Title: Body size normalization of ultrasound measured anterior upper leg muscle thickness in younger and older males and females

Michael T. Paris[1]

Kirsten E. Bell[1]

Egor Avrutin[1]

Marina Mourtzakis[1]

[1]Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada.

Corresponding author: Marina Mourtzakis, PhD, Department of Kinesiology, University of Waterloo, 200 University Ave W, Waterloo, ON, N2L 3G1, Canada. Email: mmourtzakis@uwaterloo.ca. Phone: 519-888-4567, Ext 38459.

ABSTRACT

Background: Ultrasound measurements of the anterior upper leg muscle thickness are often used to quantify muscle mass; however, the ideal normalization approach is unclear. Our primary objective was to examine how the anterior upper leg muscle thickness scales with indices of body size in younger and older adults. Our secondary objectives were to examine how normalization with body size alters the identification of low muscle thickness and associations with strength and physical function.

Methods: Younger (<45 years) males (n=38) and females (n=24) and older (≥60 years) males (n=53) and females (n=24) were evaluated for anthropometrics and anterior upper leg muscle thickness. Allometric models were used to examine how body size metrics scale with anterior upper leg muscle thickness. A subset of older males was evaluated for strength and function.

Results: Weight and BMI scaled with anterior upper leg muscle thickness with coefficients less than 1 (0.58 to 0.82, r^2=0.15 to 0.31, $p<0.05$) for both younger and older males and females. Compared to absolute anterior upper leg thickness, normalized indices identified a greater proportion of older adults with low muscle thickness ($p<0.05$). Absolute muscle thickness provided stronger associations with strength compared to weight normalized indices.

Conclusions: Scaling exponents less than 1 for weight and BMI for the anterior upper leg muscle thickness indicate that allometric normalization is the ideal approach to develop body size independent metrics. However, allometric normalization of muscle thickness increases the proportion of older adults classified as low muscle mass but decreased the associations with strength.

INTRODUCTION

Comparison of body composition compartments (e.g. skeletal muscle) between individuals often requires normalization to height, to develop metrics which are independent of body size (1). For example, adult body weight scales proportionally with the square of height, which establishes body mass index (BMI) as a height-independent measure of body size (2). BMI is easy to measure and moderately associated with adiposity, but it cannot distinguish specific body composition compartments, such as skeletal muscle (3), limiting its applicability in the classification of sarcopenia. Ultrasound has emerged as a useful tool to quantify skeletal muscle mass, as the thickness of several limb muscles are strongly associated with muscle mass measured with reference modalities (4,5). Using ultrasound, we (6), and others (7,8), have observed that the thickness of the anterior quadriceps are particularly susceptible to ageing-related skeletal muscle atrophy in comparison to several other limb muscles or appendicular lean tissue mass. While some groups have normalized ultrasound measures of muscle thickness to height (9), weight (10) and BMI (8), it is uncertain how it scales with different indices of body size. Furthermore, it is unclear how these different normalization approaches alter the identification of older adults with low skeletal muscle mass (in relation to healthy young adults) or their associations with muscle strength and physical performance.

Allometric models are frequently used to evaluate how body composition scales with different metrics of body size (1,11). Allometry is the study of biological scaling, which is often used to examine the relative proportion of different body compartments during growth. For example, from infancy to young adulthood, many organs (e.g., heart) increase in a 1:1 ratio (i.e., ratio scaling) with body size, whereas other organs (e.g., brain) do not scale linearly with body mass (i.e., allometric growth) (12). When using allometric scaling to create body-size independent metrics, the body composition compartment is normalized (compartment/body size $^{\text{scaling exponent}}$) by the scaled body size metric, which creates a null correlation between these variables. Scaling exponents are derived through allometric modelling of the body composition compartment and body size metric of interest. Understanding how different scaling factors are influence by age is critical to ensure that normalization approaches are valid across younger and older adults.

Here, our primary objective was to evaluate how the anterior thigh muscle thickness scales with indices of body size (height, weight, BMI, and limb length) in younger and older males and females. As a secondary objective we examined how normalizing the anterior upper leg muscle thickness with body size indices alters the identification of low skeletal muscle mass and correlates with strength and physical performance in older adults. The younger adult cohort was used as the reference cohort for identification of low muscle thickness in older adults.

