Poor Glycemic Control Is a Major Factor in the Overestimation of Glomerular Filtration Rate in Diabetic Patients

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OBJECTIVE

Serum creatinine levels are lower in diabetic patients compared with their nondiabetic counterparts. Therefore, estimated glomerular filtration rate (eGFR) is higher in the former than in the latter group. Factors associated with overestimation of renal function in diabetic patients were examined, and new formulae reflecting precise eGFR were created.

RESEARCH DESIGN AND METHODS

Eighty subjects (age 56.5 ± 15.4 years; 35 males [43.8%]; 40 patients with diabetes and 40 nondiabetic subjects) were enrolled. GFR was evaluated by inulin clearance (C_{in}). eGFR values were calculated based on serum creatinine and/or serum cystatin C levels. The factors related to the dissociation between eGFR and C_{in} in diabetic patients and the agreement among each of three eGFR and C_{in} were compared.

RESULTS

Although C_{in} was not significantly different between the diabetic and nondiabetic subjects ($P = 0.2866$), each of three eGFR measures from the diabetic patients was significantly higher than that of the nondiabetic subjects ($P < 0.01$). There were significant and positive correlations between the ratio of each eGFR/C_{in}, hemoglobin A_{1c}, and glycated albumin. The intraclass correlation coefficients in diabetic patients were weaker than those in the nondiabetic subjects, and the intercepts of the regression lines between each eGFR measure and C_{in} in the diabetic patients were significantly higher than those of the nondiabetic subjects. New formulae for the calculation of eGFR corrected by the glycemic control indices were better than the original eGFR, particularly in diabetic patients.

CONCLUSIONS

eGFR overestimates C_{in} as glycemic controls worsen. eGFR corrected by hemoglobin A_{1c} is considered to be clinically useful and feasible.

Diabetes Care 2014;37:596–603 | DOI: 10.2337/dc13-1899

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Received 10 August 2013 and accepted 10 October 2013.

With the continuing increase in the number of patients with end-stage renal disease, diabetic nephropathy, particularly resulting from type 2 diabetes mellitus (DM), has become the single most common cause of end-stage renal disease in Japan (1), as well as in the U.S. and Europe (2,3). Therefore, early diagnosis and strict glycemic control has emerged as a key issue to prevent the development of diabetic renal disease or at least to slow down the disease process (4,5). However, some diabetic patients often need to undergo renal replacement therapies at a relatively higher glomerular filtration rate (GFR) level, even before meeting the criteria for dialysis (6).

In addition to microalbuminuria, evaluation of the GFR by a creatinine-based formula is widely believed to provide a clinically useful index for the assessment of the progression of renal dysfunction and cardiovascular risk in diabetic nephropathy (7,8). Although serum creatinine is widely used to estimate GFR, serum creatinine is affected by muscle mass, sex, and age. Serum creatinine levels are significantly lower in the diabetic patients (9,10). Low serum creatinine levels were associated with type 2 DM in a recent study of nonobese middle-aged Japanese men (11), leading to the hypothesis that low creatinine might reflect low muscle mass volume (12,13). Low serum creatinine may also be related to significantly higher estimated GFR (eGFR) when calculated based on serum creatinine. Low serum creatinine levels in patients with diabetic nephropathy who require dialysis treatment are likely due to their low muscle volume (13). We previously demonstrated a significant decrease of serum creatinine in anuric diabetic hemodialysis patients compared with their nondiabetic counterparts (9), which was due to a reduction in the amount of creatine in muscle mass in the diabetic hemodialysis patients (9). Alternatively, it is increasingly recognized that the fraction of creatinine excreted from the renal tubuli that is not attributable to glomerular creatinine filtration might lead to overestimation of renal function when assessed by serum creatinine, particularly when renal function has deteriorated (10).

It is known that cystatin C is filtered by the glomeruli and metabolized in proximal tubular cells by Megalin in a Ca^{2+}-dependent manner (14). Cystatin C is not affected by muscle mass, sex, or age. Accordingly, serum cystatin C levels and equations that estimate GFR based on serum cystatin C have recently been proposed as better markers of GFR (15–18).

The gold standard in the determining GFR is the measurement of inulin clearance (C_{in}). To date, no data exist regarding the relationship between glycemic control and C_{in} or eGFR in type 2 diabetic patients. Thus, we evaluated the correlation among three eGFR measures (i.e., eGFR based on serum creatinine [$eGFR_{cr}$], serum cystatin C [$eGFR_{cys}$], and both serum creatinine and cystatin C [$eGFR_{cr\text{-}cys}$]) and C_{in} in both diabetic patients and nondiabetic subjects. We developed new formulae for the calculation of eGFR corrected by the glycemic control indices to more precisely assess renal function in diabetic nephropathy, after elucidating the degree to which renal function is overestimated in diabetic patients.

