Age-related alterations in muscle architecture are a signature of sarcopenia: the ultrasound sarcopenia index

Marco Narici[1,2]* (iD), Jamie McPhee[3] (iD), Maria Conte[4] (iD), Martino V. Franchi[1] (iD), Kyle Mitchell[5], Sara Tagliaferri[6] (iD), Elena Monti[1] (iD), Giuseppe Marcolin[1] (iD), Philip J. Atherton[7] (iD), Kenneth Smith[7] (iD), Bethan Phillips[7] (iD), Jonathan Lund[8] (iD), Claudio Franceschi[4] (iD), Marcello Maggio[9] (iD) & Gillian S. Butler-Browne[10] (iD)

[1]Department of Biomedical Sciences, University of Padova, Padova, Italy; [2]CIR-MYO Myology Center, University of Padua, Padua, Italy; [3]Department of Sport and Exercise Sciences, Manchester Metropolitan University, Manchester, UK; [4]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy; [5]Dorsey County Hospital NHS Foundation Trust, Dorchester, UK; [6]Department of Medicine and Surgery, University of Parma, Parma, Italy; [7]MRC/Versus Arthritis Centre for Musculoskeletal Ageing Research and National Institute for Health Research Nottingham Biomedical Research Centre, University of Nottingham, Derby, UK; [8]Department of Surgery and University of Nottingham School of Medicine, Royal Derby Hospital, Derby, UK; [9]Department of Internal Medicine and Biomedical Sciences, Section of Geriatrics, University of Parma, Parma, Italy; [10]Center for Research in Myology UMRS974, Sorbonne Université, INSERM, Myology Institute, Paris, France

Abstract

Background   The assessment of muscle mass is a key determinant of the diagnosis of sarcopenia. We introduce for the first time an ultrasound imaging method for diagnosing sarcopenia based on changes in muscle geometric proportions.
Methods   Vastus lateralis muscle fascicle length (L_f) and thickness (T_m) were measured at 35% distal femur length by ultrasonography in a population of 279 individuals classified as moderately active elderly (MAE), sedentary elderly (SE) ($n = 109$), mobility impaired elderly (MIE) ($n = 43$), and in adult young controls (YC) ($n = 60$). The ratio of L_f/T_m was calculated to obtain an ultrasound index of the loss of muscle mass associated with sarcopenia (USI). In a subsample of elderly male individuals ($n = 76$) in which corresponding DXA measurements were available (MAE, $n = 52$ and SE, $n = 24$), DXA-derived skeletal muscle index (SMI, appendicular limb mass/height^2) was compared with corresponding USI values.
Results   For both young and older participants, USI values were found to be independent of sex, height and body mass. USI values were 3.70 ± 0.52 for YC, 4.50 ± 0.72 for the MAE, 5.05 ± 1.11 for the SE and 6.31 ± 1.38 for the MIE, all significantly different between each other ($P < 0.0001$). Based on the USI Z-scores, with reference to the YC population, the 219 elderly participants were stratified according to their muscle sarcopenic status. Individuals with USI values within a range of $3.70 < USI \geq 4.23$ were classified as non-sarcopenic (prevalence 23.7%), those with USI values within $4.23 < USI \geq 4.76$ were classified as pre-sarcopenic (prevalence 23.7%), those with USI values within $4.76 < USI \geq 5.29$ were classified as moderately sarcopenic (prevalence 15.1%), those with USI values within range $5.29 < USI \geq 5.82$ were classified as sarcopenic (prevalence 27.9%), and those with USI values >5.82 were classified as severely sarcopenic (prevalence 9.6%). The DXA-derived SMI was found to be significantly correlated with USI ($r = 0.61$, $P < 0.0001$). Notably, the USI cut-off value for moderate sarcopenia (4.76 a.u.) was found to coincide with the DXA cut-off value of sarcopenia (7.26 kg/m^2).
Conclusions   We propose a novel, practical, and inexpensive imaging marker of the loss of muscle mass associated with sarcopenia, called the ultrasound sarcopenic index (USI), based on changes in muscle geometric proportions. These changes provide a useful 'signature of sarcopenia' and allow the stratification of individuals according to the presence and severity of muscle sarcopenia. We are convinced that the USI will be a useful clinical tool for confirming the diagnosis of sarcopenia, of which the assessment of muscle mass is a key-component.

