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Biological Variation of Creatinine, Cystatin C, and eGFR over 24 Hours

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BACKGROUND: Estimated glomerular filtration rate (eGFR) is widely used in clinical practice. This study assessed the within-subject biological variation (CV_I) of different eGFR equations in people with chronic kidney disease (CKD) and people without CKD. The aims of this study were (a) to determine the 24-h biological variation profiles of creatinine, cystatin C, and eGFR and (b) to determine whether CV_I of creatinine, cystatin C, and eGFR changes on deterioration of glomerular filtration.

METHODS: Hourly blood samples were analyzed from 37 individuals (17 without CKD, 20 with CKD) during 24 h. Creatinine (enzymatic method) and cystatin C were measured using a Cobas 8000 (Roche Diagnostics). eGFR was estimated using the Modification of Diet in Renal Disease and the Chronic Kidney Disease Epidemiology Collaboration based on creatinine and/or cystatin C. Plasma samples were stored at -80°C before analysis. Outlier and homogeneity analyses were checked before performing a nested ANOVA to determine biological variation.

RESULTS: CV_I of creatinine was higher in people without CKD than in those with CKD (6.4% vs 2.5%) owing primarily to the more profound effect of meat consumption on creatinine variability in individuals with lower baseline creatinine concentrations. Unlike creatinine, cystatin C concentrations were unaffected by meat consumption. Cystatin C showed some diurnal rhythmic variation and less in people with CKD. Reference change values (RCVs) of all eGFR equations were within 13% to 20% in both study groups.

CONCLUSIONS: Despite differences in CV_I of creatinine, the CV_I and RCV of the eGFR equations were relatively similar for people with or without CKD.

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The estimated glomerular filtration rate (eGFR)⁴ is widely applied for the diagnosis of kidney disease and the monitoring of renal function in patients with chronic kidney disease (CKD) (1). CKD is defined as the presence of kidney damage or a glomerular filtration rate (GFR) $<60\text{ mL/min/1.73 m}^2$ for ≥ 3 months, irrespective of the cause (1, 2). The Chronic Kidney Disease–Epidemiology Collaboration equation (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) are the equations most commonly used in clinical practice to estimate the GFR (3, 4). Although the equations are constructed differently, the calculation of both CKD-EPI and MDRD is based on the 2 renal biomarkers: creatinine and cystatin C (3–5). Serum creatinine and cystatin C concentrations can fluctuate during the day, either from true circadian differences in GFR or from random biological variation, without reflecting real changes (6, 7). These fluctuations could affect the interpretation of eGFR values based on creatinine and/or cystatin C. Insight regarding the magnitude of diurnal fluctuations may prevent physicians from erroneously interpreting random variations as clinically relevant changes (8).

Biological variation studies allow a systematic assessment of random diurnal variation of biomarkers. Thus far, most studies that have assessed the biological variation of renal biomarkers and eGFR reported on between-day variation (6, 9–17), but data on within-day biolog-

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⁴ Nonstandard abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; GFR, glomerular filtration rate; CKD-EPI, Chronic Kidney Disease–Epidemiology Collaboration; MDRD, Modification of Diet in Renal Disease; CV_A , analytical coefficient of variation; CV_I , intraindividual coefficient of variation; CV_G , group coefficient of variation; RCV, reference change value; IL, index of individuality.

ical variation are scarce (18). Moreover, most studies focused on either healthy volunteers or people with impaired renal function, but data from a direct comparison of people with and without CKD are not available (6, 15, 19, 20). In this study, we aimed to construct 24-h profiles of creatinine and cystatin C concentrations, as well as GFR estimations that are based on creatinine and/or cystatin C. In addition, we assessed whether variation over the day is of similar magnitude in people with CKD and people without CKD.

Materials and Methods

STUDY DESIGN AND POPULATION

This study on 24-h variability in renal function and eGFR was performed between January 2013 and October 2015, as described previously (21, 22). It conformed to the principles of the Declaration of Helsinki (23) and was approved by the Institutional Review Board and the ethics committee at Maastricht University Medical Center. Each participant provided written informed consent.

