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Decreased haemoglobin levels are associated with lower muscle mass and strength in kidney transplant recipients

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Abstract

Background Post-transplant anaemia and reduced muscle mass and strength are highly prevalent in kidney transplant recipients (KTRs). Decreased haemoglobin levels, a marker of anaemia, could adversely affect muscle mass and strength through multiple mechanisms, among others, through diminished tissue oxygenation. We aimed to investigate the association between haemoglobin levels with muscle mass and strength in KTRs.

Methods We included stable KTRs from the TransplantLines Biobank and Cohort study with a functional graft ≥1 year post-transplantation. Muscle mass was assessed using 24 h urinary creatinine excretion rate (CER) and bioelectrical impedance analysis (BIA). Muscle strength was assessed with a handgrip strength test using a dynamometer and, in a subgroup (n = 290), with the five-times sit-to-stand (FTSTS) test. We used multivariable linear and logistic regression analyses to investigate the associations of haemoglobin levels with muscle mass and strength.

Results In 871 included KTRs [median age 58 (interquartile range (IQR), 48–66)] years; 60% men; eGFR 51 ± 18 mL/min/1.73 m^2) who were 3.5 (1.0–10.2) years post-transplantation, the mean serum haemoglobin level was 13.9 ± 1.8 g/dL in men and 12.8 ± 1.5 g/dL in women. Lower haemoglobin levels were independently associated with a lower CER (std. β = 0.07, P = 0.01), BIA-derived skeletal muscle mass (std. β = 0.22, $P < 0.001$), handgrip strength (std. β = 0.15, $P < 0.001$), and worse FTSTS test scores (std. β = −0.17, P = 0.02). KTRs in the lowest age-specific and sex-specific quartile of haemoglobin levels had an increased risk of being in the worst age-specific and sex-specific quartile of CER (fully adjusted OR, 2.09; 95% CI 1.15–3.77; P = 0.02), handgrip strength (fully adjusted OR, 3.30; 95% CI 1.95–5.59; $P < 0.001$), and FTSTS test score (fully adjusted OR, 7.21; 95% CI 2.59–20.05; $P < 0.001$).

Conclusions Low haemoglobin levels are strongly associated with decreased muscle mass and strength in KTRs. Future investigation will need to investigate whether maintaining higher haemoglobin levels may improve muscle mass and strength in KTRs.

Keywords Haemoglobin levels; 24 h urinary creatinine excretion; Handgrip strength; Kidney transplant recipients

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Introduction

Kidney transplant recipients (KTRs) generally have an impaired skeletal muscle strength and exercise capacity.[1,2] Loss of muscle mass is an important factor adversely influencing muscle strength. Previous studies have shown that reduced muscle mass, impaired muscle strength, and low physical activity are associated with an increased risk of mortality and worse graft outcomes in KTRs.[3–5] Many factors may contribute to reduced muscle mass and strength after transplantation, including physical inactivity, co-morbidities, long-term dialysis prior to transplantation, and the use of immunosuppressive medications, particularly corticosteroids.[6] In addition, the presence of anaemia may contribute to impaired muscle mass and strength, as has been suggested in a cohort study among the general population in the northern Netherlands.[7]

Post-transplantation anaemia, affecting 12–39% of KTRs,[8,9] can be caused by a wide variety of factors, including low-grade inflammation, iron deficiency, erythropoietin deficiency, and the use of immunosuppressive medication, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.[10,11] In KTRs, anaemia has been associated with an increased risk of cardiovascular and all-cause mortality as well as allograft failure.[12–15] Recent studies in non-transplant populations, such as the general population[7] and the elderly,[16] have linked lower haemoglobin levels to reduced muscle mass. In patients with chronic kidney disease, it is well appreciated that anaemia is related to a decreased exercise capacity,[17] but whether decreased haemoglobin levels, a marker of anaemia, are associated with lower muscle mass and strength in KTRs has not been well characterized.

Therefore, the aim of the present study was to elucidate whether haemoglobin levels are associated with muscle mass, reflected by 24 h urinary creatinine excretion rate (CER) and by assessment of appendicular skeletal muscle mass (ASMM) with bioelectrical impedance analysis (BIA), and with muscle strength, determined by handgrip strength and the five times sit-to-stand (FTSTS) test.

