

GFR Estimation Using Cystatin C Is Not Independent of Body Composition

Jamie Macdonald, BSc, Samuele Marcora, PhD, Mahdi Jibani, FRCP,
Gareth Roberts, MRCP, Mick Kumwenda, FRCP, Ruth Glover, BSc,
Jeffrey Barron, MMED, and Andrew Lemmey, PhD

● **Background:** Cystatin C (CysC) is an endogenous marker of glomerular filtration rate (GFR) that is claimed to be unaffected by body composition. In this study, we tested this speculation. **Methods:** In 77 patients with chronic kidney disease (mean age, 65.1 ± 11.9 [SD] years; mean indexed GFR, 45.7 ± 28.6 mL/min/1.73 m² [0.76 ± 0.48 mL/s]), we evaluated kidney function (GFR) by means of inulin clearance. CysC level was determined by using a particle-enhanced turbidimetric immunoassay. Total lean (LM) and fat masses were measured by means of dual-energy x-ray absorptiometry. Multiple regression was used to analyze relationships between absolute GFR, LM, fat mass, demographic and anthropometric variables (age, sex, height, and weight), and CysC levels. Then prediction equations were built that included only CysC level or CysC level and LM. Their performance to predict absolute GFR was evaluated in a subset of patients with extreme body composition (LM or fat mass $> \pm 1$ SD of the entire sample). **Results:** Only absolute GFR and LM significantly explained variance in CysC levels, and an equation including LM explained more variance in absolute GFR than an equation including CysC level alone. Consequently, the equation including LM performed better than the equation with only CysC level, especially in patients with extreme body composition, showing reduced bias and improved limits of agreement and accuracy (71.4% versus 51.4% of patients' predicted GFR did not deviate by $>30\%$ of GFR). **Conclusion:** LM is a previously unrecognized, but important, factor affecting CysC level, and GFR estimation improves when including LM. CysC level is not independent of body composition, as previously assumed, and hence accounting for body composition improves CysC-based GFR estimation. *Am J Kidney Dis* 48:712-719.

© 2006 by the National Kidney Foundation, Inc.

INDEX WORDS: Chronic kidney disease (CKD); body cell mass; body composition; cystatin C; dual-energy x-ray absorptiometry; glomerular filtration rate (GFR).

Editorial, p. 842

THE IDEAL METHOD to measure glomerular filtration rate (GFR) has yet to be determined. Despite the requirement to assess

From the School of Sport, Health and Exercise Sciences, University of Wales-Bangor; Renal Unit, Ysbyty Gwynedd, Penrhosgarnedd, Bangor; Gwynedd; Renal Unit, Ysbyty Glan Clwyd, Rhyl, Denbighshire; and Department of Chemical Pathology and Metabolism, St Helier Hospital, Carshalton, Surrey, UK.

Received May 30, 2006; accepted in revised form July 10, 2006.

Originally published online as doi:10.1053/j.ajkd.2006.07.001 on September 7, 2006.

Support: This study was supported by a startup grant from Kidney Research UK (RP34/1/2004). Our research group receives a proportion of its funding from local NHS executives. Potential conflicts of interest: None.

Address reprint requests to Jamie Macdonald, BSc, School of Sport, Health and Exercise Sciences, University of Wales-Bangor, George Bldg, Bangor, Gwynedd, LL57 2PZ, UK. E-mail: j.h.macdonald@bangor.ac.uk

© 2006 by the National Kidney Foundation, Inc.

0272-6386/06/4805-0003\$32.00/0

doi:10.1053/j.ajkd.2006.07.001

kidney function for both clinical and research applications, such current gold-standard methods as inulin clearance remain invasive, time consuming, and expensive, making them unfeasible in routine clinical use. As a viable option, current guidelines recommend the use of creatinine (Cr)-based estimation equations to predict GFR in patients with chronic kidney disease (CKD).¹ However, many investigators have questioned the accuracy of these equations, especially in patients with altered body composition.²⁻⁴

Consequently, there is interest in alternative markers of GFR, one of which is cystatin C (CysC). CysC is a low-molecular mass protein that is filtered freely through the glomerular membrane. Unlike Cr, CysC is eliminated almost exclusively from the circulation by the kidney and is affected less by renal tubular secretion, theoretically making it an ideal marker of GFR.⁵ A recent meta-analysis suggested raw CysC level was a better indicator of kidney function than raw Cr level.⁶

Furthermore, serum levels of any endogenous marker of GFR are dependent not only on the marker's removal, but also its production.

