WITHDRAWN: Biological variation of estimated glomerular filtrations rate in apparently healthy individuals within 24 h calculated using new CKD-EPI equations

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background

Glomerular filtrations rate (GFR) estimated based on serum creatinine (S-Crea) and/or serum cystatin C (S-Cys-C) levels is often used to assess renal function. The commonly used equations are the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), However, short-term studies of the corresponding GFR biological variation(BV) are scarce, and all have included race coefficients. In this study, We aimed to use the MDRD and without race coefficients of CKD-EPI new equations (CKD-EPI$_{Crea}$, CKD-EPI$_{Cys\text{-}C}$, and CKD-EPI$_{Crea+Cys\text{-}C}$) to estimate the BV of eGFR within 24 h.

Methods

30 apparently healthy subjects blood samples were collected once at 4-h intervals for 24h, Measuring S-Crea and S-Cys-C, using MDRD and CKD-EPI new equations to estimated GFR(eGFR).

Results

Based on the MDRD, CKD-EPI$_{Crea}$, CKD-EPI$_{Cys\text{-}C}$, and CKD-EPI$_{Crea+Cys\text{-}C}$ equations, the within-subject CV$_I$ (95% confidence interval (CI)) of eGFR for the 30 apparently healthy subjects were 8.39%(7.50–9.51),3.90%(3.49–4.42),6.58%(5.88–7.46)and5.03%(4.50–5.71), respectively. Further, the corresponding individual index(II) values were 0.69, 0.48, 0.51, and 0.31, respectively,and the corresponding positive and negative reference change values (RCV$_{pos/neg}$) were (29.30%,-22.66%), (12.69%,-11.26%), (20.97%,-17.33%), and (15.88%,-13.70%), respectively. Additionally, the RCV$_{pos/neg}$ values of the individual apparently healthy subjects were significantly different,indicating obvious individual characteristics.The largest corresponding individual RCVpos/neg values were (56.51%,-36.11%), (20.99%,-17.35%), (44.93%,-31.00%),and (28.83%, -22.38%), respectively, while the smallest values were (12.36%,-11.00%), (5.32%, -5.05%), (5.76%,-5.45%), and (5.01%,-4.77%),respectively.

Conclusions

The presence of BV has impact on the interpretation of GFR results, in turn affecting the CKD stage,so when using eGFRs based on MDRD and CKD-EPI equations,it is necessary to combine RCV$_{pos/neg}$ values before interpreting the results.

1. Introduction

Chronic kidney disease (CKD) with a prevalence that has been on the rise in recent years, reaching as high as 8–16%, is a global health problem.[1] Globally, glomerular filtrations rate (GFR) has been recognized as an indicator for assessing renal function in apparently healthy subjects, and 2012 the Clinical Practice guidelines for CKD Assessment and Management, Kidney Disease: Improving Global Outcomes (KDIGO) defined GFRs < 60 ml/min/1.73 m^2 and lasting more than 3 months as indicative of CKD, and based on GFR values, there are five stages of CKD.[2] In addition to staging, screening, diagnosis, and monitoring of renal function in renal diseases, GFR is also used for adjusting drug doses,[3–5]and evaluating kidney donors.[6–7]Presently, estimated GFR (eGFR) is often based on serum creatinine (S-Crea) and/or serum cystatin C (S-Cys-C) levels, and the more commonly used eGFR equations are the 2006 Modification of Diet in Renal Disease (MDRD)[8] and 2012 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) .[9] Further, the eGFR report format often contains two, one for the African-American population and the another for the non-African-American population.[8] In recent years, there has been growing concern regarding the validity of the use of such coefficients in medicine, and the dual reporting of eGFR by race is be flawed and unfair[10] given that race is a social construct rather than a biological one. Therefore, in 2021, without race coefficients of new CKD-EPI equations for determining eGFR were introduced,Compared with the 2012 CKD-EPI equation, this new equation showed comparable accuracy and its application in clinical practice was more feasible.[11]

Like other indicators, eGFR shows within-subject biological variation (CV_I), which is particularly important for distinguishing pathological and physiological changes.[12] S-Crea and S-Cys-C concentrations may fluctuate throughout the day[13]and these fluctuations may affect the interpretation of eGFR values based on S-Crea and/or S-Cys-C levels. Thus, physicians may misinterpret the observed random changes as clinically relevant, leading to wrong treatment recommendations. The reference interval corresponding to apparently healthy individuals is affected by CV_I; thus, its validity can be judged based on the individuality index (II).[14] Further, when the reference interval is not applicable, the reference change value (RCV) can be used, which is an objective tool for assessing the validity of differences between consecutive test results, can be used to judge the change status of a disease at the individual level. Notably, positive and negative RCVs ($RCV_{pos}$and RCV_{neg}, respectively) are the thresholds for an increasing or decreasing change trend between two experimental outcomes for the same individual at a given level of probability. Therefore, in evaluating the variation of test results, when the difference between two consecutive test results exceeds RCV_{pos} or RCV_{neg}, a pathological mechanism can be considered. This implies that taking RCV_{pos} and RCV_{neg} values into account can reduce the influence of clinicians' cognitive limitations and subjective biases regarding a disease.[15]

Until present, most studies have only reported long-term biological variations (BVs) for kidney injury biomarkers and eGFR,[13,15–23] while studies focusing on BVs within 24 h are limited.[24]Therefore, the aim of this study was to construct the concentration profiles of S-Crea and S-Cys-C for apparently healthy subjects within 24 h and use them to estimate GFRs. The study was conducted in strict compliance with the standards of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)[25] and collected the blood samples from 30 apparently healthy subjects at 6 time points within 24 h. Further, we also explored the CV_I, II, RCV_{pos}, and RCV_{neg} values of eGFRs corresponding to the apparently healthy subjects within 24 h to help explain future studies using these biomarkers in the context of known BV.