Methods

Patient population

We used data from the TransplantLines Biobank and Cohort study, a prospective single-centre study among solid organ transplant recipients and living organ donors (NCT03272841). The study has been described in detail previously.[18] The study protocol was approved by the medical ethical committee of the University Medical Center Groningen (METc 2014/077), conducted in accordance with the principles of the Declaration of Helsinki and consistent with the Good Clinical Practice guidelines provided by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. All participants had given their informed consent prior to enrolment. For the current study, we used stable KTRs with a functional graft for more than 1 year post-transplantation with data available on haemoglobin and 24 h urinary CER (n = 871). BIA, handgrip strength assessment and the FTSTS test were performed in 814, 854, and 290 of the included KTRs, respectively. Data for the current study were collected between June 2015 and August 2020, when the data extraction was performed.

Data collection

According to a strict protocol, all KTRs were asked to collect a 24 h urine sample during the day before their visit to the outpatient clinic.[18] Blood was drawn in the morning. Information on age, sex, height, weight, donor type (living donation vs. postmortal), and medication use was obtained from the patients' records. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Data on alcohol intake and smoking status were collected using a questionnaire. The Checklist Individual Strength (CIS) questionnaire, a tool comprising 20 questions about mental functioning and fatigue-related behaviour in the preceding 2 weeks, was used to assess subjective fatigue.

Laboratory parameters and definitions

Haemoglobin level and mean corpuscular volume (MCV) were measured using a Coulter Counter STKS sum (Coulter Corporation, Miami, FL). Serum iron was measured using a colorimetric assay, ferritin was measured using immunoassay, and transferrin was measured using an immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). Transferrin saturation (TSAT, %) was calculated as 100 × serum iron (μmol/L)/(serum transferrin (g/L) × 25).[19] CER and urinary protein and urea excretion were calculated from total urine volume and creatinine, urea, and protein concentrations. Urea excretion was used to estimate daily protein intake reflecting nutritional status.[20] To account for potential errors in the 24 h urine collections, we calculated the difference between expected and measured 24 h urine volume.[21] Potential inadequate 24 h urine collection was defined as being in the upper 5% of the difference between expected and measured 24 h urine volume. The expected 24 h urine volume was calculated with the following equation: creatinine clearance = ([urinary creatinine] × 24 h urine volume)/[serum creatinine]), where creatinine clearance was calculated using the Cockcroft–Gault equation.[22] The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[23]

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Bioelectrical impedance analysis

To estimate ASMM, we measured bioelectrical impedance analysis (BIA) resistance using a multifrequency bioelectrical impedance device (Quadscan 4,000, Bodystat, Douglas, United Kingdom), as described previously.[18] We used BIA to distinguish between water, fat, and lean body mass to assess body composition. ASMM was estimated from the equation described by Kyle *et al*.[24]:

ASMM (kg) = −4.211 + (0.267 × height (cm)^2/resistance (U)) + (0.095 × weight (kg)) + (1.909 × sex (men = 1; women = 0)) + (−0.012 × age (years)) + (0.058 × reactance(U)).

Muscle strength

Muscle strength was determined by means of handgrip strength measurement with the Jamar Hydraulic Hand Dynamometer (Patterson Medical JAMAR 5030 J1, Warrenville, Canada).[18] Handgrip strength was assessed three times per hand with 30 s intervals between attempts. For the analyses, the average of all attempts of both hands was used.

In addition, the FTSTS test was used to assess functional mobility, which includes leg strength, in a random subset of the included KTRs (n = 290). The FTSTS test was performed three times after a trial round. Subjects were asked to stand up five times as fast as possible from a sitting position with their feet flat on the ground while folding their arms across the chest. Time was measured in seconds. A higher FTSTS test score reflects worse muscle strength.

Statistical analyses

We used IBM SPSS Statistics version 23.0 (SPSS Inc., Chicago, USA) to analyse the data. Normally distributed data are presented as mean ± SD, while data with a skewed distribution are presented as median (IQR). Categorical data are expressed as number (percentage).

To explore the comparability of the different parameters reflecting muscle mass and strength, we calculated Pearson's correlation coefficients between these parameters.