Consequently, CysC often is stated to be a superior marker of GFR because of its independence from body composition.^{5,7,8} This is assumed to occur because CysC is produced by all nucleated cells,⁹ in contrast to Cr, which is produced almost exclusively by muscle.¹⁰ In the only study to investigate this hypothesis, Vinge et al¹¹ provided data that seemed to support the assumption that CysC level is independent of body composition. However, we hypothesize that this assumption is incorrect. The reasoning that CysC level is independent of body composition (ie, because CysC is produced by all nucleated cells, or the body cell mass) ignores the fact that “skeletal muscle cell mass is the largest contributor to body cell mass,”¹² and muscle mass (and consequently, body cell mass) varies in relation to demographic and anthropometric variables, diet, disease state, medication use, genetics, and activity level.¹³ Furthermore, although Vinge et al¹¹ showed that CysC level did not correlate with total lean mass (LM) in 42 healthy young people (by using simple Pearson correlation coefficient), they did not study patients with decreased renal function, for whom accounting for different body composition and thus CysC production may be more important.

If CysC level is dependent on body composition, this may help explain why performance of CysC shows only minor¹⁴ or even no¹⁵ improvement over Cr-based GFR estimation equations and why CysC was shown by some investigators to be affected by demographic and anthropometric variables.¹⁵ Therefore the aim of this study is to reevaluate the relationships between GFR, CysC level, demographic and anthropometric variables, and body composition in patients with CKD. Ultimately, we wish to determine whether CysC level is independent of body composition in patients with CKD and whether GFR estimation could be improved by accounting for body composition. This study is designed primarily to test these theoretical hypotheses, rather than generate estimation equations for immediate use in clinical practice.

METHODS

As part of a series of studies investigating the use of muscle mass to estimate GFR, we recruited 77 subjects

without diabetes with CKD. Patients had to be older than 18 years and have CKD classified as Kidney Disease Outcomes Quality Initiative stages 1 to 4. Patients with a GFR greater than 60 mL/min/1.73 m² (>1.00 mL/s/1.73 m²) had to show other evidence of kidney damage: albuminuria (2 spot urine samples with albumin-creatinine ratio > 30 µg albumin/mg Cr confirmed by overnight urine collections, with an excretion rate of 20 to 200 mg albumin [microalbuminuria] or >200 mg albumin [macroalbuminuria]), nonurological hematuria, structural abnormalities, or biopsy-proven chronic glomerulonephritis. Ethical approval was obtained from the North Central Wales Local Research Ethics Committee, and all participants gave written informed consent.

Patients initially attended their renal unit for blood tests and GFR assessment by means of single-shot bolus injection and total-body clearance of inulin, as previously described.¹⁶ The reliability of single-shot inulin clearances obtained in a group of 12 patients with CKD tested on 3 occasions, each separated by 2 weeks, was reported to be 7.1%.¹⁷ Inulin was assayed in plasma by using a double-enzyme method, and clearance was calculated by using a 2-compartment model as previously described¹⁶ (inter-assay coefficient of variation, 4.1%; intra-assay coefficient of variation, 1.2%). GFR was indexed for body surface area and standard body size by using the classic formula of Du Bois and Du Bois¹⁸ and expressed here as indexed GFR (milliliters per minute per 1.73 m²). However, indexed GFR was only used to describe our population sample. For all statistical analyses, GFR was not indexed and is expressed here as absolute GFR (milliliters per minute). This avoided inherent error associated with indexing techniques¹⁹ and reduced colinearity in multiple regression analyses. For example, using indexed GFR would result in the same factor being used to calculate the dependent variable (body surface area is calculated based on weight) and predictor variables (LM and fat mass constitute weight).

CysC level (analyzed on blood drawn immediately before GFR assessment) was determined by using a particle-enhanced turbidimetric immunoassay (DakoCytomation, Glostrup, Denmark) completed on an autoanalyzer (Advia 1650; Bayer, New York, NY). Normal range for this assay in individuals aged 1 to 50 years is 0.55 to 1.24 mg/L, and in individuals older than 50 years, 0.63 to 1.37 mg/L; inter-assay coefficient of variation is 4.9% (n = 10), and intra-assay coefficient of variation is 6.3% (n = 62, at CysC level of 6.2 mg/L).

