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Muscle mass and estimates of renal function: a longitudinal cohort study

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Abstract

Background Creatinine is the most widely used test to estimate the glomerular filtration rate (GFR), but muscle mass as key determinant of creatinine next to renal function may confound such estimates. We explored effects of 24-h height-indexed creatinine excretion rate (CER index) on GFR estimated with creatinine ($eGFR_{Cr}$), muscle mass-independent cystatin C ($eGFR_{Cys}$), and the combination of creatinine and cystatin C ($eGFR_{Cr\text{-}Cys}$) and predicted probabilities of discordant classification given age, sex, and CER index.

Methods We included 8076 adults enrolled in the PREVEND study. Discordant classification was defined as not having $eGFR_{Cr}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min/1.73 m^2. Baseline effects of age and sex on CER index were quantified with linear models using generalized least squares. Baseline effects of CER index on eGFR were quantified with quantile regression and logistic regression. Effects of annual changes in CER index on trajectories of eGFR were quantified with linear mixed-effects models. Missing observations in covariates were multiply imputed.

Results Mean (SD) CER index was 8.0 (1.7) and 6.1 (1.3) mmol/24 h per meter in male and female participants, respectively ($P_{difference} < 0.001$). In male participants, baseline CER index increased until 45 years of age followed by a gradual decrease, whereas a gradual decrease across the entire range of age was observed in female participants. For a 70-year-old male participant with low muscle mass (CER index of 2 mmol/24 h per meter), predicted baseline $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 24.7 mL/min/1.73 m^2 (and 30.1 mL/min/1.73 m^2 when creatinine was not corrected for race). Percentages (95% CI) of discordant classification in male and female participants aged 60 years and older with low muscle mass were 18.5% (14.8–22.1%) and 15.2% (11.4–18.5%), respectively. For a 70-year-old male participant who lost muscle during follow-up, $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 1.5, 5.0, 8.5, and 12.0 mL/min/1.73 m^2 (and 6.7, 10.7, 13.5, and 15.9 mL/min/1.73 m^2 when creatinine was not corrected for race) at baseline, 5 years, 10 years, and 15 years of follow-up, respectively.

Conclusions Low muscle mass may cause considerable overestimation of single measurements of $eGFR_{Cr}$. Muscle wasting may cause spurious overestimation of repeatedly measured $eGFR_{Cr}$. Implementing muscle mass-independent markers for estimating renal function, like cystatin C as superior alternative to creatinine, is crucial to accurately assess renal function in settings of low muscle mass or muscle wasting. This would also eliminate the negative consequences of current race-based approaches.

Keywords Muscle mass; Renal function; Creatinine; Cystatin C; General population; Bias

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Introduction

The glomerular filtration rate (GFR) is accepted as the best overall measure of renal function.[1] Assessment of the GFR is part of everyday medical care in both clinical and outpatient practice.[2] Current guidelines recommend using serum creatinine to obtain an estimated GFR (eGFR) as initial assessment,[3,4] which appears to be the most frequently reported proxy of renal function.[5,6] However, determinants of creatinine other than renal function may lead to imprecise estimates,[7] compromising the diagnosis and management of chronic kidney disease when eGFR is $<$60 mL/min per 1.73 m^2 of body-surface area.

One fundamental determinant of creatinine concentrations is muscle mass. Approximately 98% of circulating creatinine stems from muscle, where it is nonenzymatically converted (from creatine) and secreted at a constant rate.[8,9] In the absence of overt renal failure, creatinine is almost exclusively excreted in the urine.[9,10] Given that muscle mass typically shows no abrupt fluctuations, serum creatinine is reciprocally related to the GFR and, under steady-state conditions, creatinine production must equal excretion irrespective of serum concentrations. Based on this principle, Bürger introduced the creatinine excretion rate (CER) as accurate marker of total muscle mass.[11]

Although the influence of muscle mass on eGFR (operating through serum creatinine) is partially taken into account by correcting serum creatinine for an individual's age, sex, and race[7,12] (or more recently only age and sex without race[13]), estimating the GFR with creatinine implicitly assumes equal muscle mass in individuals of the same age, sex, and race. This unrealistic assumption leaves space for muscle mass to exert residual impact on creatinine-based eGFR—a potential pitfall that warrants further investigation. Additionally, trajectories of creatinine-based eGFR are prone to bias as muscle wasting beyond the age-associated loss of muscle mass will cause overestimation of the GFR. Notably, patients with chronic illnesses, characterized by gradual muscle wasting,[14] often require the most intensive monitoring of the GFR and overestimation will thus be most pronounced herein. We aimed to explore these limitations by quantifying effects of CER on creatinine-based eGFR ($eGFR_{Cr}$)[7,13] at baseline and of annual changes in CER on trajectories of $eGFR_{Cr}$ and compare those effects with effects on estimates of renal function based on cystatin C ($eGFR_{Cys}$)[12,13]—a marker of renal function that is independent of muscle mass[2]—and the combination of creatinine and cystatin C ($eGFR_{Cr\text{-}Cys}$)[12,13] in a cohort of community-dwelling individuals from the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study. We additionally assessed percentages of discordantly classified participants and built a model to predict future probabilities of discordant classification given age, sex, and CER index.

Methods

Study population and design

The PREVEND study prospectively investigates risk factors for and the prevalence and consequences of microalbuminuria in otherwise healthy adults in the city of Groningen (the Netherlands). We included 8076 participants with available data on serum creatinine and cystatin C. Detailed information on the objectives and study design as well as participant flow through the study is provided in the supporting information (*Method* S1 and *Figure* S1). The PREVEND study has been approved by the local medical ethics committee and was undertaken in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Laboratory measurements and definitions

Each screening comprised two visits to an outpatient clinic separated by 3 weeks. Self-administered questionnaires concerning demographics, cardiovascular and renal disease history, smoking habits, and medication use were provided by all participants prior to the first visit of each screening. Renal function was estimated with the serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations from 2009[7] and 2021[13] and the cystatin C-based CKD-EPI equations from 2012.[12] The serum creatinine-based equations from 2021 that have been used did not include a coefficient for race (black vs. non-black). Additional information on definitions is provided in *Method* S2.