Hereafter, we performed linear regression analyses to assess associations between haemoglobin levels and parameters of muscle mass or strength, that is, CER, ASMM, handgrip strength, and FTSTS test score. We first performed univariable analyses (Model 1). Then, in multivariable analyses, we adjusted for age, sex, BMI, eGFR, serum iron, urinary protein excretion, transplant vintage, donor type (living vs. post-mortal), high-sensitive C-reactive protein (hs-CRP), urinary urea excretion, CIS total score, smoking status, alcohol use, and use of calcineurin inhibitors, antiproliferative agents, systemic corticosteroids, statins, oral iron supplementation, and renin-angiotensin-aldosterone system (RAAS) inhibitors (Model 2). As age, sex, and BMI are already included in the equation by Kyle *et al*., we did not adjust the association between haemoglobin levels and ASMM for these parameters. We generated plots with locally weighted scatterplot smoothing to visualize the associations of haemoglobin levels with CER, ASMM, handgrip strength, and FTSTS test. To further adjust for the confounding effect of age and sex, on which haemoglobin levels, as well as muscle mass and strength, are strongly dependent, we also used multivariable logistic regression analyses with age-specific and sex-stratified analyses. We compared age-specific and sex-specific quartiles of haemoglobin levels with the risk of being in the lowest age-specific and sex-specific quartile of CER, as carried out previously in the general population.[7] Similarly, we assessed the risk of being in the lowest age-specific and sex-specific quartile of ASMM or handgrip strength or being in the highest (i.e. worst) age-specific and sex-specific quartile of the FTSTS test score. In a sensitivity analysis, we excluded KTRs with potential inadequate 24 h urine collection. In all analyses, skewed variables (i.e. hs-CRP, urinary protein excretion, CIS score, and transplantation vintage) were naturally log-transformed. Missing data (specified in the footnote of *Table* 1) were imputed using regressive switching. Five datasets were multiply-imputed, and results were pooled according to Rubin's rules.[25] In all analyses, a P value of ≤0.05 was considered significant.

Results

Baseline characteristics

We included 871 KTRs [median age 58 (interquartile range (IQR), 48–66) years, 60% being men]. The included KTRs were at a median 3.5 (1.0–10.2) years after transplantation. Mean eGFR was 51 ± 18 mL/min/1.73 m^2, and serum haemoglobin level was 13.9 ± 1.8 g/dL in men and 12.8 ± 1.5 g/dL in women. Additional baseline characteristics according to age-specific and sex-specific quartiles of haemoglobin levels are shown in *Table* 1.

Comparison of muscle mass and strength parameters

When comparing the different tests reflecting muscle mass and strength, we found that CER and ASMM were correlated (r = 0.58, $P < 0.001$). Furthermore, CER (r = 0.60, $P < 0.001$) and ASMM (r = 0.60, $P < 0.001$) were correlated with handgrip strength. Correlations in the subset of KTRs with FTSTS test data available showed that FTSTS test score was correlated with handgrip strength (r = −0.25, $P < 0.001$) and

