Poor glycemic control is a major factor in the overestimation of glomerular filtration rate in diabetic patients

Short running title: Overestimation of eGFR in diabetic patients.

Akihiro Tsuda, MD1, Eiji Ishimura, MD, PhD1, Yoshiteru Ohno, MD1, Mitsuru Ichii, MD, PhD1, Shinya Nakatani, MD, PhD1, Yuuichi Machida, MD, PhD2, MD, PhD1, Katsuhito Mori, MD, PhD1, Junnji Uchida, MD, PhD2, Shinya Fukumoto, MD, PhD1, Masanori Emoto, MD, PhD1, Tatsuya Nakatani, MD, PhD2, and Masaaki Inaba, MD, PhD1

1Department of Nephrology, Department of Metabolism, Endocrinology and Molecular Medicine and 2Department of Urology Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Key words: creatinine, cystatin C, type 2 diabetes, inulin clearance, eGFR corrected by HbA1c

Corresponding author: Eiji Ishimura, M.D., Department of Nephrology, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Tel: +81-6-6645-3806, Fax: +81-6-6645-3808

E-mail address: ish@med.osaka-cu.ac.jp

Word count 3993 words

2 tables and 2 figures
OBJECTIVE - Serum creatinine levels are lower in diabetic patients compared with their non-diabetic 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 diabetics and 40 non-diabetics 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 between each of three eGFR and C_{in} were compared.

RESULTS - Although C_{in} was not significantly different between the diabetic and non-diabetic subjects (p=0.2866), each of three eGFR measures from the diabetic patients was significantly higher than that of the non-diabetic subjects (p<0.01). There were significant and positive correlations between the ratio of each eGFR/C_{in}, hemoglobin A1c and glycated albumin. The intraclass correlation coefficients in diabetic patients were weaker than those in the non-diabetic 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 non-diabetic 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 A1c is considered to be clinically useful and feasible.
With the continuing increase in the number of patients with end-stage renal disease (ESRD), diabetic nephropathy, particularly resulting from type 2 diabetes mellitus (DM), has become the single most common cause of ESRD in Japan (1), as well as in the United States 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 GFR level, even before meeting the criteria for dialysis (6).

In addition to microalbuminuria, evaluation of the glomerular filtration rate (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, gender, and age. Serum creatinine levels are significantly lower in the diabetic patients (9, 10). Low serum creatinine levels were associated with type 2 diabetes mellitus in a recent study of non-obese 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 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 non-diabetic 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, gender 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 (\(C_{\text{in}}\)). To date, no data exists regarding the relationship between glycemic control and \(C_{\text{in}}\) or eGFR in type 2 diabetic patients. Thus, we evaluated the correlation between three eGFR measures, i.e., eGFR based on serum creatinine (eGFR\(_{\text{cr}}\)), serum cystatin C (eGFR\(_{\text{cys}}\)), and both serum creatinine and cystatin C (eGFR\(_{\text{cr-cys}}\)), and \(C_{\text{in}}\) in both diabetic patients and non-diabetic 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 non-diabetic subjects) were enrolled. The diagnosis of diabetes mellitus 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 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 was measured by an enzymatic method using the Lucica GA-L kit (Asahi Kasei Pharma Corp., Tokyo, Japan) (20). Glycated albumin was calculated as the percentage of glycated albumin 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 (21).

**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\text{in} in 763 Japanese subjects, which was obtained by nationwide cooperation of nephrologists from December 2006 to July 2007, as described below (22):
eGFR<sub>cr</sub> (mL/min/1.73 m<sup>2</sup>)=194 × Cr<sup>-1.094</sup> × Age<sup>-0.287</sup> (If female × 0.739)

Recently, the Japanese Society of Nephrology developed glomerular filtration rate (GFR)-estimating equations based on serum cystatin C (CKD-EPI<sub>cys</sub>, eGFR<sub>cys</sub>) (23) and serum creatinine plus serum cystatin C (CKD-EPI<sub>cr-cys</sub>, eGFR<sub>cr-cys</sub>) (15).

eGFR<sub>cys</sub> (mL/min/1.73 m<sup>2</sup>)={104 × CysC<sup>−1.019</sup> × 0.996<sup>age</sup> × 0.929 (if female)}−8

eGFR<sub>cr-cys</sub> (mL/min/1.73 m<sup>2</sup>)=92 × CysC<sup>−0.575</sup> × Cr<sup>−0.670</sup> × 0.995<sup>age</sup> × 0.784 (if female)

In the present study, cystatin C was measured in 70 patients, and eGFR<sub>cys</sub> and eGFR<sub>cr-cys</sub> was calculated.