METHODS

Participant cohorts

This study is a secondary analysis of a cohort of participants pooled from previously published studies which focused on validation of muscle thickness against dual-energy x-ray absorptiometry (DXA) (4), evaluation of image resolution on echo intensity (13), and characterizing site-specific muscle thickness differences in younger and older males (14,15). All participants were evaluated for anterior upper leg muscle thickness, and a subset of older males underwent assessment of knee extensors strength and physical performance. Participants were excluded if they had a: 1) previous history of neuromuscular disorders, 2) prosthetic joint replacement, or 3) history of cancer or cerebrovascular disease. Participants were instructed to refrain from moderate to vigorous physical activity for 48 hours and alcohol consumption for 24 hours prior to laboratory visits. All studies were approved by a human research ethics committee at the University of Waterloo (ORE41520 & ORE31468). Written informed consent was obtained from all participants in accordance with established protocols for human research.

Anthropometry and muscle thickness analysis

Weight and height were obtained using a balance beam and stadiometer, respectively. Participants were landmarked for ultrasound imaging in a supine posture, marking the two-thirds distance from the anterior superior iliac spine to the superior pole of the patella. Transverse images were obtained using B-mode ultrasound (M-turbo, Sonosite, Markham, ON) with a linear array transducer (L38xi: 5-10 MHz). Ultrasound imaging settings gain, time-gain-compensation, and dynamic range were held constant across all participants. A generous amount of ultrasound gel was used to maintain minimal compression of the ultrasound probe against the skin, as previously described (4). Ultrasound images were transferred to a personal computer and analyzed for muscle thickness using ImageJ (NIH, Bethesda, MD, version 1.53e). Muscle thickness was measured as the vertical distance from the superior aspect of the femur to the superior muscle fascia border of the rectus femoris, inclusive of the vastus intermedius and rectus femoris.

Muscle strength and physical performance

A subset of older males (n=32) underwent analysis of isometric torque of the knee extensor muscles and physical performance tests (6-min walk and 30-second sit to stand). Only a subset of participants completed torque and performance measures as these individuals completed a study which involved different outcomes in comparison to the participants from the two remaining studies. Peak isometric torque of the knee extensors was evaluated using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, New York) at 60° of knee flexion. Participants were seated against the backrest with a hip angle of 85° and straps placed tightly across the participant's waist and chest. The lateral epicondyle of the right femur was aligned with the rotation knob and the knee extension arm was attached 5 cm above the calcaneus. Participants performed 3 maximal contractions (1 minute of rest between contractions) and the highest recorded value was used for further analysis.

The 6-minute walk test was performed using two cones 20-m apart, according to a standardized protocol (16). A 30-second sit to stand test was evaluated with the participants arms cross over the chest using a chair that was approximately 46 cm from the ground.

Statistical analysis

Differences in physical characteristics for age (younger: <45 years and older: ≥60 years) and sex were evaluated using two-way ANOVAs. Pearson correlation coefficients were used to evaluate the associations between anterior upper leg muscle thickness and indices of body size (height, weight, BMI, limb length). Sample size estimations were determined based on significance of moderate ($r \geq 0.45$, minimum sample/group = 20) Pearson correlation coefficients between skeletal muscle thickness and indices of body size. Body size variables displaying significant correlations with anterior upper leg muscle thickness were further evaluated for allometric scaling.

An allometric model, $Y = \alpha X^{\beta}$, was used to evaluate the relationships between anterior upper leg muscle thickness and significant body size metrics (i.e., displays a significant correlation with anterior upper leg muscle thickness), where Y denotes the anterior upper leg muscle thickness, X indicates the body size metric, α is the proportionality constant, and β is the scaling exponent, as previously described (1,11). To fit the data using linear regression, the allometric model can be expressed in logarithmic form as $\log_e Y = \log_e \alpha + \beta \log_e X + \mathcal{E}$, where $\mathcal{E}$ is the error term. Linear regression models were fit separately for younger males, older males, younger females, and older females, using anterior upper leg muscle thickness and corresponding body size metric, expressed in logarithmic form. Differences in scaling exponents between age groups for a given sex (e.g. younger males vs older males) were evaluated by including age group and age group by body size metric interaction term in the regression model.