Table 1 Baseline characteristics of 871 kidney transplant recipients

		Age-specific and sex-specific quartiles of haemoglobin levels				
	All	Q1	Q2	Q3	Q4	*P*-value
Number of participants (%)	871	208 (24)	217 (25)	213 (24)	233 (27)	
Demographics						
Age, years	58 (48–66)	57 (46–66)	57 (47–65)	61 (48–68)	57 (49–65)	0.03
Men, *n* (%)	520 (60)	128 (62)	127 (59)	129 (61)	136 (58)	0.88
Body mass index, kg/m^2	27.3 ± 4.8	26.9 ± 5.1	27.2 ± 4.9	28.0 ± 4.8	27.3 ± 4.4	0.13
Laboratory parameters						
Haemoglobin, g/dL						
Men	13.9 ± 1.8	11.6 ± 0.9	13.3 ± 0.5	14.4 ± 0.4	16.0 ± 0.9	<0.001
Women	12.8 ± 1.5	10.7 ± 0.8	12.3 ± 0.3	13.2 ± 0.2	14.5 ± 0.8	<0.001
MCV, fL	89.3 ± 5.9	89.0 ± 7.3	89.7 ± 5.8	89.4 ± 5.1	89.0 ± 5.1	0.52
Ferritin, μg/L	91 (41–187)	105 (35–219)	99 (37–169)	85 (40–200)	87 (49–168)	0.92
Iron, μmol/L	13.9 ± 5.4	11.6 ± 5.3	13.5 ± 4.8	14.6 ± 5.4	15.3 ± 5.3	<0.001
Transferrin, g/L	2.41 ± 0.43	2.34 ± 0.52	2.41 ± 0.44	2.43 ± 0.38	2.45 ± 0.38	0.08
Transferrin saturation, %	23.8 ± 10.1	21.1 ± 10.6	23.1 ± 9.3	24.6 ± 9.9	25.8 ± 10.1	<0.001
eGFR, mL/min/1.73 m^2	51 ± 18	42 ± 17	51 ± 17	54 ± 18	57 ± 15	<0.001
hs-CRP, mg/L	1.9 (0.8–4.6)	2.4 (0.9–6.0)	1.8 (0.7–4.4)	1.6 (0.8–4.1)	1.8 (0.7–3.8)	0.03
Urinary creatinine excretion, mmol/24 h	12.4 ± 3.8	11.9 ± 3.8	12.5 ± 3.9	12.3 ± 3.7	12.7 ± 3.7	0.16
Urinary protein excretion, g/24 h	0.17 (0.12–0.30)	0.20 (0.12–0.54)	0.17 (0.13–0.26)	0.16 (0.12–0.28)	0.15 (0.11–0.25)	0.002
Urinary urea excretion, mmol/24 h	381 ± 119	357 ± 102	387 ± 124	389 ± 122	389 ± 122	0.01
Lifestyle parameters						
Alcohol intake, units/week						0.12
None, *n* (%)	350 (45)	92 (50)	89 (45)	83 (45)	86 (40)	
0–7 units per week, *n* (%)	265 (34)	60 (32)	67 (34)	70 (38)	68 (32)	
>7 units per week, *n* (%)	166 (21)	34 (18)	40 (20)	32 (17)	60 (28)	
Smoking, *n* (%)	84 (11)	14 (8)	22 (12)	22 (13)	26 (13)	0.32
CIS fatigue score	61 (42–82)	65 (43–84)	60 (41–84)	62 (42–83)	58 (42–78)	0.34
Transplant parameters						
Time since transplantation, years	3.5 (1.0–10.2)	5.0 (1.0–11.6)	2.0 (1.0–8.6)	3.1 (1.0–8.0)	4.0 (1.0–11.9)	0.06
Type of donor						0.55
Living, *n* (%)	481 (55)	113 (55)	129 (59)	113 (53)	126 (54)	
Postmortal, *n* (%)	389 (45)	94 (45)	88 (41)	100 (47)	107 (46)	
Medication use						
Calcineurin inhibitor, *n* (%)	722 (83)	182 (88)	189 (87)	177 (83)	174 (75)	0.001
Antiproliferative agent, *n* (%)	746 (86)	169 (81)	185 (85)	184 (86)	208 (89)	0.12
Prednisone, *n* (%)	849 (98)	204 (98)	211 (97)	207 (97)	227 (97)	0.93
Oral iron supplements, *n* (%)	65 (8)	36 (17)	15 (7)	10 (5)	4 (2)	<0.001
Statin, *n* (%)	497 (57)	120 (58)	116 (54)	126 (59)	135 (58)	0.65
RAAS inhibitor, *n* (%)	335 (39)	96 (46)	91 (42)	73 (34)	75 (32)	0.01
Handgrip strength, kg	34 ± 12	33 ± 12	34 ± 11	34 ± 11	35 ± 13	0.21
Appendicular skeletal muscle mass, kg	22.9 ± 5.6	22.6 ± 5.0	23.1 ± 6.3	23.5 ± 6.3	22.4 ± 4.8	0.22
Five times sit to stand test, s	12.1 ± 3.6	12.9 ± 3.9	12.0 ± 3.3	12.4 ± 4.5	11.5 ± 2.8	0.08

eGFR, estimated glomerular filtration rate; MCV, mean corpuscular volume; Hs-CRP, high sensitive C-reactive protein; RAAS, renin-angiotensin-aldosterone system.
Data are presented as mean ± standard deviation (SD), median with interquartile range (IQR), or number (*n*) with percentage (%). Data on alcohol intake were available for 781 KTRs. Data on subjective fatigue were available for 764 KTRs. Data on smoking status were available for 751 KTRs. Appendicular skeletal muscle mass was measured in 814 KTRs. Handgrip strength was determined in 854 KTRs. The five times sit to stand test was performed in 290 KTRs.

CER (r = −0.20, P = 0.001) but not with ASMM (r = −0.04, P = 0.55).