Within the same week of these assessments, patients attended the School of Sport, Health and Exercise Sciences at the University of Wales, Bangor, UK, for body composition assessment. Because CysC is produced by all nucleated cells,⁹ ideally, a measure of body cell mass would be used to determine relationships between CysC level and body composition. However, no model to determine body cell mass has been validated in patients with CKD, although measures of total LM can be obtained by using dual-energy x-ray absorptiometry.^{20,21} LM is related to body cell mass because LM is made up of body cell mass plus extracellular fluid and nonbone extracellular solids.²² Hence, in the present study, we measured LM, fat mass, and bone mineral content by means of dual-energy x-ray absorptiometry (QDR1500, soft-

ware version 5.72; Hologic, Waltham, MA), as previously described.²³

Statistics

A combination of hierarchical and stepwise multiple regression analyses was used to test whether CysC level and CysC-based GFR estimation are independent of body composition.

1. Factors explaining variance in CysC levels; absolute GFR initially was forced into a regression model (step 1). A stepwise method then was used to select any variables significantly explaining further variance in CysC levels (step 2).
2. Stepwise methods were used to generate 3 estimation equations for absolute GFR. For the first equation, only CysC level was offered for selection (GFR_{CysC}). For the second equation, CysC level and the demographic and anthropometric variables age, sex, height, and weight were offered for selection (GFR_{demo}). For the third equation, demographic and anthropometric variables plus the body composition variables LM and fat mass were offered for selection (GFR_{LM}). Weight was not entered in this final analysis because weight compromises both LM and fat mass.

Performance of these 3 equations to predict absolute GFR was assessed in 2 groups: the entire sample ($n = 77$) and a subset of this sample with extreme body composition, defined as either low or high total LM ($LM \pm 1$ SD of the entire sample) or low or high fat mass (fat mass ± 1 SD of the entire sample; $n = 35$). Performance was assessed by using repeated-measures analysis of variance, simple correlation between actual and estimated GFR (with Meng analysis for statistically comparing correlation coefficients²⁴), standard error of the estimate (SEE), and Bland-Altman plots. Accuracy was assessed as mean percentage of error and percentages of results that did not deviate by more than 30% and 50% of GFR. These analyses of equation performance were completed to provide a clinically interpretable method to determine whether GFR estimation would be improved by accounting for body composition.

For multiple regression analyses, assumptions of normality, linearity, and homoscedasticity were checked visually by plotting standardized residuals versus predicted values and statistically checked by using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Transformations were made when necessary, but for ease of interpretation of the 3 equations' performance, transformed values were inverted to raw scores. Multicollinearity was checked by ensuring that variance inflation factors did not exceed 10 and predictor variables did not have a condition index exceeding 100 or a condition index exceeding 30 when the predictor variable also contributed 50% of the variance on 2 or more regression coefficients. Influential outliers on predictor variables were identified by using leverage statistics and Cook's distances, and on the predicted variable, by standardized residuals greater than 3.0.²⁵

An n of 75 provided the study with greater than 90% statistical power to detect a 2% increase in amount of variance explained in each dependent variable (eg, from $R^2 = 0.85$ to $R^2 = 0.87$ ²⁶; a 2% increase was chosen as the

Table 1. Patient Characteristics

	Men	Women
Sex (absolute/%)	50/64.9	27/35.1
Age (y)	66.4 \pm 12.1	62.7 \pm 11.5
Height (cm)	171.0 \pm 7.1	156.9 \pm 7.0
Weight (kg)	80.8 \pm 14.2	69.6 \pm 10.9
Total LM (kg)	51.8 \pm 8.5	35.7 \pm 4.8
Fat mass (kg)	25.4 \pm 9.1	31.1 \pm 9.3
Cr (mg/dL)	2.22 \pm 0.86	2.27 \pm 1.12
CysC (mg/L)	1.79 \pm 0.57	1.80 \pm 0.56
GFR* (mL/min/1.73 m ²)	49.5 \pm 30.7	38.6 \pm 22.9

NOTE. $N = 77$. Data expressed as mean \pm SD. To convert serum Cr in mg/dL to $\mu\text{mol/L}$, multiply by 88.4; GFR in mL/min to mL/s, multiply by 0.01667.