Homoscedasticity of the residuals was confirmed via nonsignificant correlations of the absolute residuals and the natural log of the body size index. Linear regression analysis was used to examine how different scaling exponents (0 to 1.3, using 0.1 increments) alters the correlation coefficients between the normalized anterior upper leg muscle thickness (e.g. thickness/BMI$^{\beta}$) and the corresponding body size metric (e.g. BMI).

To examine how normalization of the anterior upper leg muscle thickness changes the identification of low skeletal muscle mass in older adults, the younger adult cohorts were used to derive sex-specific cutpoints (2 standard deviations [SD] below average) for absolute and body size normalized anterior upper leg muscle thickness. A Cochran's Q test was used to compare the proportion of older males or females identified as having low muscle thickness across different normalization indices. Pairwise comparisons between anterior upper leg muscle thickness normalization indices were adjusted using a Bonferroni correction. Statistical significance was set as $p<0.05$. All analyses were performed using SPSS (version 27, Chicago, IL, USA).

RESULTS

Older adults had significantly greater weight ($p=0.046$) and BMI ($p<0.001$), and lower stature ($p=0.003$), compared with younger adults (Table 1). Younger adults and males had a larger anterior upper leg muscle thickness compared with older adults ($p<0.001$) and females ($p<0.001$), respectively (Table 1).

In every group, weight and BMI were positively associated ($p\leq0.05$) with anterior upper leg muscle thickness (Table 2). Neither height nor limb length were significantly correlated ($p>0.05$) with anterior upper leg muscle thickness (Table 2).

Among younger males, weight and BMI scaled to anterior upper leg muscle thickness with an exponent of 0.58 (±0.14, full model $r^2=0.31$) and 0.62 (±0.16, full model $r^2=0.29$), respectively (Table 3).

Similar to younger males, younger females anterior upper leg muscle thickness scaled to weight and BMI with an exponent of 0.54 (±0.16, full model r^2=0.19) and 0.64 (±0.18, full model r^2=0.21), respectively (Table 3). Within older males, weight and BMI scaled to anterior upper leg muscle thickness with an exponent of 0.80 (±0.19, full model r^2=0.27) and 0.92 (±0.21, full model r^2=0.27), respectively (Table 3). Whereas in older females, weight and BMI scaled to anterior upper leg muscle thickness with an exponent of 0.82 (±0.31, full model r^2=0.24) and 0.69 (±0.34, full model r^2=0.16), respectively (Table 3). Age group and interactions between age group and either weight or BMI were not significant ($p>0.05$) within the regression models for males or females, indicating scaling exponents *w*ere not different between younger males and older males or between younger females and older females.

Different scaling exponents for weight (Figure 1A) and BMI (figure 1B) were evaluated against correlation coefficients derived between weight or BMI and normalized anterior upper leg muscle thickness.

Using the younger males and females as a reference, absolute anterior upper leg muscle thickness identified 73.6 % and 33.3 % of older males and older females as having low muscle thickness (Table 4). A significantly larger proportion of older males were identified as having low muscle thickness when the anterior upper leg was normalized to weight (92.5 %) or BMI (92.5 %). Whereas in older females, normalization using BMI (62.5 %), but not weight (50%), increased the proportion of individuals classified as having low muscle thickness (Table 4).

In a subgroup analysis (n=32 older males), absolute and BMI normalized, but not weight normalized, anterior upper leg muscle thickness displayed moderate associations with knee extensor isometric torque; however, only normalized (weight and BMI), but not absolute, anterior upper leg muscle thickness was associated with sit-to-stand performance (Table 5).