Haemoglobin levels and muscle mass

Creatinine excretion rate

Mean CER was 13.8 ± 3.7 mmol/24 h in men and 10.2 ± 2.7 mmol/24 h in women. In univariable analysis, haemoglobin levels were positively associated with CER (std. β = 0.24, $P < 0.001$; *Table* 2 and *Figure* 1). This association persisted after multivariable adjustment (std. β = 0.07, P = 0.01; *Table* 2). In multivariable logistic regression analyses, KTRs in the lowest age-specific and sex-specific quartile of haemoglobin levels had an increased risk of having a CER in the lowest age-specific and sex-specific quartile [odds ratio (OR), 2.09; 95% CI 1.15–3.77; P = 0.02; *Table* 3], independent of potential confounders.

Appendicular skeletal muscle mass

Mean ASMM was 25.8 ± 4.4 kg in men and 18.4 ± 4.1 kg in women. In univariable analysis, haemoglobin levels were

Table 2 Linear regression of the association between haemoglobin levels with different measurements of muscle mass and strength

	Creatinine excretion rate (mmol/24 h)		Appendicular skeletal muscle mass (kg)*		Handgrip strength (kg)		Five times sit to stand test score (s)**	
	N = 871		N = 814		N = 854		N = 290	
	Std. β	P value	Std. β	P value	Std. β	P value	Std. β	P value
Haemoglobin								
Univariable	0.24	<0.001	0.20	<0.001	0.31	<0.001	−0.15	0.01
Multivariable	0.07	0.01	0.22	<0.001	0.15	<0.001	−0.17	0.02

BMI, body mass index; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitive C-reactive protein; RAAS, renin-angiotensin-aldosterone system.
Model 1: Crude analysis (univariable model) Model 2: Multivariable model, adjusted for age, sex, eGFR, BMI, natural log of hs-CRP, serum iron, natural log of urinary protein excretion, urinary urea excretion, natural log of transplantation vintage, type of donor, subjective fatigue score, smoking status, alcohol intake and use of calcineurin inhibitors, antiproliferative agents, systemic corticosteroids, statins, oral iron supplements, and RAAS-inhibitors. For fatigue score, smoking status, and alcohol intake, imputed data were used.
*In case of appendicular skeletal muscle mass, age, sex, and BMI were not included in Model 2 because these variables are included in the formula of Kyle.
**A higher score reflects worse performance.

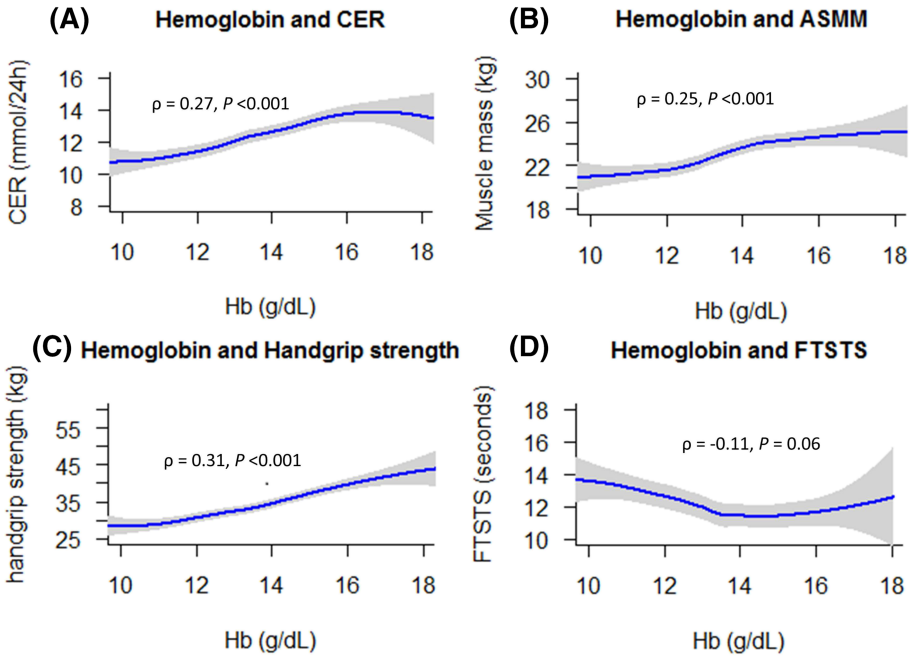

Figure 1 Haemoglobin levels and measurements of muscle mass and strength. The correlation and Spearman's rank correlation coefficient between haemoglobin levels and creatinine excretion rate (CER) *(A)*, appendicular skeletal muscle mass (ASMM) *(B)*, handgrip strength *(C)*, and five times sit to stand test score (FTSTS) *(D)*. The grey area represents the 95% confidence interval of the regression line. Data on CER available in 871 KTRs, data on ASMM available in 814 KTRs, data on handgrip strength available in 854 KTRs, and data on FTSTS available in 290 KTRs.