**Measurements of C<sub>in</sub>**

C<sub>in</sub> was determined by the constant input clearance technique with inulin.

According to the method by Horio et al., 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<sub>in</sub> by a single urine collection (24). In brief, the patients received 500 ml of water orally 15 minutes before the infusion. A 1% inulin solution (w:v in saline was infused at 300 ml/h for the first 30 minutes, and at 100 ml/min for the following 90 minutes. Patients completely voided their bladders at 45 minutes. 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<sub>in</sub> was calculated by the U<sub>in</sub>V/P<sub>in</sub> method where U<sub>in</sub> represents the urinary inulin concentration, V: urinary volume, and P<sub>in</sub>: plasma inulin concentration. P<sub>in</sub> was the mean value of the
plasma inulin concentration at the beginning and at the end of the clearance period. Plasma inulin concentration was determined colorimetrically using the N-1 naphthylethylenediamine and the anthrone method with a Corning 258 spectrophotometer (25). In 59 patients, consisting of 23 diabetic patients and 36 non-diabetic subjects, creatinine clearance (C\textsubscript{cr}) was also measured simultaneously during C\textsubscript{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\textsubscript{in} and clinical parameters. The differences of the intercepts of the regression lines between C\textsubscript{in} and eGFR were evaluated by analysis of covariance (ANCOVA). All analyses were performed using StatView 5 (SAS Institute Inc., Cary, NC, USA) on a Windows computer. The level of statistical significance was set at \( p<0.05 \). The agreement between C\textsubscript{in} and each eGFR measure with and without correction by hemoglobin A1c was evaluated using intraclass correlation coefficient (ICC).

RESULTS

Clinical characteristics and renal function

The baseline characteristics of the 80 subjects enrolled in the present 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\textsubscript{cr}, eGFR\textsubscript{cys}, eGFR\textsubscript{cr-cys} and
C_in were 1.0±0.5 mg/dl, 1.2±0.7 mg/dl, 64.1±23.7 ml/min/1.73m², 68.9±26.8 ml/min/1.73m², 67.7±27.3 ml/min/1.73m² and 65.3±23.8 ml/min/1.73m², respectively. The diabetic patients were significantly older than the non-diabetic subjects. There was no significant difference in C_in between the diabetic patients and the non-diabetic subjects (p=0.2866). Serum creatinine levels in the diabetic patients were significantly lower than the non-diabetic subjects (p<0.0001). Serum cystatin C in the diabetic patients was significantly lower than the non-diabetic subjects (p<0.0001). eGFR_cr, eGFR_cys, eGFR_cr-cys in the diabetic patients were all significantly higher than those in the non-diabetic subjects. These data indicated that the assessment of renal 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-cys) and C_in in diabetic patients and non-diabetic subjects**

As shown in Figure1, there was a significant and positive correlation between eGFR_cr and C_in in both diabetic patients (r=0.683, p<0.0001) and non-diabetic 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 in non-diabetic subjects (r=0.845, p<0.0001). There was also a significant and positive correlation between eGFR_cr-cys and C_in in both diabetic patients (r=0.712, p<0.0001) and in non-diabetic subjects (r=0.930, p<0.0001).

In each eGFR measure of eGFR_cr, eGFR_cys, and eGFR_cr-cys, the correlation coefficients for the non-diabetic 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 non-diabetic subjects (eGFR_{cr} ; p<0.0001, eGFR_{cys} ; p=0.0027, and eGFR_{cre-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-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, gender, body mass index or blood pressure, there were significant and positive correlations between each eGFR/C_{in} ratio and the glycemic control indices of hemoglobin A1c (eGFR_{cr}/C_{in} : r=0.605, p<0.0001, eGFR_{cys}/C_{in} : r=0.340, p=0.0042, eGFR_{cre-cys}/C_{in} : r=0.603, p<0.0001), glycated albumin (eGFR_{cr}/C_{in} : r=0.565, p<0.0001, eGFR_{cys}/C_{in} : r=0.372, p=0.0092, eGFR_{cre-cys}/C_{in} : r=0.548, p <0.0001) (Figure 2).