Keywords   Sarcopenia; Ultrasound; Ageing; Skeletal muscle

Introduction

Sarcopenia is the most prominent phenotypic feature of ageing of the musculoskeletal system. While the definition of sarcopenia is an evolving concept that started with a classification based on muscle mass alone,[1] this has progressively moved to a more operational definition that includes the loss not only of muscle mass but also of muscle strength,[2] with a risk of adverse outcomes such as physical disability, poor quality of life, and even death.[3] However, as recognized by the latest definition of sarcopenia by the European Working Group on Sarcopenia in Older People (EWSOP2), low muscle mass or quality is a determinant factor for confirming sarcopenia in the presence of low muscle strength,[4] otherwise known as dynapenia.[5]

Hence, the measurement of muscle mass remains a key requirement for the clinical diagnosis of sarcopenia. Typically, this has been achieved using dual X-ray absorptiometry (DXA),[6] MRI,[7] or bioelectrical impedance.[8] These techniques provide a skeletal muscle index calculated as the ratio of appendicular skeletal muscle mass (ASM) to the square of body height (ASM/ht^2). The use of these methods has been widespread and has been instrumental for the diagnosis of sarcopenia in clinical settings.[9]

In 2003, we reported for the first time that the loss of muscle mass associated with sarcopenia not only entails a decrease in muscle cross-sectional area and volume but also alterations in the spatial arrangement of muscle fibres within the muscle (specifically, fibre fascicle length, pennation angle, and muscle thickness), known as 'muscle architecture'.[10] Knowledge of the spatial arrangement of muscle fibres within a muscle is particularly important because muscle architecture is one of the most important determinants of muscle force and velocity and of its excursion characteristics.[11]

Using ultrasonography, we were able to show, for several locomotor muscles,[12–14] that the key parameters of muscle architecture are significantly altered in sarcopenic muscle. Importantly, these original findings showed that as muscle volume, cross-sectional area, and muscle thickness decrease with ageing, also fibre fascicle length and pennation angle become smaller. This spatial rearrangement of muscle fibres is expected to reflect a change in sarcomere number[15]: a decrease in fascicle length predicting a loss of sarcomere in-series[16,17] and a decrease in pennation angle predicting a loss of sarcomeres arranged in parallel.[18]

If changes in muscle architecture were to scale harmonically with the decrease in muscle volume due to sarcopenia, one would expect the ratio of fascicle length (Lf) to muscle thickness (Tm) to remain constant. However, muscle length (and thus fascicle length) is constrained by its connections into the proximal and distal tendons that insert into bony structures. Although fascicle length has been found to decrease with ageing,[10,19] this effect should be limited by the proximal and distal tendon insertions into bone, unless tendons were to elongate, which is most unlikely. Hence, the reduction in muscle mass with ageing should be due more to a decrease in muscle thickness than in fascicle length, that is, it should involve a greater loss of sarcomeres in parallel than in series. Recent observations made in our laboratory in different populations of older individuals (active, sedentary, and mobility impaired) seem to confirm this assumption: with increasing degree of sarcopenia, the decrease in muscle thickness (Tm) exceeds that of fascicle length (Lf).

These findings prompted us to formulate the hypothesis that the Lf/Tm ratio, which we shall refer to as 'ultrasound sarcopenia index' (USI), may be used as a marker of the loss of muscle mass associated with sarcopenia. An important advantage of using a marker based on an anatomical ratio rather than on absolute values is its independence from gender and body dimensions. This is relevant because fascicle length and pennation angle (and thus muscle thickness) have been shown to be greater in men than women because of the different body height and body mass.[20] Instead, the use of the ratio Lf/Tm would thus circumvent this problem as it inherently compensates for allometric scaling.

Moreover, there are several important advantages to be considered regarding the use of ultrasound for assessing muscle architecture, both for clinical and practical purposes. Ultrasound is commonly available in most clinical settings, is portable, simple and quick to perform, even at the bed site, it can be delivered at a fraction of the cost of MRI, and is not subject to extensive exclusions such as implanted metalwork. Most of all, ultrasound has a very good reliability and reproducibility when performed by properly trained personnel [intraclass correlation coefficient (ICC) of 0.97–0.99 for muscle thickness parameter and 0.97–0.99 for fascicle length),[21–24] and more importantly, it does not involve exposure to ionizing radiations such as DXA.

Therefore, the goal of the present study was to exploit the age-related alterations in muscle geometric proportion (differential decrease in fascicle length and of muscle thickness) to obtain an ultrasound-based index of the loss of muscle mass associated with sarcopenia.

To accomplish this goal, we set out the following objectives:

 i to establish whether USI values of young and older individuals were independent of sex, height, and body mass;
 ii to compare the USI values in populations of elderly individuals with different degrees of physical activity, from mobility impaired to moderately active, with respect to those of young adult controls; and
 iii to identify cut-off values for diagnosing sarcopenia using the USI.

