Muscle-related determinants of serum filtration markers and their association with GFR estimating equation performance in older adults

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Abstract

Introduction: Accurate assessment of GFR is essential in older adults, where chronic kidney disease and sarcopenia are highly prevalent. Previous work has shown that estimated GFR using creatinine and cystatin-C provide the most accurate assessment of measured GFR (mGFR) in community-dwelling older adults, and new GFR estimating equations which include novel low-molecular-weight protein and metabolite filtration markers have demonstrated similar accuracy in a diverse cohort. However, each of the filtration markers used in these estimating equations may be affected by muscle mass or function independently of mGFR, leading to biased or imprecise estimates in the subgroup of older adults with low muscle mass.

Methods: This cross-sectional analysis used data from participants enrolled in the renal substudy of the Age, Gene/Environment Susceptibility Study (AGES-Kidney), a population-based study of community dwelling older adults residing in Reykjavik, Iceland. Serum levels of creatinine, cystatin-C, beta-trace protein (BTP), beta-2-microglobulin (B2M), N-acetylthreonine, pseudouridine, phenylacetylglutamine, and tryptophan were measured, and GFR was measured using clearance of iohexol. Muscle mass was assessed using computed tomography of the thigh, and muscle function was assessed using six-meter walk speed and handgrip strength. Linear regression models were created with log-transformed serum filtration marker levels as outcome and including each muscle-related factor normalized to its interquartile range (IQR), mGFR, GFR measurement error, age, and sex as covariates. The performance of CKD-EPI GFR estimating equations was assessed using established metrics for bias, precision, and accuracy in subgroups defined by thigh muscle area, gait speed, and handgrip strength.

Results: Participants had a mean age of 80 (SD 3.8) years, and a mean mGFR of 63 (SD 16) ml/min/1.73m^2. After adjusting for mGFR, all filtration markers had a residual association with thigh muscle area and handgrip strength, but this was not seen with gait speed in all markers. Only for BTP did further adjustment for age and sex abrogate the association. Creatinine had the greatest strength of association with all muscle-related factors, but this difference decreased after further adjustment for age and sex. When comparing participants in the lowest quintile of thigh muscle area to the upper four quintiles, all GFR estimating equations had a greater magnitude of bias except for the 2020 CKD-EPI panel eGFR using cystatin-C, BTP, and B2M, and the 2009 and 2012 CKD-EPI equations using creatinine and cystatin-C had a higher percentage of estimates exceeding 30% of mGFR. These patterns were not observed between the lowest and upper quintiles of handgrip strength and gait speed.

Conclusions: In community-dwelling older adults, muscle mass is associated with serum filtration marker levels independently of mGFR, an effect which was greatest with creatinine and persisted for most filtration markers after adjustment for age and sex. This relationship may be partially responsible for the increased bias seen in estimated GFR for the subgroup of older adults with low muscle mass.

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List of Abbreviations

1 - P30	Percentage of GFR estimates that exceed mGFR by greater than 30%
AGES-RS	Age/Gene Environment Reykjavik Study
AGES-Kidney	Kidney substudy of AGES-RS
ARIC	Atherosclerosis Risk in Communities
B2M	Beta-2 microglobulin
BMI	Body Mass index
BP	Blood Pressure
BTP	Beta-trace protein
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Cr	Creatinine
CT	Computed tomography
CV	Coefficient of variation
Cys	Cystatin
DXA	Duel-energy x-ray absorptiometry
eGFR	Estimated glomerular filtration rate
GFR	Glomerular filtration rate
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
kg	Kilograms
kV	Kilovolt
LC-MS/MS	Tandem liquid chromatography and mass spectrometry
MDRD	Modification of diet in renal disease
mGFR	Measured glomerular filtration rate
mmHg	Millimeters of mercury
MRI	magnetic resonance imaging
NIST	National institute of standards and technology
Q 1	Lowest quintile
Q 2-5	Upper four quintiles
RMSE	Root mean square error
SD	Standard deviation
UACR	Urine albumin creatinine ratio

Chapter 1. Introduction

Chronic kidney disease (CKD) is an important global public health problem, affecting up to 15% of the world's population, and leading to cardiovascular disease, kidney failure, or premature death for tens of millions of people each year.[1,2] In 2002 the National Kidney Foundation's Kidney Disease Outcome Quality Initiative Clinical practice guidelines on Definition, Stratification and Evaluation of CKD, defined CKD in terms of either signs of kidney damage on analysis of imaging, pathology, blood or urine sample, or a reduced glomerular filtration rate (GFR) for at least three months.[3] These guidelines also classified CKD severity by strata of GFR, and showed that lower strata were associated with a higher risk of complications. Widely adopted and later revised and expanded in 2013, the guidelines cemented GFR as the primary measure by which kidney function is quantitatively assessed.

Accurate determination of GFR is necessary to properly diagnose and treat CKD, as well as make important clinical decisions in all medical fields.[4] For instance, GFR is necessary to recognize complications of CKD such as anemia and metabolic bone disease.[5] It is used to determine prognosis for risk of progression to kidney failure, cardiovascular disease and mortality, and inclusion in clinical trials in all domains, such as oncology. GFR is also used to identify patients at risk of contrast induced acute kidney injury from imaging studies and administer prophylactic treatments, and adjust the dose of medications which are cleared by the kidney to avoid toxicity.[6–10]

In routine clinical practice, GFR is most commonly estimated using creatinine measured in blood, an endogenous filtration marker. GFR estimates based on creatinine

($eGFR_{cr}$) are highly accurate in many, but because its levels are also determined by factors other than GFR such as muscle mass and diet, $eGFR_{cr}$ can be inaccurate in people who have very different muscle mass or dietary protein intake than the average population. GFR estimates other than creatinine are being evaluated and in some cases introduced into clinical practice to overcome the key limitation of creatinine.

Older adults have a high prevalence of CKD as well as comorbid conditions, requiring many clinical decisions that require GFR. It is therefore important to maximize the accuracy of GFR assessment in this population. Up to one-third of older adults also suffer from sarcopenia, a progressive skeletal muscle disorder defined by declines in muscle mass, strength, and performance, and associated with significant morbidity and reduced quality of life.[11,12] GFR assessment may be particularly susceptible to error in this population owing to the association between creatinine and muscle mass. This study will examine the relationship between measures of muscle mass and function and the filtration markers used to estimate GFR and evaluate the performance of GFR estimating equations in subpopulations defined by muscle-based differences.

1.1. Glomerular Filtration Rate

1.1.1. Measured GFR (reference standard)

The "true" GFR is the physiological function of the kidney and refers to simultaneous action of the million or so glomeruli in the kidney. It cannot be measured directly, but can be assessed using urinary or plasma clearance of exogenous filtration markers such as inulin, ^{125}I-iothalamate, iohexol, or ^{99m}Tc-diethylenetriamine pentaacetic acid.[13] These are referred to as measured GFR (mGFR) and are considered the gold

standard methods for assessment of true GFR. However, all mGFR methods are cumbersome and costly, and cannot be performed in routine clinical practice or even most research settings. Thus, for most purposes, GFR is derived from serum levels of endogenous filtration markers using estimating equations to give an "estimated GFR" (eGFR).

1.1.1. Estimated GFR

The relationship between the serum level of an endogenous filtration marker and GFR depends not only on the clearance by the kidney of that marker but also on its generation through metabolism or diet as well as its extrarenal elimination. Since, in a steady state, the generation of a particular filtration marker is equal to its total excretion, GFR can be expressed as the inverse of the serum concentration of that marker multiplied by a set of factors collectively termed the "non-GFR determinants" of that filtration marker. The most common filtration marker used in current practice is creatinine. For creatinine, generation from muscle is the most significant non-GFR determinant. [14] In other words, differences in muscle mass between individuals is responsible for differences in serum creatinine, independently of GFR. Cystatin-C, a low-molecular weight protein produced in all nucleated cells and freely filtered by the kidney, is an alternative filtration marker also available in clinical practice.[15] There has been much enthusiasm for it as it does not appear to be determined by muscle and was a better predictor of adverse outcomes than creatinine.[16,17] However, subsequent studies showed that there are non-GFR determinants which include inflammation, smoking, obesity, and diabetes.[5,18]

1.1.2. GFR estimating equations using creatinine and cystatin C

GFR estimating equations account for non-GFR determinants using demographic surrogates such as age, sex, and race, which allows them to estimate mGFR more accurately when compared to measurements of serum levels of filtration markers alone.