RESEARCH DESIGN AND METHODS

Subjects and Assays

The study protocol was approved by the Ethics Committee of Osaka City University Graduate School of Medicine (#1444), Osaka, Japan, and the study was performed between January 2009 and March 2013. Written informed consent was obtained from each patient. The design was a single-center, randomized study that was conducted at Osaka City University Hospital. Eighty subjects (age 56.6 ± 15.4 years; 35 males and 45 females; 40 diabetic patients and 40 nondiabetic subjects) were enrolled. The diagnosis of DM was based on a history of diabetes or criteria according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (19). The patients enrolled were restricted to those with chronic kidney disease (CKD) stage 1–5 and were not on dialysis. Patients who were taking drugs that might affect tubular creatinine secretion and who had thyroid dysfunction that might affect cystatin C metabolism were excluded from the study.

Glycated albumin (GA) was measured by an enzymatic method using the Lucica GA-L kit (Asahi Kasei Pharma Corp., Tokyo, Japan) (20). GA was calculated as the percentage of GA relative to total albumin, which was measured in the same serum sample using a new bromocresol purple method (20). Serum cystatin C was measured by a colloidal gold immunoassay (Alfresa Pharma) that was traceable to ERM-DA471/IFCC or calibrated to the previously reported colloidal gold immunoassay (15).

Assessment of Renal Function by eGFR Based on Serum Creatinine, Serum Cystatin C, and the Combination of Serum Creatinine–Cystatin C

To develop an accurate eGFR estimation equation for the Japanese population, the Japanese Society of Nephrology launched a specific formula of eGFR based on serum creatinine, with the simultaneous measurement of C_{in} in 763 Japanese subjects, which was obtained by nationwide cooperation of nephrologists from December 2006 to July 2007, as described below (21):

$$eGFR_{cr}\left(mL/min/1.73\,m^2\right) = 194 \times Cr^{-1.094} \times Age^{-0.287}\left(\text{If female} \times 0.739\right)$$

Recently, the Japanese Society of Nephrology developed GFR-estimating equations based on serum cystatin C (CKD-EPI_{cys}, $eGFR_{cys}$) (22) and serum creatinine plus serum cystatin C (CKD-$EPI_{cr\text{-}cys}$, $eGFR_{cr\text{-}cys}$) (15).

$$eGFR_{cys}\left(mL/min/1.73\ m^2\right) = \left\{104 \times CysC^{-1.019} \times 0.996^{age} \times 0.929\ \left(\text{if female}\right)\right\} - 8$$

$$eGFR_{cr\text{-}cys}\left(mL/min/1.73\ m^2\right) = 92 \times CysC^{-0.575} \times Cr^{-0.670} \times 0.995^{age} \times 0.784\ \left(\text{if female}\right)$$

In the current study, cystatin C was measured in 70 patients, and $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$ were calculated.

Measurements of C_{in}

C_{in} was determined by the constant input clearance technique with inulin. According to the method by Horio et al.

(23), continuous intravenous infusion of inulin from a forearm antecubital vein was performed in the morning under a fasting state (i.e., a simple method of determining C_{in} by a single urine collection). In brief, the patients received 500 mL of water orally 15 min before the infusion. A 1% inulin solution (weight/volume) in saline was infused at 300 mL/h for the first 30 min and at 100 mL/min for the following 90 min. Patients completely voided their bladders at 45 min. Blood samples for the measurement of plasma inulin were collected at the same time. To maintain hydration, 180 mL of water was provided orally at the time of voiding the bladder. Blood and urine samples were taken at the end of the clearance period to measure plasma and urine inulin, respectively. A urine collection period of 90 min was set in order to increase the accuracy of the clearance study. C_{in} was calculated by the $U_{in}V/P_{in}$ method, where U_{in} represents the urinary inulin concentration, V the urinary volume, and P_{in} the plasma inulin concentration. P_{in} was the mean value of the plasma inulin concentration at the beginning and end of the clearance period. P_{in} was determined colorimetric ally using the *N*-1 naphthylethylenediamine and the anthrone method with a Corning 258 spectrophotometer (Corning) (24). In 59 patients, consisting of 23 diabetic patients and 36 nondiabetic subjects, creatinine clearance (C_{cr}) was also measured simultaneously during C_{in} measurement.

Statistical Methods

The data are expressed as the mean ± SD. Correlations between two variables were examined using simple regression analysis. Multiple regression analyses were performed to evaluate the relationships between eGFR/C_{in} and clinical parameters. The differences of the intercepts of the regression lines between C_{in} and eGFR were evaluated by ANCOVA. All analyses were performed using StatView 5 (SAS Institute Inc., Cary, NC) on a Windows computer (Microsoft, Redmond, WA). The level of statistical significance was set at $P < 0.05$. The agreement between C_{in} and each eGFR measure with and without correction by hemoglobin A_{1c} was evaluated using intraclass correlation coefficient (ICC).