2. Materials And Methods

2.1 Study population and protocols

Thirty apparently healthy subjects (13 females and 17 males) were recruited for this study at Pidu District Traditional Chinese Medicine Hospital, Chengdu, China from September to November 2019(A total of 31 healthy subjects were recruited, and one subject was not included in the statistical analysis because only one result was left after excluding the outliers). There were no statistically significant differences between the subjects with age, blood pressure, and heart rate ($P > 0.05$). The inclusion criteria were as follows: no chronic diabetes, hypertension, goiter, cardiovascular, and cerebrovascular diseases, no history of drugs, and a stable lifestyle. All the apparently healthy subjects completed questionnaires for the verification of their health status, provided relevant lifestyle information, and also underwent relevant physical examinations to ensure that the inclusion criteria.[26]Thereafter, the eligible apparently healthy subjects were required to follow a normal meal time on the day of blood sample collection (08:00, 12:00, and 19:00). The study protocol was approved by the Ethics Committee of Pidu District Hospital of Traditional Chinese Medicine, Chengdu, and written informed consent was obtained from all the participants.

2.2 Sample collection and handling

Blood samples were collected from the 30 apparently healthy subjects at 6 time points (04:00, 08:00, 12:00, 16:00, 20:00, 24:00) within 24 h, and thereafter, maintained at room temperature (26°C) for 30–90 min to allow for serum to naturally separate from whole blood. Next, the samples were centrifuged at 3000g for 10 min, and the sera samples thus collected were transferred into Eppend tubes and stored at -70°C until further analysis. After thawing at room temperature, each sample was analyzed twice simultaneously using a Hitachi 7180 automatic biochemical analyzer (Hitachi, Tokyo, Japan). The analyzer was calibrated according to the manufacturer's instructions prior to testing. S-Crea level was determined using the highly specific sarcosine oxidase method, which can be traced back to the highest standard ID-MS method for S-Crea determination, while S-Cys-C level was determined via latex immunoturbidimetry, with performance that meets clinical requirements. For internal quality control, two quality control materials, S-Crea, batch number 48811/45813 and S-Cys-C, batch number 68912/68913, provided by Bio-rad (Hercules, CA, USA) were used. Further, the reagents for the determination of S-Crea and S-Cys-C levels were provided by Maccura Industries (Sichuan, China).

2.3 Statistical analysis

All statistical analyses were performed using SPSS software version 27.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Further, the standard deviation method was used to remove outliers and ANOVA was performed to calculate BV. To determine whether the data collected was normally distributed(S-Crea, S-Cys-C and eGFR), the Shapiro-Wilk test was performed; the Mann-Whitney U test was also performed for non-normally distributed sex-related variables. Furthermore, to determine differences between males and females at each time point as well as differences between individuals of the same sex at each time point, the Kruskal Wallis test was performed. Statistical significance was set at $P < 0.05$.

2.4 GFR, II, and RCV calculation

GFR was estimated by the MDRD[8] and CKD-EPI equations based on S-Crea and/or S-Cys-C levels.[11] Further, II and RCV values were calculated as follows: II = $(CV_A^2 + CV_I^2)^{1/2}/CV_G$,[27]RCV = $Z \times 2^{1/2} \times (CV_A^2 + CV_I^2)^{1/2}$,[28] $RCV_{pos/neg} = 100\% * (\exp(\pm Z*2^{1/2} (SD_A^2 + SD_I^2)^{1/2} - 1)$, $SD_A^2 = \ln(CV_A^2 + 1)$, $SD_I^2 = \ln(CV_I^2 + 1)$; [29]where the bilateral value of Z is 1.96 under 95% probability. Further, CV_A, CV_G represent analytical variation and between-subject variation, respectively.

3. Results

3.1 Baseline characteristics

A total of 180 sera samples were collected from the 30 apparently healthy subjects within 24 h, and a total of 162 samples were included in the statistical analysis after the exclusion of outliers. Except for one apparently healthy subject’s blood sample was not collected at 12:00, other apparently healthy subjects collected samples as required. The baseline characteristics of the apparently healthy subjects as well as the S-Crea, S-Cys-C, and eGFR data obtained are shown in Table 1.

Table 1
Characteristics of the study population

Characteristic	all	males	females	p
n	30	17	13	/
age(year)	33(18–54)	30(21–54)	33(18–48)	0.711
Systolic pressure ,mmHg	101.5(92–125)	103(92–125)	100(92–118)	0.563
Diastolic pressure,mmHg	74.5(67–86)	73(67–86)	75(69–86)	0.869
Heart rate, bpm	73.5(65–92)	74(65–90)	73(69–92)	0.563
Height, cm	162(148–178)	170(158–178)	158(148–162)	<0.001
Weight, kg	62(44–80)	68(58–80)	51(44–60)	<0.001
Body mass index, kg/m^2	23.15(17.09–29.30)	23.31(20.24–29.30)	20.96(17.09–27.39)	0.012
S-Crea, mg/dl	0.82(0.45–1.14)	0.88(0.59–1.14)	0.61(0.45–0.81)	<0.001
S-Cys-C, mg/l	0.90(0.59–1.22)	0.97(0.68–1.22)	0.83(0.59–0.93)	<0.001
MDRD GFR, ml/min/1.73 m^2	105.35(67.82-152.33)	100.88(67.82-152.33)	117.19(82.94-149.93)	<0.001
CKD-EPI$_{Crea}$ GFR, ml/min/ 1.73 m^2	119.44(78.93-134.61)	117.43(78.93-129.87)	121.78(100.83-134.61)	<0.001
CKD-EPI$_{Cys-C}$ GFR, ml/min / 1.73 m^2	96.45(61.16-125.91)	89.85(61.16-123.93)	103.56(90.20-125.91)	<0.001
CKD-EPI$_{Crea+Cys-C}$,GFR, ml/min /1.73 m^2	96.13(59.84-131.96)	87.10(59.84-121.19)	115.98(103.50-131.96)	<0.001

MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GFR, glomerular filtration rate ; eGFR, estimated glomerular filtration rate ; S-Crea, Serum Creatinine ; S-Cys-C, Serum Cystatin C. Corresponding values for continuous data are shown as median (range). Laboratory median data (S - Crea, S-Cys- C and e GFR) are calculated using all values over the the 24 hours.

3.2 CV$_I$, II, and RCV$_{pos/neg}$ values

The CV$_I$ (95% CI) of the eGFR values obtained based on the MDRD, CKD-EPI$_{Crea}$, CKD-EPI$_{Cys-C}$, and CKD-EPI$_{Crea+Cys-C}$ equations were 8.39% (7.50–9.51), 3.90% (3.49–4.42), 6.58% (5.88–7.46), and 5.03% (4.50–5.71), respectively. Further, the corresponding II and RCV$_{pos/neg}$ values were 0.69, 0.48, 0.51, and 0.31, respectively, and (29.30%,-22.66%), (12.69%,-11.2 6%), (20.97%,-17.33%), and (15.88%,-13.70%), respectively (Table 2). Additionally, the $RCV_{pos/neg}$ values of eGFR were the highest and lowest for the MDRD and $CKD\text{-}EPI_{Crea}$ equations, respectively.

Table 2
Summary of components of variation for S- Crea and S-Cys-C and eGFR

Components	**CV_A (95% CI)**	**CV_I (95% CI)**	**CV_G (95% CI)**	**II**	**RCV (%)**	**RCV_{POS} (%)**	**RCV_{Neg} (%)**
S-Crea	4.05(3.76–4.39)	6.52(5.83–7.40)	19.59 (15.60-26.34)	0.39	21.27	23.68	-19.15
S-Cys-C	1.61(1.49–1.74)	5.81(5.20–6.59)	12.79 (10.19–17.20)	0.47	16.72	18.18	-15.38
MDRD	3.98(3.7–4.32)	8.39(7.50–9.51)	13.37(10.65–17.97)	0.69	25.73	29.30	-22.66
$CKD\text{-}EPI_{Crea}$	1.84(1.70–1.99)	3.90(3.49–4.42)	8.92(7.10-11.99)	0.48	11.95	12.69	-11.26
$CKD\text{-}EPI_{Cys-C}$	1.99(1.85–2.16)	6.58(5.88–7.46)	13.48 (10.74–18.12)	0.51	19.05	20.97	-17.33
$CKD\text{-}EPI_{Crea+Cys-C}$	1.73(1.61–1.87)	5.03(4.50–5.71)	17.33(13.80–23.30)	0.31	14.74	15.88	-13.70

CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CV_A, analytical variation; CV_G, between-subject variation; CV_I, within-subject biological variation; MDRD, Modification of Diet in Renal Disease; RCV, reference change value; RCV_{POS}, positive reference change value ; RCV_{Neg}, negative reference change value, II, index of individuality. All CV values expressed as percentages and 95% CIs were calculated using methods of Burdick and Graybill.

3.3 S-Crea, S-Cys-C, and eGFR values based on sex

The S-Crea, S-Cys-C, and eGFR values corresponding to the apparently healthy subjects as a function of sex at the different time point are shown in Fig. 1. The different time points of S-Crea and S-Cys-C levels and the eGFR values estimated based on the MDRD, $CKD\text{-}EPI_{Crea}$, $CKD\text{-}EPI_{Cys-C}$, and $CKD\text{-}EPI_{Crea+Cys-C}$ equations showed no statistically significant differences between the different sex and the same sex ($P >$ 0.05).

The individual S-Crea and S-Cys-C values as well as the MDRD and CKD-EPI based eGFR values corresponding to the 30 apparently healthy subjects are shown in Fig. 2. From this figure, it is evident that the S-Crea and S-Cys-C levels corresponding to the females were lower than males counterparts, and the eGFR values based on the MDRD, $CKD\text{-}EPI_{Crea}$, $CKD\text{-}EPI_{Cys-C}$, and $CKD\text{-}EPI_{Crea+Cys-C}$ equations were higher for females than males, and the difference was statistically significant ($P < 0.001$).

3.4 Individual $RCV_{pos/neg}$ values

$RCV_{pos/neg}$ values corresponding to the eGFRs of the individual apparently healthy subject are shown in Fig. 3, from which it is evident that the highest individual $RCV_{pos/neg}$ values based on the MDRD, CKD-EPI_{Crea}, CKD-EPI_{Cys-C}, and CKD-$EPI_{Crea+Cys-C}$ equations were (56.51%,-36.11%), (20.99%,-17.35%), (44.93%,-31.00%), and (28.83%,-22.38%), respectively, while the corresponding smallest individual $RCV_{pos/neg}$ values were (12.36%,-11.00%), (5.32%,-5.05%), (5.76%,-5.45%), and (5.01%,-4.77%), respectively.