In 1999, the Modification of Diet in Renal Disease (MDRD) Study equation was developed using stepwise regression on a large cohort of individuals with CKD and uses serum creatinine, age, sex, and race (Black or non-Black) to predict mGFR.[4] A decade later, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed a creatinine-based estimating equation ($eGFR_{cr}$) using a pooled cohort of a more diverse group individuals with and without CKD or diabetes and across a larger age range. While using the same predictor variables as the MDRD Study equation, $eGFR_{cr}$ was found to be the more accurate equation at predicting mGFR across the spectrum of GFR values.[19] This was followed in 2012 with the publication of estimating equations using serum cystatin-C as an alternative endogenous filtration marker, as well as a combined creatinine-cystatin-C equation.[15]

As cystatin-C had been shown to be less affected by muscle mass than creatinine, it was thought that a GFR estimating equation based on cystatin-C would be more accurate than one using creatinine. This was not shown to be true. While the combined creatinine-cystatin-C equation ($eGFR_{cr\text{-}cys}$) was more accurate than either single-marker equation, the cystatin-C equation ($eGFR_{cys}$) was no more accurate than its creatinine-based counterpart.[15] Current guidelines set forth by the Kidney Disease: Improving Global Outcomes (KDIGO) work group recommend use of $eGFR_{cr}$ for initial assessment

of eGFR with $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$ used as confirmatory tests when more accurate GFR estimation would aid in clinical decision-making.[5]

1.1.3. GFR estimating equations using novel endogenous filtration markers

Since demographic variables in GFR estimating equations reflect only average values for the development population, application to an outside population or heterogeneity within demographic groups may lead to increased bias and decreased precision. They may also incompletely account for the variation in filtration marker levels due to their non-GFR determinants. These pitfalls have resulted in a search for additional filtration markers that obviate the need for demographics while providing GFR estimates that are at least as accurate as currently used equations.[20] Any single candidate filtration marker is likely to have non-GFR determinants which are non-negligible, but in theory, a panel of multiple filtration markers whose non-GFR determinants do not correlate will result in increased precision by minimizing the impact of the non-GFR determinants of any individual marker.[21]

To date, this search has resulted in identification of two sets of filtration markers. The first are low molecular weight proteins beta-2-microglobulin (B2M) and beta-trace protein (BTP), which have been shown to correlate with mGFR and are strongly associated with an increased risk of mortality and adverse kidney outcomes.[22] Recently, a set of estimating equations have been published using all three low molecular weight proteins (cystatin-C, BTP, B2M) with or without creatinine and which both do not require use of race[23]. The four-marker panel eGFR was shown in a large and diverse population to be as accurate as $eGFR_{cr\text{-}cys}$.[23] The second set was identified by screening

hundreds of serum metabolites for those that strongly correlated with mGFR.[24] A GFR estimating equation was created from a panel of four of these metabolites (acetylthreonine, pseudouridine, phenylacetylglutamine, and tryptophan), without creatinine and cystatin-C or demographics.[25]

1.2. Errors in GFR estimating equations.

The accuracy of GFR estimates is typically described in terms of bias and precision.[26] Bias refers to the average difference between the estimated and measured GFR within a population. Since estimating equations can either overestimate or underestimate mGFR, bias indicates systematic estimation error in a population. In the development population, the bias is necessarily zero since these equations are developed using regression methods, but bias may be elevated in two scenarios: firstly, in external populations for estimating equations that are not highly generalizable, and secondly, in subgroups of both development and external populations that differ from the overall development population according to one or more non-GFR determinants.[26] Precision refers to variability in the difference between estimated and measured GFR and is typically expressed as the interquartile range of these differences.

Measurements of GFR or of filtration markers themselves may be imprecise owing to measurement error or measurements taken outside of the steady state. GFR estimates may also be imprecise because the way that demographics are included in an estimating equation does not fully capture their relationship with the non-GFR determinants.[26] (For example, a hypothetical interaction between age and sex not captured in an estimating equation would lead to imprecision in older age groups owing

to the greater spread between male and female estimates). Metrics of overall accuracy, which combine bias and precision, include the percentage of estimates within K percent of mGFR (P_K) and the square root of the mean squared error (RMSE).[26]

1.3. CKD in older adults

CKD is common in older adults. The prevalence of CKD increases with age, and data from the National Health and Nutrition Examination Survey (NHANES) from 2013-2016 show that nearly one third of participants aged 60 and over had CKD.[27] Polypharmacy is common in the elderly and more common in frail older adults relative to their non-frail counterparts, including medications cleared by the kidney.[28,29] Among frail community-dwelling older adults in the Atherosclerosis Risk in Communities (ARIC) study, 45% had $eGFR_{cr}$ < 60 ml/min/1.73m^2 compared with 77% with $eGFR_{cys}$ < 60ml/min/1.73m^2 suggesting an overestimation of mGFR in this group by equations which rely on serum creatinine measurements.[28] However, while researchers have repeatedly expressed concern about the use of creatinine in elderly individuals due to its relationship with muscle, no studies have directly examined the relationship between muscle mass, muscle function, and serum filtration marker levels or GFR estimating equation performance in the elderly.

1.4. Sarcopenia

Sarcopenia is a state of muscle failure and is highly prevalent in the elderly. Individuals with sarcopenia have difficulty performing activities of daily living, are more likely to fall or have a mobility disorder, and have higher rates of cardiovascular disease, pulmonary disease, cognitive decline, and mortality than non-sarcopenic

individuals.[11] The current consensus definition of sarcopenia includes measures of muscle mass, strength and function.[11,30] Currently, there are many ways to assess each of these elements; for example, muscle mass may be assessed using dual-energy x-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), magnetic resonance imaging (MRI), or computed tomography (CT).

1.5. The Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS)

The study population is a substudy of the Age, Gene/Environment Susceptibility Reykjavik Study (AGES-RS). It provides a unique opportunity to examine the relationship between body composition, muscle function, and measures of kidney function.[31] AGES was designed as a comprehensive population-based study aimed at examining risk factors for disease and disability in an elderly Icelandic cohort. Muscle mass was measured using CT and muscle function was assessed using grip strength and gait speed measurements (collectively termed muscle-related factors).[31] Those enrolled in the AGES-Kidney substudy also had GFR measured as well as serum levels of each of the filtration markers mentioned previously.[32]

1.6. Study Aims

The aim of this investigation is to examine the relationship between measures of muscle mass and muscle function and serum filtration markers, as well as the performance of GFR estimating equations using these filtration markers. The first aim will examine the performance of GFR estimating equations among participants in AGES-Kidney defined by differences in muscle mass and muscle function. The second aim will determine the strength of the residual association between each muscle-related factor and

serum levels of each filtration marker after accounting for the effects of GFR, an analysis that will highlight any potential relationship between muscle and the non-GFR determinants of those filtration markers. The results will provide insights into which GFR estimating equations will exhibit a higher bias in subpopulations defined by muscle mass, and help clinicians identify patients in whom to obtain confirmatory GFR testing.

Collaboration statement: All content in this chapter is based solely on the work of Erin Flanagin.

Chapter 2. Methods and materials

2.1. Sources of data

Participants were enrolled in a substudy of the Age, Gene/Environment, Susceptibility Reykjavik Study (AGES-RS)[31]. AGES-RS was a follow up study of the Reykjavik Study which examined adults born between 1907-1935 in Reykjavik, Iceland to investigate the associations of middle life risk factors with the risk of disease later in life, including cardiovascular, neurocognitive, musculoskeletal, and metabolic factors. It was comprised of two visits between 2002-2006 and 2007-2011, respectively. A total of 3,411 people participated in the second visit of AGES-RS. A sub-study, AGES-Kidney (n=805), was performed after the second AGES-RS visit to measure GFR and obtain blood samples to measure levels of serum filtration markers.[32] Patients enrolled in the AGES-Kidney study who had complete information for all serum filtration markers and muscle-related factors were included in the present analysis. The study was approved by the National Bioethics Committee in Iceland, and the institutional review boards of the United States National Institute of Aging and Tufts Medical Center. All participants gave written informed consent.

2.2. Measurements of GFR and filtration markers

The AGES-Kidney visit occurred a median of 65 (IQR, 32-376) days after measurement of muscle-related factors during the second AGES-RS visit date.[32] GFR was calculated on the day of the AGES-Kidney visit from plasma clearance of iohexol as described previously.[32] Filtration marker levels were measured from stored serum samples from the AGES-Kidney visit. Creatinine was measured using the Roche

enzymatic method with a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corp; coefficient of variation [CV] 2.3%) and calibrated using a National Institute of Standards and Technology (NIST) standard traceable to reference material SRM 909b using isotope-dilution mass spectrometry.[15]. Cystatin-C was measured using the Gentian turbidimetric method (Gentian AS) with a Roche cobas 6000 chemistry analyzer (Roche Diagnostics; CV 4.3% at 0.75 mg/L and 3.2% at 3.83 mg/L) using a NIST standard traceable to reference material.[15,33]. BTP was measured using immunonephelometric methods on the Siemens ProSpec nephelometer (Siemens Healthcare Diagnostics; CV 10.6% at 0.618 mg/L and 7.4% at 1.852 mg/L) and B2M was measured on the Roche cobas 6000 chemistry analyzer (Roche Diagnostics; CV 3.2% at 1.63 mg/L and 4.3% at 0.63 mg/L).[34] N-acetylthreonine, pseudouridine, phenylacetylglutamine, and tryptophan were measured using an ultra-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with internal standards that was developed and analytically validated using Clinical and Laboratory Standards Institute guidelines on 3 identical LC-MS/MS systems as described previously.[25]

2.3. Measurement of muscle related factors

Participants underwent computed tomography of the thigh using a Siemens Sensation four-detector CT system (Siemens Medical Systems). Thigh total muscle cross sectional area (in square centimeters) was determined from a single 10 mm thick axial section at a 120 kV peak through the mid-thigh in each leg and the average value was obtained.[35] Positioning for the mid-thigh section was determined by calculating the center point of the femur from its total length on an anterio-posterior scout image and muscle cross-sectional area was segmented manually along the fascial plane between

muscle and subcutaneous fat using a contouring program.[36] Grip strength (in kilograms) was measured in the dominant hand using a Good Strength dynamometer attached to a schair with the elbow flexed at 90 degrees and the shoulders relaxed. Three 4-5 second trials were performed, each separated by half a minute rest, and the maximum value was used.[35] Gait speed (in meters per second) was calculated from the time taken to walk a 6-meter course according to a standardized protocol, using a stopwatch, and instructing participants to walk at their usual walking pace.[35]

2.4. Statistical analysis

The goal of Aim 1 was to compare the performance of the CKD-EPI GFR estimating equations in subgroups defined by differences in muscle mass and function. Estimated GFR was calculated using the CKD-EPI 2009 creatinine and 2012 cystatin-C equations; the CKD-EPI panel GFR equations that include BTP and B2M in combination with cystatin C with or without creatinine; and the panel eGFR equation that includes four novel metabolites.[15,19,23,25]. See Table 2.1 for a list of the equations used.