RESULTS

Clinical Characteristics and Renal Function

The baseline characteristics of the 80 subjects enrolled in the current study are shown in Table 1. The average age of all subjects was 56.6 ± 15.4 years; 35 (43.8%) were male. Mean serum creatinine, serum cystatin C, $eGFR_{cr}$, $eGFR_{cys}$, $eGFR_{cr\text{-}cys}$, and C_{in} were 1.0 ± 0.5 mg/dL, 1.2 ± 0.7 mg/dL, 64.1 ± 23.7 mL/min/1.73 m^2, 68.9 ± 26.8 mL/min/1.73 m^2, 67.7 ± 27.3 mL/min/1.73 m^2, and 65.3 ± 23.8 mL/min/1.73 m^2, respectively. The diabetic patients were significantly older than the nondiabetic subjects. There was no significant difference in C_{in} between the diabetic patients and nondiabetic subjects ($P = 0.2866$). Serum creatinine levels in the diabetic patients were significantly lower than the nondiabetic subjects ($P < 0.0001$). Serum cystatin C in the diabetic patients was significantly lower than the nondiabetic subjects ($P < 0.0001$). $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ in the diabetic patients were all significantly higher than those in the nondiabetic subjects. These data indicated that the assessment of renal

Table 1—Baseline characteristics and eGFR of all subjects and diabetic and nondiabetic subjects

	All subjects	Diabetic	Nondiabetic	*P* value
N	80	40	40	
Age (years)	56.5 ± 15.4	64.8 ± 9.5	48.3 ± 15.8	<0.0001
Sex (male/female)	35/45	16/24	19/21	NS
BMI (kg/m^2)	24.8 ± 4.2	25.4 ± 3.4	24.3 ± 4.7	NS
Mean blood pressure (mmHg)	87.8 ± 10.8	89.2 ± 11.4	86.3 ± 10.0	NS
Systolic pressure (mmHg)	121.8 ± 17.8	126.1 ± 18.5	117.5 ± 16.2	NS
Diastolic pressure (mmHg)	70.8 ± 9.4	70.8 ± 9.6	70.8 ± 9.4	NS
Fasting plasma glucose (g/dL)	108.4 ± 30.4	121.5 ± 33.5	95.7 ± 20.4	<0.0001
Hemoglobin A_{1c} (%)	6.7 ± 1.8	8.1 ± 1.5	5.4 ± 0.5	<0.0001
GA (%)	19.7 ± 5.8 (*n* = 53)	21.9 ± 5.0 (*n* = 39)	13.4 ± 2.0 (*n* = 14)	<0.0001
Serum creatinine (mg/dL)	1.0 ± 0.5	0.7 ± 0.3	1.2 ± 0.6	<0.0001
Serum cystatin C (mg/dL)	1.2 ± 0.673 (*n* = 70)	0.9 ± 0.3 (*n* = 37)	1.5 ± 0.8 (*n* = 33)	0.0005
$eGFR_{cr}$ (mL/min/1.73 m^2)	64.1 ± 23.7	75.0 ± 20.2	53.2 ± 22.0	<0.0001
$eGFR_{cr}$ corrected by hemoglobin A_{1c} (mL/min/1.73 m^2)	64.2 ± 22.6	67.5 ± 18.6	60.8 ± 25.8	0.1841
$eGFR_{cr}$ corrected by GA (mL/min/1.73 m^2)	64.9 ± 21.2 (*n* = 53)	67.0 ± 19.7 (*n* = 39)	59.3 ± 25.3 (*n* = 39)	0.2493
$eGFR_{cys}$ (mL/min/1.73 m^2)	68.9 ± 26.8 (*n* = 70)	80.6 ± 22.4 (*n* = 37)	60.0 ± 29.6 (*n* = 33)	0.0015
$eGFR_{cys}$ corrected by hemoglobin A_{1c} (mL/min/1.73 m^2)	62.1 ± 24.2 (*n* = 70)	66.3 ± 18.3 (*n* = 37)	57.6 ± 29.1 (*n* = 33)	0.1393
$eGFR_{cys}$ corrected by GA (mL/min/1.73 m^2)	64.3 ± 20.7 (*n* = 49)	66.2 ± 18.8 (*n* = 37)	58.4 ± 25.7 (*n* = 12)	0.2571
$eGFR_{cr\text{-}cys}$ (mL/min/1.73 m^2)	67.7 ± 27.3 (*n* = 70)	82.0 ± 22.4 (*n* = 37)	54.2 ± 25.1 (*n* = 33)	<0.0001
$eGFR_{cr\text{-}cys}$ corrected by hemoglobin A_{1c} (mL/min/1.73 m^2)	62.3 ± 23.6 (*n* = 70)	67.7 ± 18.6 (*n* = 37)	56.8 ± 27.6 (*n* = 30)	0.0562
$eGFR_{cr\text{-}cys}$ corrected by GA (mL/min/1.73 m^2)	64.9 ± 21.0 (*n* = 49)	67.6 ± 19.3 (*n* = 37)	56.6 ± 24.4 (*n* = 12)	0.1149
C_{in} (mL/min/1.73 m^2)	65.3 ± 23.8	68.1 ± 20.6	62.4 ± 26.8	0.2866

P value, diabetic patients versus nondiabetic subjects.

function on the basis of serum creatinine and cystatin C might lead to overestimation of renal function in diabetic patients.