The design of this study complied with the current checklists regarding biological variation as much as possible (24, 25).

In total, 44 individuals were included and divided into 2 study groups: The first study group consisted of 24 individuals without clinically diagnosed CKD (79% males and 21% females), and the second group (70% males and 30% females) consisted of 20 patients (participant numbers 25–44) with clinically diagnosed CKD stage 3 or higher (eGFR, <60 mL/min/1.73 m²) (2). This number of individuals per group afforded sufficient power to make reliable estimations about biological variation (26). All individuals were white and between 39 and 83 years of age. Diabetes was defined as fasting plasma glucose ≥ 126 mg/dL and/or HbA1c $\geq 6.5\%$ ($= 47$ mmol/mol) (27). Exclusion criteria were current dialysis treatment, an acute myocardial infarction in the 12 months before the study, active cardiac disease (angina pectoris, cardiomyopathy, or myocarditis), and anemia (hemoglobin, <10.5 g/dL).

SAMPLE COLLECTION AND HANDLING

Creatinine and cystatin C were measured on a Cobas® 8000 (Roche Diagnostics) using the following Roche reagents: CREP2 (Creatinine plus ver.2, code 05168589) and CYSC2 (Tina-quant Cystatin C Gen.2, code 06600239). We used an enzymatic method to measure creatinine, based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde, and hydrogen peroxide. We used Bio-Rad controls for creatinine and Cystatin C Control Set Gen. 2 for cystatin C (Roche Diagnostics).

The eGFR was calculated using MDRD and CKD-EPI formulae (3, 5). Three CKD-EPI estimations were

calculated: CKD-EPI_{creatinine}, CKD-EPI_{cystatin C}, and CKD-EPI_{creatinine–cystatin C} (4).

Hourly blood samples were drawn in EDTA-containing tubes (8 mL). The plasma samples were centrifuged immediately (centrifugation at 2700g for 12 min at room temperature) after collection, and plasma was stored at -80 °C until analysis. The plasma samples were then thawed, and all samples from the same individual were analyzed within a single run.

To estimate analytical variation (CV_A), samples from 8 randomly selected individuals (4 individuals without CKD and 4 individuals with CKD, 18% of all samples) were analyzed in duplicate.

STUDY PROCEDURE

To study the within-day biological variability, blood samples were drawn every hour during 24 h using an intravenous cannula. The participants arrived at 8 AM at the laboratory after an overnight fast. During the test day, participants were restricted to the laboratory under sedentary conditions. Mealtimes were standardized at 8:30 AM (breakfast), 12:30 PM (lunch), and 6 PM (dinner). The participants could select from approximately 4 dinner options. The majority of dinner options consisted of dishes containing meat. The meal options and meat content were not standardized. Breakfast and lunch consisted of sandwiches with cheese, ham, or sweet toppings. The participants went to bed at 11:30 PM and got up the next morning at 7 AM. During the night, an extension line was attached to the cannula to prevent sleep disturbance during blood sampling.

STATISTICAL ANALYSIS

As recommended for biological variation studies, we performed outlier analyses on 3 levels (analytical, within-subject, and between-subject) (7, 26). The Cochran test was used to identify outliers on the analytical and within-subject level, and the Dixon–Reed criterion was performed to identify outliers on the between-subject level (7, 8, 28, 29). People with outlying within-subject variances were rejected from calculations because of their heterogeneity of variance (24). Because data on the analytical, within-subject, and between-subject levels followed a gaussian distribution (Shapiro–Wilk test), transformation of the data was not required. For the analytical normality check, we used data of 8 individuals that were measured in duplicate (192 replicates). For the within-subject level, we verified normality for each participant. For the between-subject level, we verified normality for the means of each individual. The variation on 3 levels, the between-subject variation (CV_G), within-subject biological variation (CV_I), and CV_A , were calculated using a nested ANOVA with 95% CIs determined according to the method of Burdick and Graybill (8, 26, 30).