DISUCSSION

Here, our primary objective was to characterize how body size indices (weight and BMI) allometrically scale with anterior upper leg muscle thickness in younger and older males and females. We observed that the scaling exponents for weight and BMI were not statistically different between younger and older adults. Importantly, there were significant differences in the proportion of older adults classified as having low skeletal muscle mass when using absolute compared with weight or BMI normalized indices of anterior upper leg muscle thickness. In a subset of older males, the absolute anterior upper leg muscle thickness displayed the strongest associations with measures of muscle strength, however, in gravity-dependent physical performance tests, such as 30-second sit to stand, body size normalized indices provided stronger associations compared to absolute muscle thickness.

Low skeletal muscle mass in older adults is typically identified using cutpoints established in healthy young adults reference cohort (2 SD below the average) (17). These measures of skeletal muscle mass are often normalized to height, to achieve cutpoints which are independent of stature. While the anterior thigh muscle thickness is increasingly being used for identification of low skeletal muscle mass in older adults (8,18), the ideal approach for normalization has not been comprehensively evaluated. Trunk and anterior thigh muscles measured using ultrasound have been adjusted for height (9), weight (10) and BMI (8) using both allometric and ratio (e.g., 1:1 thickness/weight) normalization. Nuzzo et al. (2013) (10) observed that the anterior, lateral, and posterior trunk muscles displayed scaling exponents with body weight that were less than 1 (0.348 to 0.775) in male career firefighters. Furthermore, they observed that erector spinae cross-sectional area is positively associated with body weight (r=0.49), but when they normalized the cross-sectional area to weight using a ratio adjustment (i.e., muscle cross-sectional area/weight), an inverse association (r= -0.42) was observed; indicating that use of ratio normalization may not be optimal. Whereas allometric normalization (i.e., muscle cross-sectional area/weight$^{0.51}$) displayed no association with weight (r=0.00) (10).

Here, we observed similar strength associations with weight or BMI and anterior upper leg muscle thickness across younger and older adults. Similar to Nuzzo et al. (2013) (10), we observed scaling exponents less than 1 using allometric modelling between the anterior upper leg muscle thickness and either weight or BMI, further supporting that ratio normalization for muscle thickness may not be the ideal approach. Recently, Kara et al. (2020) (8) normalized the anterior upper leg muscle thickness with BMI in 326 community dwelling adults, denoted as sonographic thigh adjustment ratio (STAR), which demonstrated a linear decline beginning at ~45 years of age in males and females. However, it is difficult to establish if ageing-related declines in STAR is due to skeletal muscle atrophy, increases in adiposity, or a combination or both. However, if the objective of these indices are for characterizing low skeletal muscle mass for the identification of sarcopenia, it may be more relevant to examine how different normalization indices relate to strength and functional capacity. The previously established STAR metric displayed stronger associations with knee extensors strength and functional capacity in comparison to traditional whole-body measures of muscle mass (8). We observed that weight or BMI normalized anterior upper leg muscle thickness was more strongly associated with gravity-dependent functional tests (e.g. 30-second sit to stand); however, absolute anterior upper leg muscle thickness displayed the strongest associations with muscle strength. However, it should be noted that our findings are rather limited in comparison to strength and functional capacity, as our analysis was limited to a small subset of older males.

Using the younger adults as a reference cohort, we observed substantial differences in the proportion of older adults identified as having low skeletal muscle mass using either absolute (74% males and 33% females) or normalized indices (93% males and 50-63% females) of muscle thickness. However, using even absolute anterior thigh muscle thickness, arguably too large of a proportion of the older adults were classified as low skeletal muscle mass, which further increased when using weight or BMI normalized indices. The substantial proportion of older adults being classified as having low muscle thickness using the normalized indices questions the applicability of these metrics in the assessment of sarcopenia. Even the use of absolute anterior thigh muscle thickness appears to classify too large of a proportion of older adults, which has also been observed by others (>70% prevalence) (19), suggesting that using a cutpoint of 2SD below a young reference cohort may need to be reconsidered for this muscle group.

