ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2019; **10**: 621–629
Published online 25 March 2019 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12399

Muscle mass determined from urinary creatinine excretion rate, and muscle performance in renal transplant recipients

Suzanne P. Stam[1,2]*, Michele F. Eisenga[1,2], Antonio W. Gomes-Neto[1,2], Marco van Londen[1,2], Vincent E. de Meijer[3,4], André P. van Beek[1,5], Ron T. Gansevoort[1,2] & Stephan J.L. Bakker[1,2]

[1]*Department of Internal Medicine, University of Groningen, Groningen, The Netherlands,* [2]*Division of Nephrology, University Medical Center Groningen, Groningen, The Netherlands,* [3]*Department of Surgery, University of Groningen, Groningen, The Netherlands,* [4]*Division of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, Groningen, The Netherlands,* [5]*Division of Endocrinology, University Medical Center Groningen, Groningen, The Netherlands*

Abstract

Background Muscle mass, as determined from 24-h urinary creatinine excretion rate (CER), is an independent predictor for mortality and graft failure in renal transplant recipients (RTR). It is currently unknown whether CER is comparable with healthy controls after transplantation and whether it reflects muscle performance besides muscle mass. We aimed to compare urinary CER and muscle performance between RTR and healthy controls and to investigate whether urinary CER is associated with muscle performance in RTR.

Methods We included RTR, transplanted between 1975 and 2016 in the University Medical Center Groningen. Healthy controls were subjects screened for kidney donation. CER was calculated from a 24-h urine collection. Muscle performance was assessed by handgrip strength, sit-to-stand test, and 2-min walk test. Statistical analyses were performed using linear regression analyses.

Results We included 184 RTR (mean age 56.9 ± 11.9 years, 54% male recipient) and 78 healthy controls (age 57.9 ± 9.9, 47% male recipient). RTR were at a median time of 4.0 (1.1–8.8) years after transplantation. Mean CER was lower in RTR compared to healthy controls (11.7 ± 4.0 vs. 13.1 ± 5.2 mmol/24 h; $P = 0.04$). Significantly poorer results in muscle performance were found in RTR compared to controls for the handgrip strength (30.5 [23.7–41.1] N vs. 38.3 [29.3–46.0] N, $P < 0.001$) and the 2-min walk test (151.5 ± 49.2 m vs. 172.3 ± 12.2 m, $P < 0.001$) but not for the sit-to-stand (12.2 ± 3.3 m vs. 11.9 ± 2.8 m, $P = 0.46$). In RTR, CER was significantly associated with handgrip strength (std. β 0.33; $P < 0.001$), independent of adjustment for potential confounders. In RTR, CER was neither associated with the time used for the sit-to-stand test (std. β −0.09; $P = 0.27$) nor with the distance covered during the 2-min walk test (std. β 0.07; $P = 0.40$).

Conclusions Muscle mass as measured by CER in RTR is lower compared to controls. CER is positively associated with muscle performance in RTR. The results demonstrate that CER does not only reflect muscle mass but also muscle performance in this patient setting. Determination of CER could be an interesting addition to the imaging technique armamentarium available and applied for evaluation of muscle mass in clinical intervention studies and observational studies.

Keywords Creatinine excretion rate; Muscle mass; Muscle performance; Renal transplant recipients

Received: 31 May 2018; Revised: 26 October 2018; Accepted: 31 December 2018
**Correspondence to: Suzanne P. Stam, Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen. P.O. Box 30.001, Groningen 9700 RB, The Netherlands. Phone: 0031 050 361 01 37. Fax: 0031 50 361 9310, Email: s.p.stam@umcg.nl*

Introduction

Survival after renal transplantation outweighs survival on dialysis, offering better quality of life, against lower cost, making transplantation the treatment of choice for patients with end-stage renal disease.[1,2] This survival benefit is mainly the result of the advancement in tissue matching techniques and the development of better immunosuppressive therapy leading to a remarkable improvement in short-term survival after renal transplantation.[3] Unfortunately, this improvement in short-term survival has not yet been translated in improved long-term survival.[4] It has been hypothesized that the development of numerous complications after renal transplantation including post-transplant obesity, new-onset diabetes after transplantation, cardiovascular disease (CVD), and accelerated senescence are the cause of this impaired long-term survival. The latter of these complications are often characterized by a low muscle mass, or sarcopenia, and a progressive decline in muscle strength.[5–7] The severity of the latter complication is substantiated by study results which show muscle mass to be inversely associated with mortality and graft failure in renal transplant recipients (RTR).[8]

A non-invasive method to estimate total body muscle mass is urinary creatinine excretion rate (CER). In a steady state, creatinine is produced at a constant rate, depending on the quantity of muscle mass, as creatinine is formed from the non-enzymatic conversion of creatine and creatine phosphate in muscle.[9] As a result, CER is an established method to study total body muscle mass in healthy populations and transplant recipients.[10–12] Moreover, a recent study demonstrated an association between CER and frailty and frailty-related markers in patients suffering from chronic kidney disease, indicating that CER is not only a marker for muscle mass, but may also reflect muscle performance.[13] Consequently, muscle performance may at least partially explain the association of muscle mass with mortality, as muscle strength has been demonstrated to be an even stronger predictor of mortality.[14,15]

As is it currently unknown, we aim to assess whether muscle mass as reflected by CER in RTR is comparable with healthy controls. Furthermore, this study aims to determine whether urinary CER, as marker for muscle mass, is associated with muscle performance in RTR. We hypothesize that muscle mass, as reflected by CER, is positively associated with muscle function in RTR.

