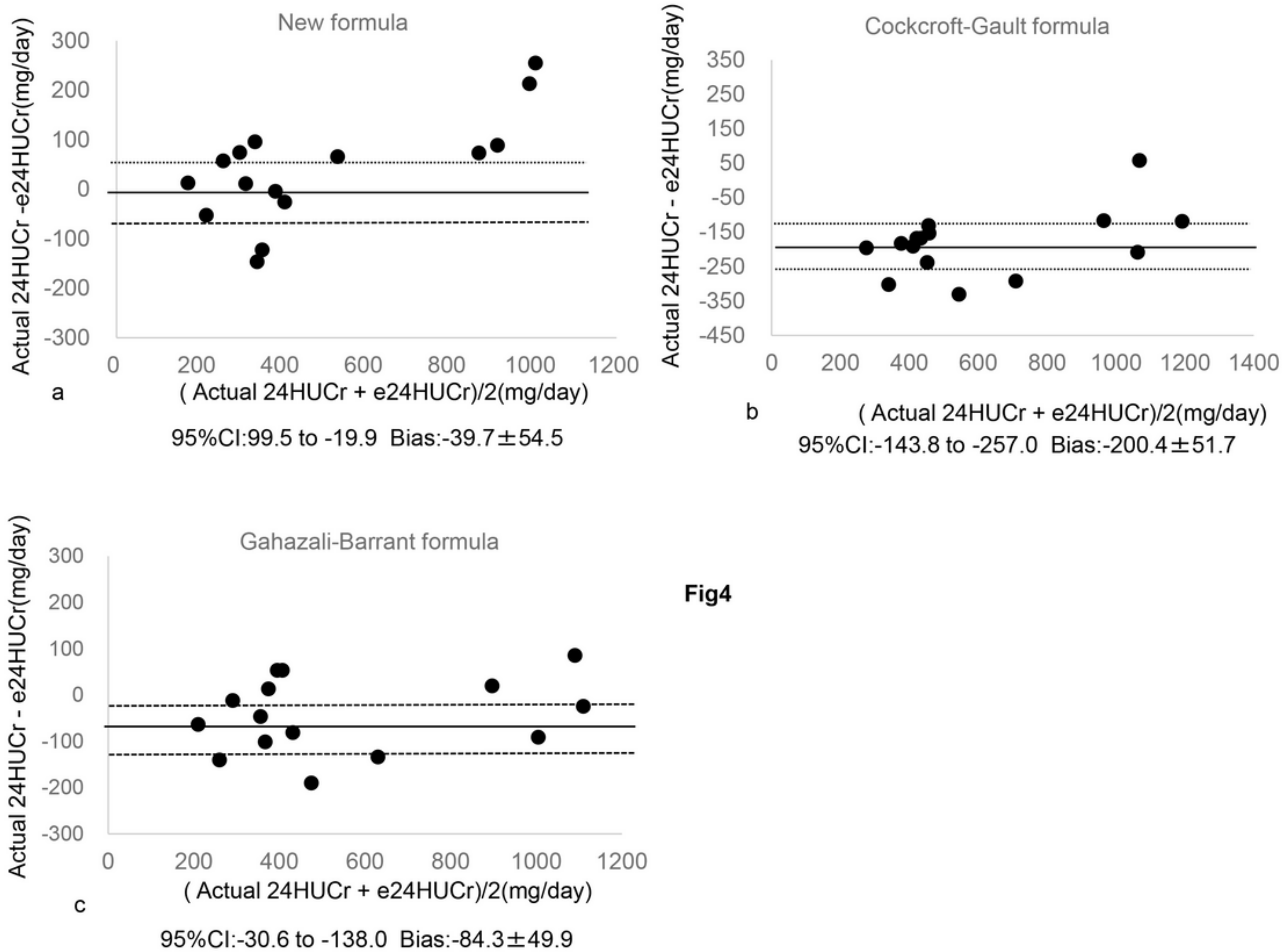

Figure 4

Brand-Altman plot. Difference versus average actual 24HUCr extraction and estimated 24HUCr form each three formulas in Normal muscle mass subjects.