Multiple regression analyses were performed to examine whether the glycemic control indices of hemoglobin A1c and glycated albumin were associated with eGFR/C_{in} ratio of eGFR_{cr}/C_{in}, eGFR_{cys}/C_{in} and eGFR_{cre-cys}/C_{in} after adjustment for age, gender, body mass index and blood pressure. Glycemic control indices of hemoglobin A1c and glycated albumin 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_{cre-cys}/C_{in} (n=70) after the adjustment.
Relationship between the secretory component of C\textsubscript{cr} (SF\textsubscript{cr}) and glycemic control indices

We next evaluated the mechanism of overestimation of the renal function as evaluated by eGFR\textsubscript{cr} in 59 subjects (23 diabetic patients and 36 non-diabetic subjects), in whom C\textsubscript{cr} was measured concomitant with the C\textsubscript{in} measurements. To quantify the secretory component of C\textsubscript{cr} (SF\textsubscript{cr}), SF\textsubscript{cr} was calculated as follows (26):

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

Relationships between the SF\textsubscript{cr} and glycemic control indices of hemoglobin A1c and glycated albumin were examined. There were significant positive correlations between SF\textsubscript{cr} and hemoglobin A1c ($r=0.359$, $p=0.0053$), and between SF\textsubscript{cr} and glycated albumin ($r=0.536$, $p=0.0009$).

The formulae for eGFR corrected by the glycemic control index

From the regression line of Figure 2, the formulae for each eGFR measure corrected by the glycemic control indices of hemoglobin A1c (HbA1c) and glycated albumin (GA) were considered. A linear function, $y=ax + c$, was applied to elucidate eGFR corrected by each glycemic control indices of HbA1c and GA, where $y=$each eGFR/C\textsubscript{in}, $x=$HbA1c or GA, $a=$slope, and $b=$intercept.

Based upon the results of Figure 2, when using HbA1c, $eGFR/C_{in}=slope \times Hba1c + intercept$; then, $C_{in}=eGFR / (slope \times Hba1c + intercept)$; the calculated $C_{in}$ was then considered to be the eGFR corrected by HbA1c.

Based upon the results of Figure 2; when using GA, $eGFR/C_{in}=slope \times GA + intercept$; then, $C_{in}=eGFR / (slope \times Hba1c + intercept)$; 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 HbA1c and GA.

1) \( \text{eGFR}_{\text{cr}} \) corrected by HbA1c = \( \frac{\text{eGFR}_{\text{cr}}}{(0.428 + 0.085 \times \text{HbA1c})} \)

2) \( \text{eGFR}_{\text{cr}} \) corrected by GA = \( \frac{\text{eGFR}_{\text{cr}}}{(0.525 + 0.028 \times \text{GA})} \)

3) \( \text{eGFR}_{\text{cys}} \) corrected by HbA1c = \( \frac{\text{eGFR}_{\text{cys}}}{(0.734 + 0.059 \times \text{HbA1c})} \)

4) \( \text{eGFR}_{\text{cys}} \) corrected by GA = \( \frac{\text{eGFR}_{\text{cys}}}{(0.785 + 0.020 \times \text{GA})} \)

5) \( \text{eGFR}_{\text{cr-cys}} \) corrected by HbA1c = \( \frac{\text{eGFR}_{\text{cr-cys}}}{(0.490 + 0.089 \times \text{HbA1c})} \)

6) \( \text{eGFR}_{\text{cr-cys}} \) corrected by GA = \( \frac{\text{eGFR}_{\text{cr-cys}}}{(0.633 + 0.027 \times \text{GA})} \)

\( C_{\text{in}}, \text{eGFR}_{\text{cr}}, \text{eGFR}_{\text{cys}}, \text{eGFR}_{\text{cr-cys}}, \) and each eGFR measure corrected by hemoglobin A1c and glycated albumin in diabetic patients and non-diabetic subjects.