The population was divided into sex-specific quintiles of muscle-related factors using threshold values listed in Table 2.2 and performance was evaluated for the lowest quintile and for the upper four quintiles separately. The lowest quintile of each muscle-related factor was used because previous studies in this population used this threshold to identify adults with sarcopenia.[35] Performance was evaluated using established metrics for bias, precision, and accuracy.[26] Bias was calculated as the median difference between measured and estimated GFR. Precision was calculated as the difference between the 75^{th} and 25^{th} quantile (the interquartile range, IQR) of the difference between measured

Table 2.1 List of equations used in the study.

Equation Name	Formula	Variable definitions
$eGFR_{cr}$[19]	$GFR = 141 * \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} * \max\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} * 0.993^{Age} * 1.018\ [if\ female] * 1.159[if\ Black]$	κ is 0.7 for females and 0.9 for males α is -0.329 for females and -0.411 for males
$eGFR_{cys}$[15]	$GFR = 133 * \min\left(\frac{Scys}{0.8}, 1\right)^{-0.499} * \max\left(\frac{Scys}{0.8}, 1\right)^{-1.328} * 0.996^{Age} * 0.932\ [if\ female]$	
$eGFR_{cr\text{-}cys}$[15]	$GFR = 135 * \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} * \max\left(\frac{Scr}{\kappa}, 1\right)^{-0.601} * \min\left(\frac{Scys}{0.8}, 1\right)^{-0.375} * \max\left(\frac{Scys}{0.8}, 1\right)^{-0.711} * 0.995^{Age} * 0.969\ [if\ female] * 1.08\ [if\ Black]$	κ is 0.7 for females and 0.9 for males α is -0.248 for females and -0.207 for males
$eGFR_{cys\text{-}BTP\text{-}B2M}$[23]	$GFR = 120 * \min\left(\frac{Scys}{0.8}, 1\right)^{0.416} * \max\left(\frac{Scys}{0.8}, 1\right)^{0.498} * \min\left(\frac{BTP}{0.6}, 1\right)^{1.038} * \max\left(\frac{BTP}{0.6}, 1\right)^{0.784} * 0.814^{B2M} * 0.999^{Age} * 0.922\ [if\ female]$	
$eGFR_{cr\text{-}cys\text{-}BTP\text{-}B2M}$[23]	$GFR = 131 * \min\left(\frac{Scr}{\kappa}, 1\right)^{\alpha} * \max\left(\frac{Scr}{\kappa}, 1\right)^{0.624} * \min\left(\frac{Scys}{0.8}, 1\right)^{0.595} * \max\left(\frac{Scys}{0.8}, 1\right)^{0.655} * \min\left(\frac{BTP}{0.6}, 1\right)^{0.996} * \max\left(\frac{BTP}{0.6}, 1\right)^{0.838} * 0.902^{B2M} * 0.996^{Age} * 0.937\ [if\ female]$	κ is 0.7 for females and 0.9 for males α is 0.784 for females and 0.745 for males
$eGFR_{metabolites}$[25]	$GFR = 28.106 * Pseudouridine^{-0.805} * Phenylacetylglutamine^{-0.047} * Acetylthreonine^{-0.075} * Tryptophan^{0.266}$	

Scr refers to serum creatinine. Scys refers to serum cystatin-C. Min indicates the minimum of the two quantities, and max indicates the maximum of the two quantities.

Table 2.2 Sex-specific 20th percentiles of average thigh muscle area, handgrip strength, and gait speed.

Muscle-related factor	Male	Female
Thigh muscle area (square centimeters)	125	91.3
Handgrip Strength (kilograms)	34.7	18.9
Gait Speed (meters per second)	0.859	0.779

and estimated GFR. Accuracy was calculated as the square root of the mean squared error (RMSE) and as a percentage of the estimates differing by more than 30% of the measured GFR ($1 - P_{30}$). The 95% confidence intervals for all metrics were calculated using bootstrapping with 1000 iterations. Formal statistical testing was not performed between subgroups to avoid type I error caused by multiple comparisons.

The goal of Aim 2 was to examine the relationship between measures of muscle mass and function and the serum filtration markers used in CKD-EPI GFR estimating equations that is independent of GFR. All serum filtration marker levels and measured GFR were log transformed to capture their relationship with each of the predictor variables. Muscle related factor values were normalized to the IQR of the included participants to allow for comparison between variables. To identify the relationship between muscle related factors and serum filtration marker levels that is independent of GFR, a set of nested linear regression models were created with the log transformed serum filtration marker level as the outcome variable. For each muscle-related factor, the initial univariable model included only that factor as a covariate. A second multivariable model adjusted for measured GFR, and a third further corrected for GFR measurement error using errors-in-variables regression. As has been done previously, the variance of log-transformed GFR measurements was estimated to be 0.015.[18,37] A final model further adjusted for age and sex. Parameter estimates were calculated from regression coefficients as ($e^{coefficient}$-1) * 100% in order to represent the geometric mean difference in

the serum filtration marker associated with a single IQR increase in the muscle related factor.

R version 3.6.2 for Windows and RStudio version 1.2.5019 (RStudio, Inc.) was used for the analysis. The "eivtools" package in R was used to compute the errors-in-variables models.

Collaboration statement: This is a post-hoc analysis using a dataset provided by the AGES-RS research group, who recruited the study population and collected the data. The GFR estimating equations listed in Table 2.1 were developed by CKD-EPI. All statistical analyses were performed by Erin Flanagin. The contents of Table 2.2 are based on results of these analyses.

Chapter 3. Results

3.1. Study population

Table 3.1 shows population characteristics of AGES-Kidney participants stratified by inclusion versus exclusion due to missing data. Details of selection and exclusion are provided in Figure 5.1. A total of 265 out of 805 AGES-Kidney participants were excluded due to missing data, a majority of whom were excluded due to missing metabolite measurements. Overall, there were not clinically relevant differences in measured patient characteristics between participants who were included versus excluded.

Of the included patients, mean (standard deviation, SD) age was 80 (3.84) years old and 57% were women (Table 3.2). Mean (SD) mGFR was 63 (15.83) ml/min/1.73m^2. Participants in the lowest quintile of all muscle-related facters were older and had lower mGFR (i.e., 60 versus 64 ml/min/1.73m2 for thigh muscle area) compared with those in the upper four quintiles. Participants in the lowest quintile of thigh muscle area also had lower BMI (23.8 versus 28.0 kg/m^2). Participants in the lowest quintile of handgrip strength and gait speed had lower reported frequency of physical activity and higher rates of stroke and congestive heart failure. BMI was similar between groups of handgrip strength, and was higher in participants in the lowest quintile of gait speed.

3.2. Performance of GFR estimating equations

The performance of each of the six CKD-EPI GFR estimating equations (eGFRcr, eGFRcys, eGFRcr-cys, eGFRcys-BTP-B2M, eGFRcr-cys-BTP-B2M, and

Table 3.1 Population characteristics of AGES-Kidney participants included or excluded due to missing data.

	Included	Excluded	P	Missing
No. of participants	540	265		
Age (years)	80.08 (3.84)	80.70 (4.32)	0.039	
Female	306 (56.7)	144 (54.3)	0.583	
BMI (kg/m^2)	27.17 (4.06)	27.75 (4.68)	0.071	
Smoking			0.032	
Never	279 (55.1)	112 (45.7)		
Former	197 (38.9)	120 (49.0)		
Current	30 (5.9)	13 (5.3)		
Physical activity			0.421	
Never	205 (38.4)	111 (42.7)		
Rarely-Occasionally	50 (9.4)	26 (10.0)		
Moderate-High	279 (52.2)	123 (47.3)		
Systolic BP (mmHg)	143.07 (20.10)	141.65 (20.39)	0.349	
Diastolic BP (mmHg)	69.18 (10.65)	69.80 (10.36)	0.436	
Diabetes	55 (10.2)	30 (11.3)	0.711	
Previous stroke	33 (6.1)	22 (8.3)	0.313	
Congestive heart failure	22 (4.1)	15 (5.7)	0.406	
Atrial fibrillation	27 (5.3)	10 (4.0)	0.545	
Measured GFR (ml/min/1.73m^2)	63.14 (15.83)	60.80 (17.59)	0.058	
UACR (mg/g)	7.86 [5.08, 17.35]	8.33 [5.17, 19.19]	0.316	
Gait speed (m/s)	0.98 [0.85, 1.10]	0.96 [0.82, 1.08]	0.053	10
Handgrip strength (kg)	28.48 [22.62, 39.45]	28.84 [21.87, 37.99]	0.384	34
Average thigh muscle area (cm^2)	117.32 [100.21, 137.57]	118.81 [100.13, 137.40]	0.595	10
Creatinine (mg/dL)	0.98 (0.36)	1.03 (0.39)	0.061	
Cystatin-C (mg/dL)	1.17 (0.36)	1.23 (0.39)	0.032	
BTP (mg/L)	0.91 (0.39)	0.97 (0.40)	0.047	41
B2M (mg/L)	2.86 (1.22)	3.06 (1.38)	0.051	41
Acetylthreonine (mg/L)	0.12 (0.05)	0.14 (0.08)	0.011	232
Phenylacetyl-glutamine (mg/L)	1.42 (1.10)	2.14 (3.01)	0.002	232
Pseudouridine (mg/L)	1.15 (0.51)	1.41 (0.97)	0.007	232
Tryptophan (mg/L)	14.41 (2.43)	13.80 (2.77)	0.161	232

Values represent mean (SD), median [IQR], or count (%).