Relationship Between Each eGFR Measure ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$) and C_{in} in Diabetic Patients and Nondiabetic Subjects

As shown in Fig. 1, there was a significant and positive correlation between $eGFR_{cr}$ and C_{in} in both diabetic patients (r = 0.683; $P < 0.0001$) and nondiabetic subjects (r = 0.930; $P < 0.0001$). There was a significant and positive correlation between $eGFR_{cys}$ and C_{in} in both diabetic patients (r = 0.584; $P < 0.0001$) and nondiabetic subjects (r = 0.845; $P < 0.0001$). There was also a significant and positive correlation between $eGFR_{cr\text{-}cys}$ and C_{in} in both diabetic patients (r = 0.712; $P < 0.0001$) and nondiabetic subjects (r = 0.930; $P < 0.0001$).

In each eGFR measure of $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, the correlation coefficients for the nondiabetic subjects were greater than those of the diabetic patients, and the intercepts of the regression line for diabetic patients were significantly higher than those of the nondiabetic subjects ($eGFR_{cr}$, $P < 0.0001$; $eGFR_{cys}$, P = 0.0027; and $eGFR_{cre\text{-}cys}$, $P < 0.0001$, respectively). These findings indicated significant overestimation of renal function in diabetic patients on the basis of each of the three eGFRs measurements.

Correlation of the $eGFR_{cr}/C_{in}$, $eGFR_{cys}/C_{in}$, and $eGFR_{cr\text{-}cys}/C_{in}$ With Clinical Parameters

We next evaluated the factors associated with the overestimation of renal function in diabetic patients. Since the dissociation of the regression line between each eGFR measure and C_{in} could be represented by the $eGFR/C_{in}$ ratio, we first examined a simple regression analysis of each $eGFR/C_{in}$ ratio with the various clinical parameters. Although there were no significant correlations between each $eGFR/C_{in}$ ratio and age, sex, BMI, or blood pressure, there were significant and positive correlations between each $eGFR/C_{in}$ ratio and the glycemic control indices of hemoglobin A_{1c} ($eGFR_{cr}/C_{in}$: r = 0.605, $P < 0.0001$; $eGFR_{cys}/C_{in}$: r = 0.340, P = 0.0042; and $eGFR_{cr\text{-}cys}/C_{in}$: r = 0.603, $P < 0.0001$) and GA ($eGFR_{cr}/C_{in}$: r = 0.565, $P < 0.0001$; $eGFR_{cys}/C_{in}$: r = 0.372, P = 0.0092; and $eGFR_{cr\text{-}cys}/C_{in}$: r = 0.548, $P < 0.0001$) (Fig. 2).

Multiple regression analyses were performed to examine whether the glycemic control indices of hemoglobin A_{1c} and GA were associated with $eGFR/C_{in}$ ratio of $eGFR_{cr}/C_{in}$, $eGFR_{cys}/C_{in}$, and $eGFR_{cr\text{-}cys}/C_{in}$ after adjustment for age, sex, BMI, and blood pressure. Glycemic control indices of hemoglobin A_{1c} and GA were significantly and positively associated with each $eGFR/C_{in}$ ratio of $eGFR_{cr}/C_{in}$ (n = 80), $eGFR_{cys}/C_{in}$ (n = 70), and $eGFR_{cr\text{-}cys}/C_{in}$ (n = 70) after the adjustment.

Relationship Between the Secretory Component of C_{cr} and Glycemic Control Indices

We next evaluated the mechanism of overestimation of the renal function as evaluated by $eGFR_{cr}$ in 59 subjects (23 diabetic patients and 36 nondiabetic subjects), in whom C_{cr} was measured concomitant with the C_{in} measurements. To quantify the secretory component of C_{cr} (SF_{cr}), SF_{cr} was calculated as follows (25):

$$SF_{cr} = (C_{cr} - C_{in})/C_{cr}$$

Relationships between the SF_{cr} and glycemic control indices of hemoglobin A_{1c} and GA were examined. There were significant positive correlations between SF_{cr} and hemoglobin A_{1c} (r = 0.359; P = 0.0053) and between SF_{cr} and GA (r = 0.536; P = 0.0009).

The Formulae for eGFR Corrected by the Glycemic Control Index

From the regression line of Fig. 2, the formulae for each eGFR measure corrected by the glycemic control indices of hemoglobin A_{1c} and GA were considered. A linear function, y = ax + b, was applied to elucidate eGFR corrected by each of the glycemic control indices of HbA_{1c} and GA, where y = each $eGFR/C_{in}$, x = HbA_{1c} or GA, a = slope, and b = intercept.

Based on the results of Fig. 2, when using HbA_{1c}, $eGFR/C_{in}$=slope × HbA_{1c} + intercept, then, C_{in} = eGFR/(slope × $HbA1_c$ + intercept); the calculated C_{in} was then considered to be the eGFR corrected by HbA_{1c}.