*Actual GFR determined by using renal clearance of inulin indexed to body surface area¹⁸ and standard body size.

minimum improvement in explanation of variance needed for clinical significance). All analyses were performed on a statistical computer package (SPSS, version 12.0; SSPS Inc, Chicago, IL), and statistical significance is assumed for P less than 0.05. Values are expressed as mean \pm SD.

RESULTS

Patient characteristics are listed in Table 1 and are suggestive that our convenience sample is representative of a normal CKD population. Relationships between CysC level and absolute GFR and LM are shown in Figs 1 and 2. As expected, CysC level showed a curvilinear relationship with GFR. Also as expected, CysC level showed an orthogonal relationship with LM because in this simple scatterplot, GFR had not been adjusted for.

Factors Explaining Variance in Serum CysC Levels

By using multiple regression, a significant model was built to explain the variance in CysC levels ($R^2 = 0.546$; $F_{2,74} = 44.461$; $P < 0.001$; Table 2). Absolute GFR explained 48.6% ($\beta = -0.834$) of the variance in CysC levels (hierarchical method, step 1; Table 2). When absolute GFR had been accounted for, LM was selected in preference to other demographic and anthropometric variables and explained an extra 6.0% ($\beta = 0.280$) of the variance in CysC levels (stepwise method, step 2; Table 2). This extra explained variance attained both statistical significance ($P < 0.005$) and clinical significance (3 times the required minimum increase in vari-

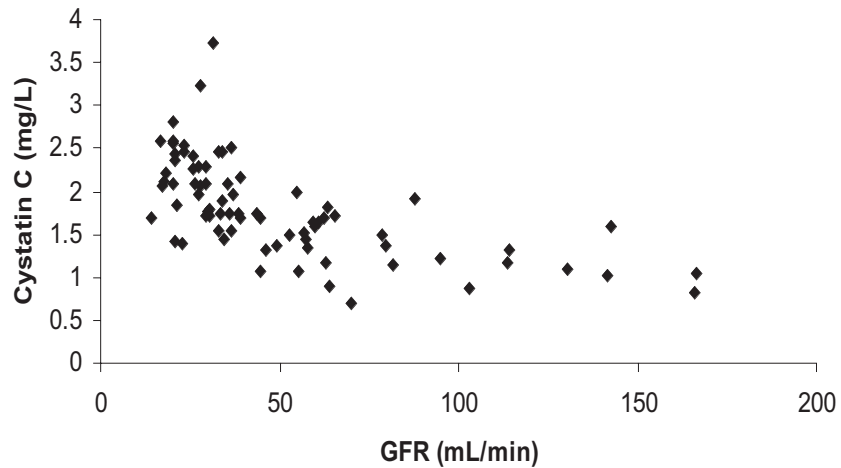


Fig 1. Relationship between CysC level and absolute GFR; actual absolute GFR was determined by means of renal clearance of inulin. To convert GFR in mL/min to mL/s, multiply by 0.01667.

ance of 2.0% set by us to be of clinical importance). Excluded variables were sex ($P = 0.378$), height ($P = 0.388$), fat mass ($P = 0.420$), age ($P = 0.507$), and weight ($P = 0.583$). The relationship between CysC levels and both LM and fat mass is explained further in Table 3. Although fat mass did not contribute to variance in CysC levels at all, LM did when absolute GFR had been accounted for. As expected, this relationship was weakened after adjustment for sex because sex is a strong predictor of body composition.

Prediction Equations for GFR

Multiple regression was used to build the following models to predict GFR^t , where GFR^t is \log_{10} transformed GFR (absolute) by inulin clearance (milliliters per minute); $CysC^t$ is $\text{square root transformed}$ serum

CysC concentration (milligrams per liter); weight^t is $\text{square root transformed}$ weight (kilograms); sex is 0 for male and 1 for female; and LM is total LM (kilograms):

$$GFR_{CysC} = 2.764 + (-0.867 \times CysC^t)$$

($R^2 = 0.486$; $F_{1,75} = 70.984$; $P < 0.001$). $CysC^t$ accounted for 48.6% ($P < 0.001$; $\beta = -0.697$) of the variance in GFR^t .

$$GFR_{demo} = 1.846 + (-0.805 \times CysC^t) + (0.09979 \times \text{weight}^t) + (-0.0993 \times \text{sex})$$

($R^2 = 0.652$; $F_{3,73} = 45.539$; $P < 0.001$). $CysC^t$ accounted for 48.6% ($P < 0.001$; $\beta = -0.648$) of the variance in GFR^t , whereas weight^t and sex accounted for 13.7% ($P < 0.001$; $\beta = 0.302$) and 2.8% ($P < 0.05$; $\beta = -0.183$), respectively.