As shown in Table 1, although there were no significant differences in the actual \( C_{\text{in}} \) between the diabetic patients and non-diabetic subjects, there were significant differences in the original eGFR\(_{\text{cr}}\) (\( p<0.0001 \)). However, as expected, eGFR\(_{\text{cr}}\) corrected by the glycemic control indices of hemoglobin A1c and glycated albumin did not differ significantly between the diabetic patients and the non-diabetic subjects (\( p=0.1841 \) and \( p=0.2493 \), respectively). Furthermore, although the original eGFR\(_{\text{cys}}\) was significantly different between the diabetic patients and the non-diabetic subjects (\( p=0.0017 \)), eGFR\(_{\text{cys}}\) corrected by the glycemic control indices of hemoglobin A1c and glycated albumin did not differ between groups (\( p=0.1393 \) and \( p=0.2571 \), respectively). Finally, although the original eGFR\(_{\text{cr-cys}}\) was significantly different between the diabetic patients and the non-diabetic subjects (\( p<0.0001 \)), eGFR\(_{\text{cr-cys}}\) corrected by the glycemic control indices of hemoglobin A1c and glycated albumin 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-cys}) and each eGFR measure corrected by the glycemic control indices and C_{in}

Correlations and intraclass correlation coefficients (ICC) between C_{in}, each of the uncorrected eGFR measures (eGFR_{cr}, eGFR_{cys}, and eGFR_{cr-cys}), and each eGFR measure corrected by hemoglobin A1c were examined, in order to validate the suitability of the correction. As shown in Table 2, the correlation coefficients and ICC between C_{in} and each eGFR measure corrected by hemoglobin A1c were stronger than those between C_{in} and the original eGFR measures in all subjects. The correlation coefficients and ICC between C_{in} and each eGFR measure corrected by hemoglobin A1c 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 each eGFR measure corrected by glycated albumin 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 present study, we demonstrated that the estimation of renal function by eGFR using serum creatinine alone (eGFR_{cr}), serum cystatin C alone (eGFR_{cys}), and combination of serum creatinine and cystatin C (eGFR_{cr-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 non-diabetic subjects, and that the intercepts of the regression lines
in diabetic patients were significantly higher than those in non-diabetic subjects, indicating that each of three eGFR measurements in diabetic patients are more inaccurate than in the non-diabetic subjects. Further, the values of each of the three eGFR/C_{in} ratios (eGFR_{cr}/C_{in}, eGFR_{cys}/C_{in}, and eGFR_{cr-cys}/C_{in}) correlated significantly with the glycemic control indices of hemoglobin A1c and glycated albumin, suggesting that the three values of eGFR estimation (i.e., eGFR_{cr}, eGFR_{cys}, and eGFR_{cr-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, 26-28). This phenomenon has been reported to be caused by creatinine secretion from the renal tubuli (26, 29), in addition to glomerular filtration of creatinine. To overcome the weakness of C_{cr} compared with C_{in}, the MDRD formula of eGFR was developed in 2006, in consideration of serum creatinine, age, gender, and race (30, 31). The eGFR equation based on serum creatinine, age, and gender was also constructed by the Japanese Society of Nephrology, by directly measuring C_{in} in 763 Japanese subjects (22). Furthermore, the CKD-EPI formula was recently developed (15). However, since serum creatinine is affected by muscle mass, gender, 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 (32). It is known that cystatin C is filtered by the glomeruli and is not affected by muscle mass, gender, 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. recently reported eGFR equations by using serum cystatin C, and eGFR by using the combination of serum creatinine and cystatin C (15). Recently, Lesley et al. reported that the equation with the combination of serum creatinine and cystatin C performed better than that with either of these markers alone (33). Consistent with the findings of Lesley et al. (33), in our study, the ICC of eGFR\textsubscript{cr-cys} was stronger than that of eGFR\textsubscript{cr} and eGFR\textsubscript{cys} in the non-diabetic subjects, when compared with C\textsubscript{in}.