4. Discussion

The monitoring of BV, which requires the strict time and resource management, is a very challenging to study. As we all know, this study is the first to report eGFR values corresponding to apparently healthy subjects within 24 h obtained using the new CKD-EPI equations, which exclude race coefficients. Further, in this study, blood samples were collected from 30 apparently healthy subjects over a 24-h period following a standardized study design.[25] Thereafter, S-Crea and S-Cys-C levels were measured and estimated GFR based on the MDRD, CKD-EPI_{Crea}, CKD-EPI_{Cys-C}, and CKD-$EPI_{Crea+Cys-C}$ equations. The BV of the individual subjects and the entire study population were also obtained.

Except for the age, blood pressure, and heart rate, all other characteristics of the subjects were significantly different. After eliminating outliers, we observed that S-Crea and S-Cys-C levels were higher for males than females, consistent with previously reported findings.[30]However, the eGFR of the individuals based on the new CKD-EPI equations were higher for females than males. This observation was found to be different from previously reported findings based on the old CKD-EPI equations. Further, males show a higher in eGFR values than females (males,106.75 ml/min/1.73 m^2 ;females,95.6 ml/min/1.73 m^2).[30] This may be due to the inconsistency of the estimation equation and the distribution of the study population. For example, Rowe[12] and Hilderink[24] studied eGFR values based on the old equations, which included race coefficients, using a different population size and study cohort. The eGFR values corresponding to the apparently healthy subjects included in this study were higher than those reported for patients with CKD and individuals without CKD[24] in these previous studies.[12, 24]

- Our results also indicated that the S-Crea-based CV_I and between-subject variation(CV_G) values of the apparently healthy subjects were 6.52% and 19.59%, respectively, higher than those based on EFLM data (CV_I,4.50%(4.20–5.70); CV_G,14.10%(7.00-17.40).[31] Hilderink[24] reported similar CV_I and CV_G values (6.40% 6.00-6.90 and 21.20% 15.70–32.90 , respectively) for subjects without CKD; however, their values for subjects with CKD were lower (4.40%(3.70–5.30) and 2.50% 2.40–2.70 , respectively).[12, 24] S-Crea concentrations are affected by a various factors, including sex, age, body weight, diet,[32] blood collection time interval, meal time, and region of study subjects, and subjects without CKD have lower baseline S-Crea levels.[24] Therefore, S-Crea levels have a considerable impact on CV_I and CV_G. and then the values reported in the EFLM database were calculated by meta-analysis and excluding short-term experiments.In this study, the S-Cys-C-based CV_I and CV_G values for the apparently healthy subjects were 5.81% and 12.79%, respectively. Compared with the values based on EFLM data (CV_I,4.00% 3.90–8.60 ; CV_G,12.10% 12.00-15.10),[31] the CV_I value obtained in this study was slightly higher, while the CV_G value was similar. However, our CV_I and CV_G values were higher and lower than previously reported values (CKD:4.00% 3.40–3.90 and 19.00% 14.40–28.20,[12]respectively; CKD: 3.20% 3.00-3.40 and 27.20% 20.40–40.80, respectively; and without CKD: 4.10% 3.80–4.40 and 15.30% 11.30–23.20 ,[24] respectively). Reportedly, the rate of S-Cys-C production is constant, and the influencing factors are slightly fewer compared with the S-Crea. Notwithstanding, S-Cys-C production is affected by a number of factors, including GFR, inflammation, hormone use, and thyroid function.[33] This study involved apparently healthy subjects with a median age of 33 years. However, the baseline GFR obtained was higher than those reported by Rowe[12] and Hilderink,[24] whose studies involved subjects aged over 60 years; this possibly resulted in the observed differences.Meanwhile the exclusion of outlier criteria in this study was inconsistent with the our previous study[26], where the S-Crea, S-Cys-C, and MDRD and CKD-EPI need to be met simultaneously with those within the X ± 3SD. Therefore, the BV results corresponding to S-Crea and S-Cys-C are slightly different.

The use of eGFR to determine renal disease progression is as reliable as the use of directly measured GFR values.[34]however, eGFR values obtained using different equations for the same apparently healthy subjects at different time periods may differ, and this may mislead physicians in making clinical decisions.[35]

Most foreign laboratories use MDRD to estimate GFR.[36] Notably, the CV_I values of eGFR obtained in this study based on the MDRD equation was the highest (8.39%), and was also higher than that those reported by Rowe[12] and Hilderink[24](CKD: 5.00%(4.30–6.10)[12], subjects were an elderly individuals and samples were collected once a week for 4 weeks; CKD:5.50% 5.20–5.90 , without CKD:6.10% 5.70–6.60 ,[24] subjects were elderly and samples were collected at 1-h intervals for 24 h). CV_I estimates obtained for different populations (individuals with different disease status, sex, age, etc.) and at different sampling times (short, medium, and long-term) vary considerably,[37–43]while MDRD, which was developed for a group with known renal disease status, tends to be less accurate at higher levels of renal function[34]; thus, for apparently healthy subjects, the MDRD based results obtained are not very accurate.