Table 1. Baseline characteristics.^a

	People without CKD (n = 17)	People with CKD (n = 20)
Age, years	72 ± 7	66 ± 12
Male sex	14 (82)	14 (70)
Body mass index, kg/m ²	26 ± 3	28 ± 4
Diabetes mellitus ^b	6 (35)	7 (35)
Cholesterol concentration, mg/dL	176 ± 35	157 ± 33
Systolic blood pressure, mmHg	140 ± 15	136 ± 19
Diastolic blood pressure, mmHg	68 ± 8	86 ± 14
Creatinine, mg/dL	1.0 ± 0.2	3.3 ± 1.0
Cystatin C, mg/L	1.0 ± 0.2	2.8 ± 0.8
MDRD, mL/min/1.73 m ²	73.4 ± 18.5	19.2 ± 6.4
CKD-EPI _{creatinine} , mL/min/1.73 m ²	72.9 ± 17.2	18.9 ± 6.6
CKD-EPI _{cystatin C} , mL/min/1.73 m ²	74.2 ± 17.2	20.2 ± 8.5
CKD-EPI _{creatinine-cystatin C} , mL/min/1.73 m ²	74.2 ± 17.2	19.0 ± 7.0
CKD	0 (0)	20 (100)
Glomerular disease	NA ^c	7 (35)
Tubulointerstitial disease	NA	4 (20)
Vascular disease	NA	8 (40)
Cystic and congenital disease	NA	1 (5)

^a Continuous data are presented as mean ± SD, and categorical data are presented as n (%).

^b Diabetes was defined as fasting plasma glucose ≥126 mg/dL and/or HbA1c ≥6.5% (= 47 mmol/mol) (27).

^c NA, not applicable.

Reference change values (RCVs) (Z-score of 1.96) and the index of individuality (II) were calculated according to the method of Fraser and Harris (8):

$$RCV = \sqrt{2} \times Z \times \sqrt{(CV_I^2 + CV_A^2)}$$

$$II = \sqrt{(CV_I^2 + CV_A^2)} / CV_G$$

We used an *F*-test to monitor whether the degree of renal impairment was stable in the people with CKD. To investigate this, we examined the eGFR 1 week, 1 month, and 3 months after the initial test day. *P* values <0.05 were considered statistically significant.

To visualize the diurnal rhythm of cystatin C, 24-h concentration curves were fitted for both study groups using cosinor rhythmometry.

All statistical calculations were performed using SPSS for Windows version 23 (IBM SPSS Statistics).

Results

ELIGIBILITY OF PARTICIPANTS

A total of 44 individuals, 24 without CKD and 20 with CKD, participated in this biological variation study. Two participants (1 and 8) left the study prematurely, and 2 other participants (15 and 23) had missing data because

of problems with the venous cannula overnight. To maintain a balanced design, and in line with the statistical conditions for a nested ANOVA, these people were considered not eligible and excluded from further analyses (31). Additionally, participant 20 was excluded because this participant developed a severe cold during the test day; therefore, the clinical situation of this participant was unstable. Although participants 10 and 18 were not clinically diagnosed with CKD, these participants were excluded because the creatinine concentrations were not appropriate for the non-CKD group (mean concentrations, 1.8 ± 0.2 mg/dL and 2.0 ± 0.08 mg/dL, respectively). In Fig. 1 of the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol64/issue5>, a flowchart of the study is shown. Eventually, 37 individuals were considered eligible for analyses. Baseline characteristics of the 2 study groups are shown in Table 1.