Methods

Study design and participants

In total of 279 participants were recruited for this study: 60 young controls (30 female and 30 male) aged 19–32 years, 67 moderately active elderly aged 65≥ years (65–82 years) (59 female and 8 male), 109 sedentary elderly (76 female and 33 male), and 43 mobility impaired elderly (MIE) aged 65≥ year (65–86 years old, 24 female and 19 male). These distinct populations were recruited by three different academic institutions collaborating to this study. The populations of young controls and moderately active elderly were recruited by the University of Nottingham and by Manchester Metropolitan University, the population of sedentary elderly was recruited by the University of Parma, while the mobility impaired elderly were recruited by the University of Bologna.

Participants defined as moderately active elderly (MAE) were those individuals who, for the past 3 months or more, had completed only one session or less per week in any physical activities designed to improve health and fitness other than their usual activities of daily living, and were rarely active in their daily lives to such an extent as to work up a sweat. Participants defined as sedentary elderly (SE) were those individuals not engaged in any physical activities designed to improve health and fitness other than their usual activities of daily living. The mobility impaired elderly (MIE) were individuals, awaiting hip surgery but not hospitalized. The young controls (YC) participants were university students, not engaged in sports at a competitive level.

All participants provided informed written consent to this study, which was approved by the local Ethics Committees of the University of Padova, University of Bologna, Manchester Metropolitan University, University of Nottingham, and the University of Parma, and complied with the Declaration of Helsinki.

Inclusion criteria

Inclusion criteria for the elderly populations were (i) age ≥65 years, (ii) BMI 20–30 kg/m^2, (iii) signed informed consent, (iv) willingness and ability to comply with the protocol. Inclusion criteria for the young control population were age >18 and <35 years and BMI 20–30 kg/m^2.

Exclusion criteria

For the MAE and SE participants, exclusion criteria were the active presence of (i) neurologic diseases with mobility impairment; (ii) dementia or moderate-severe cognitive impairment identified by score <24 of Mini-Mental State Examination Test (MMSE), according to Folstein et al.[25]; (iii) severe cardiopulmonary diseases; (iv) cancer, osteoarthritis, and/or orthopaedic diseases with severe mobility impairment.

For the MIE, exclusion criteria were the active presence of (i) neurologic diseases with mobility impairment; (ii) dementia or moderate-severe cognitive impairment identified by score <24 of Mini-Mental State Examination Test (MMSE), according to Folstein et al.[25]; (iii) severe cardiopulmonary diseases; (iv) cancer.

Ultrasound measures

In all participants, vastus lateralis (VL) muscle architecture was measured at rest by ultrasonography. Each recruiting centre collaborating to this study received specific training for the muscle ultrasound measures involved in this investigation. This required the implementation of a standardized ultrasound protocol, and each operator received extensive familiarization with the ultrasound imaging procedures. Accreditation of each ultrasound operator was given on the basis of his or her ability to obtain high-quality ultrasound images with high inter-day reliability tested in repeated examinations performed 2 days apart. The quality of each image was judged by an experienced ultrasound investigator and a high inter-day reliability was defined as an intraclass correlation coefficient (ICC) >0.90, considered excellent,[26] for each of the two key ultrasound parameters: fascicle length and muscle thickness. Training of the ultrasound operators also involve the assessment of inter-operator reliability through repeated scanning on the same individual by each operator from the three recruiting centers; only ICC values >0.90 were considered acceptable.

Measurements were performed with the participant lying supine on an examination couch, using a portable digital ultrasonographer (MyLab25, Esaote, Genoa, Italy), fitted with a 7–10 MHz, 4.7 cm linear array transducer. Scans were acquired at the distal third (defined as 35% of the distance between the between the caudal part of the trochanter major and the distal boundary of the lateral femoral condyle) of the VL muscle upwards, along the mid sagittal axis of the VL identified as that axis located at mid distance

between the proximal and medial borders of the muscle measured by ultrasound (*Figure* 1). Briefly, the 35% of the total femur length was marked on the skin: thereafter, the transducer was placed with its distal border aligned to the 35% femur length mark. This particular site was chosen because it coincides with the region of VL recommended for biopsy muscle sampling as it is associated with the lowest risk of neurovascular damage.[27,28] This approach seems particularly relevant because it is often necessary to relate ultrasound muscle architectural measurements to the morphological, contractile, and biochemical properties of muscle fibres.[29–32]

In each ultrasound image, the fascicular path was determined as the interspaces between echoes coming from the perimysial tissue surrounding the fascicle. Fascicle length (Lf), defined as the distance between the fascicle insertions into the superior and deep aponeuroses (*Figure* 1), and muscle thickness (Tm), defined as the orthogonal distance between the deep and superficial aponeuroses measured at mid-image,[33] were assessed using the public domain NIH software 'ImageJ' (version 1.42q, National Institute of Health, USA, http://rsb.info.nih.gov/ij). Where the fascicle extended beyond the image, the non-visible part was estimated by extrapolation.[13] This was achieved by increasing the image canvas size to enable to extend the superficial and deep aponeuroses beyond the boundaries set by the probe length as described by Ticinesi *et al.*[13] The length of the fascicle, beyond its visible portion, was then extrapolated until it met the superficial or deep aponeurosis. Fascicle length extrapolation was limited to those fascicles for which at least 50% of the entire length was visible.[13]