- The CV_I values based on the CKD-EPI_{Crea}, CKD-EPI_{Cys-C}, and CKD-$EPI_{Crea+Cys-C}$ equations (with race coefficients) reported by Rowe[12] and Hilderink[24] are very close (5.30%(4.50–6.40), 5.30%(4.50–6.40), and 5.00%(4.30–6.20),[12] respectively, without CKD:5.30%(5.10–5.60), 5.50%(5.20–5.90), and 4.6%(4.30-5.00), respectively, CKD:5.20%(4.90–5.60),7.30%(6.80–7.80), and 5.40%(5.00-5.80), respectively.[24]). In this study, the CV_I values of the eGFR obtained using the new CKD-EPI_{Crea}, CKD-EPI_{Cys-C}, and CKD-$EPI_{Crea+Cys-C}$ equations (without race coefficients) were 3.90% (3.49–4.42), 6.58%

(5.88–7.46) and 5.03% (4.50–5.71), respectively. Further, the CKD-EPI$_{Crea}$ based CV$_I$ value was the lowest, while that based on the CKD-EPI$_{Cys-C}$ equation was the highest. It is also worth noting that even though the values based on the CKD-EPI$_{Crea}$ and CKD-EPI$_{Cys-C}$ equations were slightly different from those reported in previous studies,[12, 24] that based on the CKD-EPI$_{Crea+Cys-C}$ equation was very similar to the previously reported values (5.03% vs. 5.0%[12] and 5.03% vs. 4.6%[24]). This observation may be attributed to non-GFR-determined S-Crea and S-Cys-C variable factors, which are independent of each other, and combining the eGFR based on these two factors can reduce the equation variance.[44] In this study, we used the new CKD-EPI equation of eliminate ethnic parameters to estimate GFR, standardize laboratory reports, and reduce the variability associated with the use of eGFR in clinical decision-making, while improving the quality of care for apparently healthy subjects and favoring ethnic harmony.[45]

The influence of the circadian rhythm on S-Crea and S-Cys-C concentrations[28, 45]affects the interpretation of eGFR values based on S-Crea and/or S-Cys-C levels. This can mislead clinicians to erroneously interpret random changes as clinically relevant changes.[46]In this study, no significant differences in S-Crea and S-Cys-C levels as well as eGFR values at different time point. Possibly, this resulted from the inconsistency that characterized the study population and the specimen collection time.

Individual variations affect the clinical application of reference interval. Reportedly, II is an indicator of the validity of the reference interval of a discriminant test item.[16] Specifically, II values > 1.4 imply that the CV$_G$I, Thus, any minor changes in the physiological state of apparently healthy subjects may immediately lead to the test value exceeding the reference interval. While the reverse was true at II values < 0.6.The II values obtained in this study using the MDRD, CKD-EPI$_{Crea}$, CKD-EPI$_{Cys-C}$, and CKD-EPI$_{Crea+Cys-C}$ equations were higher than the previously reported values (0.69, 0.48, 0.51, and 0.31 vs CKD: 0.20, 0.20, 0.20, and 0.20; without CKD: 0.20, 0.20, 0.30, and 0.20[12]; 0.30, 0.30, 0.20, and 0.30[24]). This may be related to the high variability of CV$_A$ and the age and health status of the subjects during laboratory analysis Our results also showed that the CV$_A$ based on S-Cys-C levels was < 1/2 CV$_I$, and the contribution of the analysis factor to its total change was < 12%.[28]This S-Crea CV$_A$ value > 1/2 CV$_I$ may be related to the time interval between the collection of blood samples, meal time, or the regional diet as well as the demographics of the study subjects.[24] However, the CV$_A$ of GFR estimated using the CKD-EPI$_{Crea}$ and CKD-EPI$_{Crea+Cys-C}$ equations basically conformed to the ideal ratio between CV$_I$ and CV$_A$, which is 1:2.[28] In this study, all the II value of eGFR based the MDRD and CKD-EPI equation were<1.4;Therefore, it is important for physicians to combine RCV when making clinical decisions using MDRD and CKD-EPI estimated GFR.[24]

There are biological and analytical variation on the results of the two tests performed for each sample, the numerical differences between the results could be considered as the sum of the inherent variation values, known as RCV.[47–48] Additionally, when evaluating the results of two consecutive tests, it is important to take into account changes in the RCV threshold as these have important clinical implications. Notably, RCV_{pos} and RCV_{neg}, respectively, are the thresholds for an increase or decrease between two experimental outcomes for the same individual at a given level of probability.[49] Further, in evaluating the variation of test results, when the difference between two consecutive test results exceeds the RCV_{pos} or RCV_{neg} values, a pathological mechanism can be considered.[15] In this study, the $RCV_{pos/neg}$ values of MDRD, $CKD\text{-}EPI_{Crea}$, $CKD\text{-}EPI_{Cys-C}$, $CKD\text{-}EPI_{Crea+Cys-C}$ equations were (29.30%,-22.66%), (12.69%,-11.26%), (20.97%,-17.33%), and (15.88%,-13.70%), respectively, which the MDRD and $CKD\text{-}EPI_{Crea}$ equations showing the highest and lowest values, respectively. Compared with the $RCV_{pos/neg}$ values previously reported by Rowe[12] based on the use of the 2006 MDRD and 2012 CKD-EPI equations for patients with CKD, the $RCV_{pos/neg}$ values for MDRD and $CKD\text{-}EPI_{Cys-C}$ obtained in this study were higher than the previously reported values(MDRD,15.10%,-13.10%[12]; $CKD\text{-}EPI_{Cys-C,}$ 15.90%,-13.80%[12]); however, that based on the $CKD\text{-}EPI_{Crea}$ equation obtained in this study was lower than the reported value (15.90,-13.70%),[12] while that based on the $CKD\text{-}EPI_{Crea+Cys-C}$ equation was comparable (15.10%,-13.10%).[12] Further, eGFR based on the MDRD equation showed the largest $RCV_{pos/neg}$ differences, probably because the diets of the study subjects were not standardized. Further, MDRD was developed for a group of subjects with known kidney disease, and this probably affected the reliability of the results for apparently healthy subjects.[34]