Table 3.2 Population characteristics stratified by quintiles of muscle-related factors.

	Overall	Average thigh muscle area		Handgrip Strength		Gait speed	
		Q1	Q2-5	Q1	Q2-5	Q1	Q2-5
N	540	109	431	109	431	110	430
Age (years)	80.08 (3.84)	81.72 (4.43)	79.66 (3.57)	82.17 (4.41)	79.55 (3.50)	82.28 (4.05)	79.51 (3.58)
Female	306 (56.7)	62 (56.9)	244 (56.6)	62 (56.9)	244 (56.6)	63 (57.3)	243 (56.5)
BMI (kg/m^2)	27.17 (4.06)	23.84 (2.96)	28.01 (3.87)	27.00 (4.01)	27.21 (4.08)	27.90 (4.05)	26.98 (4.05)
Smoking							
Never	279 (55.1)	60 (58.8)	219 (54.2)	54 (51.4)	225 (56.1)	61 (57.0)	218 (54.6)
Former	197 (38.9)	35 (34.3)	162 (40.1)	46 (43.8)	151 (37.7)	40 (37.4)	157 (39.3)
Current	30 (5.9)	7 (6.9)	23 (5.7)	5 (4.8)	25 (6.2)	6 (5.6)	24 (6.0)
Physical activity							
Never	205 (38.4)	52 (47.7)	153 (36.0)	62 (57.9)	143 (33.5)	65 (59.6)	140 (32.9)
Rarely-Occasionally	50 (9.4)	7 (6.4)	43 (10.1)	13 (12.1)	37 (8.7)	11 (10.1)	39 (9.2)
Moderate-High	279 (52.2)	50 (45.9)	229 (53.9)	32 (29.9)	247 (57.8)	33 (30.3)	246 (57.9)
Systolic BP (mmHg)	143.07 (20.10)	141.81 (21.11)	143.38 (19.86)	138.97 (20.75)	144.08 (19.83)	144.33 (23.89)	142.75 (19.05)
Diastolic BP (mmHg)	69.18 (10.65)	66.93 (10.74)	69.74 (10.57)	67.91 (11.59)	69.49 (10.39)	68.94 (12.25)	69.24 (10.22)
Diabetes	55 (10.2)	7 (6.4)	48 (11.1)	14 (12.8)	41 (9.5)	12 (10.9)	43 (10.0)
Previous stroke	33 (6.1)	10 (9.2)	23 (5.3)	14 (12.8)	19 (4.4)	10 (9.1)	23 (5.3)
Congestive Heart Failure	22 (4.1)	7 (6.4)	15 (3.5)	9 (8.3)	13 (3.0)	8 (7.3)	14 (3.3)
Atrial fibrillation	27 (5.3)	5 (5.0)	22 (5.4)	7 (6.5)	20 (5.0)	8 (7.5)	19 (4.8)

(continued)	Overall	Average thigh muscle area		Handgrip Strength		Gait speed	
		Q1	Q2-5	Q1	Q2-5	Q1	Q2-5
Measured GFR (ml/min/1.73m^2)	63.14 (15.83)	59.98 (16.26)	63.94 (15.64)	57.89 (17.71)	64.47 (15.05)	56.99 (16.68)	64.72 (15.23)
UACR (mg/g)	7.86 [5.08, 17.35]	8.80 [5.42, 18.66]	7.69 [5.05, 17.23]	8.80 [5.84, 15.98]	7.55 [5.01, 17.57]	8.20 [5.50, 21.37]	7.78 [5.00, 16.00]
Average thigh muscle area (cm2)	117.32 [100.21, 137.57]	90.50 [84.38, 111.96]	125.32 [105.23, 141.79]	108.24 [94.86, 131.88]	119.30 [102.31, 139.93]	111.56 [96.50, 135.27]	119.11 [101.61, 138.20]
Handgrip strength (kg)	28.48 [22.62, 39.45]	24.39 [19.65, 35.06]	29.24 [23.60, 40.42]	18.38 [15.21, 28.81]	31.13 [24.43, 41.55]	25.41 [19.06, 34.33]	29.19 [23.38, 40.90]
Gait speed (m/s)	0.98 [0.85, 1.10]	0.96 [0.81, 1.05]	1.00 [0.86, 1.12]	0.87 [0.73, 0.99]	1.01 [0.88, 1.13]	0.73 [0.64, 0.77]	1.03 [0.94, 1.13]

Values represent mean (SD), median [IQR], or count (%). Q 1 refers to the subgroup of participants with values for each muscle-related factor in the lowest sex-specific quintile of values for the study population. Q 2-5 refers to the subgroup in the upper four quintiles.

eGFRmetabolites) in the overall cohort is shown in Table 5.1 (appendix), and the performance of the GFR estimating equations in the lowest quintile of each of the three muscle-related factors compared to the upper four quintiles is shown in Figure 3.1 and Table 5.1.

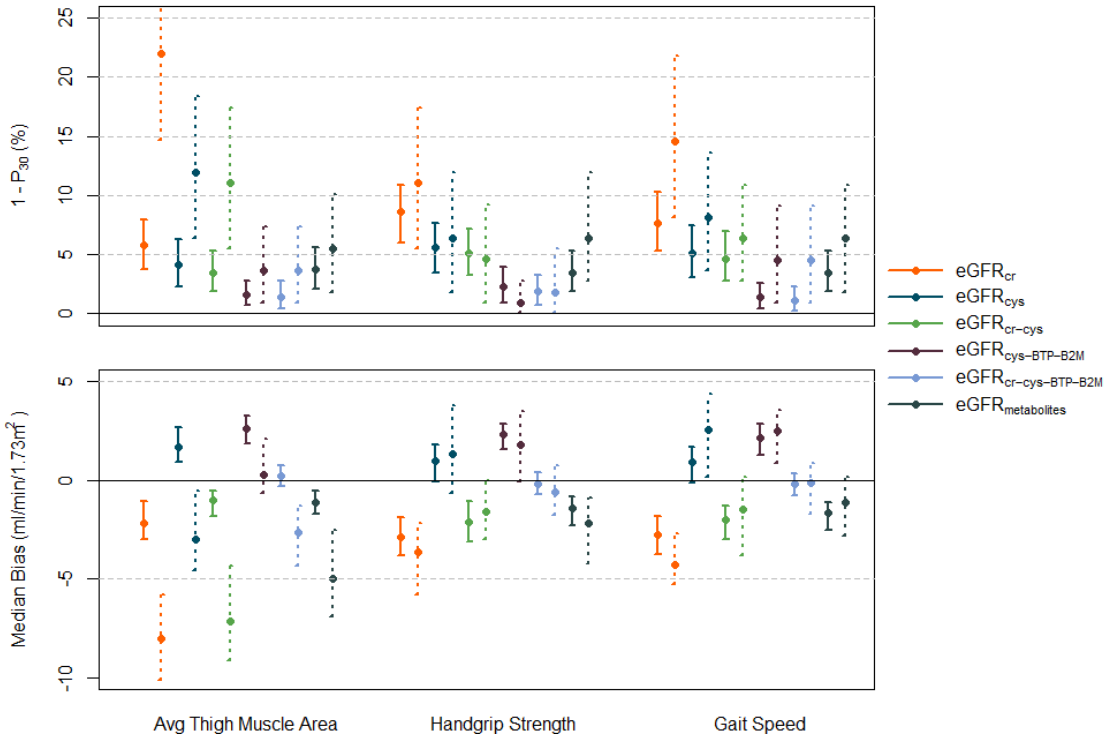

Figure 3.1 Performance of GFR estimating equations stratified by quintiles of muscle-related factors. Solid lines indicate the upper four quintiles and the dashed lines indicate the lowest quintile. Lines represent 95% confidence intervals.

Except for $eGFR_{cys\text{-}BTP\text{-}B2M}$, a greater magnitude of bias was seen in all estimating equations in the lowest quintile of thigh muscle area compared with patients in the upper four quintiles. For example, in the lowest quintile of thigh muscle area, the bias of $eGFR_{cr\text{-}cys}$ was -7.2 (95% confidence interval [CI], -9.1 to -4.3) ml/min/1.73m^2, while in the upper four quintiles it was -1.0 (95% CI -1.8 to -0.5). Similarly, the $1 - P_{30}$ was higher in participants in the lowest quintile of average thigh muscle area, though there was very similar $1 - P_{30}$ for the upper and lower quintiles for $eGFR_{cys\text{-}BTP\text{-}B2M}$, $eGFR_{cr\text{-}cys\text{-}BTP\text{-}B2M}$,

and $eGFR_{metabolites}$. The difference between quintiles was smaller for handgrip strength and gait speed, with $eGFR_{cr}$ again showing the biggest difference.