Participants collected two consecutive 24-h urine specimens after thorough oral and written instruction. During collection, participants were asked to refrain from heavy exercise and instructed to postpone urine collection in case of urinary tract infection, menstruation, or fever. Collected urine was subsequently stored cold (4°C) for a maximum of 4 days before the second visit. Blood samples were drawn between 8:00 and 10:00 AM from all participants, and aliquots were immediately stockpiled at −80°C until analysis. Serum creatinine was measured with an enzymatic method on a Roche Modular analyser, using reagents and calibrators from Roche (Roche Diagnostics, Mannheim, Germany). Serum cystatin C was measured with the Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Roche Modular analyser and was calibrated directly with the standard supplied by the manufacturer. The coefficients of variation for total imprecision at 0.85 and 2.97 mg/L were 1.8% and 3.0%, respectively. Urinary creatinine was measured by dry chemistry (Eastman Kodak, Rochester, USA). Urinary albumin was measured by nephelometry (Dade Behring BNII, Marburg, Germany). Concentrations of urine markers were multiplied by urine volume to obtain a value in units per 24 h. The mean value of the paired 24-h

urine collections was calculated for each screening round. Additional information on the coefficients of variation for total imprecision is provided in *Method* S2.

Statistical analyses

Several participants had missing observations for one or more covariates (*Table* S1). To account for and reduce potential bias due to missing data,[15] multiple imputation of incomplete covariates using fully conditional specification was performed to obtain 10 imputed data sets. Analyses were performed in each of the data sets and results were pooled using Rubin's rules.[16] Details on the imputation procedure are provided in *Method* S3A. Only participants for whom data on serum creatinine and cystatin C was available at baseline were included in the cross-sectional analyses. For longitudinal analyses, all participants were included. Baseline characteristics are expressed as mean (SD), median (interquartile range), or number (percentage) for normally distributed, skewed, and categorical data, respectively. The CER was indexed by height, because muscle mass highly depends on body size. This height-indexed CER is hereafter referred to as 'CER index'. *P* for sex difference in baseline CER index was computed using an unequal variances *t*-test.

Baseline effects of age and sex on CER index were quantified with linear models using generalized least squares, specifying sex and a non-linear effect of age as main effects as well as their product term. Details on these models are provided in *Method* S3B. Effects of CER index on $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ at baseline were investigated using quantile regression models for the conditional median eGFR, specifying the main effects of age and sex and a non-linear effect of CER index, all two-way interactions, and the three-way interaction. These models were additionally adjusted for various potential confounders, which are listed in *Method* S3C. Model parameters were computed in each of the 10 imputed data sets. The standard errors, confidence intervals, and *P* values of the various model terms were computed using a bootstrapping procedure.[17] Details on these models are provided in *Method* S3C. Model parameters along with their confidence intervals and *P* values are presented in *Tables* S3 and S4). The results are displayed visually to facilitate their interpretation.

Discordant classification was defined as not having $eGFR_{Cr}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min per 1.73 m^2. The 25th and 75th percentiles of age and CER index were used as cut points to obtain three categories of these covariates for which sex-specific numbers and percentages of discordantly classified participants per category were assessed. In sensitivity analyses, discordant classification was defined as not having $eGFR_{Cr\text{-}Cys}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min per 1.73 m^2 using the same categories of age and CER index. To predict probabilities of discordant classification given age, sex, and CER index, a logistic regression model was built, specifying sex and non-linear effects of age and CER index as main effects. Natural cubic splines with two degrees of freedom were used to model potential non-linear effects of age and CER index. Model parameters along with their confidence intervals and *P* values are presented in *Table* S7.

Annual changes in CER index were obtained using subject-specific slope estimates of a linear mixed model for CER index with fixed and random effects for intercept and time. *P* for sex difference in annual changes in CER index was computed using an unequal variances *t*-test. Effects of annual changes in CER index on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ were quantified using linear mixed-effects models. These models were adjusted for various potential confounders, which are listed in *Method* S3D. Model parameters along with their confidence intervals and *P* values are presented in *Tables* S8 and S9. Statistical analyses were performed with R Version 4.1.2 (Vienna, Austria). A two-sided $P < 0.05$ was considered to indicate statistical significance.

Results

Baseline characteristics

Baseline characteristics of imputed cohort data are shown in *Table* 1. These characteristics were almost identical to the baseline characteristics of unimputed cohort data (*Table* S1). Mean (SD) age of the 7943 participants (49.7% male) was 49.7 (12.7) years. Mean CER index was 8.0 (1.7) and 6.1 (1.3) mmol/24 h per meter in male and female participants, respectively (*P* for sex difference < 0.001; *Figure* 1A). In male participants, baseline CER index increased until 45 years of age, after which it gradually decreased, whereas a gradual decrease across the entire range of age was observed in female participants (*Figure* 1B; *Table* S2). Mean $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ was 96.4 (16.1), 92.5 (19.3), and 94.8 (17.2) mL/min per 1.73 m^2, respectively. Notably, 188 (2.4%) had $eGFR_{Cr}$ <60 mL/min per 1.73 m^2, whereas 472 (5.9%) and 258 (3.2%) had $eGFR_{Cys}$ and $eGFR_{Cr\text{-}Cys}$ <60 mL/min per 1.73 m^2, respectively.

Baseline effects of muscle mass on estimates of renal function

Model parameters along with their confidence intervals and *P* values are presented in *Tables* S3 and S4). Higher CER index was associated with lower $eGFR_{Cr}$ and higher $eGFR_{Cys}$ (*Figure* 2A,B). This discrepancy was largest for low CER indices and older age. The regression line that depicts the effect of CER index on $eGFR_{Cr\text{-}Cys}$ runs almost exactly in between the re-

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Table 1 Characteristics of the imputed cohort data at baseline[a]