In this study, significant differences were also observed between the $RCV_{pos/neg}$ values corresponding to the individual eGFRs. The individual maximum $RCV_{pos/neg}$ values of the eGFR of the 30 apparently healthy subjects based on the MDRD, $CKD\text{-}EPI_{Crea}$, $CKD\text{-}EPI_{Cys-C}$, and $CKD\text{-}EPI_{Crea +Cys-C}$ equations were (56.51%,-36.11%), (20.99%,-17.35%), (44.93%,-31.00%), and (28.83%,-22.38%), respectively, while the individual minimum values were (12.36%,-11.00%), (5.32%,-5.05%), (5.76%,-5.45%), and (5.01%,-4.77%), respectively. For example, when using the individual maximum RCV_{pos} for a patient with an eGFR baseline of 40 ml/min/1.73 m^2, the MDRD increased to > 62.60 and the $CKD\text{-}EPI_{Crea}$ increased to > 48.40. Under such conditions, evaluating CKD using the eGFR based on the MDRD equation may lead to the classification of CKD-G3b as CKD-G2. Conversely, if the $CKD\text{-}EPI_{Crea}$ equation is used, CKD-G3b may then be classified as G3a, which may mislead clinicians to change the patient's treatment regimen, thereby worsening the patient's condition. Similarly, when using individual maximum RCV_{neg} value for a patient with an eGFR baseline of 90 ml/min/1.73 m^2, the MDRD decreased to < 57.50, while the $CKD\text{-}EPI_{Crea}$ decreased to < 74.39. This may lead to the misclassification of the apparently healthy subjects as having CKD-G3a when using eGFR based on the MDRD equation for CKD staging, and if the eGFR based on the $CKD\text{-}EPI_{Crea}$ equation is used, the subjects may be misclassified as patients with CKD-G2, meanwhile patients with different CKD stages have different treatment plans. Therefore, incorrect staging can lead to untimely treatment or excessive medical treatment, thereby increasing pain as well as the economic burden on the patients. It has also been observed that the $RCV_{pos/neg}$ value corresponding to a population of apparently healthy subjects is quite different from that corresponding to individual subjects. Therefore, the correct use of $RCV_{pos/neg}$ can prevent clinicians from misinterpreting random errors as clinically relevant changes owing to cognitive limitations; this is particularly important for analyzing and judging patients' conditions.[15]

The strengths of this study include the construction of the 24-h concentration profiles of S-Crea and S-Cys-C and their use to estimate GFR values based on the new 2021 CKD-EPI equation for apparently healthy subjects. Further, by studying the eGFR of apparently healthy subjects, this study provides a BV reference for the clinical use of the new CKD-EPI equations to estimate GFR.

This study had some limitations. First, even though the study involved apparently healthy subjects with a uniform meal time (08:00, 12:00, and 19:00), the meal content is not standardized. Second, the sample size was very small, and the geographical distribution of the subjects was relatively limited. Therefore, in future, it would be necessary to validate the findings of this study by recruiting a greater number of subjects representing a wider geographical region. Third, even though this study involved the estimation of GFR within 24 h, the blood sample collection interval was 4 h, i.e., blood samples were not collect at 1-h intervals. Thus, the results obtained do not accurately reflect the 24-h eGFR changes in the body. Finally, all the subjects included in this study were apparently healthy subjects; therefore, in subsequent studies, it would be necessary to include stable CKD subjects.

5. Conclusion

we observed that the CV_I value of eGFR estimated using the new $CKD\text{-}EPI_{Crea}$ equation was the smallest, while that estimated using the MDRD equation was the largest. Further, the RCV_{pos} and RCV_{neg} values obtained using the MDRD equation had a wider range than those obtained using the new CKD-EPI equations. We also observed that the II values obtained based on the MDRD and CKD-EPI equation were<1.4;Furthermore, our results indicated a significant difference between the apparently healthy subject population and individual subjects with respect to $RCV_{pos/neg}$ values.So when using eGFR values based on MDRD and CKD-EPI equations in clinical practice, it is necessary to take the $RCV_{pos/neg}$ values into account when interpreting eGRF data and analyzing kidney disease.

Declarations

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Author's contributions

ChaoQiong Zhou, Qianrong Xie drafted the manuscript and analyzed the data. HuaLi Wang, Feng Wu and DaHai He revised the manuscript. Ying Huang, Ying He, ShiRong Dai and Jie Chen collected the data. Yan

Zhang and Lirui Kong designed this work and revised the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Pidu District Hospital of Traditional Chinese Medicine, Chengdu, and written informed consent was obtained from all the participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

All participants agree to the publication of the article.

Competing interests

Authors state no conflict of interest.