Table 3 Age-specific and sex-specific quartiles of haemoglobin levels and the risk of being in the worst age-specific and sex-specific quartile of different measures of muscle mass and strength

	Lowest quartile of creatinine excretion rate (mmol/24 h)		Lowest quartile of appendicular skeletal muscle mass (kg)*		Lowest quartile of handgrip strength (kg)		Highest quartile of five times sit to stand test score (s)**	
	N = 871		*N* = 814		*N* = 854		*N* = 290	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age-specific and sex-specific quartile of haemoglobin level								
Univariable								
Quartile 1	2.10 (1.35–3.26)	0.001	0.92 (0.58–1.45)	0.72	2.00 (1.31–3.07)	0.001	5.07 (2.19–11.77)	<0.001
Quartile 2	1.54 (0.98–2.41)	0.06	1.05 (0.68–1.62)	0.84	1.17 (0.75–1.83)	0.49	3.09 (1.30–7.34)	0.01
Quartile 3	1.05 (0.66–1.69)	0.83	0.94 (0.60–1.47)	0.79	1.22 (0.78–1.90)	0.39	2.54 (1.01–6.40)	0.05
Quartile 4	Reference		Reference		Reference		Reference	
Multivariable								
Quartile 1	2.09 (1.15–3.77)	0.02	1.38 (0.80–2.37)	0.25	3.30 (1.95–5.59)	<0.001	7.21 (2.59–20.05)	<0.001
Quartile 2	1.94 (1.12–3.39)	0.02	1.55 (0.95–2.52)	0.08	1.51 (0.92–2.48)	0.11	3.12 (1.20–8.08)	0.02
Quartile 3	1.12 (0.63–1.99)	0.70	1.04 (0.64–1.67)	0.89	1.27 (0.78–2.06)	0.34	2.33 (0.86–6.30)	0.10
Quartile 4	Reference		Reference		Reference		Reference	

BMI, body mass index; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitive C-reactive protein; RAAS, renin-angiotensin-aldosterone system.
Odds ratios and corresponding 95% confidence intervals are presented for the being in the lowest age-specific and sex-specific quartile of CER, appendicular skeletal muscle mass or handgrip strength or in the highest age-specific and sex-specific quartile of FTSTS test score. Model 1: Crude analysis (univariable model). Model 2**: Multivariable model, adjusted for eGFR, BMI, natural log of hs-CRP, serum iron, natural log of urinary protein excretion, urinary urea excretion, natural log of transplantation vintage, type of donor, subjective fatigue score, smoking status, alcohol intake and use of calcineurin inhibitors, antiproliferative agents, systemic corticosteroids, statins, oral iron supplements, and RAAS inhibitors. For fatigue score, smoking status, and alcohol intake, imputed data were used.
*In case of appendicular skeletal muscle mass, age, sex and BMI were not included in Model 2 because these variables are included in the formula of Kyle.
**A higher score reflects worse performance.

positively associated with ASMM, estimated using BIA resistance measurements (std. β = 0.20, $P < 0.001$; *Table* 2 and *Figure* 1). After adjustment for potential confounders, this association was confirmed (std. β = 0.22; $P < 0.001$; *Table* 2). In multivariable logistic regression analyses, KTRs in the lowest age-specific and sex-specific quartile of haemoglobin levels did not have a significantly increased risk of being in the lowest quartile of ASMM (*Table* 3).

Haemoglobin levels and muscle strength

Handgrip strength

Mean handgrip strength was 40 ± 10 kg in men and 25 ± 7 kg in women. In line with muscle mass parameters, haemoglobin levels were associated with handgrip strength upon univariable (std. β = 0.31; $P < 0.001$; *Table* 2 and *Figure* 1) and multivariable analyses (std. β = 0.15; $P < 0.001$; *Table* 2). In multivariable logistic regression analyses, we observed that KTRs in the lowest age-specific and sex-specific quartile of haemoglobin levels had an increased risk of having a mean handgrip strength in the lowest age-specific and sex-specific quartile (OR, 3.30; 95% CI 1.95–5.59; $P < 0.001$), independent of potential confounders (*Table* 3).