Characteristic	Total (n = 7943)
Sociodemographic characteristics	
Age, mean (SD), years	49.7 (12.7)
Categories of age, no. (%), years	
<40	2165 (27.3)
40–50	2170 (27.3)
50–60	1611 (20.3)
60–70	1409 (17.7)
>70	588 (7.4)
Male sex, no. (%)	3950 (49.7)
Race, no. (%)	
Caucasian	7589 (95.5)
Negroid	79 (1.0)
Asian	167 (2.1)
Other	108 (1.4)
Education, no. (%)	
Low	3591 (45.2)
Middle	1991 (25.1)
High	2361 (29.7)
Current smoking, no. (%)	2689 (33.9)
Prevalent type 2 diabetes, no. (%)	285 (3.6)
Prevalent cardiovascular disease, no. (%)	408 (5.1)
Body composition	
Height-indexed CER, mean (SD), mmol/24 h per meter	7.0 (1.8)
Male participants	8.0 (1.7)
Female participants	6.1 (1.3)
Estimated 10-year change in CER index, mean (SD), mmol/24 h per meter	−0.22 (0.23)
Male participants	−0.19 (0.26)
Female participants	−0.24 (0.19)
Waist circumference, mean (SD), cm	88.4 (13.0)
Male participants	93.8 (11.1)
Female participants	83.1 (12.7)
Haemodynamics	
Diastolic blood pressure, mean (SD), mmHg	128.8 (20.2)
Systolic blood pressure, mean (SD), mmHg	73.9 (9.7)
Lipid spectrum	
Total cholesterol, mean (SD), mmol/L	5.6 (1.1)
High-density lipoprotein cholesterol, mean (SD), mg/dL	1.3 (0.4)
Total cholesterol/high-density lipoprotein cholesterol ratio, mean (SD)	4.7 (1.8)
Triglycerides, median (IQR), mg/dL	1.15 (0.84–1.68)
Renal function parameters[b]	
$eGFR_{Cr}$, mean (SD), mL/min per 1.73 m^2	96.4 (16.1)
$eGFR_{Cys}$, mean (SD), mL/min per 1.73 m^2	92.5 (19.3)
$eGFR_{Cr\text{-}Cys}$, mean (SD), mL/min per 1.73 m^2	94.8 (17.2)
$eGFR_{Cr}$ < 60 mL/min per 1.73 m^2, no. (%)	188 (2.4)
$eGFR_{Cys}$ < 60 mL/min per 1.73 m^2, no. (%)	472 (5.9)
$eGFR_{Cr\text{-}Cys}$ < 60 mL/min per 1.73 m^2, no. (%)	258 (3.2)
Urinary albumin excretion, median (IQR), mg/24 h	9.4 (6.3–17.6)
Categories of urinary albumin excretion, no. (%), mg/24 h	
<15	5600 (70.5)
15–29.9	1161 (14.6)
30–300	1060 (13.4)
>300	121 (1.5)
Medication, no. (%)	
Antihypertensive drugs	1232 (15.5)
Lipid-lowering drugs	505 (6.4)
Attendance of screening rounds, no. (%)[c]	
Baseline	7943 (100)
Second	6397 (80.5)
Third	5513 (69.4)
Fourth	4655 (58.6)
Fifth	3247 (40.9)

CER, creatinine excretion rate; $eGFR_{Cr}$, creatinine-based estimated glomerular filtration rate; $eGFR_{Cr\text{-}Cys}$, creatinine-cystatin C-based estimated glomerular filtration rate; $eGFR_{Cys}$, cystatin C-based estimated glomerular filtration rate.

[a]Percentages may not total 100 because of rounding.

[b]Estimates of GFR were based on the serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation from 2009[7] and the cystatin C-based CKD-EPI equations from 2012.[12]

[c]Percentages of participants are with respect to the total number of participants that attended the first screening round.

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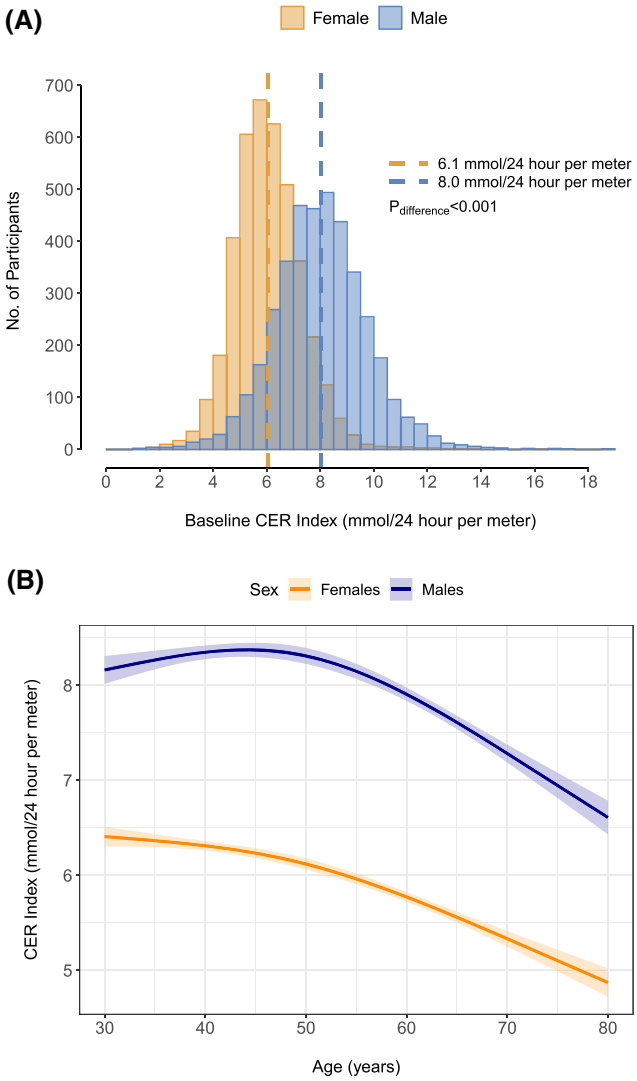

Figure 1 Distribution and conditional mean of height-indexed creatinine excretion rate according to age and sex. (*A*) Histogram plots showing that the distribution of male participants (blue) is shifted towards the right compared with female participants (orange), indicating that male participants had a higher muscle mass at baseline ($P < 0.001$). The respective means are given by the dashed vertical lines. (*B*) Effects of age and sex on height-indexed creatinine excretion rate (CER index). Effect estimates were obtained with a generalized least squares regression model.

gression lines that depict the effects of CER index on $eGFR_{Cr}$ and $eGFR_{Cys}$, which is consistent with the fact that the equation for $eGFR_{Cr\text{-}Cys}$ is based both on serum creatinine and cystatin C. For a 50-year-old and 70-year-old male participant with a CER index of 2 mmol/24 h per meter (indicating low muscle mass), the expected median $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 19.3 and 24.7 mL/min per 1.73 m^2, respectively (*Figure* 2A,B). Point estimates were of comparable magnitude after adjustment for sociodemographic and cardiovascular risk factors (*Table* S3). The disagreement between the expected median $eGFR_{Cr}$ and $eGFR_{Cys}$ was largest when the GFR was estimated with the most recent estimating equation from 2021 (which does not consider the effect of race on serum creatinine).[13] For 50-year-old and 70-year-old male participants with a CER index of 2 mmol/24 h per meter, the expected median $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 26.0 and 30.1 mL/min per 1.73 m^2, respectively (*Figure* S3A,B). To further illustrate the contrast between $eGFR_{Cr}$ and $eGFR_{Cys}$ in settings of low muscle mass, the predicted ages at which $eGFR_{Cr}$ and $eGFR_{Cys}$ would have crossed the diagnostic threshold of 60 mL/min per 1.73 m^2 for detecting CKD were 102.8 and 73.0 (difference: 29.8) years, respectively. When the GFR was estimated with the estimating equation from 2021,[13] the predicted age at which $eGFR_{Cr}$ would have crossed the threshold of 60 mL/min per 1.73 m^2 increased to 115.9 (difference with $eGFR_{Cys}$: 42.9) years.