There were several limitations to our investigation. Our sample size was relatively small, particularly in comparison to other studies conducting allometric modelling for body composition using DXA or computed tomography (1,20). This small sample size is further compounded by having a non-normal (bimodal) distribution for age (younger and older adults), making it challenging to account for the influence of age across males or females, as has been previously described (11). Furthermore, our cohorts of younger and older males and females were heterogenous in terms of body composition, physical activity status, and comorbidities, which may alter the ideal scaling coefficients for body size normalization. Lastly, our analysis focused solely on the anterior upper leg thickness, which may not be applicable to other muscle groups or analysis of cross-sectional area. Furthermore, we evaluated the thickness of the anterior upper leg muscle at a single landmark, which may not be applicable across other landmarks. Therefore, our comparisons with those by Kara et al. (2020) (8), which utilized a slightly different anterior thigh landmark, may be confounded.

In conclusion, we observed that scaling exponents for weight and BMI for the anterior upper leg muscle thickness is less than 1 for older and younger adults, indicating that ratio normalization may overcorrect for the influence of body size. Weight and BMI normalized anterior thigh muscle thickness may identify too large of a proportion of older adults as having low skeletal muscle mass for the classification of sarcopenia using standard approaches (i.e., 2 SD below young reference group).

Conflicts of interest: The authors declare no conflicts of interest.

Authorship: MTP and MM designed the research; MTP, KEB, and EA conducted all data collections; MTP performed all statistical analysis; MTP drafted the manuscript; all authors have responsibility for the final content and read and approved the final manuscript.

Funding: This research was funded by the Network for Aging Research at the University of Waterloo. MTP was supported by a CIHR Doctoral research award and KEB was supported by a CIHR Fellowship.

REFERENCES

1. Heymsfield SB, Heo M, Thomas D, Pietrobelli A. Scaling of body composition to height: Relevance to height-normalized indexes. Am J Clin Nutr. 2011;93(4):736–40.

2. Heymsfield SB, Peterson CM, Thomas DM, Heo M, Schuna JM, Hong S, et al. Scaling of adult body weight to height across sex and race/ethnic groups: Relevance to BMI. Am J Clin Nutr. 2014;100(6):1455–61.

3. Gonzalez MC, Correia MITD, Heymsfield SB. A requiem for BMI in the clinical setting. Curr Opin Clin Nutr Metab Care [Internet]. 2017;20(5):314–21. Available from: http://insights.ovid.com/crossref?an=00075197-900000000-99286

4. Paris MT, Lafleur B, Dubin JA, Mourtzakis M. Development of a bedside viable ultrasound protocol to quantify appendicular lean tissue mass. J Cachexia Sarcopenia Muscle. 2017;8(5):713–26.

5. Sanada K, Kearns CF, Midorikawa T, Abe T. Prediction and validation of total and regional skeletal muscle mass by ultrasound in Japanese adults. Eur J Appl Physiol [Internet]. 2006 Jan [cited 2014 Oct 16];96(1):24–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16235068

6. Paris, Letofsky N, Mourtzakis M. Site-specific skeletal muscle echo intensity and thickness differences in subcutaneous adipose tissue matched older and younger adults. Clin Physiol Funct Imaging. 2020;1–15.

7. Abe T, Loenneke JP, Thiebaud RS, Fukunaga T. Age-related site-specific muscle wasting of upper and lower extremities and trunk in Japanese men and women. Age (Dordr) [Internet]. 2014 Apr [cited 2015 Jan 13];36(2):813–21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24243442

8. Kara M, Kaymak B, Ata AM, Özkal Ö, Kara Ö, Baki A, et al. STAR - Sonographic Thigh Adjustment Ratio: A Golden Formula for the Diagnosis of Sarcopenia. Am J Phys Med Rehabil. 2020;99(10):902–8.

9. Wilson D V., Moorey H, Stringer H, Sahbudin I, Filer A, Lord JM, et al. Bilateral Anterior Thigh Thickness: A New Diagnostic Tool for the Identification of Low Muscle Mass? J Am Med Dir Assoc. 2019;20(10):1247-1253.e2.