CLINICAL STABILITY OF PEOPLE WITH CKD

To verify that the people with CKD were clinically stable and not rapidly progressive in terms of their CKD, additional blood samples were taken at 1 week, 1 month, and 3 months after the test day at the same time as the baseline measurement on the initial test day (8:30 AM). The eGFR

values (calculated with MDRD, CKD-EPI_{creatinine}, CKD-EPI_{cystatin C}, and CKD-EPI_{creatinine-cystatin C}) did not decrease significantly during this follow-up period (minimum–maximum follow-up concentration ranges: MDRD, 18.7–19.3 mL/min/1.73 m², $P = 0.63$; CKD-EPI_{creatinine}, 18.3–18.9 mL/min/1.73 m², $P = 0.99$; CKD-EPI_{cystatin C}, 20.1–20.3 mL/min/1.73 m², $P = 0.49$; CKD-EPI_{creatinine-cystatin C}, 18.6–19.0 mL/min/1.73 m², $P = 0.99$). Therefore, the participants with CKD were considered as having a stable chronic disease.

24-H VARIABILITY PROFILES

Figs. 1 and 2 show 24-h variability profiles of creatinine, cystatin C, and all eGFR equations for both study groups. The mean creatinine and cystatin C concentrations were, by definition, significantly lower in people without CKD than in those with CKD (creatinine: 1.0 ± 0.3 mg/dL vs 3.3 ± 1.0 mg/dL, $P < 0.01$; cystatin C: 1.0 ± 0.3 mg/L vs 2.7 ± 0.8 mg/L, $P < 0.01$). Consequently, all average eGFR values were significantly higher in people without CKD compared with the people with CKD (MDRD: 75 ± 21 mL/min/1.73 m² vs 20 ± 9 mL/min/1.73 m², $P < 0.01$; CKD-EPI_{creatinine}: 74 ± 18 mL/min/1.73 m² vs 20 ± 10 mL/min/1.73 m², $P < 0.01$; CKD-EPI_{cystatin C}: 77 ± 17 mL/min/1.73 m² vs 24 ± 14 mL/min/1.73 m², $P < 0.01$; CKD-EPI_{creatinine-cystatin C}: 76 ± 17 mL/min/1.73 m² vs 21 ± 12 mL/min/1.73 m², $P < 0.01$).

Creatinine concentrations increased up to 50% (0.46 mg/dL) in people without CKD after dinner (participant 11, individual 24-h profile of this participant was not shown). The increase was numerically similar in people with CKD (0.40 mg/dL) compared with people without CKD, but as a percentage, the increase was substantially lower (18%) because of the high baseline concentrations of creatinine in the people with CKD.

Unlike creatinine, cystatin C concentrations were not affected by dinner. Interestingly, however, cystatin C showed a small, but evident, diurnal rhythm, with decreasing cystatin C concentrations during daytime and increasing concentrations during the evening and night. This circadian rhythm of cystatin C was less prominent in people with CKD (Figs. 1 and 2). In Fig. 2 of the online Data Supplement, the diurnal rhythm of cystatin C was visualized by fitting a cosinor model through the average data points. For people without CKD, the amplitude of the diurnal cosinor rhythm was $4.9 \pm 0.8\%$, whereas the amplitude was reduced to $1.6 \pm 0.5\%$ for people with CKD.

COMPONENTS OF VARIATION IN PEOPLE WITH AND WITHOUT CKD

Variation components of all parameters in both study groups are shown in Table 2. CV_G was of similar magnitude for the study groups. In both groups, CV_G was

significantly larger than CV_I for all parameters. Because of the relatively high CV_G values, all indexes of individuality were low (0.1–0.3).

In Fig. 3, the minimum–maximum concentration ranges of creatinine and cystatin C are presented for the people without and with CKD. The people without CKD (Fig. 3A) exhibit a higher relative range in creatinine concentrations than the people with CKD (Fig. 3C). This difference in concentrations contributes to a significantly higher CV_I expressed as percent in people without CKD (6.4% vs 2.5%); also, the RCV was significantly higher in people without CKD (18% vs 8%). The CV_I values of cystatin C, CKD-EPI_{cystatin C}, and CKD-EPI_{creatinine-cystatin C} were also significantly different between the groups. However, these differences were smaller than for creatinine.