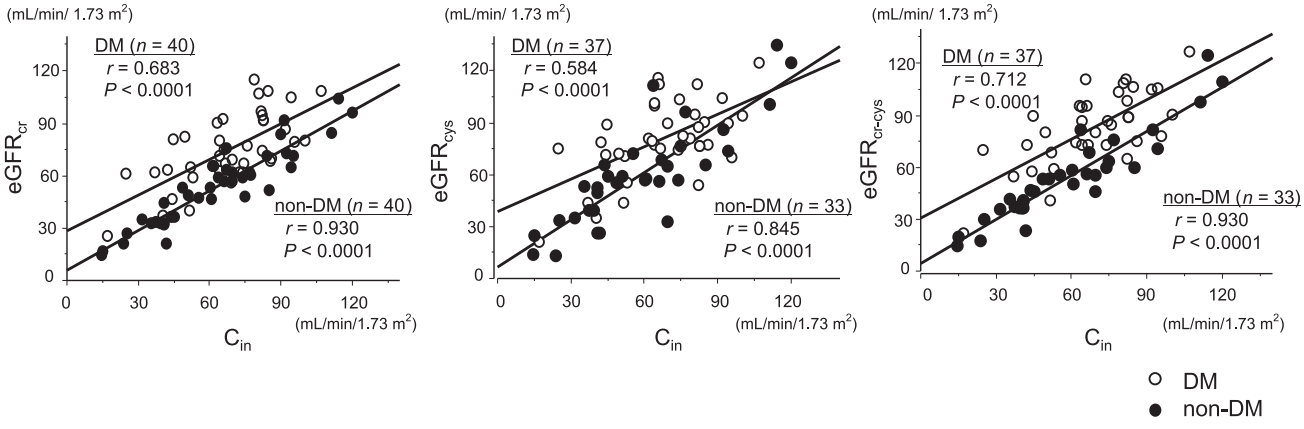

Figure 1—Relationships between C_{in} and $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$. There was a significant and positive correlation between $eGFR_{cr}$ and C_{in} in both diabetic patients (DM) and nondiabetic subjects (non-DM), between $eGFR_{cys}$ and C_{in} in both DM and non-DM, and between $eGFR_{cr\text{-}cys}$ and C_{in} in both DM and non-DM. Intercepts of the regression line between C_{in} and each eGFR measure ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, respectively) were significantly higher in DM than in non-DM ($P < 0.0001$, P = 0.0027, and $P < 0.0001$, respectively).

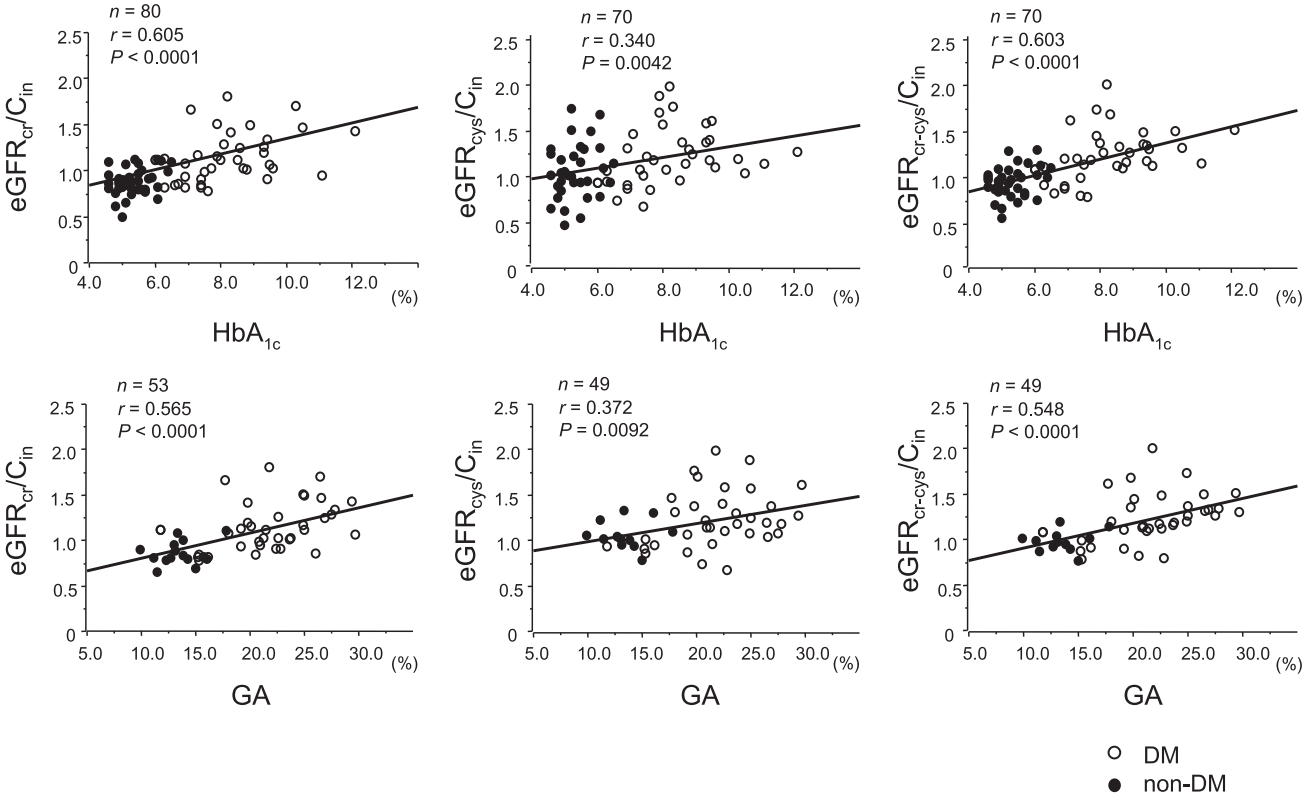

Figure 2—Relationships between HbA_{1c} and the ratio of each $eGFR/C_{in}$ and between GA and the ratio of each $eGFR/C_{in}$. There were significant and positive correlations between the ratio of each $eGFR/C_{in}$ and HbA_{1c} and between the ratio of each $eGFR/C_{in}$ and GA.