Figure 5.2 (appendix) shows the bias in each equation compared to thigh muscle area, handgrip strength, and gait speed. $eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$, but not $eGFR_{cys}$, overestimated mGFR on average at lower values of each of the three muscle-related factors, and this trend was attenuated in $eGFR_{cr\text{-}cys\text{-}BTP\text{-}B2M}$. All eGFR underestimated mGFR at the higher end of thigh muscle area, handgrip strength, and gait speed with $eGFR_{metabolites}$ having the lowest underestimate.

3.3. Associations of muscle-related factors with filtration markers

Associations of muscle-related factors with filtration markers with and without adjustment for mGFR, GFR measurement error, age, and sex are shown in Figure 3.2 and Table 3.3. We observed that thigh muscle area and handgrip strength had residual associations with all filtration markers after adjustment for mGFR and GFR measurement error, and that the strength of association with creatinine was the greatest across all muscle-related factors. For example, after adjustment for mGFR and GFR measurement error, a single IQR increase in thigh muscle area (corresponding to a value of 37 cm^2) was associated with a 22.4% (95% CI, 20.3-24.7%) increase in serum creatinine levels among study participants, and a 6.9% (95% CI, 5.3-8.7%) increase in serum cystatin-C levels. Gait speed was more weakly associated with filtration marker levels compared with thigh muscle area and handgrip strength. After adjustment for age and sex, the strength of association of all muscle-related factors with creatinine declined, and the association between thigh muscle area and BTP was lost. Adjustment for age and sex did

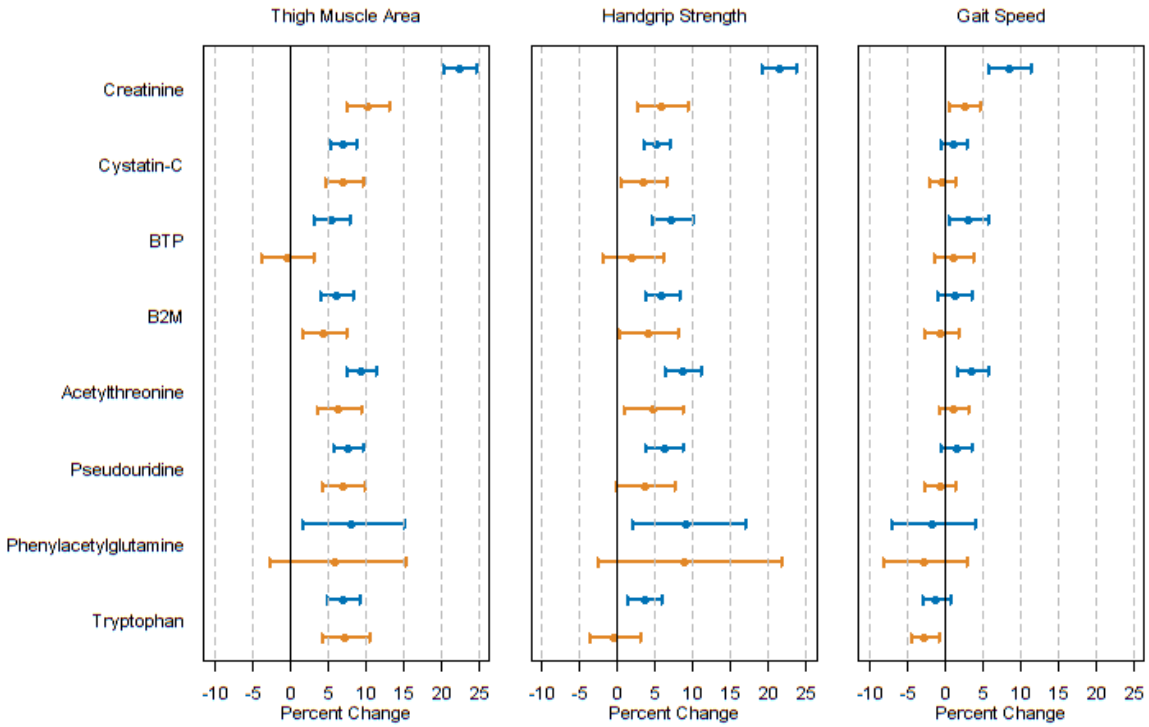

Figure 3.2 Percent difference in levels of serum filtration markers per IQR increase in muscle-related factors. Models shown in blue adjust for mGFR and GFR measurement error. Models in orange adjust for mGFR, GFR measurement error, age, and sex. Lines reflect 95% confidence intervals.

not appear to affect the strength of association between the muscle-related factors and other filtration markers. Associations of phenylacetylglutamine with all muscle-related factors exhibited high variability in all models.

Collaboration statement: All content in this chapter is based on the experimental results and analysis of Erin Flanagin.

Table 3.3 Percent difference in levels of serum filtration markers per IQR change in muscle-related factors. Log serum values of the filtration markers were outcome variables in all models. Model 1 includes only the muscle-related factor. Model 2 includes the muscle-related factor, measured GFR, and GFR measurement error. Model 3 adds age and sex to the variables in model 2. 95% confidence intervals are in parentheses.

	Creatinine	Cystatin-C	BTP	B2M
Thigh Muscle Area				
Model 1	16.5 (12.4, 20.6)	1.9 (-1.3, 5.3)	-0.2 (-4.2, 4.0)	0.2 (-3.7, 4.3)
Model 2	22.4 (20.3, 24.7)	6.9 (5.3, 8.7)	5.6 (3.2, 8.0)	6.1 (3.9, 8.2)
Model 3	10.3 (7.5, 13.2)	7.0 (4.6, 9.5)	-0.4 (-3.8, 3.0)	4.4 (1.5, 7.4)
Handgrip				
Model 1	10.7 (6.7, 14.8)	-3.4 (-6.5, -0.2)	-3.0 (-6.9, 1.0)	-4.4 (-8.1, -0.5)
Model 2	21.4 (19.1, 23.7)	5.2 (3.4, 7.0)	7.2 (4.5, 10.0)	5.9 (3.7, 8.2)
Model 3	5.9 (2.6, 9.4)	3.4 (0.4, 6.4)	1.9 (-2.1, 6.1)	4.0 (0.3, 7.9)
Gait speed				
Model 1	-1.3 (-4.6, 2.1)	-7.6 (-10.2, -4.9)	-7.4 (-10.7, -3.9)	-9.1 (-12.2, -5.8)
Model 2	8.6 (5.8, 11.5)	1.2 (-0.6, 3.0)	3.1 (0.6, 5.8)	1.3 (-0.9, 3.6)
Model 3	2.6 (0.6, 4.7)	-0.4 (-2.1, 1.5)	1.1 (-1.5, 3.8)	-0.5 (-2.7, 1.8)
	Acetylthreonine	**Pseudouridine**	**Phenylacetylglutamine**	**Tryptophan**
Thigh Muscle Area				
Model 1	3.5 (-0.3, 7.5)	1.5 (-2.5, 5.7)	2.3 (-4.5, 9.5)	7.9 (5.7, 10.2)
Model 2	9.3 (7.3, 11.4)	7.7 (5.8, 9.7)	8.1 (1.6, 15.0)	7.0 (4.9, 9.1)
Model 3	6.4 (3.6, 9.3)	7.0 (4.2, 9.9)	5.9 (-2.8, 15.3)	7.3 (4.2, 10.4)
Handgrip				
Model 1	-1.5 (-5.1, 2.3)	-4.5 (-8.2, -0.6)	-1.2 (-7.7, 5.7)	5.2 (3.0, 7.4)
Model 2	8.7 (6.3, 11.1)	6.2 (3.8, 8.6)	9.2 (2.0, 16.9)	3.6 (1.4, 5.8)
Model 3	4.6 (0.8, 8.6)	3.6 (-0.2, 7.6)	8.9 (-2.6, 21.8)	-0.5 (-3.8, 3.0)
Gait speed				
Model 1	-6.6 (-9.8, -3.4)	-9.2 (-12.4, -6.0)	-11.2 (-16.5, -5.7)	0.8 (-1.2, 2.8)
Model 2	3.6 (1.6, 5.7)	1.5 (-0.5, 3.6)	-1.7 (-7.0, 4.0)	-1.1 (-3.0, 0.7)
Model 3	1.1 (-0.9, 3.1)	-0.6 (-2.6, 1.5)	-2.7 (-8.2, 3.0)	-2.7 (-4.5, -0.9)

Chapter 4. Discussion

4.1. Key results and implications

In this study of community-dwelling Icelandic older adults, we found that muscle mass as measured by thigh muscle area is associated with serum filtration marker levels independent of mGFR, and that GFR estimating equations performed less accurately in those with less muscle mass. Specifically, in the lowest quintile of thigh muscle area, all GFR estimating equations had a greater magnitude of bias except for $eGFR_{cys\text{-}BTP\text{-}B2M}$, and $1 - P_{30}$ was higher for $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$ compared to the upper four quintiles. We also found that handgrip strength and gait speed were associated with changes in serum levels of filtration markers independent of mGFR, but GFR estimating equations were not less accurate for participants in the lowest quintile of these measures of muscle function.