In people without CKD, the CKD-EPI based on the combination of creatinine and cystatin C (CKD-EPI_{creatinine-cystatin C}) was the eGFR equation with lowest CV_I. In people with CKD, the CKD-EPI based on creatinine (CKD-EPI_{creatinine}) demonstrated the lowest CV_I over the day. However, in this group, the difference with CKD-EPI_{creatinine-cystatin C} was minimal (5.2% vs 5.4%, with overlapping CIs).

CV_A was low in both study groups (maximum 2.0% for people without CKD and 2.6% for people with CKD). All CV_A values met the desirable ratio between CV_I and CV_A, which is 1:2. A ratio < 0.5 ensures that the analytical noise contributes $< 12\%$ to the total variation (7).

SENSITIVITY ANALYSIS

According to the Cochran within-subject outlier test, the after-dinner increase of creatinine demanded the exclusion of some individuals (participants 14 and 30, respectively; see Table 1 and Fig. 3 in the online Data Supplement). However, this strict statistical perspective may neglect the fact that the creatinine increases are in fact physiological changes that also occur in hospitalized patients. We believe that excluding these participants may be too stringent and can lead to an underestimation of biological variation data. A sensitivity analysis included individuals who were excluded in the primary analysis because of heterogeneity of variance. In this analysis, slightly larger CV_I values were revealed for most biomarkers (see Table 2 in the online Data Supplement). The difference was more pronounced for creatinine than for cystatin C (which lacks the after-dinner increase), and the effect was stronger in people without CKD than in people with CKD.

Discussion

In the current study, we present 24-h variation profiles of creatinine, cystatin C, and their derived estimates of GFR

Fig. 1. Twenty-four-hour variation profiles in people without CKD. Values represent mean (SE). Participants slept between 11:30 PM and 7 AM (shaded area).

Table 2. Components of biological variation, IIs, and RCVs for renal biomarkers and different eGFR equations in both study groups.^a

	n	Mean	CV _A	CV _I	CV _G	II	RCV
People without CKD							
Creatinine	16	1.0 mg/dL	1.1 (1.0–1.3)	6.4 (6.0–6.9)	21.2 (15.7–32.9)	0.3	18.2
Cystatin C	17	1.0 mg/dL	1.1 (1.0–1.3)	4.1 (3.8–4.4)	15.3 (11.3–23.2)	0.3	11.6
MDRD	16	75.3 mL/min/1.73 m ²	1.6 (1.4–1.9)	6.1 (5.7–6.6)	27.3 (20.2–42.3)	0.2	16.9
CKD-EPI _{creatinine}	16	73.8 mL/min/1.73 m ²	1.2 (1.0–1.4)	5.3 (5.1–5.6)	24.3 (18.0–37.7)	0.2	14.6
CKD-EPI _{cystatin C}	17	76.7 mL/min/1.73 m ²	2.0 (1.8–2.3)	5.5 (5.2–5.9)	21.2 (15.8–32.3)	0.3	15.3
CKD-EPI _{creatinine-cystatin C}	16	75.2 mL/min/1.73 m ²	1.2 (1.1–1.4)	4.6 (4.3–5.0)	20.3 (16.5–34.5)	0.2	13.2
People with CKD							
Creatinine	19	3.3 mg/dL	1.3 (1.1–1.5)	2.5 (2.4–2.7)	28.1 (21.2–41.6)	0.1	7.9
Cystatin C	18	2.7 mg/L	0.8 (0.7–1.0)	3.2 (3.0–3.4)	27.2 (20.4–40.8)	0.1	9.1
MDRD	19	20.2 mL/min/1.73 m ²	2.4 (2.1–2.8)	5.5 (5.2–5.9)	28.4 (21.5–42.0)	0.2	13.3
CKD-EPI _{creatinine}	19	20.1 mL/min/1.73 m ²	2.6 (2.3–3.0)	5.2 (4.9–5.6)	30.0 (22.7–44.4)	0.2	14.4
CKD-EPI _{cystatin C}	18	23.8 mL/min/1.73 m ²	1.2 (1.1–1.4)	7.3 (6.8–7.8)	38.7 (29.1–58.1)	0.2	20.1
CKD-EPI _{creatinine-cystatin C}	17	20.9 mL/min/1.73 m ²	1.6 (1.4–1.8)	5.4 (5.0–5.8)	35.2 (26.4–52.9)	0.2	15.1