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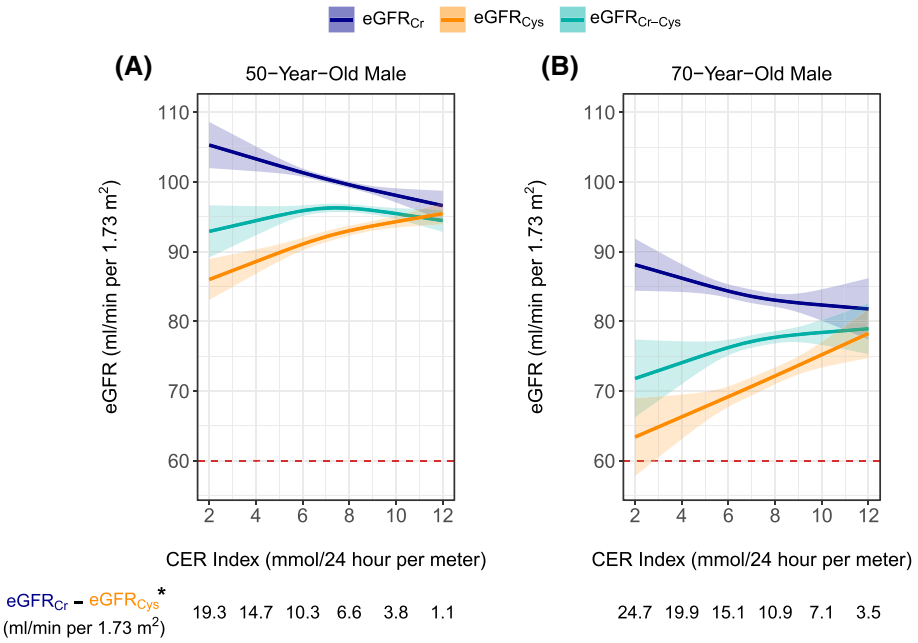

Figure 2 Effects of height-indexed creatinine excretion rate on creatinine-based, cystatin C-based, and creatinine-cystatin C-based eGFR at baseline. (*A*) Effects of height-indexed creatinine excretion rate (CER index) on creatinine-based eGFR ($eGFR_{Cr}$), cystatin C-based eGFR ($eGFR_{Cys}$), and creatinine-cystatin C-based eGFR ($eGFR_{Cr\text{-}Cys}$) for a 50-year-old male participant. (*B*) Effects of CER index on $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 70-year-old male participant. Effect estimates were obtained with quantile regression models for the median $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$. The red dashed line refers to the threshold of eGFR 60 ml/min per 1.73 m^2 for the detection of chronic kidney disease. *Expected numerical differences between $eGFR_{Cr}$ and $eGFR_{Cys}$ at CER indices of 2, 4, 6, 8, 10, and 12 mmol/24 h per meter.

Observed percentage and expected probability of discordant classification

Lower CER index was associated with higher percentages of participants discordantly classified as not having $eGFR_{Cr}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min per 1.73 m^2 (*Figure* 3A; *Table* S5). Importantly, when age was taken into account, discordant classification almost exclusively occurred in participants aged 60 years and older. In participants aged 40 years and younger, percentages of discordant classification were approximately 0%, irrespective of CER index and sex. These percentages (95% CI) slightly increased to 2.8% (1.1–4.4%) in male participants and 1.1% (0.1–2.1%) in female participants aged 40 to 60 years only when muscle mass was low (i.e. CER index below the 25th percentile). In the highest age category, percentages of discordant classification were highly dependent on CER index. In participants aged 60 years and older with low muscle mass, the percentage of discordant classification was 18.5% (14.8–22.1%) in male participants and 15.2% (11.4–18.5%) in female participants. However, when muscle mass was high (i.e. CER index above the 75th percentile), these percentages were considerably lower, namely, 8.0% (4.0–12.1%) in male participants and 9.3% (4.1–14.6%) in female participants (*Figure* 3A). When the definition of discordant classification was changed to not having $eGFR_{Cr\text{-}Cys}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min per 1.73 m^2, percentages of discordant classification only slightly lowered in magnitude, but the underlying pattern persisted (*Figure* S2; *Table* S6). Expected probabilities of discordant classification for any given age, sex, and CER index are shown in *Figure* 3B. The corresponding model parameters along with their confidence intervals and *P* values are presented in *Table* S7. Individuals aged 40 years and younger are not expected to be discordantly classified, as probabilities of discordant classification are approximately 0%, irrespective of sex and CER index (*Figure* 3B). However, beyond the age of 40, probabilities of discordant classification considerably rise with older age and higher CER indices. Besides, discordant classification is expected to occur more often in male participants than female participants (*Figure* 3B).

Effects of changes in muscle mass with trajectories of renal function

For each 10 years of follow-up, the estimated mean (SD) decrease in CER index (indicating muscle wasting) was 0.19 (0.26) and 0.24 (0.19) mmol/24 h per meter in male and female participants, respectively (*P* for sex difference < 0.001; *Figure* S3A). Annual changes in CER index did not substantially differ with age (*Figure* S3B). Model parameters along with their confidence intervals and *P* values are presented