However, in the present study, eGFR\textsubscript{cr}, as well as eGFR\textsubscript{cys} and eGFR\textsubscript{cr-cys}, were higher in diabetic patients compared with the non-diabetic subjects. This result suggests that sustained elevations of plasma glucose might lead to increased eGFR in diabetic patients. Accordingly, in the present study, eGFR/C\textsubscript{in} ratios were examined in relation to glycemic control indices of hemoglobin A1c and glycated albumin. We found that there were significant and positive correlations between each of three eGFR/C\textsubscript{in} ratios and hemoglobin A1c, and between each of three eGFR/C\textsubscript{in} ratios and glycated albumin, as shown in Figure 2. Serum creatinine is excreted into urine via glomerular filtration and tubular secretion (26). It is considered that C\textsubscript{cr} represents the net effect of glomerular creatinine clearance and tubular creatinine secretion (26). C\textsubscript{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\textsubscript{cr} \((\text{SF}_{\text{cr}}; \frac{(C\text{cr} - C\text{in})}{C\text{cr}})\) is considered to be a ratio of tubular creatinine secretion (26). The secretory components of C\textsubscript{cr} correlated significantly and positively with glycemic control indices of hemoglobin A1c and glycated albumin, 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 (34), are
modulated by high glucose in rats, via the increased oxidative stress of advanced glycation end-products (35). The results of the present study may reflect these experimental findings. Thus, from the findings of the present 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-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-cys} in the diabetic patients were significantly higher than those in non-diabetic subjects, the eGFR measures after correction by hemoglobin A1c and glycated albumin were not significantly different between the diabetic patients and the non-diabetic subjects, which was consistent with the result of C_{in}, which was not significantly different between diabetic patients and non-diabetic subjects (Table 1). With correction of each eGFR measure by hemoglobin A1c, the correlation coefficients and the ICCs between C_{in} and each eGFR measure corrected by hemoglobin A1c 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 A1c are among the laboratory data that we generally measure in clinical practice, compared with serum
cystatin C and glycated albumin, we consider that eGFR_{cr} corrected by hemoglobin A1c is the most feasible and useful in the evaluation of renal function, particularly in diabetic patients. From the present study, the formula, eGFR_{cr} / (0.428 + 0.085 \times HbA1c), 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 A1c. Rognant et al. evaluated the three equations for glomerular filtration rate: i.e., the Cockcroft and Gault (CG) equation, Modification of Diet in Renal Disease (MDRD) equations, and Chronic Kidney Disease Epidemiology (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 (36). In the present study, we evaluated renal function in 40 diabetic patients and 40 non-diabetic subjects by inulin clearance (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-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 utilized hemoglobin A1c or glycated albumin, and showed that these equations utilizing hemoglobin A1c or glycated albumin were better than the original eGFR equations, particularly in diabetic patients. Although we created additional formulae for correction by hemoglobin A1c and glycated albumin 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 (37). 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. reported that urinary megalin was increased in correlation with the severity of type 2 diabetic nephropathy (38), 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 A1c or glycated albumin, 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 A1c.

In conclusion, in CKD patients, each of three eGFR measures (eGFR_cr, eGFR_cys, and eGFR_cr\_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 A1c are better than the original eGFR. We consider that eGFR$c_r$ corrected by hemoglobin A1c is clinically feasible and useful.

Acknowledgement.

A.T. and E.I. researched data and wrote the manuscript. A.T., E.I., Y.O., M.I., S.N., Y.M., K.M., J.U., S.F., M.E., T.N., and M.I., contributed to the discussion, and reviewed manuscript. A.T. and E.I. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the reported research. This study was not funded by any grant from a funding agency in the public, commercial or not-for-profit sector.
FIGURE LEGENDS

Figure 1. Relationships between inulin clearance ($C_{in}$) and each glomerular filtration rate estimated based on serum creatinine (eGFR$_{cr}$), serum cystatin C (eGFR$_{cys}$) and the combination of serum creatinine and cystatin C (eGFR$_{cr-cys}$). There was a significant and positive correlation between eGFR$_{cr}$ and $C_{in}$ in both diabetic patients (DM) and non-diabetic subjects (non-DM), between eGFR$_{cys}$ and $C_{in}$ in both DM and non-DM, and between eGFR$_{cr-cys}$ and $C_{in}$ in both DM and non-DM subjects. Intercepts of the regression line between $C_{in}$ and each eGFR measure (eGFR$_{cr}$, eGFR$_{cys}$, and eGFR$_{cr-cys}$, respectively) were significantly higher in DM than in non-DM subjects ($p<0.0001$, $p=0.0027$, and $p<0.0001$, respectively).