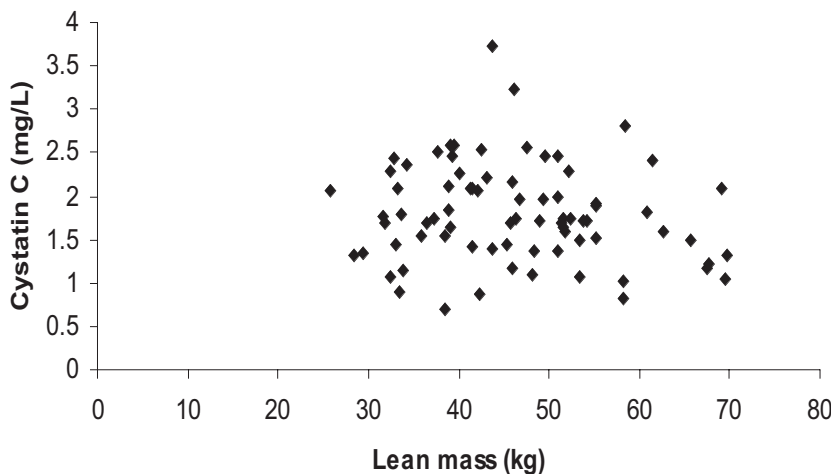


Fig 2. Relationship between CysC level and LM (before adjustment for absolute GFR).

Table 2. Multiple Regression Analysis of Serum CysC Levels

Step	R^2	R^2_{change}	F_{change}	$\text{Sig}_{\text{change}}$	β	Sig P
1. GFR* (mL/min)	0.486	0.486	70.984	0.000	-0.834	0.000
2. Total LM (kg)	0.546	0.060	9.702	0.003	0.280	0.003

NOTE. Step 1, hierarchal method, and step 2, stepwise method. R^2 is accumulative explained variance in GFR; R^2_{change} , F_{change} , and $\text{Sig}_{\text{change}}$ are independent variance explained in GFR by predictor variable with associated statistics; and β and Sig P are standardized coefficient and associated statistic.

*GFR, actual absolute GFR determined by renal clearance of inulin.

Excluded variables were age ($P = 0.189$) and height ($P = 0.618$).

$$\text{GFR}_{\text{LM}} = 2.222 + (-0.802 \times \text{CysC}^t) + (0.009876 \times \text{LM})$$

($R^2 = 0.649$; $F_{2,74} = 68.401$; $P < 0.001$). CysC^t accounted for 48.6% ($P < 0.001$; $\beta = -0.645$) of the variance in GFR^t, whereas LM accounted for 16.3% ($P < 0.001$; $\beta = 0.407$). This extra explained variance by LM attained both statistical significance ($P < 0.001$) and clinical significance (8 times the required minimum increase in variance of 2.0% set by us to be of clinical importance). Excluded variables were fat mass ($P = 0.062$), age ($P = 0.830$), height ($P = 0.892$), and sex ($P = 0.896$).

Performance of the equations when estimating absolute GFR in the entire sample and the extreme body composition subset is listed in Tables 4 and 5. Although analysis of variance showed no significant difference between kidney function measured by using absolute GFR and that estimated by using GFR_{CysC} , GFR_{demo} , and GFR_{LM} ($F_{1,7,132,6} = 2.172$; $P = 0.125$), correlations were statistically stronger between estimated and actual GFR when including both demographic and anthropometric variables or LM compared with CysC level alone. Further-

more, including demographic and anthropometric variables or LM improved estimation performance: SEE was lower, bias was reduced, and both limits of agreement and accuracy were improved. Moreover, performance of the prediction method including a direct measure of body composition (GFR_{LM}) generally was better than using just demographic and anthropometric variables (GFR_{demo}).