10. Nuzzo JL, Mayer JM. Body mass normalisation for ultrasound measurements of lumbar multifidus and abdominal muscle size. Man Ther [Internet]. 2013;18(3):237–42. Available from: http://dx.doi.org/10.1016/j.math.2012.10.011

11. Heymsfield SB, Hwaung P, Ferreyro-Bravo F, Heo M, Thomas DM, Schuna JM. Scaling of adult human bone and skeletal muscle mass to height in the US population. Am J Hum Biol. 2019;31(4):1–12.

12. Moore K. The developing human: Clinically oriented embryology. Philadelphia: WB Saunderes; 1988. 207 p.

13. Paris M, Bell KE, Avrutin E, Mourtzakis M. Ultrasound image resolution influences analysis of skeletal muscle composition. Clin Physiol Funct Imaging. 2020;40(4):277–83.

14. Paris MT, Bell KE, Avrutin E, Mourtzakis M. Older males exhibit reduced anterior upper leg and anterior abdominal muscle thickness compared to younger males when matched for relative appendicular lean tissue. Arch Gerontol Geriatr. 2021 Sep;96:104483.

15. Paris MT, Bell KE, Avrutin E, Mourtzakis M. Associations between skeletal muscle echo intensity and thickness in relation to glucose homeostasis in healthy and glucose impaired older males. Exp Gerontol. 2021;154(June):111547.

16. Crapo R, Casaburi R, Coates A, Enright P, MacIntrye N, McKay R, et al. American Thoracic Society ATS Statement : Guidelines for the Six-Minute Walk Test. Am J Respir Crit Care Med. 2002;166:111–7.

17. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis. Age Ageing. 2018;39(4):412–23.

18. Rustani K, Kundisova L, Capecchi PL, Nante N, Bicchi M. Ultrasound measurement of rectus femoris muscle thickness as a quick screening test for sarcopenia assessment. Arch Gerontol Geriatr [Internet]. 2019;83(November 2018):151–4. Available from: https://doi.org/10.1016/j.archger.2019.03.021

19. Minetto MA, Caresio C, Menapace T, Hajdarevic A, Marchini A, Molinari F, et al. Ultrasound-Based Detection of Low Muscle Mass for Diagnosis of Sarcopenia in Older Adults. PM R [Internet]. 2016;8(5):453–62. Available from: http://dx.doi.org/10.1016/j.pmrj.2015.09.014

20. Derstine BA, Holcombe SA, Ross BE, Wang NC, Su GL, Wang SC. Optimal body size adjustment of L3 CT skeletal muscle area for sarcopenia assessment. Sci Rep [Internet]. 2021;11(1):1–10. Available from: https://doi.org/10.1038/s41598-020-79471-z

Table 1. Physical characteristics

	Younger males (n=38)	Older males (n=53)	Younger females (n=49)	Older females (n=24)	Sex p-value	Age p-value
Age, y	27.0 (4.5)	74.7 (7.2)	27.5 (7.4)	72.7 (5.8)	0.489	<0.001
Height, m	1.75 (0.06)	1.75 (0.07)	1.66 (0.07)	1.59 (0.05)	<0.001	0.003
Weight, kg	78.4 (10.9)	81.9 (13.2)	64.3 (11.3)	68.4 (10.1)	<0.001	0.046
BMI, kg/m^2	25.6 (3.2)	26.7 (3.8)	23.3 (3.8)	26.9 (3.8)	0.093	<0.001
Limb length, cm	46.6 (2.1)	46.5 (2.3)	44.2 (2.3)	43.5 (1.9)	<0.001	0.265
Anterior upper leg thickness, cm	4.38 (0.63)	2.73 (0.62)	3.21 (0.61)	2.11 (0.49)	<0.001	<0.001

Values are presented as mean (±SD). BMI, body mass index.

Table 2. Correlation coefficients between anterior upper leg muscle thickness and body size metrics

	Younger males (n=38)	Older males (n=53)	Younger females (n=49)	Older females (n=24)
Height	0.15	0.10	0.10	0.23
Weight	**0.57**	**0.54**	**0.42**	**0.51**
BMI	**0.53**	**0.55**	**0.42**	**0.42**
Limb length	0.25	-0.09	0.15	0.10

Bolded correlation coefficients indicate statistical significance. BMI, body mass index.