Figure 2. Relationships between hemoglobin A1c (HbA1c) and the ratio of each eGFR/$C_{in}$, and between glycated albumin (GA) and the ratio of each eGFR/$C_{in}$. There were significant and positive correlations between the ratio of each eGFR/$C_{in}$ and HbA1c and between the ratio of each eGFR/$C_{in}$ and GA.
References


12. Hjelmesaeth J, Roislien J, Nordstrand N, Hofso D, Hager H, Hartmann A: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men:
a cross-sectional study. BMC Endocr Disord 2010;10:6


<table>
<thead>
<tr>
<th></th>
<th>all subjects</th>
<th>diabetic</th>
<th>non-diabetic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>number</strong></td>
<td>80</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>age (years)</strong></td>
<td>56.5±15.4</td>
<td>64.8±9.5</td>
<td>48.3±15.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>gender (male/female)</strong></td>
<td>35 / 45</td>
<td>16 / 24</td>
<td>19 / 21</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>body mass index (kg/m²)</strong></td>
<td>24.8±4.2</td>
<td>25.4±3.4</td>
<td>24.3±4.7</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>mean blood pressure (mmHg)</strong></td>
<td>87.8±10.8</td>
<td>89.2±11.4</td>
<td>86.3±10.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>systolic pressure (mmHg)</td>
<td>121.8±17.8</td>
<td>126.1±18.5</td>
<td>117.5±16.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>diastolic pressure (mmHg)</td>
<td>70.8±9.4</td>
<td>70.8±9.6</td>
<td>70.8±9.4</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>fasting plasma glucose (g/dl)</strong></td>
<td>108.4±30.4</td>
<td>121.5±33.5</td>
<td>95.7±20.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>hemoglobin A1c (%)</strong></td>
<td>6.7±1.8</td>
<td>8.1±1.5</td>
<td>5.4±0.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>serum creatinine (mg/dl)</strong></td>
<td>1.0±0.5</td>
<td>0.7±0.3</td>
<td>1.2±0.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>serum cystatin C (mg/dl)</strong></td>
<td>1.2±0.67 (n=70)</td>
<td>0.9±0.3 (n=37)</td>
<td>1.5±0.8 (n=33)</td>
<td>0.0005</td>
</tr>
<tr>
<td><strong>eGFR&lt;sub&gt;cr&lt;/sub&gt; (ml/min./1.73m²)</strong></td>
<td>64.1±23.7</td>
<td>75.0±20.2</td>
<td>53.2±22.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cr&lt;/sub&gt; corrected by hemoglobin A1c (ml/min./1.73m²)</td>
<td>64.2±22.6</td>
<td>67.5±18.6</td>
<td>60.8±25.8</td>
<td>0.1841</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cr&lt;/sub&gt; corrected by glycated albumin (ml/min./1.73m²)</td>
<td>64.9±21.2 (n=53)</td>
<td>67.0±19.7 (n=39)</td>
<td>59.3±25.3 (n=39)</td>
<td>0.2493</td>
</tr>
<tr>
<td><strong>eGFR&lt;sub&gt;cys&lt;/sub&gt; (ml/min./1.73m²)</strong></td>
<td>68.9±26.8 (n=70)</td>
<td>80.6±22.4 (n=37)</td>
<td>60.0±29.6 (n=33)</td>
<td>0.0015</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cys&lt;/sub&gt; corrected by hemoglobin A1c (ml/min./1.73m²)</td>
<td>62.1±24.2 (n=70)</td>
<td>66.3±18.3 (n=37)</td>
<td>57.6±29.1 (n=33)</td>
<td>0.1393</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cys&lt;/sub&gt; corrected by glycated albumin (ml/min./1.73m²)</td>
<td>64.3±20.7 (n=49)</td>
<td>66.2±18.8 (n=37)</td>
<td>58.4±25.7 (n=12)</td>
<td>0.2571</td>
</tr>
<tr>
<td><strong>eGFR&lt;sub&gt;cr&lt;/sub&gt;&lt;sub&gt;cys&lt;/sub&gt; (ml/min./1.73m²)</strong></td>
<td>67.7±27.3 (n=70)</td>
<td>82.0±22.4 (n=37)</td>
<td>54.2±25.1 (n=33)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cr&lt;/sub&gt;&lt;sub&gt;cys&lt;/sub&gt; corrected by hemoglobin A1c (ml/min./1.73m²)</td>
<td>62.3±23.6 (n=70)</td>
<td>67.7±18.6 (n=37)</td>
<td>56.8±27.6 (n=30)</td>
<td>0.0562</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cr&lt;/sub&gt;&lt;sub&gt;cys&lt;/sub&gt; corrected by glycated albumin (ml/min./1.73m²)</td>
<td>64.9±21.0 (n=49)</td>
<td>67.6±19.3 (n=37)</td>
<td>56.6±24.4 (n=12)</td>
<td>0.1149</td>
</tr>
<tr>
<td><strong>inulin clearance (ml/min./1.73m²)</strong></td>
<td>65.3±23.8</td>
<td>68.1±20.6</td>
<td>62.4±26.8</td>
<td>0.2866</td>
</tr>
</tbody>
</table>