4.2. Physiology of non-GFR determinants and implications for using $eGFR_{cr}$

Serum levels of every filtration marker are determined by factors other than GFR. This results in systematic bias of eGFR in populations defined by differences in these non-GFR determinants. It also results in imprecision of eGFR in populations where non-GFR determinants are highly heterogeneous. Muscle is a well-known determinant of serum creatinine levels, and we observed that creatinine was highly associated with all muscle-related factors independently of mGFR. The strength of association between thigh muscle area and serum creatinine decreased significantly after adjusting for age and sex, demonstrating the common association of both factors with these demographic variables, but it did not disappear entirely. This provides additional justification in a separate cohort for the inclusion of age and sex in $eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$ as surrogates for

creatinine generation by muscle, but it also suggests that they do not account entirely for the association. A similar pattern was seen with handgrip strength and gait speed.

We also found that $eGFR_{cr}$ and $eGFR_{cr\text{-}cys}$ demonstrated increased bias (specifically, systemic underestimation of mGFR) in participants in the lowest quintile of thigh muscle area compared with the upper four quintiles. For $eGFR_{cr}$, while the magnitude of bias was similar between the upper four quintiles and the estimating equation's original validation cohort (2.1 and 2.5 ml/min/1.73m^2, respectively), the magnitude of bias for the low thigh muscle area subgroup was greater (8.0 ml/min/1.73m^2).[19] Similarly, for $eGFR_{cr\text{-}cys}$, while the magnitude of bias in the upper four quintiles of thigh muscle area was less than that of the original validation cohort (1.0 versus 3.9 ml/min/1.73m^2, respectively), bias was greater in the lowest quintile (7.2 ml/min/1.73m^2). [15] Additionally, over a fifth of estimates by $eGFR_{cr}$ in the low thigh muscle area group exceeded 30% of mGFR ($1 - P_{30}$ of 22%) compared with roughly one in twenty in the higher thigh muscle area group ($1 - P_{30}$ of 5.8%). By comparison, $1 - P_{30}$ in the original validation cohort of $eGFR_{cr}$ was 15.9%.[19] As such, our data indicate that older adults with low muscle mass are likely to have eGFR that are inaccurate compared to mGFR, whereas those with higher muscle mass are likely to have accurate estimates. Clinicians should therefore exercise caution in estimating GFR using $eGFR_{cr}$ in elderly patients with decreased muscle mass.

4.3. Cystatin-C

We observed that an increase in thigh muscle area was associated with higher serum cystatin-C levels after adjustment for mGFR, age, and sex, though not as strongly as with serum creatinine. Though a common belief among clinicians is that cystatin-C is

independent of muscle mass, it has previously been shown to be associated with lean mass assessed using dual-energy x-ray absorptiometry in individuals with CKD, and our observations implicate muscle in the non-GFR determinants of cystatin-C as well.[38] Consistent with these results, we also found that $eGFR_{cys}$ was less accurate for participants in the lowest quintile of thigh muscle area compared to those in the upper four quintiles. It has previously been shown in this population that an increase in BMI is associated with a decreased $eGFR_{cys}$ independent of mGFR, and these results implicate a specific change in body composition (that is, muscle mass) which is at least partially responsible for that association.[34]

4.4. Low molecular weight proteins, metabolites, and panel GFR

We found that an increase in thigh muscle area was associated with higher serum levels of all filtration markers studied. Such a finding is undesirable when trying to design GFR estimating equations relying on a panel of filtration markers, since one hopes that the non-GFR determinants of each filtration marker do not correlate with one another. This presents a potential hurdle: since most filtration markers are bound to be either low-molecular-weight proteins or small-molecule metabolites produced by cells, many are bound to be correlated positively with metrics of muscle mass. To minimize bias, filtration markers are needed which are minimally or negatively associated with muscle mass, and evaluation of filtration marker candidates should include examination of their association with muscle.

BTP is a 168 amino acid glycoprotein that catalyzes conversion of prostaglandin $H_2$to prostaglandin D_2. Though the details of its origin are unclear, BTP is hypothesized to be produced in the central nervous system, by myocytes, and by endothelial cells.[39] In

the present study, it was found to have a residual association with thigh muscle area after accounting for mGFR, but this difference was abolished after accounting for age and sex. This is consistent with previous data showing a high residual association between age, sex, and BTP after accounting for mGFR.[34]. It suggests that BTP levels do not correlate with muscle and differences in serum BTP seen with variations in muscle mass may be accounted for by the association of muscle mass with age and sex. Furthermore, BTP may play an important role in a panel GFR that includes other filtration markers likely to be influenced by muscle.

B2M is a 100 amino acid protein subgroup of the human leukocyte antigen class I molecule found on the surface of most nucleated cells that is freely filtered by the glomerulus and catabolized in the proximal tubule.[40] B2M was found to have a residual association with thigh muscle area after accounting for mGFR, and this association was not found to change after adjustment for age and sex in the AGES population. Previously, Liu et al. found only weak associations between B2M, age and sex that differed significantly between studies included in their pooled cohort.[37] Further work is needed to understand these associations, since if B2M levels are largely independent of age and sex, then it may be an important component in GFR estimating equations that do not include demographic variables.

This is the first study to examine a potential non-GFR determinant of small-molecule metabolites using measured GFR. After adjustment for mGFR, each had a weak residual association with thigh muscle area. We also observed an increase in bias in $eGFR_{metabolites}$ in older adults with lower thigh muscle area. After further adjusting for age and sex, we did not observe a large change in residual association of these filtration

markers with thigh muscle area. Notably, in its validation cohort, $eGFR_{metabolites}$ was not found to perform less accurately with or without demographic variables.[25]

Pseudouridine is a modified uridine nucleoside, and phenylacetylglutamine and acetylthreonine are modified amino acids, which have all be found to be associated with progression to end stage kidney disease.[41,42] Tryptophan is an essential amino acid positively correlated with GFR, a finding that is perhaps due to increased metabolism in chronic kidney disease.[43] Little is known about their filtration or tubular handling, though some evidence suggests that pseudouridine may be partially reabsorbed by the tubule.[44] The high variability of estimates for the association of muscle-related factors with phenylacetylglutamine is due to the high variability in measurements of phenylacetylglutamine in serum. This may be due to measurement error, owing to a newly developed method without a reference standard or certified reference materials, or it may be due to heterogeneity in non-GFR determinants of phenylacetylglutamine within the study population.

4.5. Handgrip strength and gait speed

We found that after adjusting for mGFR, the residual association of handgrip strength with filtration markers appeared similar to that of thigh muscle area. However, while bias and $1 - P_{30}$ of $eGFR_{cr}$ was higher among those in the lowest quintile of handgrip strength and gait speed compared to the upper four quintiles, the difference was not as large. Lean mass using dual-energy x-ray absorptiometry and hand strength have been previously shown to be weakly associated with eGFR, though without accounting for mGFR.[45] It was hoped that handgrip strength and gait speed could serve as surrogates for muscle mass as they are easy to perform in a clinical setting. If so, they

could be used to identify elderly patients with low muscle mass in situations where accurate estimates of GFR are needed. The findings of this study show that they are imperfect in this regard. Gait speed also had little to no residual association with filtration markers independent of mGFR. While previous studies have shown a modest correlation between thigh muscle area and gait speed in healthy older women, it is a measure of physical performance which involves the central and peripheral nervous systems, bones, joints, and perception in addition to muscle function, and it is likely affected significantly by conditions which affect these other factors.[46,47] This would confound any association with muscle mass.

4.6. Implications for research and clinical practice

Current KDIGO guidelines recommend use of $eGFR_{cys}$ and $eGFR_{cr\text{-}cys}$ as confirmatory tests in individuals where $eGFR_{cr}$ is suspected to be inaccurate due to extremes of muscle mass, but we observed that these confirmatory tests were also less accurate in participants with lower muscle mass. This analysis adds to the growing body of literature that suggests we must move beyond GFR estimation that is primarily creatinine-based. At the same time, the newly developed panel eGFR equations appeared to maintain a high degree of accuracy in this subgroup. These results support efforts to increase the use of the panel GFR equations. Important steps will include making BTP measurement available in clinical laboratories, and establishing a standardized reference material for B2M.[37] Meanwhile, further research is needed to replicate these findings in a larger and more diverse patient population, and to further elucidate other non-GFR determinants of the individual filtration markers including diet, medication use, and

comorbid conditions. Finally, clinical tools are needed which can identify individuals with low muscle mass to help guide decision-making about GFR estimation.

4.7. Strengths and limitations

Strengths of this study include a population-based cohort that includes both gold standard measures of GFR and muscle along with measurements of multiple filtration markers and multiple estimates of GFR and other functional muscle tools.

This study has several limitations. Firstly, significant number of participants of AGES-Kidney were excluded due to missing data. Those excluded were older with lower mGFR than those included, and as the GFR estimating equations included in this study are more accurate at lower GFR, these differences may bias the results away from the null hypothesis. However, there were no differences in muscle-related factors. Also, all participants in AGES-Kidney were white and of Icelandic descent, limiting the generalizability of our findings. Further studies are needed in a large and diverse population such as US clinicians may encounter. AGES-Kidney was included in the development population of $eGFR_{cys\text{-}BTP\text{-}B2M}$, $eGFR_{cr\text{-}cys\text{-}BTP\text{-}B2M}$, and $eGFR_{metabolites}$, which will make these estimating equations appear relatively unbiased compared to $eGFR_{cr}$, $eGFR_{cys}$, and $eGFR_{cr\text{-}cys}$, which were not developed with participants from AGES-Kidney. Comparisons of performance should not be made between these two sets of estimating equations.