Methods and materials

Study design and population

This study is part of the TransplantLines Biobank and Cohort Study of the University Medical Center Groningen. In brief, all RTR (aged ≥18 years) with a functioning graft who visited the outpatient clinic of the University Medical Center Groningen between June 2015 and July 2016 were invited to participate. RTR with missing baseline data on CER were excluded. Written informed consent was acquired of all eligible RTR prior to inclusion, and the TransplantLines study protocol was approved by the institutional research board (METc 2014/077), adhering to the declaration of Helsinki. Furthermore, the study protocol is in concordance with the principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.[16]

Healthy controls

To compare the analyses with a healthy control group, we included 78 subjects who were evaluated for living kidney donation. All subjects participated in the TransplantLines cohort study (METc 2014/077). All subjects were deemed healthy controls, as all subjects were healthy enough to undergo kidney donation in the University Medical Center Groningen, The Netherlands. None had history of kidney disease, diabetes, or cardiovascular events. Hypertension, if present, was treated with a maximum of one antihypertensive drug.

Data collection and measurements

Gender, date of birth, primary renal disease, weight, height, transplantation date, dialysis parameters, and data on medication use were extracted from the electronic hospital records. Body mass index was defined as weight divided by height squared (kg/m^2). Body surface area (BSA) was calculated using the DuBois formula.[17] Additional body composition measurements were collected utilizing multifrequency Bio-Impedance Analyses (Quadscan4000, Bodystat Ltd, Douglas, British Isles). Data on fat mass and dry lean weight were extracted for analyses. A positive history of CVD was defined as a clinically diagnosed myocardial infarction, stroke, and/or peripheral arterial disease. Protein intake was calculated using an equation based on urinary urea excretion.[18] The estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[19]

Data on haemoglobin levels, mean corpuscular volume, haematocrit levels, haptoglobin, glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triglycerides, high sensitivity C-reactive protein (hs-CRP), serum albumin, and serum creatinine were extracted from the hospital laboratory system. Levels of total serum testosterone and androstenedione were measured using liquid-chromatography-tandem mass spectrometry

(TQ-S Xevo, Waters, Milford, MA, USA, and Symbiosis Pharma, Spark-Holland, Emmen, NL). To ensure measurement of total serum testosterone levels, a protein disruptor was added.

All patients received strict instructions to assure adequate 24-h urine collection. At the start of collection, all patients were instructed to start by discarding a void and to subsequently collect all urine for the next 24-h, including a void at exactly 24-h after the collection start. Data on 24-h urinary CER, proteinuria, and urinary urea excretion were extracted from our hospital laboratory system.

Immunosuppressive regiments

Immunosuppressive medication was regulated by standardized protocols which have been previously described.[20] Variations to the standard regimes were present and were due to treatment of allograft rejection or side effects.

The cumulative dose of prednisolone was calculated by multiplying the time since transplantation by the prescribed dose of prednisolone and adding the dose of prednisolone and/or methylprednisolone required for treatment of acute rejection. A conversion factor of 1.25 was utilized to convert methylprednisolone dose to prednisolone dose.

Physical function tests

Handgrip strength (HGS) was chosen as a marker for muscle strength in the upper limbs. HGS was measured with a hydraulic handheld dynamometer (Patterson Medical JAMAR 5030J1, Warrenville, Canada). Participants were asked to perform this test, while sitting with their shoulders in adduction and their arms rotated into neutral position. Elbows were flexed to 90°, and forearms and wrists moved into neutral position. Participants were instructed and stimulated to perform a maximal isometric contraction. Measurements were alternately repeated between both hands with an interval of 30 s. The average of three consecutive measurements were calculated for analyses.

To measure muscle strength of the lower limbs, a sit-to-stand test was performed. A straight-backed chair with a hard seat was stabilized by the investigator. Participants were asked to sit down, subsequently put their feet flat on the floor, and to fold their arms across the chest. Furthermore, participants were instructed to fully stand up before sitting down again without using their arms. Measurements were started in seated position, and upon command, patients stood up and returned sitting. This routine was done five times as rapidly as possible. The average of two consecutive measurements were calculated for analyses.

The 2-min walk test (2MWT) was performed to test endurance. For this test, participants were asked to walk as fast as possible without running for a consecutive time of 2 min. To calculate the distance covered by the participants, two markers were set 15 m apart.[16]

Statistical analyses

Statistical analyses were performed using IBM Statistics SPSS version 23.0 (IBM Inc. Chicago, IL, USA) and R version 3.2.3 (Vienna, Austria). A two-sided *P*-value of ≤ 0.05 was considered to be statistically significant. A total of 220 RTR were included. Data on CER were missing or incomplete in 36 RTR, leaving 184 RTR eligible for analyses. Distribution of variables was assessed by histograms and probability plots. Normally distributed variables are presented as mean ± SD, skewed distributed variables are presented as median (IQR), and categorical variables are presented as a number (percentage).

To identify differences in CER and muscle performance between RTR and healthy controls, *t*-test for independent samples were performed when variables were normally distrubuted. Mann–Whitney *U* tests were performed when variables were skewedly distributed and chi-squared tests when variables were categorical. Potential differences across sex-stratified tertiles of CER were assessed, using one-way analysis of variance tests were performed for normally distributed variables, Kruskal–Wallis test when variables were skewedly distributed, and chi-squared tests were used for categorical variables.

Percentage difference in CER and percentage difference in protein intake between RTR and healthy controls were calculated as $((CER_{RTR} - CER_{healthy\ controls})/(CER_{healthy\ controls})) \times 100\%$ and $((\text{Protein intake}_{RTR} - \text{Protein intake}_{healthy\ controls})/(\text{Protein intake}_{healthy\ controls})) \times 100\%$, respectively.

To study the association of CER with HGS, sit-to-stand test, and 2MWT, multivariable linear regression analyses were performed. First, a crude analysis was performed (Model 1). We then proceeded with adjustments for age and sex (Model 2). Model 3 was additionally adjusted for renal function, time after transplantation, donor type, and BSA. Thereafter, we additionally adjusted for cardiovascular risk factors, including hypertension, glucose levels, and a history of CVD in Model 4, LDL-cholesterol, serum albumin, hs-CRP, androstenedione, and protein intake in Model 5. Lastly, in Model 6, we additionally adjusted for medication use, including cumulative prednisolone dose, use of a calcineurin inhibitor, lipid lowering drugs, and insulin use.