DISCUSSION

The principle finding of the present study is that CysC level is not independent of body composition, as previously assumed.^{5,7,8,11} Intuitively, this is not surprising. When previous investigators stated that CysC level is independent of body composition, this statement often was supported by the argument that CysC is produced at a constant rate by all nucleated cells (or the body cell mass). This argument ignores that 60% of body cell mass is skeletal muscle cell mass,¹² which varies in relation to demographic and anthropometric variables, nutritional intake, disease state, medication use, genetics, and activity level.¹³

Our data contradict the only other study to directly investigate the relationship between CysC level and body composition.¹¹ In that study, the relationship between CysC level and LM by means of dual-energy x-ray absorptiometry was investigated by using simple correlation only. Lack of significant correlation led the investigators to conclude that CysC level is independent of body composition. Although we found a similar relationship between CysC level and LM in the present study (Fig 2), the level of any endogenous marker in serum is the balance between its appearance and its removal. Thus, in the present study, we also accounted for removal by entering absolute GFR before CysC level in a multiple regression model. We then found that LM signifi-

Table 3. Relationship Between Serum CysC Levels and Body Composition

Predictor Variable	CysC	CysC With Adjustment for GFR	CysC With Adjustment for GFR and Sex
LM	0.129 (0.264)	0.060 (0.003)	0.016 (0.108)
Fat mass	0.005 (0.550)	0.000 (0.857)	0.090 (0.238)

NOTE. Values are R^2 change (P for significance of R^2 change) from hierarchal regression analyses. GFR is actual absolute GFR determined by renal clearance of inulin (milliliters per minute).

Table 4. Performance of CysC-Based Estimation Equations Compared With Actual GFR for Entire Data Set

	GFR (mL/min)	Correlation (SEE [mL/min])	Bias (mL/min)	LoA (mL/min)	Mean Error (%)	Accuracy (%)	
						Within 30% Error	Within 50% Error
GFR	50.0						
GFR _{CysC}	44.9	0.689 (25.6)	-5.0	50.8	9.5	51.9	77.9
GFR _{demo}	46.6	0.830* (19.7)	-3.44	39.2	6.5	62.3	85.7
GFR _{LM}	46.5	0.851* (18.6)	-3.6	37.9	6.6	66.2	85.7

NOTE. N = 77. To convert GFR in mL/min to mL/s, multiply by 0.01667.

Abbreviations: GFR, actual absolute GFR determined by renal clearance of inulin; correlation, Pearson correlation coefficient between actual and estimated GFR, ie, mean difference between estimated and actual GFR; LoA, limits of agreement, width of the SD of the mean bias \times 1.96; accuracy, percentage of results that did not deviate by more than 30% and 50% of actual GFR.

*Statistical difference of regression slopes versus GFR_{CysC} by Meng¹² analysis.

cantly (both statistically and clinically) explained the variance in CysC levels (Table 2). Furthermore, analysis of standardized β coefficients (β values detail the contribution of each individual predictor variable when all model steps in the multiple regression model have been entered) suggested that LM contributed positively and substantially to variance in CysC levels, and by using a stepwise regression technique, LM was selected in preference to demographic and anthropometric variables, suggesting greater importance for directly measured body composition in explaining the variance in CysC levels. Other potential reasons for our contrasting findings to the previous study¹¹ include the wider variation in body composition in our sample: eg, LM in our men with CKD showed a greater SD (8.5 versus 6.8 kg). Furthermore, in healthy persons, as studied previously,¹¹ variations in CysC production may have less

influence on serum concentration because of high GFR.

Having established that CysC level is dependent on total LM, we wished to determine the relationships of CysC level, body composition, and demographic and anthropometric variables with GFR. Including LM significantly (both statistically and clinically) explained additional variance in absolute GFR than using CysC level alone, and LM was selected by using stepwise regression in preference to demographic and anthropometric variables to estimate absolute GFR. As we previously showed for Cr,²⁷ these data suggest that body composition mediates the relationship between traditional demographic and anthropometric variables and CysC-estimated GFR in patients with CKD. Hence, LM appears to be an important and previously unrecognized factor that, if included in estimation equations, may improve GFR prediction.