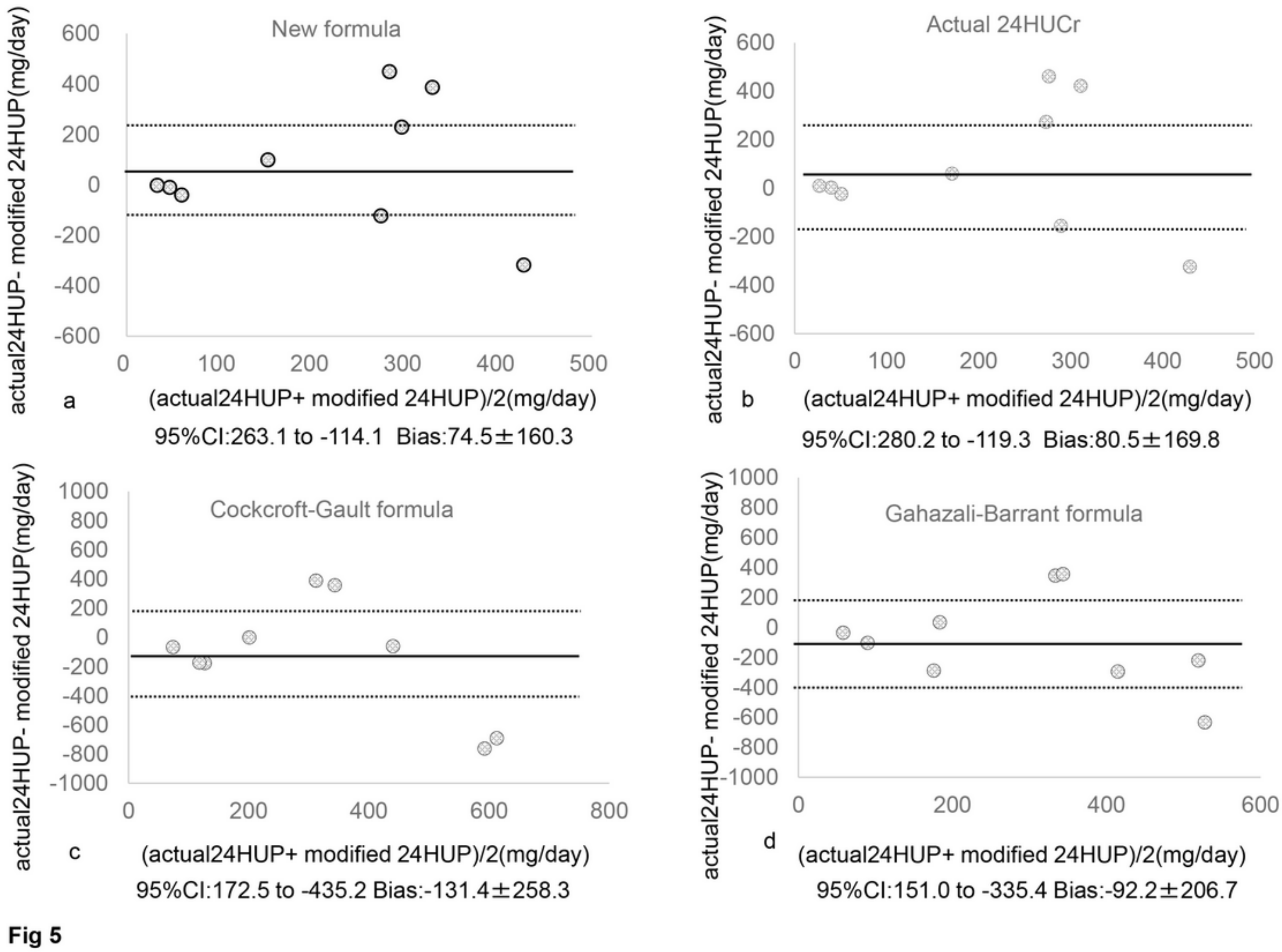

Figure 5

Brand-Altman plot. Difference and versus average 24HUP modified with various 24HUCr vs actual24HUP in patients with decreased muscle mass patients.