Results

Comparison of renal transplant recipients with healthy controls

In both groups, there was a similar number of male subjects and age did not differ between the two groups (data

DOI: 10.1002/jcsm.12399

presented in Table 1). Healthy controls had higher levels of CER compared to RTR. In addition, healthy controls had a greater HGS than RTR and performed better at the 2MWT. No difference in performance of the sit-to-stand test was observed. Percentagewise, RTR had a 10.7% lower CER and 1.5% lower protein intake compared to healthy controls.

Baseline characteristics

Out of the 184 RTR eligible for analyses, 54% was male with a mean (±SD) age of 56.9 ± 11.9 years. Median (IQR) time after transplantation was 4.0 [1.1–8.8] years. Because there is a physiological difference in the quantity of muscle mass between men and women, RTR were divided into sex-stratified tertiles of CER. Mean CER was 12.9 ± 4.0 mmol/24-h for men and 9.5 ± 2.9 mmol/24-h for women. Baseline characteristics are presented in Table 2 according to sex-stratified tertiles. RTR in the lowest tertile were older and had more often a history of CVD. Furthermore, BSA, levels of androstenedione, and protein intake gradually increased over the tertiles. Causes of primary renal disease, kidney function, hs-CRP, testosterone, and cumulative prednisolone dose did not differ across tertiles.

Association of urinary creatinine excretion rate with muscle strength and endurance

To investigate whether urinary CER is associated with muscle performance, standardized βs for the association of CER with HGS, sit-to-stand-test, and 2MWT were calculated for RTR and were presented in Table 3.

Univariate analysis of the association of CER, measured as continuous variable, with HGS showed a significant association with a standardized β of 0.64, $P < 0.001$ (Table 3, Model 1) and is graphically depicted for men in Figure 1A and for women in Figure 1B. Subsequently, we proceeded with multivariable analyses in which adjustments for age and sex were made, std. β 0.49, $P < 0.001$ (Table 3, Model 2). Further adjustments for estimated glomerular filtration rate, time after transplantation, donor type, and BSA resulted in a standardized β of 0.41 $P < 0.001$ (Table 3, Model 3). Additional adjustments for cardiovascular risk factor did not materially change the results (std. β 0.41 $P < 0.001$) (Table 3, Model 4). Further adjustment for androstenedione, LDL-cholesterol, serum albumin, hs-CRP, and protein intake showed a significant association with a standardized β of 0.32 $P < 0.001$ (Table 3, Model 5). Finally, additional adjustments for medication use in Model 6 ($R^2 = 0.76$) did not materially change the association of CER with HGS, std. β 0.33 $P < 0.001$ (Table 3). univariate analysis of the association of CER with the sit-to-stand test showed a borderline significant association with a standardized β of −0.19, $P = 0.06$ (Table 3, Model 1). After additional adjustments for age and sex in Model 2 and kidney function, time after transplantation, donor status, and BSA in Model 3, an association between CER and the sit-to-stand test was uncovered (std. β −0.19, $P = 0.03$). Adjusting for a history of CVD, hypertension, and glucose levels did not materially change the association (std. β −0.18, $P = 0.04$). However, the association was lost after additional adjustment for the potential confounders in Models 5 and 6 (std. β −0.08, $P = 0.30$ and −0.09, $P = 0.27$, respectively). To investigate the association of CER with 2MWT, a crude analysis was first performed and did not reveal a significant association (std. β 0.14, $P = 0.16$). Adjustment for all potential confounders did not uncover an otherwise existing association of CER with 2MWT (std. β 0.07, $P = 0.40$).

Discussion

In the current study, we demonstrated that despite all effort, muscle mass, HGS, and endurance in RTR are still not at a comparable level to healthy controls. Moreover, 24-h urinary CER was found to be a marker not only for total body muscle mass but also for muscle performance in RTR. The results of this study support the previously reported hypothesis of a positive association of muscle mass and muscle performance.

To the best of our knowledge, this study is the first to show a positive association between urinary CER and muscle

Table 1 Differences of CER and muscle performance between RTR and healthy controls

	RTR	Healthy controls	*P*-value
Male, *n* (%)	99 (53.8)	37 (47.4)	0.35
Age, years	56.9 ± 11.9	57.9 ± 9.9	0.51
BMI, kg/m^2	26.8 ± 4.8	26.5 ± 4.1	0.64
BSA, m^2	1.93 ± 0.2	1.89 ± 0.4	0.44
CER, mmol/24-h	11.7 ± 4.0	13.1 ± 5.2	0.04
Handgrip strength, N	30.5 (23.7–41.1)	38.3 (29.3–46.0)	<0.001
Sit to stand, s	12.2 ± 3.3	11.9 ± 2.8	0.46
2-min walk test, m	151.5 ± 49.2	172.3 ± 12.2	<0.001
Hs-CRP, mg/L	1.9 (0.8–5.0)	1.1 (0.7–2.6)	0.02
Protein intake, g/day	79.6 ± 8.1	80.8 ± 10.8	0.39

BMI, defined as weight divided by height squared (kg/m^2); BSA, body surface area; CER, creatinine excretion rate; hs-CRP, high sensitivity C-reactive protein.