Table 5. Performance of CysC-Based Estimation Equations Compared With Actual GFR for Subjects With Extreme Body Composition

	GFR (mL/min)	Correlation (SEE [mL/min])	Bias (mL/min)	LoA (mL/min)	Mean Error (%)	Accuracy (%)	
						Within 30% Error	Within 50% Error
GFR	57.3						
GFR _{CysC}	49.6	0.691 (31.0)	-7.7	62.0	13.0	51.4	80.0
GFR _{demo}	52.8	0.862* (21.7)	-4.5	43.6	8.6	68.6	88.6
GFR _{LM}	52.6	0.891* (19.5)	-4.7	41.7	9.3	71.4	85.7

NOTE. N = 35. Extreme body composition indicates total LM or fat mass $> \pm 1$ SD of the entire sample. To convert GFR in mL/min to mL/s, multiply by 0.01667.

Abbreviations: GFR, actual absolute GFR determined by renal clearance of inulin; correlation, Pearson correlation coefficient between actual and estimated GFR, ie, mean difference between estimated and actual GFR; LoA, limits of agreement, width of the SD of the mean bias \times 1.96; accuracy, percentage of results that did not deviate by more than 30% and 50% of actual GFR.

*Statistical difference of regression slopes versus GFR_{CysC} by Meng¹² analysis.

We tested this speculation by building regression equations using only CysC level, CysC level and demographic and anthropometric variables, or CysC level and LM. It should be noted that these GFR estimation equations were built simply to test our hypothesis and not necessarily for immediate use in clinical practice. Performance of equations using CysC level alone (as advocated in recently published equations)^{14,28,29} improved substantially if LM was included, evidenced by statistically stronger correlations between actual and predicted absolute GFR, lower SEE, reduced bias, reduced error, and improved accuracy. These improvements were evident whether the entire group was analyzed or just patients presenting with extreme body composition (ie, those with high or low LM and/or fat mass) were investigated. Of note, further improvements in GFR estimation may be obtained should more direct markers of body cell mass be developed because LM compromises not only body cell mass, but also extracellular water,²² and because overhydration of the CKD population is likely, total LM may overestimate body cell mass. In this regard, 9 of our 77 patients were overhydrated (assessed by hydration of the combined total LM and bone mineral compartments), although results were no different whether these patients were included in multiple regression analyses or not (data not shown).

Regarding the use of demographic and anthropometric variables with CysC level, data currently are equivocal about whether age, sex, height, and weight should be included to estimate GFR.¹⁵ Although some studies found that equations using CysC level to estimate GFR were not improved by inclusion of demographic and anthropometric variables,^{14,28,29} in the present study, including these variables improved CysC-based equation performance. We speculate that these differences may be caused in part by the body composition of patients studied: although we used a convenience sample to select our patients, more than half our patients were either muscle wasted or obese. Thus, our population may include more patients with altered body composition than previous studies, although it is difficult to evaluate this because detailed body composition data were not provided in these reports. For example, comparison of body mass index of patients in our study ($n = 77$; body mass

index = 27.9 ± 4.4 kg/m²; range, 20.9 to 44.3 kg/m²) with patients studied by Grubb et al¹⁴ (2005; $n = 451$; body mass index = 25.0 kg/m², no SD provided; range, 18 to 39 kg/m²) suggests a greater proportion of patients were overweight in the present study. However, it should be remembered that our sample is still representative of a typical CKD population, many of whom present with extreme body composition.

In conclusion, our results show that CysC level is not independent of body composition, as previously advocated. Consequently, when using CysC level, including demographic and anthropometric variables or, even better, a direct measure of body composition, such as total LM, improves GFR estimation. Even greater improvements in GFR estimation may be realized as more direct markers of body cell mass are developed and validated for use in patients with CKD. An obvious avenue of future research thus is to develop quick and inexpensive methods to estimate body cell mass in clinical practice (perhaps by using bioelectrical impedance spectroscopy) and include these measures in larger studies to generate GFR estimation equations for use in clinical practice. For now, caution is required when using current CysC-based GFR estimation equations in patients with altered body composition.