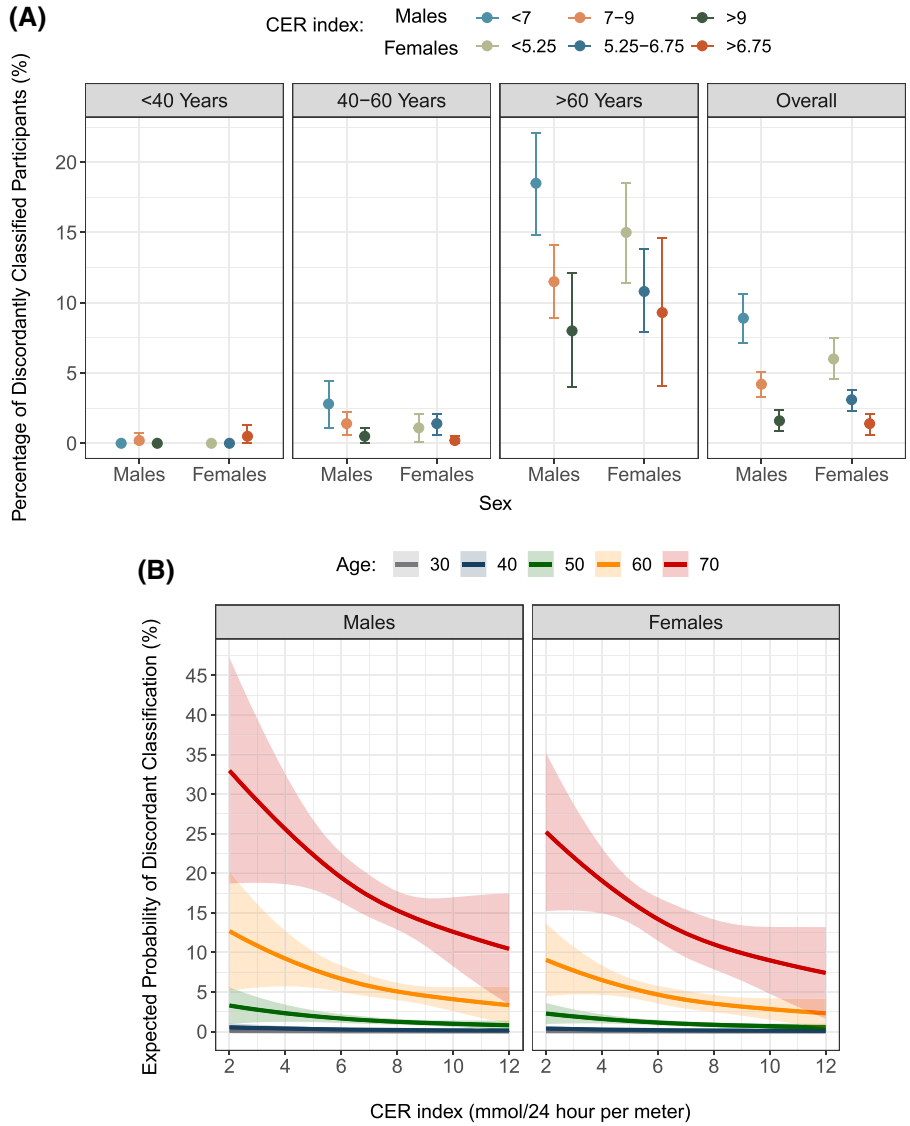

Figure 3 Observed percentages and expected probability of discordant classification according to age, sex, and height-indexed creatinine excretion rate. (*A*) Dot plot showing the observed percentages of discordantly classified participants according to categories of age, sex, and cut-off values of height-indexed creatinine excretion rate (CER index). Cut-off values were based on the 25th and 75th percentiles of CER index in male and female participants separately. The error bars about the dots represent the 95% confidence intervals. (*B*) Expected probability of discordant classification based on age, sex, and CER index. The lines represent the expected probability of discordant classification for any given age, sex, and CER index and were obtained with logistic regression. The shaded areas about the lines are the corresponding 95% pointwise confidence intervals. Discordant classification was defined as not having a creatinine-based eGFR <60 mL/min per 1.73 m^2 when cystatin C-based eGFR was <60 mL/min per 1.73 m^2.

in *Tables* S8 and S9. Muscle wasting was associated with decreased deterioration in $eGFR_{Cr}$ and increased deterioration in $eGFR_{Cys}$ (*Figure* 4A–F). This discrepancy became especially apparent when the deterioration in eGFR was expressed as percentage difference between eGFR at baseline and 15 years of follow-up, that is $(eGFR_{0\ years} - eGFR_{15\ years})/eGFR_{0\ years} \times 100\%$. For a 50-year-old male participant who experienced muscle wasting, had stable muscle mass, or gained muscle during follow-up, the percentage difference for $eGFR_{Cr}$ increased from 9.5% to 11.4% to 12.4% in the respective scenarios, whereas the percentage difference for $eGFR_{Cys}$ decreased from 14.8% to 12.1% to 10.7% (*Figure* 4A–C). The discrepancy between $eGFR_{Cr}$ and $eGFR_{Cys}$ concerning the percentage differences aggravated with increasing age. For a 70-year-old male participant who lost muscle mass, had stable muscle mass, or gained muscle mass during follow-up, the percentage difference for $eGFR_{Cr}$ increased from 11.3% to 13.6% to 14.8% in the respective scenarios, whereas the percentage difference for $eGFR_{Cys}$ decreased from 25.2% to 22.2% to 20.5% (*Figure* 4D–F). Estimating

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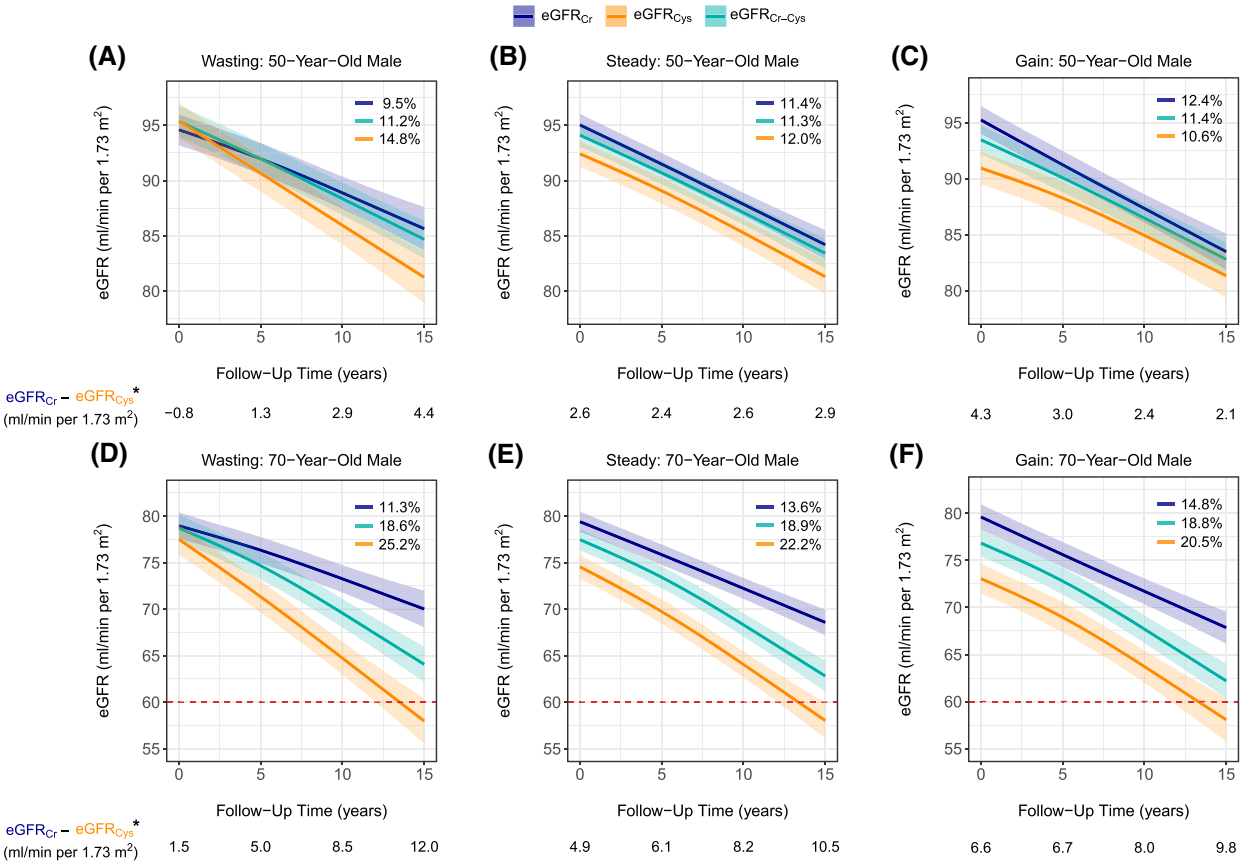