^a Except where stated otherwise, the data are percentage (%) values; 95% CIs are shown between brackets.

(MDRD, CKD-EPI_{creatinine}, CKD-EPI_{cystatin C}, and CKD-EPI_{creatinine-cystatin C}) in people without CKD and in people with CKD. In addition, separate variation components were calculated and compared between both study groups.

An important finding of this study is that the CV_I of creatinine is significantly higher in people without CKD than in people with CKD. In our study, the effect of the postdinner creatinine spike on CV_I was substantial, especially for people without CKD, given that these people have low baseline creatinine concentrations (mean, 1.0 mg/dL). For people with CKD, the effect on CV_I because of higher baseline creatinine concentrations (mean, 3.3 mg/dL) was smaller. This difference was even slightly more pronounced in the sensitivity analysis that included the individuals with a (physiological) high postdinner creatinine increase, likely because of meat consumption, increasing their CV_I and marking them for removal based on statistical criteria. However, the variation in creatinine concentrations caused by dinner content is of interest for clinical practice, given that patients often have dinner with unknown meat and other content. For clinical practice, this variation might ideally be included, even if this variation is statistically large and seemingly divergent. Rivara et al. performed a similar sensitivity analysis in which they showed a slight increase of the CV_I of creatinine after including individuals who were excluded in the primary analysis because of heterogeneity of variances (19).

The CV_I for creatinine in this study was higher than reported in the EUBIVAS project (6.2% vs 4.4%), which

can likely be attributed to the fact that the creatinine concentrations measured in the EUBIVAS project were between-day CV_I values based on fasting blood samples collected once per day at the same hour between 8 AM and 10 AM (20, 32). Therefore, creatinine increase after meat consumption is not integrated in the EUBIVAS CV_I value. Because within-day CV_I and between-day CV_I are different concepts, the results of this study are not directly comparable with those of the EUBIVAS project.

The design of this study complies with the current checklists regarding biological variation as much as possible (24, 25). Only on item 7 of the most recent biological variation checklist, which is about the steady-state condition, do we deviate from the checklist by not including meat consumption in the model for the concentration of creatinine to create a steady state. Whether meat consumption is included in a model for the concentration of creatinine has consequences for the amount of variation left unexplained, and with that, the size of the CV_I. In a clinical setting, neither the time of dinner of a patient nor the meat content of the dinner (or other meal) is known to the physician interpreting the creatinine laboratory results. Hence, for clinical purposes, variation resulting from meat consumption should not be excluded from the model and should be included in the CV_I estimate to allow for this additional uncertainty. Because most people consume meat in their diet, our CV_I estimate for a 24-h period can be considered to reflect typical physiologic conditions facing interpretation of creatinine and eGFR results.

Because this study was not originally designed to investigate the influence of meat consumption on creatinine concentrations, no detailed individual data of the participants regarding their meat consumption during the day were available. However, the study of Nair et al. standardized the meat consumption of all participants and compared their results with a nonmeat meal. The Nair study described a significant increase of creatinine concentration after a meat meal for all study participants (33). For healthy volunteers and individuals with CKD stage 1 or 2, the study reported statistically significant creatinine increases of 5 $\mu\text{mol/L}$ (0.06 mg/dL) and 8 $\mu\text{mol/L}$ (0.09 mg/dL), respectively. We observed a similar average increase of 0.07 mg/dL (6.2 $\mu\text{mol/L}$) between 6 PM and 10 PM, presumably related to dinner composition. For people with CKD, the Nair study reported maximum creatinine increases of up to 0.25 mg/dL (22 $\mu\text{mol/L}$), which is more pronounced than the

average increase of 0.13 mg/dL (12 $\mu\text{mol/L}$) we observed in the people with CKD.