Based on the results of Fig. 2; when using GA, $eGFR/C_{in}$ = slope × GA + intercept; then, C_{in} = eGFR/(slope × HbA_{1c} + intercept); the calculated C_{in} was then considered to be the eGFR corrected by GA.

Using the above calculation, the following formulae were considered for each eGFR measure corrected by the glycemic control indices of HbA_{1c} and GA.

1. $eGFR_{cr}$ corrected by HbA_{1c} $= eGFR_{cr}/(0.428 + 0.085 \times HbA_{1c})$
2. $eGFR_{cr}$ corrected by GA $= eGFR_{cr}/(0.525 + 0.028 \times GA)$
3. $eGFR_{cys}$ corrected by HbA_{1c} $= eGFR_{cys}/(0.734 + 0.059 \times HbA_{1c})$
4. $eGFR_{cys}$ corrected by GA $= eGFR_{cys}/(0.785 + 0.020 \times GA)$
5. $eGFR_{cr\text{-}cys}$ corrected by HbA_{1c} $= eGRF_{cr\text{-}cys}/(0.490 + 0.089 \times HbA_{1c})$
6. $eGFR_{cr\text{-}cys}$ corrected by GA $= eGFR_{cr\text{-}cys}/(0.633 + 0.027 \times GA)$

C_{in}, $eGFR_{cr}$, $eGFR_{cys}$, $eGFR_{cr\text{-}cys}$, and Each eGFR Measure Corrected by Hemoglobin A_{1c} and GA in Diabetic Patients and Nondiabetic Subjects

As shown in Table 1, although there were no significant differences in the actual C_{in} between the diabetic patients and nondiabetic subjects, there were significant differences in the original $eGFR_{cr}$ ($P < 0.0001$). However, as expected, $eGFR_{cr}$ corrected by the glycemic control indices of hemoglobin A_{1c} and GA did not differ significantly between the diabetic patients and the nondiabetic subjects ($P = 0.1841$ and $P = 0.2493$, respectively). Furthermore, although the original $eGFR_{cys}$ was significantly different between the diabetic patients and the nondiabetic subjects ($P = 0.0017$), $eGFR_{cys}$ corrected by the glycemic control indices of hemoglobin A_{1c} and GA did not differ between groups ($P = 0.1393$ and $P = 0.2571$, respectively). Finally, although the original $eGFR_{cr\text{-}cys}$ was significantly different between the diabetic patients and the nondiabetic subjects ($P < 0.0001$), $eGFR_{cr\text{-}cys}$ corrected by the glycemic control indices of hemoglobin A_{1c} and GA did not differ between groups ($P = 0.0562$ and $P = 0.1149$, respectively).

Relationship and Agreement Between Each eGFR Measure ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$) and Each eGFR Measure Corrected by the Glycemic Control Indices and C_{in}

Correlations and ICCs between C_{in}, each of the uncorrected eGFR measures ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$) and each eGFR measure corrected by hemoglobin A_{1c} were examined in order to validate the suitability of the correction. As shown in Table 2, the correlation coefficients and ICCs between C_{in} and each eGFR measure corrected by hemoglobin A_{1c} were stronger than those between C_{in} and the original eGFR measures in all subjects. The correlation coefficients and ICCs between C_{in} and each eGFR measure corrected by hemoglobin A_{1c} became stronger than those between C_{in} and the original eGFR measures in the diabetic patients. Similarly, the correlation coefficients and ICCs between C_{in} and

Table 2—Correlation coefficients and ICCs between C_{in} and each eGFR measure with and without correction by hemoglobin A_{1c} (HbA_{1c})

		C_{in}					
		All subjects		Diabetic		Nondiabetic	
		r	ICC (95% CI)	*r*	ICC (95% CI)	*r*	ICC (95% CI)#
$eGFR_{cr}$	Uncorrected	0.781#	0.802 (0.700–0.873)#	0.683#	0.669 (0.442–0.816)#	0.930#	0.847 (0.717–0.921)#
	Corrected by HbA_{1c}	0.872#	0.881 (0.873–0.957)#	0.759#	0.768 (0.593–0.874)#	0.931#	0.938 (0.879–0.969)#
$eGFR_{cys}$	Uncorrected*	0.746#	0.727 (0.594–0.822)#	0.584#	0.492 (0.202–0.703)#	0.845#	0.845 (0.712–0.920)#
	Corrected by HbA_{1c}*	0.777#	0.788 (0.679–0.863)#	0.636#	0.647 (0.411–0.803)#	0.847#	0.849 (0.719–0.992)#
$eGFR_{cr\text{-}cys}$	Uncorrected*	0.799#	0.793 (0.686–0.866)#	0.712#	0.568 (0.301–0.753)#	0.930#	0.916 (0.873–0.957)#
	Corrected by HbA_{1c}*	0.875#	0.881 (0.815–0.924)#	0.777#	0.781 (0.614–0.882)#	0.927#	0.927 (0.859–0.963)#

#$P < 0.0001$. *$n = 70$.

each eGFR measure corrected by GA became stronger than those between C_{in} and the original each eGFR measure in all subjects, particularly in diabetic patients (data not shown).