While GFR measurement and blood sampling for filtration marker measurement were performed simultaneously, thigh muscle area measurement and other metrics of muscle function were not, with the two separated by a median of 65 (IQR 32-376) days,

as stated earlier. Changes in muscle mass, function, GFR, or non-GFR determinants for any of the filtration markers occurring differentially between quintile groups have the potential to bias the results away from the null hypothesis. However, as muscle mass is generally observed to decline with age, this would also likely lead to misclassification of individuals with low thigh muscle area at the time of GFR assesment as having higher thigh muscle area, decreasing the difference in equation performance seen between the two groups. In the analysis of changes in serum filtration marker levels per IQR change in thigh muscle area, a greater interim decline in muscle mass in individuals with lower thigh muscle area would lead to an underestimation of IQR and an increase in the apparent strength of association.

4.8. Conclusion

Among community dwelling older adults, those with low muscle mass as measured by thigh muscle area on CT had higher bias and imprecision in GFR estimates than those with higher muscle mass, and muscle mass was shown to be associated with every major serum filtration marker independently of mGFR. While a panel GFR presents a promising direction toward the goal of improving the accuracy of GFR estimates, future studies should consider muscle-related determinants of any potential new serum filtration markers.

Collaboration statement: All content in this chapter was written by Erin Flanagin.

Chapter 5. Appendix

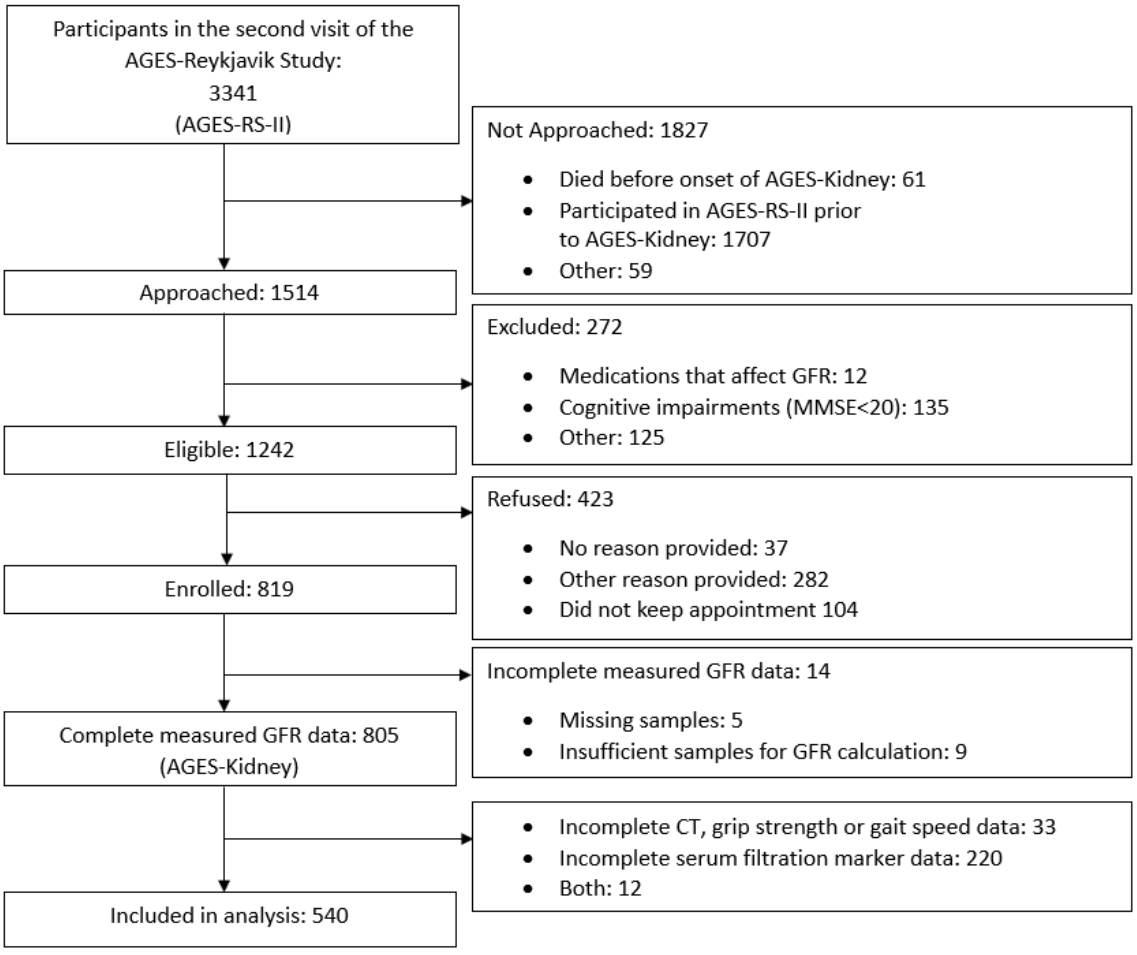

Figure 5.1 AGES-Kidney study participant recruitment and inclusion in analysis.

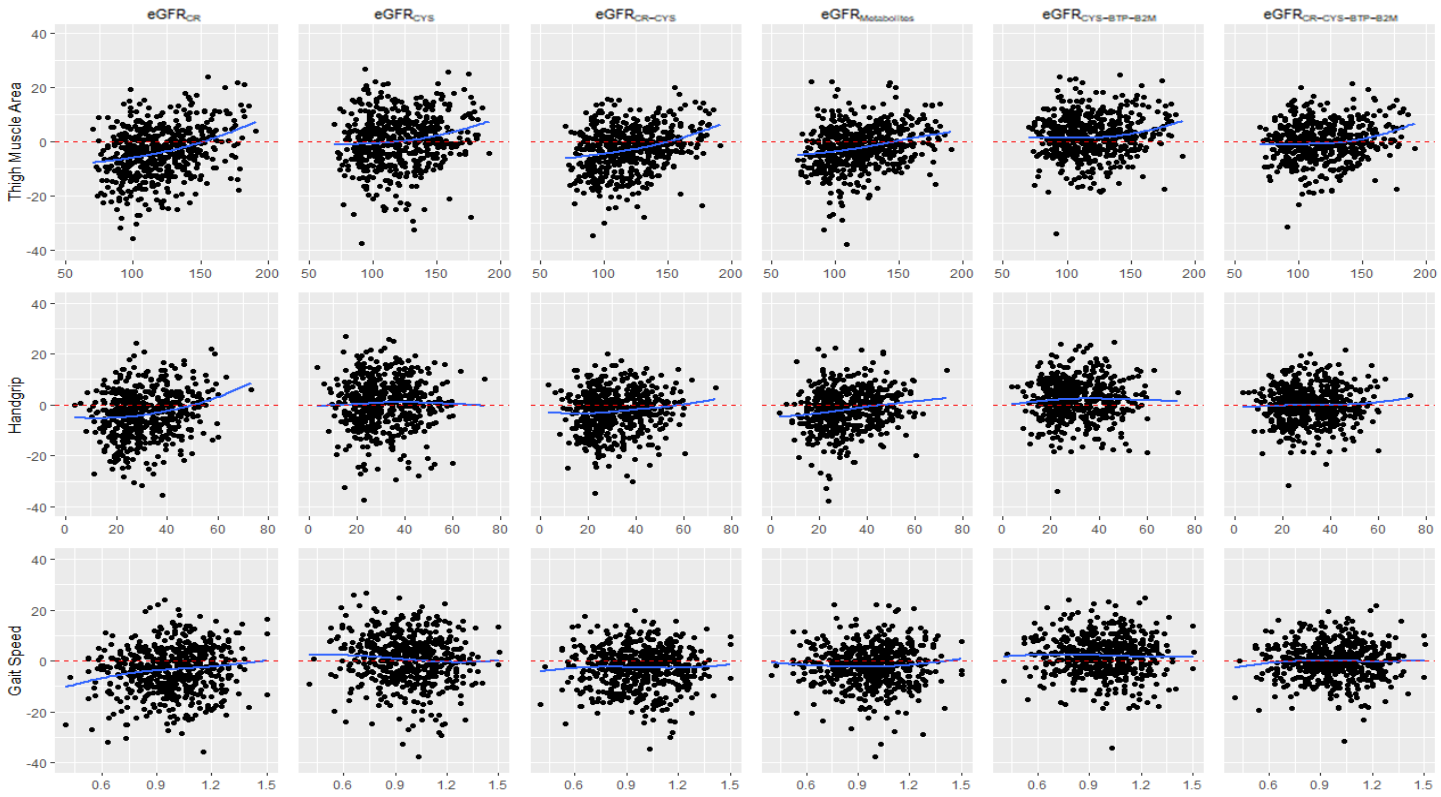

Figure 5.2 Bias of CKD-EPI equations according to thigh muscle area, handgrip strength, and gait speed in AGES-Kidney participants. Each panel shows the associations of eGFR by one muscle-related factor. Each column shows the associations for one estimating equation and each row shows the data for one muscle-related factor. The Y axes are expressed as the difference between measured and estimated GFR in units of ml/min/1.73m2. Positive values therefore represent an underestimate of the measured GFR, and negative values represent an overestimate. For thigh muscle area, the X axes are expressed as square centimeters. For handgrip strength, the X axes are expressed as kilograms. For gait speed, the X axes are expressed as meters per second. The blue lines depict loess curves, and the dotted red line represents zero bias, that is, when measured GFR and estimated GFR are equal. Each black dot represents a single participant.