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Figures

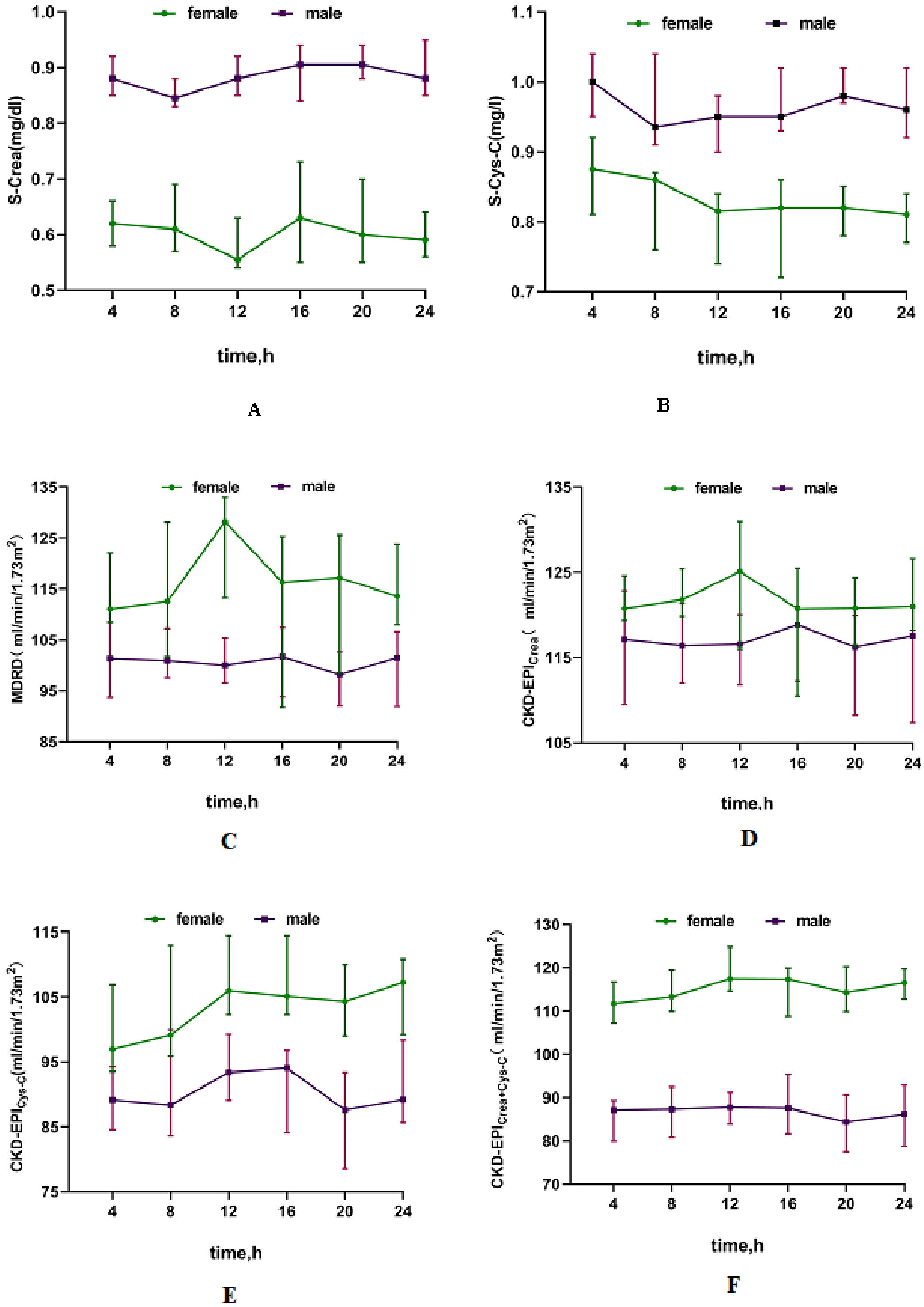

Figure 1

Distribution of variation profiles by sex

(Corresponding values for continuous data are shown as median (95%CI))