Table 3. Allometric modelling of anterior upper leg muscle thickness with weight and body mass index

	Younger males (n=38)		Older males (n=53)		Younger females (n=49)		Older females (n=24)	
	Exponent (β)	R^2	Exponent (β)	R^2	Exponent (β)	R^2	Exponent (β)	R^2
Weight, kg	0.58 (0.14)	0.31	0.80 (0.19)	0.27	0.54 (0.16)	0.19	0.82 (0.31)	0.24
BMI, kg/m^2	0.62 (0.16)	0.29	0.92 (0.21)	0.27	0.64 (0.18)	0.21	0.69 (0.34)	0.16

The general model for each outcome is $\log_e Y = \log_e \alpha + \beta \log_e X$. Values are presented as β (±SE). Coefficient of determination refers to explained variance of the full model. All models are $p<0.001$. AUL, anterior upper leg; BMI, body mass index.

Table 4. Comparison of anterior upper leg muscle thickness normalization in the identification of low muscle thickness

	Older males (n=53)	Older females (n=24)
Low anterior upper leg, n (%)	39 (73.6)[a]	8 (33.3)[a]
Low anterior upper leg /weight $^{\text{males: 0.58, females: 0.54}}$, n (%)	49 (92.5)[b]	12 (50)[a,b]
Low anterior upper leg /BMI $^{\text{males: 0.62, females: 0.64}}$, n (%)	49 (92.5)[b]	15 (62.5)[b]

Normalization indices that do not share a letter are statistically different within a given sex. Cutpoints for identification of indices of low AUL were derived using sex-specific 2SD below younger adult cohort. For males, cutpoints were 3.11 cm, 0.26 cm/kg$^{0.58}$, and 0.45 cm/(kg/m^2)$^{0.62}$ for absolute, weight normalized, and BMI normalized, respectively. For females, cutpoints were 1.98 cm, 0.22 cm/kg$^{0.54}$, and 0.28 cm/(kg/m^2)$^{0.64}$ for absolute, weight normalized, and BMI normalized, respectively. BMI, body mass index.

Table 5. Subgroup (n=32 older males) correlation coefficients of anterior upper leg muscle thickness indices and strength and physical performance

	anterior upper leg	anterior upper leg /weight$^{0.8}$	anterior upper leg /weight	anterior upper leg /BMI$^{0.92}$	anterior upper leg /BMI
Knee extensors isometric torque, Nm	**0.50**	0.34	0.28	**0.43**	**0.42**
6 min distance, m	0.04	0.09	0.09	0.21	0.22
Sit to stand, n	0.17	**0.38**	**0.41**	**0.36**	**0.37**

Bolded correlation coefficients indicate statistical significance. BMI, body mass index.