Table 5.1 Performance of GFR estimating equations, overall and stratified by quintiles of muscle-related factors. Q 1 refers to the subgroup of participants with values for each muscle-related factor in the lowest sex-specific quintile of values for the study population. Q 2-5 refers to the subgroup in the upper four quintiles. Bias was calculated as the median difference between measured and estimated GFR and is expressed in ml/min/1.73m^2. The interquartile range (IQR) was calculated as the difference between the 75th and 25th quantile of the difference between measured and estimated GFR and is expressed in ml/min/1.73m^2. Accuracy was calculated as the square root of the mean squared error (RMSE) and as a percentage of the estimates differing by more than 30% of the measured GFR ($1 - P_{30}$). The 95% confidence intervals are in parentheses.

Overall	**Bias**	**IQR**	**1 - P_{30}**	**RMSE**
eGFR$_{CR}$	-3.0 (-3.9, -2.3)	12.2 (10.9, 13.7)	9.1 (6.9, 11.7)	0.161 (0.150, 0.173)
eGFR$_{CYS}$	1.1 (0.5, 1.8)	11.8 (10.7, 13.0)	5.7 (3.9, 8.0)	0.162 (0.150, 0.172)
eGFR$_{CR-CYS}$	-1.9 (-2.9, -1.2)	10.0 (8.9, 11.4)	5.0 (3.3, 6.7)	0.137 (0.128, 0.148)
eGFR$_{CYS-BTP-B2M}$	2.2 (1.4, 2.8)	9.7 (8.7, 10.6)	2.0 (0.9, 3.3)	0.131 (0.121, 0.142)
eGFR$_{CR-CYS-BTP-B2M}$	-0.2 (-0.7, 0.3)	8.2 (7.2, 9.3)	1.9 (0.9, 3.0)	0.115 (0.106, 0.125)
eGFR$_{METABOLITES}$	-1.6 (-2.3, -1.1)	10.2 (8.8, 11.0)	4.1 (2.4, 5.7)	0.139 (0.124, 0.156)

Thigh muscle area		**Bias**	**IQR**	**1 - P_{30}**	**RMSE**
eGFR$_{CR}$	Q 2-5	-2.1 (-3.0, -1.1)	11.6 (10.1, 12.9)	5.8 (3.7, 7.9)	0.148 (0.136, 0.160)
	Q 1	-8.0 (-10.1, -5.8)	12.9 (9.6, 15.5)	22.0 (14.7, 30.3)	0.206 (0.178, 0.232)
eGFR$_{CYS}$	Q 2-5	1.7 (0.9, 2.7)	11.5 (10.4, 12.9)	4.2 (2.3, 6.3)	0.157 (0.146, 0.168)
	Q 1	-3.0 (-4.5, -0.6)	11.6 (9.5, 14.0)	11.9 (6.4, 18.3)	0.178 (0.147, 0.208)
eGFR$_{CR-CYS}$	Q 2-5	-1.0 (-1.8, -0.5)	9.4 (7.9, 10.5)	3.5 (1.9, 5.3)	0.126 (0.116, 0.136)
	Q 1	-7.2 (-9.1, -4.3)	11.2 (8.8, 14.4)	11.0 (5.5, 17.4)	0.175 (0.150, 0.202)
eGFR$_{CYS-BTP-B2M}$	Q 2-5	2.6 (1.8, 3.3)	9.6 (8.6, 10.8)	1.6 (0.7, 2.8)	0.129 (0.120, 0.139)
	Q 1	0.3 (-0.6, 2.1)	9.0 (7.2, 10.6)	3.7 (0.9, 7.3)	0.140 (0.112, 0.169)
eGFR$_{CR-CYS-BTP-B2M}$	Q 2-5	0.3 (-0.3, 0.8)	8.2 (7.0, 9.0)	1.4 (0.5, 2.8)	0.109 (0.101, 0.118)
	Q 1	-2.7 (-4.3, -1.3)	8.3 (6.9, 10.1)	3.7 (0.9, 7.3)	0.136 (0.111, 0.164)
eGFR$_{METABOLITES}$	Q 2-5	-1.1 (-1.7, -0.5)	9.3 (8.2, 10.2)	3.7 (2.1, 5.6)	0.133 (0.115, 0.154)
	Q 1	-5.0 (-6.9, -2.5)	9.7 (8.2, 12.1)	5.5 (1.8, 10.1)	0.160 (0.136, 0.186)

Table 5.1 Continued.

Handgrip strength		Bias	IQR	1 - P_{30}	RMSE
$eGFR_{CR}$	Q 2-5	-2.9 (-3.8, -1.9)	12.8 (11.3, 14.2)	8.6 (6.0, 10.9)	0.157 (0.145, 0.169)
	Q 1	-3.7 (-5.8, -2.2)	10.2 (8.2, 13.7)	11.0 (5.5, 17.4)	0.177 (0.150, 0.206)
$eGFR_{CYS}$	Q 2-5	1.0 (-0.1, 1.8)	11.9 (10.6, 13.4)	5.6 (3.5, 7.7)	0.160 (0.147, 0.172)
	Q 1	1.3 (-0.7, 3.8)	11.4 (9.2, 13.6)	6.4 (1.8, 11.9)	0.169 (0.148, 0.191)
$eGFR_{CR-CYS}$	Q 2-5	-2.1 (-3.1, -1.1)	9.9 (8.6, 11.4)	5.1 (3.2, 7.2)	0.136 (0.124, 0.147)
	Q 1	-1.6 (-3.0, 0.0)	10.0 (8.3, 13.3)	4.6 (0.9, 9.2)	0.145 (0.124, 0.164)
$eGFR_{CYS-BTP-B2M}$	Q 2-5	2.3 (1.6, 2.8)	9.6 (8.5, 10.5)	2.3 (0.9, 3.9)	0.132 (0.120, 0.144)
	Q 1	1.8 (-0.1, 3.5)	10.3 (7.8, 12.2)	0.9 (0.0, 2.8)	0.129 (0.115, 0.143)
$eGFR_{CR-CYS-BTP-B2M}$	Q 2-5	-0.2 (-0.7, 0.4)	7.9 (6.9, 9.5)	1.9 (0.7, 3.2)	0.114 (0.103, 0.125)
	Q 1	-0.6 (-1.8, 0.7)	8.9 (6.5, 11.0)	1.8 (0.0, 5.5)	0.122 (0.104, 0.139)
$eGFR_{METABOLITES}$	Q 2-5	-1.4 (-2.3, -0.8)	10.2 (8.8, 11.4)	3.5 (1.9, 5.3)	0.137 (0.119, 0.158)
	Q 1	-2.1 (-4.2, -0.9)	9.1 (6.9, 10.9)	6.4 (2.7, 11.9)	0.147 (0.124, 0.169)

Gait speed		Bias	IQR	1 - P_{30}	RMSE
$eGFR_{CR}$	Q 2-5	-2.7 (-3.8, -1.8)	12.9 (11.5, 14.3)	7.7 (5.3, 10.2)	0.156 (0.144, 0.167)
	Q 1	-4.3 (-5.3, -2.7)	11.0 (7.7, 13.0)	14.5 (8.2, 21.8)	0.182 (0.151, 0.212)
$eGFR_{CYS}$	Q 2-5	0.9 (-0.1, 1.7)	12.1 (10.6, 13.4)	5.1 (3.0, 7.4)	0.156 (0.144, 0.169)
	Q 1	2.5 (0.1, 4.4)	11.6 (9.1, 14.3)	8.2 (3.6, 13.6)	0.180 (0.159, 0.201)
$eGFR_{CR-CYS}$	Q 2-5	-2.0 (-3.0, -1.3)	9.7 (8.3, 11.4)	4.7 (2.8, 7.0)	0.133 (0.122, 0.145)
	Q 1	-1.5 (-3.8, 0.2)	10.6 (8.6, 14.4)	6.4 (2.7, 10.9)	0.152 (0.131, 0.174)
$eGFR_{CYS-BTP-B2M}$	Q 2-5	2.1 (1.3, 2.9)	9.6 (8.4, 10.4)	1.4 (0.5, 2.6)	0.128 (0.117, 0.140)
	Q 1	2.5 (0.9, 3.6)	10.2 (7.7, 11.9)	4.5 (0.9, 9.1)	0.143 (0.125, 0.160)
$eGFR_{CR-CYS-BTP-B2M}$	Q 2-5	-0.2 (-0.8, 0.4)	7.9 (6.8, 9.3)	1.2 (0.2, 2.3)	0.112 (0.101, 0.123)
	Q 1	-0.1 (-1.7, 0.9)	9.3 (6.9, 11.5)	4.5 (0.9, 9.1)	0.128 (0.109, 0.146)
$eGFR_{METABOLITES}$	Q 2-5	-1.7 (-2.5, -1.1)	10.5 (9.1, 11.8)	3.5 (1.9, 5.3)	0.141 (0.123, 0.162)
	Q 1	-1.1 (-2.8, 0.1)	8.3 (6.3, 10.7)	6.4 (1.8, 10.9)	0.131 (0.111, 0.151)

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