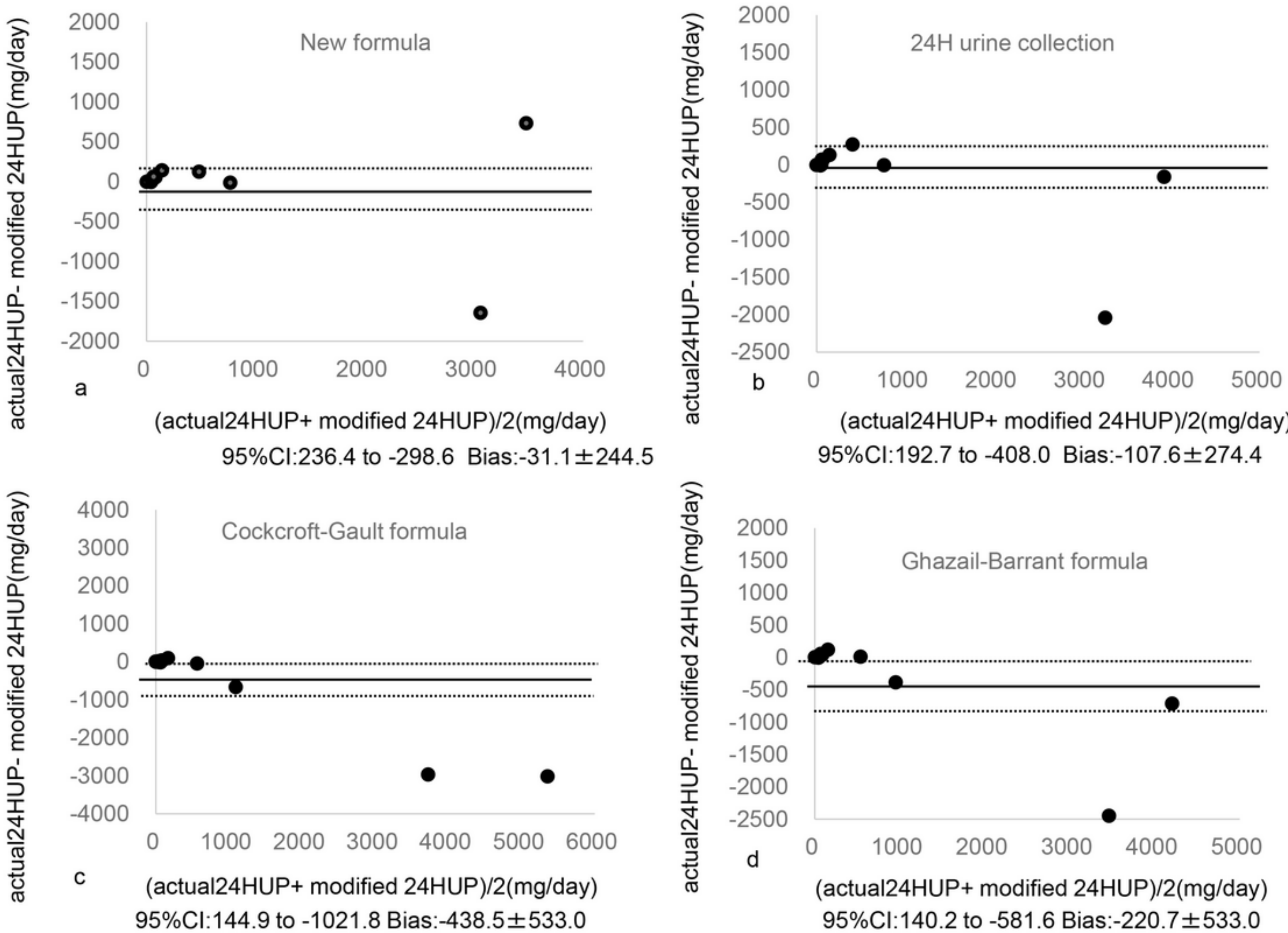

Fig 6

Figure 6

Brand-Altman plot. Difference and versus average 24HUP modified with various 24HUCr vs actual24HUP in patients with normal muscle mass patients.

Supplementary Files

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- TableBMCNephrol.pdf