Figure 1

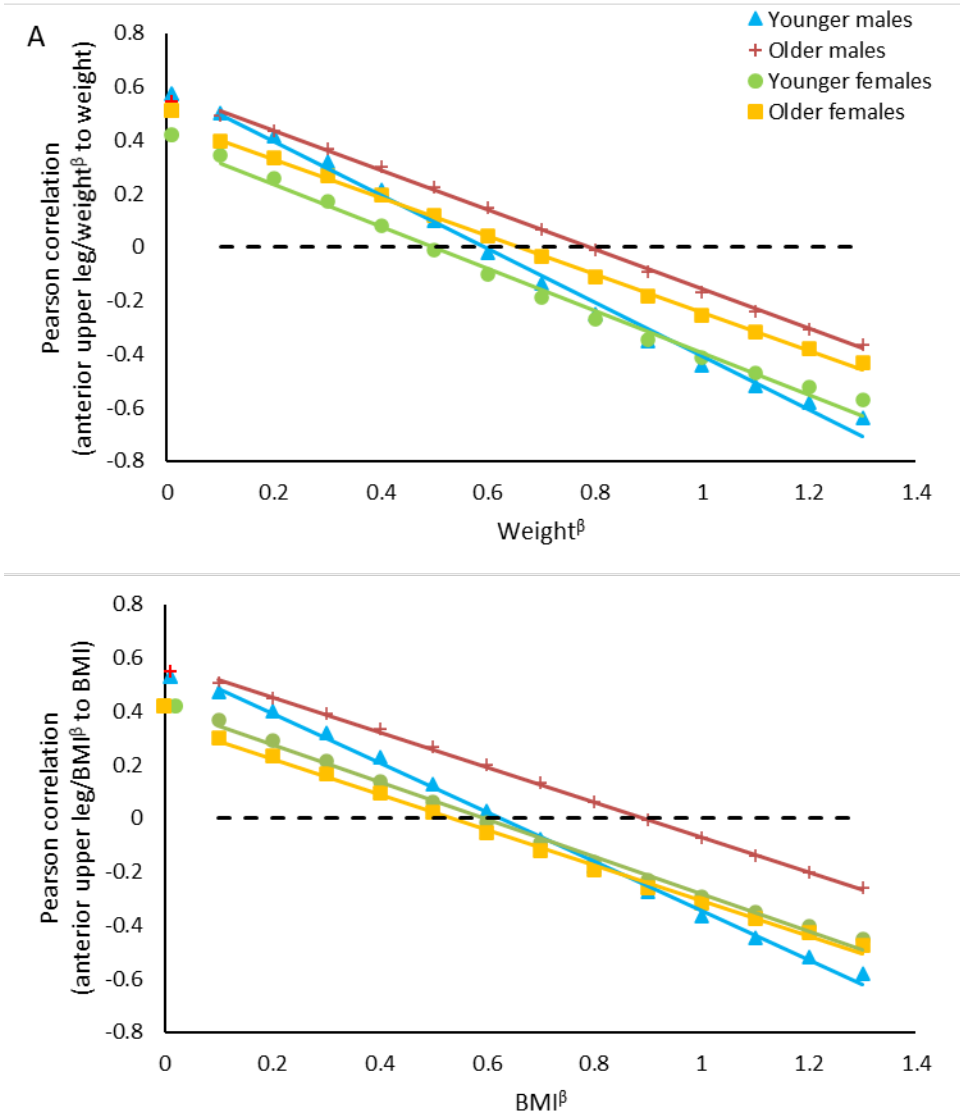
A
Younger males
Older males
Younger females
Older females
Pearson correlation
(anterior upper leg/weight$^{\beta}$ to weight)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Weight$^{\beta}$
Pearson correlation
(anterior upper leg/BMI$^{\beta}$ to BMI)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
0
0.2
0.4
0.6
0.8
1
1.2
1.4
BMI$^{\beta}$

Figure legends

Figure 1. Correlation coefficients for anterior upper leg scaling factors. Correlation coefficients for the regression of A) anterior upper leg muscle thickness/weight$^{\beta}$ with weight or B) anterior upper leg muscle thickness/BMI$^{\beta}$ with BMI, ranging from 0.0 (no normalization) to 1.3 in increments of 0.1. This figure demonstrates how different scaling factors (x-axis) influence the Pearson correlation (y-axis) between normalized anterior upper leg muscle thickness and the corresponding body size metric. Fitted lines cross the horizontal dashed line indicate the scaling factor that is associated with a null Pearson correlation. Blue triangles denote younger males, red crosses denote older males, green circles denote younger females, and orange squares denote older females. Linear regression analysis generated lines for A) younger males: $y = -1.00x + 0.60$ ($r=0.99$, $p<0.001$), older males: $y = -0.74x + 0.58$ ($r=0.99$, $p<0.001$), younger females: $y = -0.78x + 0.39$ ($r=0.99$, $p<0.001$), and older females: $y = -0.71x + 0.47$ ($r=0.99$, $p<0.001$), and B) younger males: $y = -0.92x + 0.58$ ($r=0.99$, $p<0.001$), older males: $y = -0.65x + 0.59$ ($r=0.99$, $p<0.001$), younger females: $y = -0.70x + 0.42$ ($r=0.99$, $p<0.001$), and older females: $y = -0.66x + 0.34$ ($r=0.99$, $p<0.001$).