Figure 4 Effects of annual changes in height-indexed creatinine excretion rate on trajectories of creatinine-based, cystatin C-based, and creatinine-cystatin C-based eGFR. (*A*) Effects of muscle wasting on trajectories of creatinine-based eGFR ($eGFR_{Cr}$), cystatin C-based eGFR ($eGFR_{Cys}$), and creatinine-cystatin C-based eGFR ($eGFR_{Cr\text{-}Cys}$) for a 50-year-old male participant. (*B*) Effects of steady muscle mass on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 50-year-old male participant. (*C*) Effects of gain of muscle on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 50-year-old male. (*D*) Effects of muscle wasting on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 70-year-old male participant. (*E*) Effects of steady muscle mass on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 70-year-old male participant. (*F*) Effects of gain of muscle on trajectories of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$ for a 70-year-old male participant. The lines represent effect estimates for 50-year-old and 70-year-old male participants with all covariates at their median value, obtained with linear mixed-effects models for the multivariate outcomes of $eGFR_{Cr}$, $eGFR_{Cys}$, and $eGFR_{Cr\text{-}Cys}$. The shaded areas about the lines are the corresponding 95% pointwise confidence intervals. The three different categories of annual change in CER index (i.e. muscle wasting, steady muscle mass, and gain of muscle mass) amounted to −0.10, 0.00, and 0.05 mmol/24 h per meter, respectively. *Expected numerical differences between $eGFR_{Cr}$ and $eGFR_{Cys}$ at 0, 5, 10, and 15 years of follow-up are given below the graphs. In the top right corner of each panel, the percentage difference in renal function between baseline and 15 years of follow-up are given, which was expressed as $(eGFR_{0\ years} - eGFR_{15\text{-}years})/eGFR_{0\ years} \times 100\%$.

the GFR with the most recent estimating equation from 2021[13] did not materially affect these percentage differences (*Figure* S4A–F).

The regression lines that depict the effects of annual changes in CER index on trajectories of $eGFR_{Cr\text{-}Cys}$ run almost exactly in between the regression lines that depict the effects of annual changes in CER index on trajectories of $eGFR_{Cr}$ and $eGFR_{Cys}$, which is consistent with the fact that the equation for $eGFR_{Cr\text{-}Cys}$ is based both on serum creatinine and cystatin C. The slopes of the trajectories of $eGFR_{Cr\text{-}Cys}$ were the same regardless of whether muscle mass was lost (*Figure* 4A,D), remained stable (*Figure* 4B,E), or gained during follow-up (*Figure* 4C,F). Indeed, no statistically significant interaction between annual changes in CER index and time was found in models with $eGFR_{Cr\text{-}Cys}$ as outcome (*Table* S8).

Numerically, for a 50-year-old male participant who lost muscle mass during follow-up, $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by −0.8, 1.3, 2.9, and 4.4 mL/min per 1.73 m^2 at baseline, 5 years, 10 years, and 15 years of follow-up, respectively. For a 70-year-old male participant who lost muscle mass during follow-up, these disagreements increased substantially to 1.5, 5.0, 8.5, and 12.0 mL/min per 1.73 m^2 at baseline, 5 years, 10 years, and 15 years of follow-up, respectively (*Figure* 4A,D). Discrepancies between $eGFR_{Cr}$ and $eGFR_{Cys}$ were relatively constant when muscle mass was steady (*Figure* 4B,E) or gained during follow-up (*Figure* 4C,F). The disagreement

between $eGFR_{Cr}$ and $eGFR_{Cys}$ was largest when the GFR was estimated with the most recent estimating equation from 2021.[13] For a 50-year-old male participant who lost muscle mass during follow-up, $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 3.1, 5.4, 7.4, and 9.1 mL/min per 1.73 m^2 at baseline, 5 years, 10 years, and 15 years of follow-up, respectively. For a 70-year-old male participant who lost muscle mass during follow-up, these disagreements further increased to 6.7, 10.7, 13.5, and 15.9 mL/min per 1.73 m^2 at baseline, 5 years, 10 years, and 15 years of follow-up, respectively (*Figure* S4A,D).