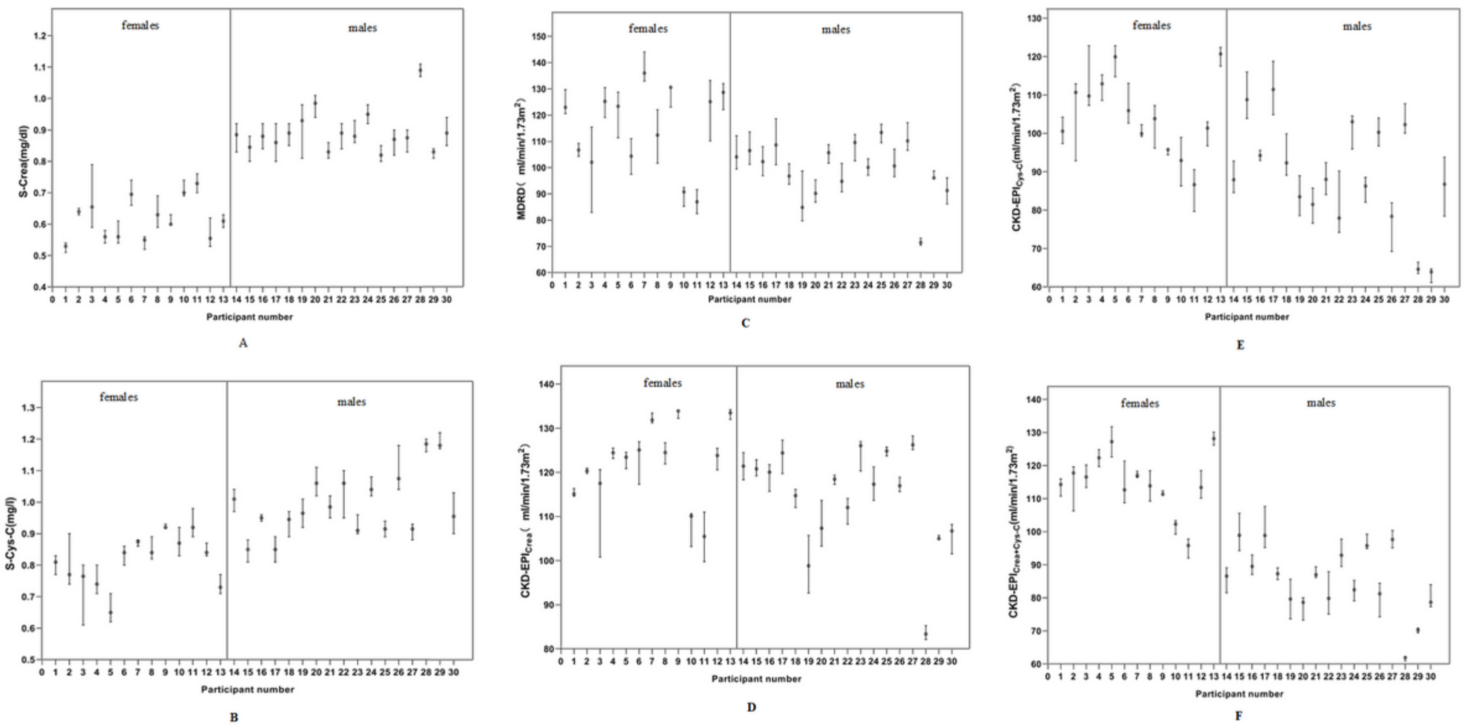

Figure 2

The median (points) and 95% confidence interval of variation profiles in 30 apparently healthy individuals.

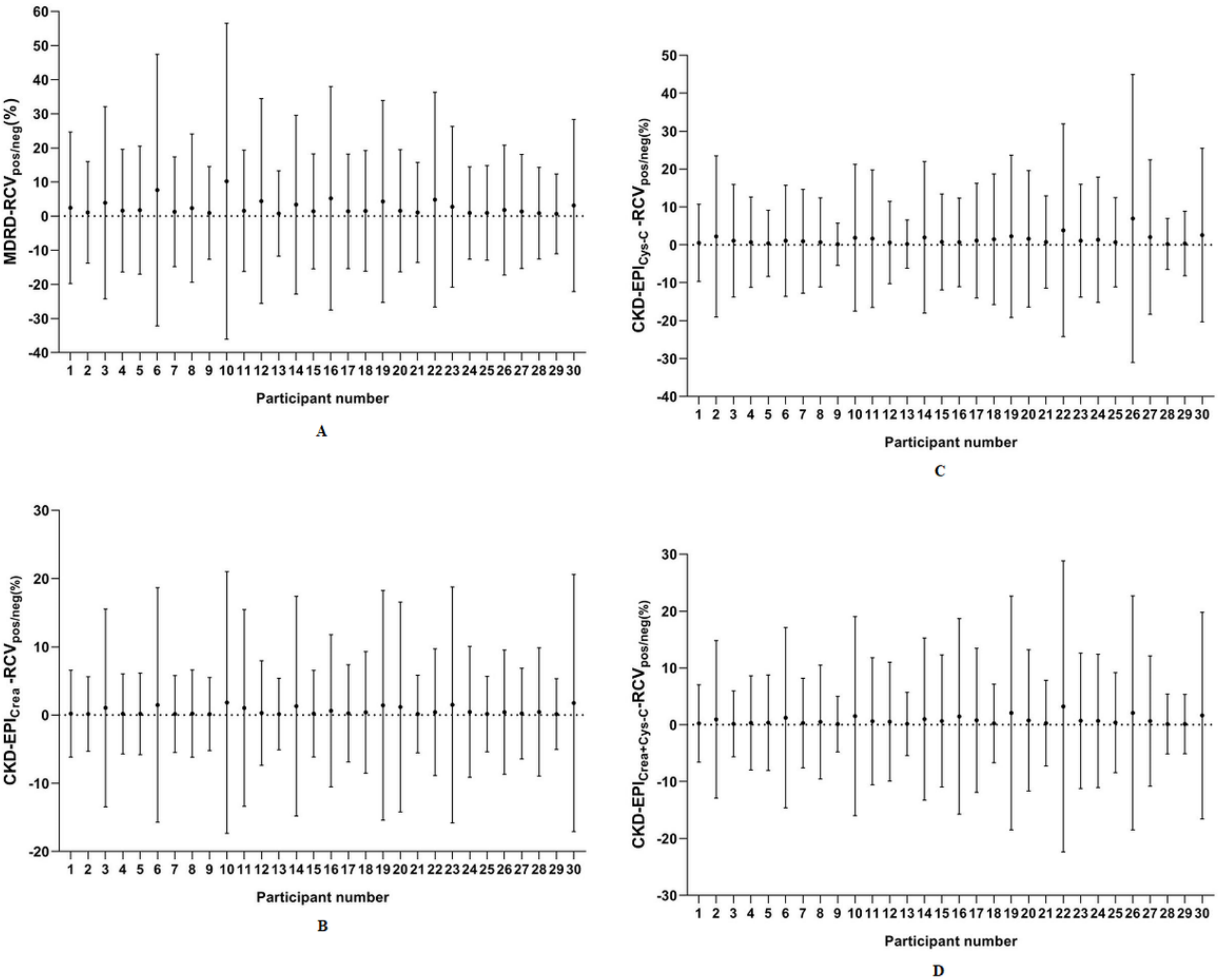

Figure 3

Positive and negative reference change values in 30 apparently healthy individuals.

(the data are shown as median (range))