P value, diabetic patients versus non-diabetic subjects
Table 2. Correlation coefficients and intraclass correlation coefficients (ICC) between $C_{in}$ and each eGFR measure with and without correction by hemoglobin A1c (HbA1c)

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>diabetic</th>
<th>non-diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>ICC (95% CI)</td>
<td>$r$</td>
</tr>
<tr>
<td><strong>eGFR_{cr}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uncorrected</td>
<td>0.781 #</td>
<td>0.802 (0.700-0.873) #</td>
<td>0.683 #</td>
</tr>
<tr>
<td>corrected by HbA1c</td>
<td>0.872 #</td>
<td>0.881 (0.873-0.957) #</td>
<td>0.759 #</td>
</tr>
<tr>
<td><strong>eGFR_{cys}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uncorrected *</td>
<td>0.746 #</td>
<td>0.727 (0.594-0.822) #</td>
<td>0.584 #</td>
</tr>
<tr>
<td>corrected by HbA1c *</td>
<td>0.777 #</td>
<td>0.788 (0.679-0.863) #</td>
<td>0.636 #</td>
</tr>
<tr>
<td><strong>eGFR_{cr-cys}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uncorrected *</td>
<td>0.799 #</td>
<td>0.793 (0.686-0.866) #</td>
<td>0.712 #</td>
</tr>
<tr>
<td>corrected by HbA1c *</td>
<td>0.875 #</td>
<td>0.881 (0.815-0.924) #</td>
<td>0.777 #</td>
</tr>
</tbody>
</table>

#, p<0.0001, *; n=70
Figure 1

DM (n = 40)
\[ r = 0.683 \]
\[ p < 0.0001 \]

non-DM (n = 40)
\[ r = 0.930 \]
\[ p < 0.0001 \]

DM (n = 37)
\[ r = 0.712 \]
\[ p < 0.0001 \]

non-DM (n = 33)
\[ r = 0.930 \]
\[ p < 0.0001 \]
Figure 2

Diabetes Care

- **eGFR\textsubscript{cr}/C\textsubscript{in}**
  - HbA1c: $n = 80$, $r = 0.605$, $p < 0.0001$
  - GA: $n = 53$, $r = 0.565$, $p < 0.0001$

- **eGFR\textsubscript{cys}/C\textsubscript{in}**
  - HbA1c: $n = 70$, $r = 0.372$, $p = 0.0092$
  - GA: $n = 49$, $r = 0.548$, $p < 0.0001$

- **eGFR\textsubscript{cr-cys}/C\textsubscript{in}**
  - HbA1c: $n = 70$, $r = 0.603$, $p < 0.0001$
  - GA: $n = 49$, $r = 0.340$, $p = 0.0042$

Legend:
- DM
- non-DM