Discussion

This study investigated effects of muscle mass, approximated by CER index, on $eGFR_{Cr}$ in community-dwelling individuals and compared those effects with effects of muscle mass on $eGFR_{Cys}$ and $eGFR_{Cr\text{-}Cys}$. Lower baseline CER index was associated with higher $eGFR_{Cr}$ and lower $eGFR_{Cys}$. Consequently, nearly one out of five male individuals and one out of six female individuals aged 60 years and older with low muscle mass were discordantly classified as not having $eGFR_{Cr}$ <60 mL/min per 1.73 m^2 when $eGFR_{Cys}$ was <60 mL/min per 1.73 m^2. When repeated measurements of eGFR and CER index were used, muscle wasting was associated with progressive disagreement between trajectories of $eGFR_{Cr}$ and $eGFR_{Cys}$. In all cases, effects of CER index on $eGFR_{Cr\text{-}Cys}$ were a compromise between effects of CER index on $eGFR_{Cr}$ and $eGFR_{Cys}$. Moreover, discrepancies between $eGFR_{Cr}$ and $eGFR_{Cys}$ were largest when the GFR was estimated using the most recent estimating equations from 2021 that do not consider the effect of race on serum creatinine.[13]

Accurate evaluation of renal function is fundamental to clinical practice, public health, and research,[2,5,6,18] because such assessments have extensive consequences for detecting, evaluating, and monitoring acute and chronic kidney disease as well as risk stratification for clinical procedures and selecting the correct dosage of drugs that are excreted by the kidney.[4,19] Our findings suggest that this purpose may be severely compromised, when $eGFR_{Cr}$ is evaluated in individuals who have low muscle mass or lose muscle mass over time, especially if the GFR is estimated without accounting for the fact that black individuals have on average higher serum creatinine than non-black individuals (irrespective of age, sex, and GFR).[20]

Based on single measurements of eGFR in a 70-year-old male subject with low muscle mass, $eGFR_{Cr}$ and $eGFR_{Cys}$ disagreed by 24.7 mL/min per 1.73 m^2 (and 30.1 mL/min per 1.73 m^2 when serum creatinine was not corrected for race). Furthermore, the predicted ages at which $eGFR_{Cr}$ and $eGFR_{Cys}$ would have crossed the diagnostic threshold of 60 mL/min per 1.73 m^2 for detecting CKD differed by 29.8 years (and 42.9 years when serum creatinine was not corrected for race). These findings strongly suggest that using creatinine as proxy of renal function leads to unacceptable overestimation of the true GFR when muscle mass is low, the consequence of which would be, for instance, selecting an inappropriately high dosage of renally cleared drugs or misclassification of individuals as not having CKD. Apart from being related to progression to end-stage renal disease, CKD is also an important risk factor for premature cardiovascular morbidity and mortality,[21–23] especially in relatively early stages of CKD (G3b–5).[22] As the application of statins has been shown to mitigate the risk of premature cardiovascular morbidity and mortality in CKD,[24] misclassification results in missing the opportunity for primary preventive therapy. The same holds true for inhibitors of sodium-glucose cotransporter 2, of which the protective effects may even extent to the kidney.[25] Finally, overestimation of the true GFR may lead to deferred initiation of dialysis or renal (re)transplantation, as the decision to do so principally relies on the eGFR.[26]

Our results corroborate prior research, posing serum creatinine as poor screening test for renal function in a myriad of patient groups, namely, geriatric,[27] critically ill,[28] heart failure,[29] type 2 diabetes,[30] liver cirrhosis,[31] and hospitalized patients,[32] among others. It should be noted, however, that results from those studies were limited to a single measurement of eGFR[27,30–32] and, more importantly, based on the population effect rather than effects of individual variations in muscle mass on serum creatinine,[29,32] if such variations were assessed at all. Besides, the greater part of those studies was conducted by merely assuming existence of a population effect of muscle mass on serum creatinine, in the absence of an actual measure of muscle mass.[27,28,30,31] Using repeated measurements of eGFR and CER index, this study extends previous literature by revealing that muscle wasting may cause profound disagreement between trajectories of $eGFR_{Cr}$ and $eGFR_{Cys}$. Decreases in muscle mass were associated with decreased deterioration in $eGFR_{Cr}$, whereas a more realistic, increased deterioration was predicted for $eGFR_{Cys}$. Of course, in reality, it is impossible for renal function to propagate in two opposite directions under the same circumstance (i.e. low muscle mass or gradual muscle wasting), which tempts to speculate on potential mechanisms underlying these discordant observations.

Muscle mass is a well-recognized, explicit indicator of nutritional status,[33] physical function,[34] frailty,[35] and overall health.[36] Although perturbances of nutritional status and physical activity invoke gradual muscle wasting, deteriorating overall health and frailty predominate as drivers of muscle wasting. Namely, compromised health and frailty directly affect muscle mass (Figure 5, path *a*), but also indirectly through impaired renal function (Figure 5, path *c*), as these conditions often coincide with impaired renal function (Figure 5, path *b*).[14,37] Indeed, impaired renal function in itself is known to cause muscle wasting, for instance through