CONCLUSIONS

In the current study, we demonstrated that the estimation of renal function by $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ led to overestimation of renal function in diabetic patients. Our study also showed that the correlation coefficients and ICCs of each of three eGFR measures and C_{in} in diabetic patients were lower than those of nondiabetic subjects and that the intercepts of the regression lines in diabetic patients were significantly higher than those in nondiabetic subjects, indicating that each of three eGFR measurements in diabetic patients are more inaccurate than in the nondiabetic subjects. Further, the values of each of the three $eGFR/C_{in}$ ratios ($eGFR_{cr}/C_{in}$, $eGFR_{cys}/C_{in}$, and $eGFR_{cr\text{-}cys}/C_{in}$) correlated significantly with the glycemic control indices of hemoglobin A_{1c} and GA, suggesting that the three values of eGFR estimation (i.e., $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$) overestimate renal function as glycemic controls worsened. Thus, the apparent increase in each of three eGFR values relative to C_{in} might be associated with poor glycemic condition in diabetic patients.

In general, C_{cr} overestimates C_{in} (10,25–27). This phenomenon has been reported to be caused by creatinine secretion from the renal tubuli (25,28), in addition to glomerular filtration of creatinine. To overcome the weakness of C_{cr} compared with C_{in}, the Modification of Diet in Renal Disease (MDRD) formula of eGFR was developed in 2006, in consideration of serum creatinine, age, sex, and race (29,30). The eGFR equation based on serum creatinine, age, and sex was also constructed by the Japanese Society of Nephrology by directly measuring C_{in} in 763 Japanese subjects (21). Furthermore, the Chronic Kidney Disease Epidemiology (CKD-EPI) formula was recently developed (15). However, since serum creatinine is affected by muscle mass, sex, and age and since the serum creatinine levels are significantly lower in diabetic patients (9,10), cystatin C has recently been proposed as a better marker of renal function. Cystatin C is produced by the nucleated cells of the body and acts as a circulating cysteine proteinase inhibitor (31). It is known that cystatin C is filtered by the glomeruli and is not affected by muscle mass, sex, and age, as is observed with serum creatinine levels (18). Serum cystatin C levels and an eGFR equation based on serum cystatin C have been recently proposed as more relevant markers of renal function (16–18). Horio et al. (15) recently reported eGFR equations by using serum cystatin C and eGFR by using the combination of serum creatinine and cystatin C. Recently, Inker et al. (32) reported that the equation with the combination of serum creatinine and cystatin C performed better than that with either of these markers alone. Consistent with the findings of Inker et al. (32), in our study, the ICC of $eGFR_{cr\text{-}cys}$ was stronger than that of $eGFR_{cr}$ and $eGFR_{cys}$ in the nondiabetic subjects, when compared with C_{in}.

However, in the current study, $eGFR_{cr}$, as well as $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$, were higher in diabetic patients compared with the nondiabetic subjects. This result suggests that sustained elevations of plasma glucose might lead to increased eGFR in diabetic patients. Accordingly, in the current study, $eGFR/C_{in}$ ratios were examined in relation to glycemic control indices of hemoglobin A_{1c} and GA. We found that there were significant and positive correlations between each of three $eGFR/C_{in}$ ratios and hemoglobin A_{1c} and between each of three $eGFR/C_{in}$ ratios and GA, as shown in Fig. 2. Serum creatinine is excreted into urine via glomerular filtration and tubular secretion (25). It is considered that C_{cr} represents the net effect of glomerular creatinine clearance and tubular creatinine secretion (25). C_{in} is considered to be equivalent to glomerular creatinine clearance, since inulin is not secreted from the renal tubuli. Accordingly, the formula of the secretory component of C_{cr} (SF_{cr}: $[C_{cr} - C_{in}]/C_{cr}$) is considered to be a ratio of tubular creatinine secretion (25). The secretory components of C_{cr} correlated significantly and positively with glycemic control indices of hemoglobin A_{1c} and GA, suggesting that increased secretion of creatinine was related to the hyperglycemic condition. Organic cation transporters, which have been reported to be associated with creatinine secretion in renal tubuli (33), are modulated by high glucose in rats via the increased oxidative stress of advanced glycation end products (34). The results of the current study may reflect these experimental findings. Thus, from the findings of the current

study, we consider that the overestimation of renal function in eGFR based on serum creatinine in diabetic patients may be caused by increased tubular secretion of creatinine.