Nevertheless, the study of Nair et al. emphasizes the finding that the relative decrease in eGFR after meat consumption is proportionately less in patients with more advanced CKD stages (33), which is consistent with our findings. Another study by Preiss et al. demonstrated that the effect of meat consumption could have an impact on diagnosis because of misclassification of CKD staging if measurements are made after consuming a cooked-meat meal (34). The authors of this study state that physicians should ensure that, when classifying the stage of CKD, samples are taken under appropriate conditions (34). We endorse this conclusion, especially in the context of a 24-h biological variation study. Creatinine was included in our study to be able to determine the calculated eGFR and not to be an indicator for GFR (35).

Unlike creatinine, the CV_I of cystatin C was in the same range for both people with or without CKD, and its 24-h profile was not characterized by a postdinner spike. Despite substantial differences in biological variation and RCVs between cystatin C and creatinine, especially in people without CKD, these differences do not translate to similar differences in the biological variation and RCVs of the eGFR equations that are derived from these biomarkers. In fact, CV_I values and RCVs of the CKD-EPI_{creatinine} equation are of the same magnitude in both study groups. However, CV_I values and RCVs of the CKD-EPI_{cystatin C} are of different magnitude. The transformation from cystatin C to CKD-EPI_{cystatin C} leads to more dispersed CKD-EPI values than the transformation from creatinine to CKD-EPI_{creatinine}.

Diurnal cystatin C profiles showed modest intrinsic diurnal rhythmicity, with a slight decrease during daytime and an increase during the evening and night. This is in line with modestly reduced glomerular filtration at nighttime compared with daytime (36, 37). A similar diurnal rhythmicity may be apparent in the creatinine profiles (at least a declining pattern from morning until evening) but is obscured by the postdinner creatinine peak. Interestingly, the diurnal cystatin C rhythm is somewhat diminished in people with CKD. This effect can be explained by reduced renal clearance in people with CKD, leading to accumulation of cystatin C, and consequent reduction in its diurnal rhythm (38).

Large rhythmic diurnal oscillations preclude calculation of overall variation components and RCVs, as they would become dependent on time of day. Instead, hour-to-hour RCVs can be calculated, which consider the structural change according to the diurnal rhythm. Such approach has been previously applied by our group and others for the calculation of RCVs for various hematological biomarkers and cardiac troponin T (39, 40). However, rhythmic variation of cystatin C in this study was so small that the calculation of hour-to-hour variation components and RCVs would have complicated their interpretation without offering substantial benefit in terms of mathematical accuracy.

Some limitations of this study merit consideration: First, the design of the study was not limited to the assessment of the biological variation of kidney biomarkers. Although mealtimes were standardized across study par-

ticipants, the content of the meals was not. Hence, the consumption of meat among meals was variable and may have accounted for the highly variable creatinine increases, especially after dinner.

Second, the ratio between men and women was skewed (77% men and 23% women), precluding robust assessment of potential sex differences. Third, the generalizability of this study to other ethnicities may be limited because all participants were white. Fourth, most individuals, including those without CKD, had comorbidities. Nevertheless, the presence of comorbidities, as well as the fact that the meals were not standardized, is representative for the average patient (both hospitalized and outpatient) and provides real world estimates of the biological variation of interest when interpreting results.

In conclusion, we show that the CV_I of creatinine is higher in people without CKD than in people with CKD. Despite differences in biological variation, RCVs of all derived estimates of GFR (MDRD, CKD-EPI_{creatinine}, CKD-EPI_{cystatin C}, and CKD-EPI_{creatinine-cystatin C}) are within the same range (13%–20%) and are similar for people with or without CKD.

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