Five times sit to stand test

Mean FTSTS test score was 12.0 ± 3.1 s in men and 12.3 ± 4.3 s in women. In univariable analysis, lower haemoglobin levels were associated with worse (higher) FTSTS test score (std. β = −0.15, $P = 0.01$; *Table* 2 and *Figure* 1). The inverse association between haemoglobin levels and FTSTS test score was confirmed after adjustment for potential confounders (std. β = −0.17, $P = 0.02$; *Table* 2). In multivariable logistic regression analyses, KTRs in the lowest age-specific and sex-specific haemoglobin quartile had an increased risk of having an FTSTS test score in the highest (i.e. worst) age-specific and sex-specific quartile (OR, 7.21; 95% CI 2.59–20.05; $P < 0.001$) (*Table* 3).

Sensitivity analysis

When we accounted for possible inadequacies in 24 h urine collection by excluding subjects in the 5% greatest difference between estimated and measured volume of 24 h urine sample, similar results to the primary analyses were found (Supporting Information, *Tables* S1 and S2).

Discussion

In this study, we show that lower haemoglobin levels are associated with lower muscle mass and strength in KTRs. Our results are consistent among two different approaches to quantify muscle mass: CER and ASMM using BIA. We show similar associations with decreased muscle strength, also assessed using two different approaches, namely, handgrip strength measurement and the FTSTS test. The present data agree with the concept that diminished haemoglobin levels may contribute to impaired muscle mass and strength, extend these findings to a novel patient setting (i.e. KTRs), and provide a rationale to investigate whether maintaining haemoglobin levels at higher concentrations improves muscle mass and strength.

Post-transplantation anaemia is highly prevalent in KTRs, affecting approximately one-third of the population.[8,9] Furthermore, it has been widely documented that post-transplant anaemia is associated with poor outcomes.[10,12–15] Post-transplantation anaemia comprises a different entity than anaemia in the non-transplant population. Aetiologically, immunosuppressive medication, chronic low-grade inflammation, and erythropoietin resistance play important roles.[10] Similarly, low muscle mass and strength are highly prevalent in KTRs.[1,2] Low muscle mass is strongly associated with an increased risk of mortality in KTRs.[3] Likewise, low muscle strength was found to be a strong and independent predictor of worse outcomes in KTRs.[5]

To our knowledge, this is the first study to investigate the relationship of haemoglobin levels with muscle mass in KTRs. In other populations, findings similar to ours have been identified. In the InCHIANTI study, involving Italian community-dwelling individuals of ≥65 years, lower haemoglobin levels corresponded to a lower muscle circumference relative to total limb circumference, assessed by calf peripheral quantitative computed tomography.[26] In the Korea National Health and Nutrition Examination Survey, the body composition of a large sample of elderly men was measured with dual-energy X-ray absorptiometry (DEXA).[16] Men with anaemia had a higher risk of having a low muscle mass. Finally, our group previously identified that lower haemoglobin levels were associated with lower muscle mass, as measured by CER, in the general population.[7] The muscle mass of our included KTRs was largely comparable with the muscle mass in the general population[7,24] and in another study of KTRs.[27]

One previous study in KTRs addressed the relationship between haemoglobin levels and muscle strength. Chan and colleagues observed that haemoglobin levels in KTRs were an independent predictor of handgrip strength.[5] However, the study involved a relatively small cohort of 128 stable KTRs and did not include different approaches to measure muscle strength, in contrast to our study. In the elderly, findings similar to ours have been identified. In the InCHIANTI study, anaemia was associated with lower ankle extension, handgrip and knee extensor strength.[28] The muscle strength of our included KTRs was largely comparable with that of the general population.[5,29,30] However, the handgrip strength scores were better than those of KTRs in two other studies, whereas the FTSTS test was performed worse compared with another

cohort of KTRs who were, on average, younger.[31] The most likely explanations for the discrepancy in handgrip strength are the inclusion of KTRs as early as 3 months after kidney transplantation in one cohort,[31] and a substantially lower mean haemoglobin level of the included KTRs in the other cohort, negatively affecting muscle strength.[5]