It should be noted that the apparent overestimation of renal function on the basis of higher eGFR, not only $eGFR_{cr}$ but also $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$, relative to C_{in} might confound the precise assessment of renal function and may thus delay the initiation of treatment when renal function declines in diabetic patients, particularly in those with poorer glycemic control. In this study, we developed new formulae that were corrected by the glycemic control indices. Although $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ in the diabetic patients were significantly higher than those in nondiabetic subjects, the eGFR measures after correction by hemoglobin A_{1c} and GA were not significantly different between the diabetic patients and the nondiabetic subjects, which was consistent with the result of C_{in}, which was not significantly different between diabetic patients and nondiabetic subjects (Table 1). With correction of each eGFR measure by hemoglobin A_{1c}, the correlation coefficients and the ICCs between C_{in} and each eGFR measure corrected by hemoglobin A_{1c} became stronger than those between C_{in} and the uncorrected eGFR measure in all subjects. The correlation coefficients and the ICCs became even stronger in the diabetic patients (Table 2). These results indicate that, in order to accurately evaluate renal function, eGFR should be corrected by the glycemic control indices in diabetic patients, particularly in those with poorer glycemic control. Since serum creatinine and hemoglobin A_{1c} are among the laboratory data that we generally measure in clinical practice, compared with serum cystatin C and GA, we consider that $eGFR_{cr}$ corrected by hemoglobin A_{1c} is the most feasible and useful in the evaluation of renal function, particularly in diabetic patients. From the current study, the formula, $eGFR_{cr}/(0.428 + 0.085 \times HbA_{1c})$, could be used for better and more accurate evaluation of renal function of diabetic patients in order to accurately evaluate renal function.

This study has some limitations. Firstly, the study was performed in a relatively small number of patients, and a further large-scale study with a greater number of patients is needed to confirm the clinical validity of eGFR correction by hemoglobin A_{1c}. Rognant et al. (35) evaluated the three equations for GFR (i.e., the Cockcroft and Gault [CG] equation, MDRD equations, and CKD-EPI collaboration equation) in 246 diabetic patients in six institutions, and they found that the simplified MDRD formula for estimating GFR performed better than the CKD-EPI and CG equations. In the current study, we evaluated renal function in 40 diabetic patients and 40 nondiabetic subjects by C_{in} in a single institution, which could prevent the variation of the values among several institution. We compared three eGFR equations, based on serum creatinine and serum cystatin C ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$), and also explored the factors that affected dissociation of eGFR and C_{in}. We found that glycemic control indices were significantly associated with the dissociations between C_{in} and three eGFR values. Furthermore, we constructed new equations, which used hemoglobin A_{1c} or GA, and showed that these equations using hemoglobin A_{1c} or GA were better than the original eGFR equations, particularly in diabetic patients. Although we created additional formulae for correction by hemoglobin A_{1c} and GA that were only applied to diabetic patients (data not shown), these measures did not differ substantially from those in all patients and subjects. Secondly, although the higher $eGFR_{cr}$ in diabetic patients was suggested to be caused by tubular creatinine secretion, we could not determine the mechanism of overestimation of the renal function in the diabetic patients when it was estimated by $eGFR_{cys}$, since we were not able to measure urine cystatin C. However, it has been reported that urinary cystatin C appears even before the increase in the classical biomarkers of diabetic nephropathy, such as albuminuria and urinary protein (36). It has also been reported that cystatin C is filtered by glomeruli and metabolized in proximal tubular cells by binding to megalin in a Ca^{2+}-dependent manner (14). Ogasawara et al. (37) reported that urinary megalin was increased in correlation with the severity of type 2 diabetic nephropathy, likely leading to reduced metabolism of cystatin C in tubuli. These reports suggest that increases in urinary cystatin C excretion in diabetic patients may be one of the mechanisms involved in the dissociation of $eGFR_{cys}$ and C_{in}. Thirdly, this was a cross-sectional study. Further studies may be needed to explore whether the dissociation of eGFR and C_{in} could be reduced by improving glycemic control. However, this is the first study in which poor glycemic control has been considered to cause the dissociation of eGFR and C_{in} in diabetic patients. Finally, our study, which developed new formulae corrected by hemoglobin A_{1c} or GA, examined Japanese subjects. Further studies are needed to construct formulae that adjust for glycemic control indices, in other races, and further large-scale studies with a greater number of patients are needed to confirm the clinical validity of eGFR correction by hemoglobin A_{1c}.

In conclusion, in CKD patients, each of three eGFR measures ($eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$) overestimated renal function when glycemic control was poor, suggesting that eGFR is needed to be corrected by the glycemic control indices. The new formulae for the correction of eGFR by hemoglobin A_{1c} are better than the original eGFR. We consider that $eGFR_{cr}$ corrected by hemoglobin A_{1c} is clinically feasible and useful.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.T. and E.I. researched data, contributed to the discussion, and wrote and reviewed the manuscript. Y.O., M.Ic., S.N., Y.M., K.M., J.U., S.F., M.E., T.N., and M.In. contributed to the discussion and reviewed the manuscript. A.T. and E.I. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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