REFERENCES

1. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: A position statement. *Clin Biochem Rev* 26:81-86, 2005
2. Froissart M, Rossert J, Jacquot C, Paillard M, Houillier P: Predictive performance of the Modification of Diet in Renal Disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrol* 16:763-773, 2005
3. Beddhu S, Samore MH, Roberts MS, Stoddard GJ, Pappas LM, Cheung AK: Creatinine production, nutrition, and glomerular filtration rate estimation. *J Am Soc Nephrol* 14:1000-1005, 2003
4. Cirillo M, Anastasio P, De Santo NG: Relationship of gender, age, and body mass index to errors in predicted kidney function. *Nephrol Dial Transplant* 20:1791-1798, 2005
5. Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A: Cystatin C as a marker of GFR—History, indications, and future research. *Clin Biochem* 38:1-8, 2005
6. Dharnidharka VR, Kwon C, Stevens G: Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. *Am J Kidney Dis* 40:221-226, 2002
7. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J: Cystatin C—A new marker of glomeru-

lar filtration rate in children independent of age and height. *Pediatrics* 101:875-881, 1998

8. Shlipak MG, Sarnak MJ, Katz R, et al: Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med* 352:2049-2060, 2005
9. Abrahamson M, Olafsson I, Palsdottir A, et al: Structure and expression of the human cystatin C gene. *Biochem J* 268:287-294, 1990
10. Levey AS, Perrone RD, Madias NE: Serum creatinine and renal function. *Annu Rev Med* 39:465-490, 1988
11. Vinge E, Lindergard B, Nilsson-Ehle P, Grubb A: Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults. *Scand J Clin Lab Invest* 59:587-592, 1999
12. Wang Z, Zhu S, Wang J, Pierson RN Jr, Heymsfield SB: Whole-body skeletal muscle mass: Development and validation of total-body potassium prediction models. *Am J Clin Nutr* 77:76-82, 2003
13. Forbes G: *Human Body Composition*. New York, NY, Springer-Verlag, 1987
14. Grubb A, Nyman U, Bjork J, et al: Simple cystatin C-based prediction equations for glomerular filtration rate compared with the Modification of Diet in Renal Disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. *Clin Chem* 51:1420-1431, 2005
15. Knight EL, Verhave JC, Spiegelman D, et al: Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 65:1416-1421, 2004
16. Soper CP, Barron JL, Hyer SL: Long-term glycaemic control directly correlates with glomerular filtration rate in early type 1 diabetes mellitus before the onset of microalbuminuria. *Diabet Med* 15:1010-1014, 1998
17. Florijn KW, Barendregt JN, Lentjes EG, et al: Glomerular filtration rate measurement by "single-shot" injection of inulin. *Kidney Int* 46:252-259, 1994
18. Du Bois D, Du Bois EF: A formula to estimate the approximate surface area if height and weight are known. *Arch Intern Med* 17:863-871, 1916
19. Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM: Indexing glomerular filtration rate for body surface area in obese patients is misleading: Concept and example. *Nephrol Dial Transplant* 20:2024-2028, 2005
20. Chumlea WC: Anthropometric and body composition assessment in dialysis patients. *Semin Dial* 17:466-470, 2004
21. Pupim LB, Ikizler TA: Assessment and monitoring of uremic malnutrition. *J Ren Nutr* 14:6-19, 2004
22. Wang Z, St Onge MP, Lecumberri B, et al: Body cell mass: Model development and validation at the cellular level of body composition. *Am J Physiol Endocrinol Metab* 286:E123-E128, 2004
23. Macdonald JH, Marcora SM, Jibani M, Phanish MK, Holly J, Lemmey AB: Intradialytic exercise as anabolic therapy in haemodialysis patients—A pilot study. *Clin Physiol Funct Imaging* 25:113-118, 2005
24. Meng X-L, Rosenthal R, Rubin DB: Comparing correlated correlation coefficients. *Psychol Bull* 111:172-175, 1992
25. Stevens JP: *Applied Multivariate Statistics for the Social Sciences* (ed 4). London, UK, Erlbaum, 2002
26. Roche A, Heymsfield SB, Lohman T: *Human body composition*. Champaign, IL, Human Kinetics, 1996
27. Macdonald JH, Marcora SM, Jibani M, Roberts G, Kumwenda MJ: The relationship between estimated glomerular filtration rate, demographic and anthropometric variables is mediated by muscle mass in non-diabetic patients with chronic kidney disease. *Nephrol Dial Transplant* (in press)
28. Hoek FJ, Kemperman FA, Krediet RT: A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant* 18:2024-2031, 2003
29. Larsson A, Malm J, Grubb A, Hansson LO: Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. *Scand J Clin Lab Invest* 64:25-30, 2004