Table 2 Baseline characteristics of 184 RTR and according to sex-stratified tertiles of urinary creatinine exertion rate

	Sex-stratified tertiles of urinary CER			
	1st	2nd	3rd	*P*-value
Men, *n*	33	33	33	
CER, mmol/24-h	9.4 ± 2.1	13.0 ± 0.9	18.0 ± 2.2	N/A
Women, *n*	28	29	28	
CER, mmol/24-h	7.2 ± 1.0	9.4 ± 0.5	12.6 ± 3.2	N/A
Demographics				
Age, years	62.7 ± 8.4	57.0 ± 10.8	51.0 ± 13.0	<0.001
History of CVD, *n* (%)	11 (18.0)	5 (8.1)	3 (4.9)	0.05
Hypertension, *n* (%)	57 (93.4)	58 (93.5)	53 (86.9)	0.09
Diabetes, *n* (%)	19 (31.1)	18 (29.0)	12 (19.7)	0.29
Dialysis vintage, months	28.5 (18.0–46.8)	32.0 (16.0–45.0)	30 (13.0–57.5)	0.92
Transplantation vintage, years	5.0 (1.0–13.0)	4.0 (1.0–7.0)	3.0 (1.0–6.5)	0.07
Living donor, *n* (%)	24 (39.3)	33 (53.2)	36 (59.0)	0.08
Primary renal disease, *n*				0.63
Glomerulonephritis/vasculitis	11 (18.0)	14 (22.6)	12 (19.7)	
Membranous glomerulopathy/FSGS	6 (9.8)	10 (16.1)	6 (9.8)	
Vascular/hypertension	6 (9.8)	6 (9.7)	4 (6.6)	
Polycystic kidney diseases	9 (14.8)	13 (21.0)	18 (29.5)	
Diabetic nephropathy	3 (4.9)	2 (3.2)	1 (1.6)	
Urological origin	3 (4.9)	4 (6.5)	2 (3.3)	
Other/unknown	23 (37.7)	13 (21.0)	18 (29.5)	
Vitals				
SBP, mmHg	137.7 ± 22.5	134.2 ± 17.3	133.7 ± 15.2	0.49
DBP, mmHg	75.0 ± 12.9	78.6 ± 10.6	81.4 ± 8.5	0.01
Heart rate, bpm	73.0 ± 10.9	71.7 ± 12.0	69.5 ± 13.0	0.33
Glucose homeostasis				
Plasma glucose, mmol/L	5.6 (5.1–6.3)	5.6 (4.9–6.5)	5.2 (4.9–6.0)	0.13
HBA1c, %	6.1 ± 0.7	6.0 ± 1.0	5.9 ± 0.8	0.39
Use of Insulin, *n* (%)	9 (14.8)	5 (8.1)	2 (3.3)	0.08
Use of oral antidiabetics, *n* (%)	7 (11.5)	8 (12.9)	6 (9.8)	0.87
Renal function				
Serum creatinine, umol/L	119.0 (103.0–141.0)	126.0 (107.8–160.0)	123.0 (107.5–154.5)	0.42
eGFR, ml/min per 1.73 m^2	49.4 ± 17.2	48.0 ± 16.6	50.5 ± 16.0	0.70
Body composition				
Weight, kg	74.7 ± 13.7	79.8 ± 14.4	85.4 ± 15.3	<0.001
BMI, kg/m^2	26.0 ± 4.2	26.6 ± 4.5	28.1 ± 5.5	0.04
BSA, m^2	1.8 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	<0.001
Fat mass, kg	23.7 ± 8.3	24.5 ± 9.7	25.6 ± 10.2	0.56
Dry lean weight, kg	11.5 ± 3.6	13.8 ± 4.2	15.9 ± 4.2	<0.001
Laboratory measurements				
Testosterone, nmol/L	5.4 (0.3–12.1)	8.3 (0.5–13.4)	9.0 (0.4–13.4)	0.75
Dihydrotestosterone, nmol/L	0.4 (0.00–1.2)	0.6 (0.02–1.3)	0.6 (0.00–1.2)	0.97
Androstenedione, nmol/L	0.8 (0.5–1.7)	1.2 (0.7–2.2)	1.3 (0.9–2.5)	0.007
Hb, mmol/L	8.1 ± 1.0	8.0 ± 1.2	8.3 ± 1.1	0.36
Haptoglobin, g/L	1.6 (1.1–2.0)	1.2 (0.9–1.8)	1.4 (1.0–1.7)	0.42
Total cholesterol, mmol/L	4.9 ± 1.1	5.0 ± 1.2	5.1 ± 1.2	0.69
LDL-cholesterol, mmol/L	2.7 ± 0.9	3.0 ± 1.0	3.1 ± 1.0	0.09
HDL-cholesterol, mmol/L	1.5 (1.2–1.9)	1.3 (1.0–1.7)	1.3 (1.1–1.7)	0.15
Triglycerides, mmol/L	1.6 (1.3–2.3)	1.6 (1.1–2.1)	1.7 (1.3–2.4)	0.60
Hs-CRP, mg/L	2.6 (0.8–7.0)	1.8 (0.7–4.2)	1.7 (0.8–4.0)	0.60
Serum albumin, g/L	42.2 ± 3.3	42.7 ± 2.6	43.7 ± 3.0	0.02
Urinary parameters				
Proteinuria, ≥0.5 g/24-h (%)	7 (11.5)	9 (14.5)	10 (16.4)	0.96
Protein intake, g/day	74.6 ± 4.4	78.9 ± 4.1	84.5 ± 7.4	<0.001
Medication				
Steroids, *n* (%)	60 (98.4)	58 (93.5)	59 (96.7)	0.37
Cumulative prednisolone, g	13.7 (1.8–39.2)	7.3 (1.8–19.4)	5.5 (1.8–21.0)	0.20
Proliferation inhibitor, *n* (%)	46 (75.4)	53 (85.5)	49 (80.3)	0.37
Calcineurin inhibitor, *n* (%)	44 (72.1)	54 (87.1)	46 (75.4)	0.11
Use of mTor inhibitor, *n* (%)	5 (8.2)	1 (1.6)	3 (4.9)	0.24
Antihypertensive drugs, *n* (%)	55 (90.2)	57 (91.9)	50 (82.0)	0.19
Lipid lowering drugs, *n* (%)	44 (72.1)	36 (58.1)	33 (54.1)	0.10

Diabetes mellitus was defined as a fasting serum glucose of ≥7.0 mmol/L or the use of antidiabetic drugs; hypertension was defined as a SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg and/or the use of antihypertensive drugs; BMI, body mass index; BSA, body surface area; CER, creatinine excretion rate; CI, calcineurin inhibitor; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; Hb, haemoglobin; HDL, high-density lipoprotein; hs-CRP; high sensitivity C-reactive protein; LDL, low-density lipoprotein; MCV, mean corpuscular volume; mTor, mammalian target of rapamycin; N/A, not applicable; PI, proliferation inhibitor; SBP, systolic blood pressure.