Mechanistically, we hypothesize that there could be a causal relation between haemoglobin levels and muscle mass and strength. Diminished haemoglobin levels could lead to poor muscle oxygenation, directly affecting its mass and strength.[32,33] This hypothesis is supported by evidence that treatment of anaemia improves myocyte metabolism. It has previously been observed that erythropoietin (EPO) treatment enhances mitochondrial function in skeletal muscle.[34] In anaemic patients, erythrocyte transfusions improved maximum skeletal muscle tissue oxygen.[35] In addition, fatigue in patients with anaemia could promote loss of muscle mass and strength, although adjustment for the CIS Score, reflecting fatigue, did not change our results.

Furthermore, several factors might affect both haemoglobin levels and muscle mass and strength and contribute to our currently identified associations. First, a potential mechanism could be underlying inflammation, compromising both haemoglobin levels and muscle mass and strength.[36] Indeed, hs-CRP levels were higher in our patients having diminished haemoglobin levels. However, adjustment for hs-CRP did not significantly affect the association between haemoglobin levels and muscle mass or strength. Second, the influence of testosterone levels could have played a role, at least in male KTRs. Direct relationships between lower androgen levels and both lower haemoglobin levels[37] and lower handgrip strength[38] in male KTRs and in elderly men have been shown. Androgenic steroids increase haematocrit as well as muscle mass.[39] Third, lower haemoglobin levels may simply reflect general frailty and poor nutritional status, resulting in a lower muscle mass and strength.[40] The observation that KTRs with diminished haemoglobin levels have a significantly lower urinary urea excretion rate, a proxy for protein consumption, compared with KTRs with higher haemoglobin levels, supports the assumption that malnutrition might play a role. However, the association between haemoglobin levels and muscle parameters remained after adjustment for BMI and urinary urea excretion rate. Finally, KTRs with lower haemoglobin levels had lower iron availability, reflected by lower serum iron levels, but comparable iron stores, reflected by serum ferritin, compared with KTRs with higher haemoglobin levels. Iron is known to be involved in the mitochondrial function and aerobic metabolism, deoxyribonucleic acid synthesis, and myoglobin production in muscle cells.[41–44] However, the relationship between haemoglobin levels and muscle parameters remained independent of adjustment for serum iron and use of oral iron supplementation.

Our study has several strengths and limitations. The main strength is the large cohort of stable KTRs in which we as first show a robust and independent association between haemoglobin levels and four different muscle parameters. The simultaneous availability of CER measurement, ASMM, handgrip strength, and FTSTS test score made it possible to meticulously assess the association between haemoglobin levels with muscle mass and strength. We also acknowledge several limitations of our study. The observational design and cross-sectional nature of our study hamper the possibility to draw any definite conclusions about causality. We used CER and BIA instead of CT imaging, which is the golden standard for estimation of (skeletal) muscle mass but is expensive and exposes patients to radiation. 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 and by exclusion of KTRs who were suspected to have inadequately collected the 24 h urine in a sensitivity analysis. Day-to-day dietary variations also affect the excretion rate of creatinine. Meat products, for example, contain creatine phosphate, which is used for muscle metabolism and which is the substrate of creatinine. A limitation of BIA is that it can be influenced by hydration status. However, BIA-derived muscle mass estimation in KTRs is strongly correlated to CT-based muscle mass estimation.[45] Another limitation is that the FTSTS test score was performed in a smaller sample size as compared with the other muscle parameters. Most likely, the smaller sample size also explains the larger identified odds ratio for patients with low haemoglobin levels of being in the highest quartile of the FTSTS test score as compared with the odds ratios of being in the worst quartile of the other muscle parameters.[46] In addition, although we adjusted for multiple confounders, residual confounding cannot be excluded. Finally, we could not include a reliable parameter of physical activity, and therefore, we cannot exclude that sedentary behaviour at least partly explains the link between diminished haemoglobin levels and reduced muscle mass and strength.

In conclusion, we identified that lower haemoglobin levels are independently associated with a decreased muscle mass and strength in KTRs. Future research is warranted to address whether maintaining haemoglobin levels at higher concentrations improves muscle mass and strength in KTRs.

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Conflict of interest

The authors declare no conflict of interest.

Online supplementary material

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

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