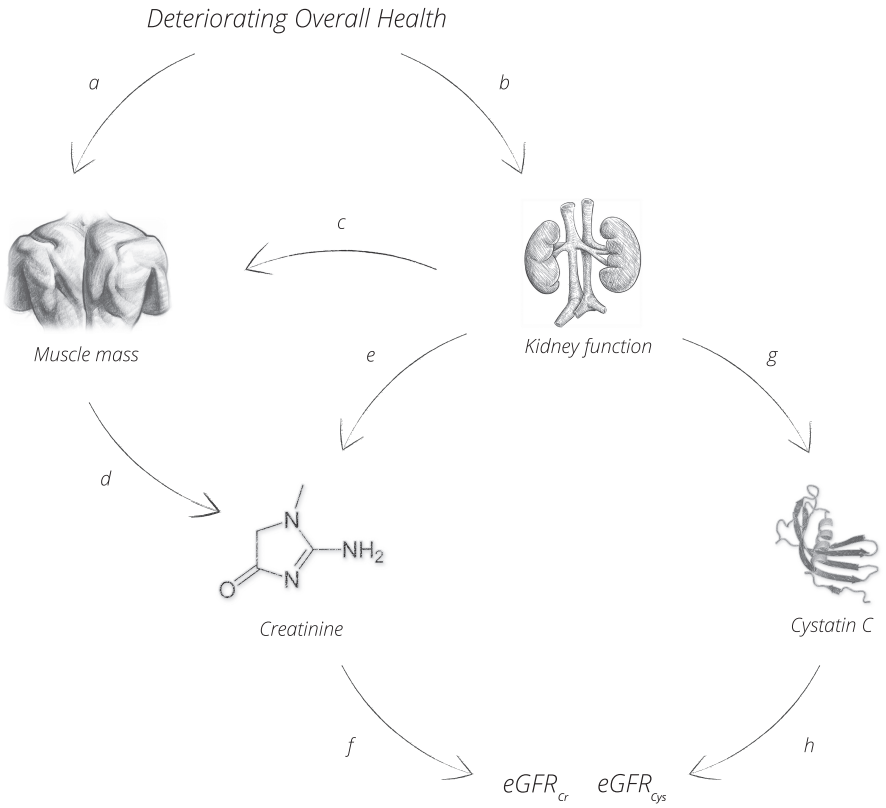

Figure 5 Schematic representation of the relationship between renal function, muscle mass, creatinine, cystatin C, and GFR estimates in the circumstance of deteriorating overall health. Deteriorating overall health is a major driver of muscle wasting (path *a*) and often coincides with impaired renal function (path *b*). Given that impaired renal function adversely affects muscle mass (path *c*), compromised health directly leads to muscle wasting (path *a*), but also indirectly through impaired renal function (path *b* and *c*). As approximately 98% of circulating creatinine originates from muscle tissue (path *d*), both reduced muscle mass and impaired renal function (operating through reduced muscle mass) translate into reduced serum creatinine. The combination of the forgoing with the fact that impaired renal function also implies increased serum creatinine (path *e*) gives rise to a paradox, namely, that impaired renal function may cause a decrease, but, at the same time, also an increase in serum creatinine (paths *c* and *e*, respectively). The mechanism underlying this paradox theoretically invalidates serum creatinine as reliable estimator of renal function in circumstances wherein low muscle mass or muscle wasting prevail (path *f*). Cystatin C, a small protein that is freely filtered by the glomerulus, is also often used as marker of renal function (path *g*). As a housekeeping gene, it is produced by all nucleated cells and therefore unrelated to muscle mass. This very property makes serum cystatin C a superior alternative to serum creatinine as estimator of the GFR (path *h*) in settings of low muscle mass or muscle wasting.

lack of appetite and anorexia, negative effects of low-grade inflammation on anabolism, or gradual loss of the ability to maintain activities of daily living.[14] These intertwined processes give rise to a paradox, namely, that impaired renal function may cause a decrease, but, at the same time, also an increase in serum creatinine (Figure 5, paths *c* and *e*, respectively).

Creatinine stems for about 98% from muscle tissue, having its origin in the non-enzymatic dehydration of creatine and phosphocreatine (Figure 5, path *d*).[8,9] As such, serum concentrations are roughly proportional to muscle mass. Provided that serum creatinine is inversely proportional to the GFR under steady-state conditions (Figure 5, path *e*),[2,9] lower muscle mass automatically implies higher $eGFR_{Cr}$ (Figure 5, paths *d* and *f*), and thus explains the association of lower baseline CER index with higher $eGFR_{Cr}$ (Figure 2). Likewise, over time, changes in muscle mass are expected to influence changes in $eGFR_{Cr}$ in the reverse direction, explaining the observation that the percentage differences between $eGFR_{Cr}$ at baseline and 15 years of follow-up increased from 11.3% to 13.6% to 14.8% when muscle mass was lost, remained stable, or gained during follow-up, respectively (Figure 4). Returning to the fact that equations to compute $eGFR_{Cr}$ correct serum creatinine for an individual's age, sex, and race[7,12] (or age and sex without race[13]), $eGFR_{Cr}$ can only be relied upon if the assumption of equal muscle mass in individuals of the same age, sex, and race is satisfied. Our results show that this assumption may be fundamentally flawed, given the observed effects of CER index on $eGFR_{Cr}$. Implementing estimating equations that are more robust to individual variations in muscle mass seems therefore warranted in settings of low muscle mass or muscle wasting.

Cystatin C, a small protein that is freely filtered by the glomerulus with negligible tubular secretion,[2] is also widely used

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in GFR estimation (Figure 5, path *g*). As a housekeeping gene, it is produced at a constant rate by all nucleated cells,[38] and serum concentrations are therefore unrelated to muscle mass. Hence, the associations of lower baseline CER index with lower $eGFR_{Cys}$ and of muscle wasting with increased deterioration of $eGFR_{Cys}$ must be a reflection of another underlying process. The fact that deteriorating overall health and frailty often coincide with impaired renal function (Figure 5, path *b*),[14,37] raises the suggestion that the associations of lower baseline CER index with lower $eGFR_{Cys}$ and of muscle wasting with increased deterioration of $eGFR_{Cys}$ were attributable to compromised health and frailty.

A multitude of processes other than the GFR affect serum creatinine concentrations (and therewith $eGFR_{Cr}$), including alterations creatinine production due to extremes in muscle mass or dietary ingestion, drug-induced inhibition of tubular creatinine secretion (trimethoprim, cimetidine, and fenofibrate), and abated extrarenal clearance by the intestinal microbiome (antibiotics).[2,39] Although current recommendations state that confirmation of the GFR is required in aforementioned conditions,[39] such recommendations were, however, not established with due regard for individual variations in muscle mass at one point in time, let alone longitudinal variations. We have shown that even subtle deviations (rather than sheer 'extremes') from the population-prevailing muscle mass could invalidate $eGFR_{Cr}$ as reliable estimator of renal function, especially when the effect of race on serum creatinine is not considered. We therefore propose that muscle mass-independent markers for estimating renal function, like cystatin C as superior alternative to creatinine, should be used to accurately assess renal function in all settings of low muscle mass or muscle wasting. This would also eliminate the negative consequences of current race-based approaches.[13,19]

Limitations

Our study has a few potential limitations. First, 24-h urine specimens might have been collected with error. Nonetheless, participants received thorough oral and written instruction prior to each screening round and the use of paired 24-h urine specimens, separated by 3 weeks, should have brought potential measurement error to a minimum. Second, the relative overrepresentation of Caucasian individuals in the current study impedes generalizability of our findings to individuals of other ethnicity and therefore requires verification in other populations. Third, although the production of cystatin C shows less person-to-person variability than creatinine,[2] circulating concentrations have also been related to factors other than renal function, including age,[40] sex,[40] C-reactive protein,[41] free thyroxine,[42] glucocorticoid use,[43] smoking status,[41] and possibly obesity.[40] In the present study, all analyses with eGFR as outcome have been adjusted for the effects of age, sex, smoking status, and waist circumference as potential confounders. However, these analyses have not been adjusted for C-reactive protein, thyroid status, and glucocorticoid use and residual confounding by these factors can therefore not be entirely ruled out. Fourth, no information on GFR measured with an exogenous filtration marker was available. Finally, because of the observational nature of our study, we cannot conclude that associations between (changes in) CER index and (trajectories of) renal function reflect cause and effect relations.

Conclusions

Low muscle mass may cause considerable overestimation of single measurements of $eGFR_{Cr}$. Muscle wasting may cause spurious overestimation of repeatedly measured $eGFR_{Cr}$. Implementing muscle mass-independent markers for estimating renal function, like cystatin C as superior alternative to creatinine, is crucial to accurately assess renal function in all settings of low muscle mass or muscle wasting. This would also eliminate the negative consequences of current race-based approaches.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Conflict of interest

The authors declare that they have no conflict of interest.

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