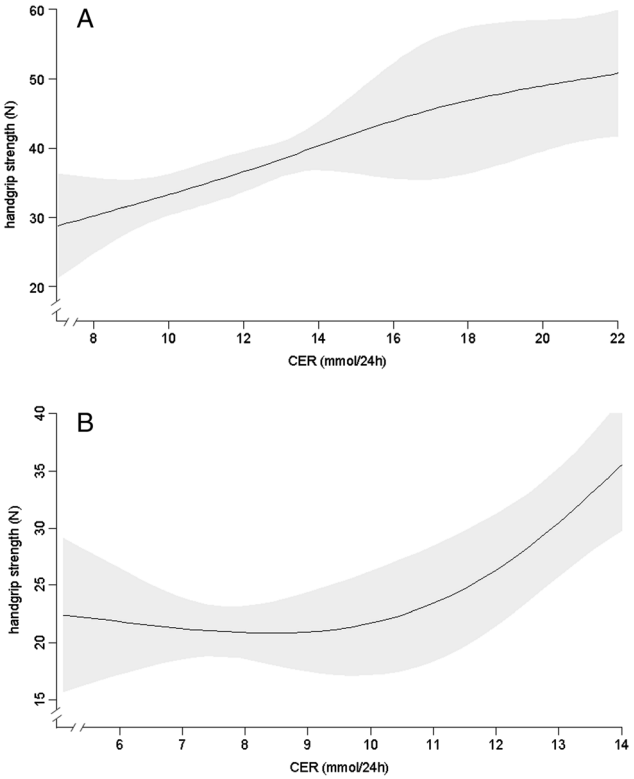
DOI: 10.1002/jcsm.12399

Table 3 Association of urinary creatinine excretion rate with muscle strength and endurance

	Std. β	Unstd. β (95% CI)	*P*-value
Handgrip strength			
Model 1	0.64	0.21 (0.17, 0.25)	<0.001
Model 2	0.49	0.16 (0.11, 0.22)	<0.001
Model 3	0.41	0.14 (0.08, 0.19)	<0.001
Model 4	0.41	0.14 (0.09, 0.19)	<0.001
Model 5	0.32	0.11 (0.06, 0.15)	<0.001
Model 6	0.33	0.11 (0.06, 0.16)	<0.001
Sit-to-stand test			
Model 1	−0.19	−0.22 (−0.45, 0.01)	0.06
Model 2	−0.08	−0.09 (−0.31, 0.12)	0.04
Model 3	−0.19	−0.22 (−0.43, −0.02)	0.03
Model 4	−0.18	−0.21 (−0.42, −0.01)	0.04
Model 5	−0.08	−0.10 (−0.26, 0.08)	0.30
Model 6	−0.09	−0.11 (−0.29, 0.08)	0.27
2MWT			
Model 1	0.14	1.56 (−0.60, 3.71)	0.16
Model 2	0.02	0.18 (−1.77, 2.12)	0.86
Model 3	0.12	1.34 (−0.53, 3.22)	0.16
Model 4	0.12	1.33 (−0.59, 3.25)	0.17
Model 5	0.06	0.63 (−0.93, 2.19)	0.43
Model 6	0.07	0.75 (−0.99, 2.49)	0.40

Model 1: Crude analysis. Model 2: Model 1 adjusted for sex and age. Model 3: Model 2 additionally adjusted for eGFR, time after transplantation, living donor, and BSA. Model 4: Model 3 additionally adjusted for history of CVD, hypertension, and glucose levels. Model 5: Model 4 additionally adjusted for androstenedione, LDL-cholesterol, albumin levels, hs-CRP, and protein intake. Model 6: Model 5 additionally adjusted for cumulative prednisolone dose, use of CI, use of lipid lowing drugs, and insulin use. Hypertension was defined as a SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg and/or the use of antihypertensive drugs. 2MWT, 2-min walk test; BSA, body surface are; CI, calcineurin inhibitor; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein.

Figure 1 Association of urinary creatinine excretion rate with handgrip strength in male (A) and female (B) renal transplant recipients.



The grey area represents the 95% CI of the correlation. CER, creatinine excretion rate.

performance in RTR. Our study results are in line with previous findings that demonstrate a positive correlation between lean body mass, as measured by dual energy X-ray absorptiometry (DEXA), and muscle strength in RTR.[21] It should be noted that lean body mass does not solely reflect muscle mass, considering lean body mass also consists of body water and organs tissue, potentially leading to inaccuracies. This was demonstrated in healthy subjects where CER, compared to DEXA, was found to be a more sensitive marker to assess changes in body composition.[11] The authors demonstrated CER to decline with age, whereas muscle mass determined by DEXA was not effected by age. Furthermore, lean body mass can easily be overestimated, because DEXA analyses and computed tomography lack the capability to differentiate between muscle mass and intramuscular fat infiltration and intramuscular oedema.[9] Fat infiltration in muscles results into a lower skeletal muscle density, which in turn was found to be a better prognostic marker for patient survival than skeletal muscle index.[21,22]

In individuals with kidney function in steady-state and not dependent on dialysis for renal replacement therapy, CER is a reliable surrogate of skeletal muscle mass, which, to a small extent, may co-vary with the amount of dietary meat intake.[8,23] It is important to note that many dialysis patients have minimal to no urine output, so that CER cannot be accurately assessed in this patient group.[23] In this subgroup of patients, in which variation in kidney function does not materially influence serum creatinine concentrations, serum creatinine is more a marker of muscle mass than of kidney function. It has indeed been shown that serum creatinine is associated with DEXA-measured lean body mass in hemodialysis patients.[23] In this line of research, it has also been shown that in hemodialysis patients, serum creatinine assessed while being on dialysis is inversely associated with rates of mortality, both on the waiting list for transplantation and after transplantation, likely because in this circumstance high serum creatinine reflects high muscle mass.[24,25]

In patients with advanced stage of chronic kidney disease, including end-stage renal disease, a low muscle mass is likely the consequence of a combination of decreased muscle protein synthesis and increased muscle protein degradation.[26] Anorexia, leading to low protein intake, in combination with reduced anabolic stimuli, particularly low physical activity has been suggested to underlie decreased muscle protein synthesis.[27,28] Increased muscle protein degradation is often a result

of catabolism induced by metabolic acidosis and chronic low-grade inflammation in this population.[26] When patients with end-stage renal disease are transplanted, transplantation is expected to at least partially reverse the tendencies for decreased muscle protein synthesis and increased muscle protein degradation. We found that in RTR, muscle mass is significantly lower compared to healthy controls, and percentagewise is more pronounced than the difference in protein intake, making it unlikely that a difference in protein intake entirely explains the difference in muscle mass. Another component that could contribute to lower muscle protein synthesis is lower physical activity. Although we did not have data on physical activity in the current study, we have previously shown that RTR maintain very low levels of physical activity, with only few RTR meeting the guidelines for regular physical activity.[29,30] It is likely that, in addition to decreased muscle protein synthesis, there is also a component of increased protein catabolism that adds. In many RTR, the immunosuppressive regimen for prevention of allograft rejection includes chronic treatment with glucocorticoids. Such treatment has been shown to increase muscle protein degradation and induce muscle atrophy.[31] As the cumulative prednisolone dose did not differ across sex-stratified tertiles of CER, it cannot explain the differences in CER between RTR, but on population level, it could contribute to the difference between RTR and healthy controls. Finally, RTR can be subjected to rejection episodes, to intercurrent diseases, do often exhibit a chronic immune response to the transplant, do often endure the inflammatory aspects of chronic atherosclerosis, and usually suffer from suboptimal kidney function, all of which can contribute to chronic low-grade inflammation and protein-energy wasting.[32–34] In the current study, levels of hs-CRP were found to be higher in RTR compared to healthy controls, making chronic low-grade inflammation leading to protein-energy wasting a plausible mechanism contributing to the difference in CER levels between RTR and healthy controls.

No association was found between CER and the sit-to-stand test or endurance. This shows the complexity of the association between muscle mass and muscle function in RTR. For this study, we selected the sit to stand as marker for lower limb strength as it is a reliable and valid clinical tool, which is least effected by age-dependent changes and commonly used to assess muscle strength and mobility.[35,36] However, in a recent study, Buckley *et al*. showed quadriceps peak torque to be more closely associated with endurance than strength.[37] This could potentially explain why both the sit-to-stand test and the endurance test were not associated with CER.

An important strength of this study is that the cohort used for analyses is a relatively large well-characterized cohort of stable RTR with extensive data on anthropometrics, biochemical measurements, and medication use. Another strength is that all measurements in RTR were put into perspective by measurements in health controls. Finally, in this study, we analysed not only muscle strength but also endurance.

The current study has some limitations that should be noted. CER as determined from 24-h urine collection is a direct measurement of muscle mass. It should, however, be noted that 24-h urine collection is prone to collection errors. In our study, minimization of collection errors was acquired by careful instruction. Another limitation is that we could not control for dietary meat or fish intake, as our study is observational in nature. Finally, due to lack of data, levels of physical activity could not be taken into account.

In a previous study, 24-h urinary CER was found to be an independent predictor for mortality and graft failure in RTR.[8] As we have now demonstrated that CER, a well-known biomarker for muscle mass, is also associated with muscle performance, CER could potentially be utilized as a modifiable factor to improve post-transplant patient and graft survival. Although, CER is a non-invasive, easily accessible, inexpensive, and direct measurement of total body muscle mass; it is often not included in the imaging technique armamentarium available and applied for evaluation of muscle mass in clinical intervention studies and observational studies.[38–47] Each of the imaging techniques that can be applied in clinical intervention and observational studies has its drawbacks, with expenses and availability as most important drawbacks for magnetic resonance imaging and computed tomography, while DEXA and muscle ultrasonography share the major disadvantage of not being able to measure intramuscular adipose tissue.[22,48] This impedes assessment of muscle quality, a measure which is nowadays more and more appraised as a more important determinant of outcome than quantification of muscle mass without taking intramuscular fat into account.[22,48] Although bioelectric impedance analysis is often advocated as a safe and inexpensive alternative to whole-body imaging, its results may easily be altered by fluid retention and general health status.[48,49] Although, for certain, application of CER has the drawback of lack of availability of a reference value in elderly people[48] its generation from the non-enzymatic conversion of creatine and creatine phosphate in muscle[9] guarantees that it is insensitive to intramuscular fat and oedema and thereby provides a direct reflection of total body muscle mass. If observational studies, like one showing that muscle quality as assessed by computed tomography is predictive of long-term survival in a cohort of elderly diffuse B-cell lymphoma patients,[21] would start including collection of 24-h urine for determination of CER, predictive properties could be compared and it could be evaluated whether CER is of additional predictive value or could even replace assessment of muscle quality as assessed by computed tomography or magnetic resonance imaging in future studies. Inclusion of a healthy control group of elderly subjects could facilitate generation of normal values and cut-off values for defining sarcopenia. If intervention studies evaluating effects of treatment on muscle mass by imaging techniques, like one that investigated the effect of espindolol on cachexia in non-small cell lung cancer or colorectal cancer[45] would start including collection of 24-h urine for determination of CER, the efficacy of CER as outcome measure insensitive to intramuscular adipose

tissue infiltration and oedema could be evaluated and provide data for power calculation for future trials with CER as outcome in addition to muscle mass and muscle quality determined by imaging techniques or even serve as an alternative.

In conclusion, 24-h urinary CER is positively associated with HGS in RTR. This implicates that urinary CER does not only reflect total body muscle mass but also muscle performance in RTR. Future research is warranted and should preferably focus on the utility of CER as modifiable component to improve long-term outcome after renal transplantation and on its potential for addition to the armamentarium of imaging techniques available and applied for evaluation of muscle mass in clinical intervention studies and observational studies.

Acknowledgement

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia, and Muscle update 2017.[50]

Conflict of Interest

R.T.G. received consultancy fees and research funding from Otsuka, Ipsen, Sanofi-Genzyme for polycystic kidney disease research. All money was paid to his institution. The other authors have no conflicts of interest to disclose.

Contribution

S.P.S. analysed the data and wrote the first draft of the paper. M.F.E., A.W.G.N., M.v.L., V.E.d.M., A.P.v.B., R.T.G., and S.J.L.B. contributed to the interpretation of the results and provided important advice and intellectual content. S.P.S., M.F.E., A.W.G.N., M.v.L., and S.J.L.B. collaborated in the data collection. All authors had access to the data, contributed to critical revision of the manuscript, and approved the final version of the manuscript.

References

1. Wolfe R, Ashby V. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *NEJM* 1999;**341**:1725–1730.
2. Collins AJ, Foley RN, Gilbertson DT, Chen S-C. United States Renal Data System public health surveillance of chronic kidney disease and end-stage renal disease. *Kidney Int Suppl* 2015;**5**:2–7.
3. Merville P. Combating chronic renal allograft dysfunction: optimal immunosuppressive regimens. *Drugs* 2005;**65**:615–631.
4. Wolfe RR. The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 2006;**84**:475–482.
5. Fried LF, Boudreau R, Lee JS, Chertow G, Kurella-Tamura M, Shlipak MG, et al. Kidney function as a predictor of loss of lean mass in older adults: health, aging and body composition study. *J Am Geriatr Soc* 2007;**55**:1578–1584.
6. Anand S, Johansen KL, Kurella Tamura M. Aging and chronic kidney disease: the impact on physical function and cognition. *J Gerontol A Biol Sci Med Sci* 2014;**69A**:315–322.
7. Roshanravan B, Patel KV, Robinson-Cohen C, De Boer IH, O'Hare AM, Ferrucci L, et al. Creatinine clearance, walking speed, and muscle atrophy: a cohort study. *Am J Kidney Dis* 2015;**65**:737–747.
8. Oterdoom LH, van Ree RM, de Vries APJ, Gansevoort RT, Schouten JP, van Son WJ, et al. Urinary creatinine excretion reflecting muscle mass is a predictor of mortality and graft loss in renal transplant recipients. *Transplantation* 2008;**86**:391–398.
9. Heymsfield SB, Olafson RP, Kutner MH, Nixon DW. A radiographic method of quantifying protein-calorie undernutrition. *Am J Clin Nutr* 1979;**32**:693–702.
10. Heymsfield SB, Arteaga C, McManus F, Smith J, Moffltt S. Perspective in nutrition measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983;**37**:478–494.
11. Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. *Am J Physiol* 1999;**277**:E489–E495.
12. Welle S, Thornton C, Totterman S, Forbes G, Welle S, Thornton C, et al. Utility of creatinine excretion in body-composition studies of healthy men and women older than 60 y. *Am J Clin Nutr* 1996;**63**:151–156.
13. Polinder-Bos HA, Nacak H, Dekker FW, Bakker SJL, Gaillard CAJM, Gansevoort RT. Low urinary creatinine excretion is associated with self-reported frailty in patients with advanced chronic kidney disease. *Kidney Int Rep* 2017;**2**:676–685.
14. Isoyama N, Qureshi AR, Avesani CM, Lindholm B, Bàràny P, Heimbürger O, et al. Comparative associations of muscle mass and muscle strength with mortality in dialysis patients. *Clin J Am Soc Nephrol* 2014;**9**:1720–1728.
15. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. *J Gerontol A Biol Sci Med Sci* 2006;**61**:72–77.
16. Eisenga MF, Gomes-Neto AW, Van Londen M, Ziengs AL, Douwes RM, Stam SP, et al. Rationale and design of TransplantLines: a prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* 2018;**8**(12):e024502.
17. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989;**5**:303.
18. Deetman PE, Said MY, Kromhout D, Dullaart RPF, Kootstra-Ros JE, J-SF S, et al. Urinary urea excretion and long-term outcome after renal transplantation. *Transplantation* 2015;**99**:1009–1015.
19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150**:604–612.
20. van Ree RM, de Vries APJ, Oterdoom LH, The TH, Gansevoort RT, van der Heide JJ H, et al. Abdominal obesity and smoking are important determinants of C-reactive protein in renal transplant recipients. *Nephrol Dial Transplant* 2005;**20**:2524–2531.
21. van den Ham ECH, Kooman JP, Schols AMWJ, Nieman FHM, Does JD, Franssen FME, et al. Similarities in skeletal muscle strength and exercise capacity between renal transplant and hemodialysis patients. *Am J Transplant* 2005;**5**:1957–1965.
22. Chu MP, Lieffers J, Ghosh S, Belch A, Chua NS, Fontaine A, et al. Skeletal muscle density is an independent predictor of diffuse large B-cell lymphoma outcomes treated with rituximab-based chemoimmunotherapy. *J Cachexia Sarcopenia Muscle* 2017;**8**:298–304.
23. Miljkovic I, Zmuda JM. Epidemiology of myosteatosis. *Curr Opin Clin Nutr Metab Care* 2010;**13**:260–264.
24. Patel SS, Molnar MZ, Tayek JA, Ix JH, Noori N, Benner D, et al. Serum creatinine as a

marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature. *J Cachexia Sarcopenia Muscle* 2013;**4**:19–29.
25. Molnar MZ, Streja E, Kovesdy CP, Bunnapradist S, Sampaio MS, Jing J, et al. Associations of body mass index and weight loss with mortality in transplant-waitlisted maintenance hemodialysis patients. *Am J Transplant* 2011;**11**:725–736.
26. Streja E, Molnar MZ, Kovesdy CP, Bunnapradist S, Jing J, Nissenson AR, et al. Associations of pretransplant weight and muscle mass with mortality in renal transplant recipients. *Clin J Am Soc Nephrol* 2011;**6**:1463–1473.
27. Wang XH, Mitch WE. Mechanisms of muscle wasting in chronic kidney diease. *Nat Rev Nephrol* 2014;**10**:504–516.
28. Atherton PJ, Smith K. Muscle protein synthesis in response to nutrition and exercise. *J Physiol* 2012;**590**:1049–1057.
29. Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. *Am J Clin Nutr* 2010;**91**:1123S–1127S.
30. Zelle DM, Corpeleijn E, Stolk RP, de Greef MHG, Gans ROB, van der Heide JJH, et al. Low physical activity and risk of cardiovascular and all-cause mortality in renal transplant recipients. *Clin J Am Soc Nephrol* 2011;**6**:898–905.
31. Dontje ML, de Greef MHG, Krijnen WP, Corpeleijn E, Kok T, Bakker SJL, et al. Longitudinal measurement of physical activity following kidney transplantation. *Clin Transplant* 2014;**28**:394–402.
32. Sato AY, Richardson D, Cregor M, Davis HM, Au ED, McAndrews K, et al. Glucocorticoids induce bone and muscle atrophy by tissue-specific mechanisms upstream of E3 ubiquitin ligases. *Endocrinology* 2017;**158**:664–677.
33. Molnar MZ, Keszei A, Czira ME, Rudas A, Ujszaszi A, Haromszeki B, et al. Evaluation of the malnutrition-inflammation score in kidney transplant recipients. *Am J Kidney Dis* 2010;**56**:102–111.
34. Stenvinkel P. Can treating persistent inflammation limit protein energy wasting? *Semin Dial* 2013;**26**:16–19.
35. Roe P, Wolfe M, Joffe M, Rosas SE. Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. *Atherosclerosis* 2010;**212**:589–594.
36. Hodes RJ, Insel TR, Landis SC. The NIH toolbox: setting a standard for biomedical research. *Neurology* 2013;**80**:S1–S1.
37. Makizako H, Shimada H, Doi T, Tsutsumimoto K, Lee S, Lee SC, et al. Age-dependent changes in physical performance and body composition in community-dwelling Japanese older adults. *J Cachexia Sarcopenia Muscle* 2017;**8**:607–614.
38. Buckley C, Stokes M, Samuel D. Muscle strength, functional endurance, and health-related quality of life in active older female golfers. *Aging Clin Exp Res* 2017;**0**:1–8.
39. Lewis A, Lee JY, Donaldson AV, Natanek SA, Vaidyanathan S, Man WDC, et al. Increased expression of H19/miR-675 is associated with a low fat-free mass index in patients with COPD. *J Cachexia Sarcopenia Muscle* 2016;**7**:330–344.
40. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. *J Cachexia Sarcopenia Muscle* 2017;**8**:639–646.
41. Fabbri E, Chiles Shaffer N, Gonzalez-Freire M, Shardell MD, Zoli M, Studenski SA, et al. Early body composition, but not body mass, is associated with future accelerated decline in muscle quality. *J Cachexia Sarcopenia Muscle* 2017;**8**:490–499.
42. Dodds RM, Granic A, Davies K, Kirkwood TBL, Jagger C, Sayer AA. Prevalence and incidence of sarcopenia in the very old: findings from the Newcastle 85+ study. *J Cachexia Sarcopenia Muscle* 2017;**8**:229–237.
43. van Vugt JLA, Levolger S, Gharbharan A, Koek M, Niessen WJ, Burger JWA, et al. A comparative study of software programmes for cross-sectional skeletal muscle and adipose tissue measurements on abdominal computed tomography scans of rectal cancer patients. *J Cachexia Sarcopenia Muscle* 2017;**8**:285–297.
44. Montano-Loza AJ, Angulo P, Meza-Junco J, Prado CMM, Sawyer MB, Beaumont C, et al. Sarcopenic obesity and myosteatosis are associated with higher mortality in patients with cirrhosis. *J Cachexia Sarcopenia Muscle* 2016;**7**:126–135.
45. Stewart Coats AJ, Ho GF, Prabhash K, von Haehling S, Tilson J, Brown R, et al. Espindolol for the treatment and prevention of cachexia in patients with stage III/IV non-small cell lung cancer or colorectal cancer: a randomized, double-blind, placebo-controlled, international multicentre phase II study (the ACT-ONE trial). *J Cachexia Sarcopenia Muscle* 2016;**7**:355–365.
46. Kazemi-Bajestani SMR, Becher H, Ghosh S, Montano-Loza AJ, Baracos VE. Concurrent depletion of skeletal muscle, fat, and left ventricular mass in patients with cirrhosis of the liver. *J Cachexia Sarcopenia Muscle* 2016;**7**:97–99.
47. Tyrovolas S, Koyanagi A, Olaya B, Ayuso-Mateos JL, Miret M, Chatterji S, et al. Factors associated with skeletal muscle mass, sarcopenia, and sarcopenic obesity in older adults: a multi-continent study. *J Cachexia Sarcopenia Muscle* 2016;**7**:312–321.
48. Tosato M, Marzetti E, Cesari M, Savera G, Miller RR, Bernabei R, et al. Measurement of muscle mass in sarcopenia: from imaging to biochemical markers. *Aging Clin Exp Res* 2017;**29**:19–27.
49. Morley JE, Abbatecola AM, Argiles JM, Baracos V, Bauer J, Bhasin S, et al. Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc* 2011;**12**:403–409.
50. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the journal of cachexia, sarcopenia and muscle: update 2017. *J Cachexia Sarcopenia Muscle* 2017;**8**:1081–1083.