In each scan, the average Lf of three fascicles and the average of three Tm were used for analysis. Because the fascicles measured were limited to those for which at least 50% of the entire length was visible, these were inevitably found in the central portion of the image. In this image portion, at this specific region of interest (i.e. VL distal third), both pennation angles and fascicle lengths are usually uniform, as opposed to the periphery of the image where pennation angles, and thus, fascicle length may differ from the central region.[34]

The ratio of fascicle length to muscle thickness (Lf/Tm) was used as 'ultrasound sarcopenia index'.

Appendicular skeletal muscle mass determination by dual X-ray absorptiometry

In a subsample of MAE and SE individuals (n = 114), appendicular skeletal muscle mass (ASM) was assessed by DXA as described by McPhee *et al.*[35] and correlated to SMI values determined by ultrasound.

In brief, ASM was assessed using a Lunar Prodigy Advance DXA scanner (version EnCore 10.50.086). A whole body scan was performed with the participant lying supine with legs and arms fully extended. Appendages were isolated from the trunk and head by using DXA regional computer-generated default lines, with manual adjustment. The appendicular skeletal muscle mass (ASM) was calculated as the combined lean mass of each of the four limbs as measured by DXA. The ASM was then normalized to body height^2 (ASM/ht^2) to obtain the skeletal muscle index (SMI) as described by Baumgartner *et al.*[6]
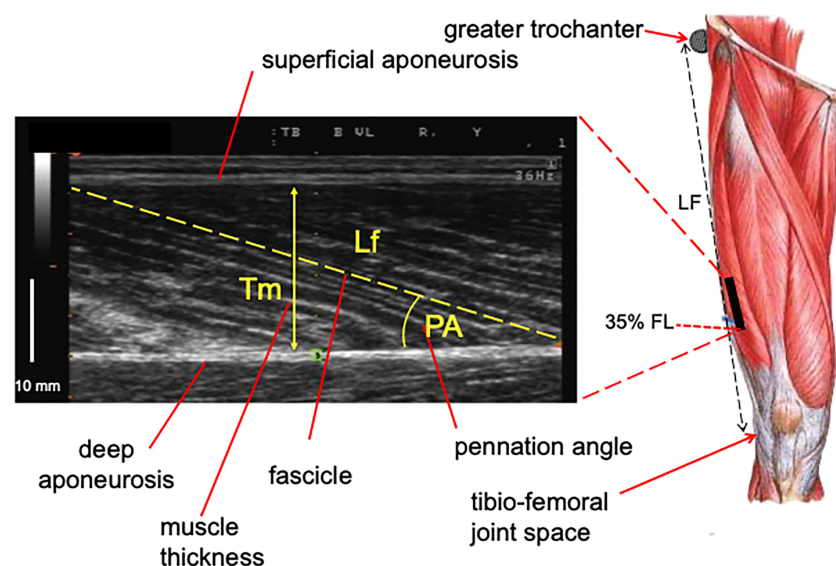
Figure 1 Sagittal ultrasound image of the vastus lateralis muscle obtained at the distal 35% of femur length (LF), showing fascicle length (Lf), muscle thickness (Tm), and pennation angle (PA).

Statistics

Data were analysed using Prism 8 (version 8.4.3) GraphPad Software (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± standard deviation.

The data were checked for normality of distribution using the D'Agostino and Pearson test. Differences amongst means were tested using a one-way ANOVA followed by the Bonferroni's multiple comparison test. Statistical difference was set at $P < 0.05$. In order to test the independence of USI from sex of the young controls and of the elderly participants, the unpaired, Student's t-test was used separately for the two populations. Moreover, independence of USI values of young and elderly participants with height and body mass was tested using the Pearson's correlation.

USI score of each patient was compared with the USI mean value of the young reference group (YC), and a Z-score was calculated as

$$USI\,Z\,score = \frac{USI\,value \; - \; mean\,USI\,YC}{SD\,USI\,YC}$$

Pearson's correlation (r) and simple linear regression were calculated to determine relationships between the USI and the SMI data.

Results

Values of VL fascicle length (Lf) and muscle thickness (Tm), Lf/Tm ratio (USI) in the studied populations are reported in *Table* 1.

When ratio of Lf to Tm (USI) was calculated, the resulting USI was 3.70 ± 0.52 for the YC, 4.50 ± 0.72 for the MEA, 5.05 ± 1.11 for the SE, and 6.31 ± 1.38 for the MIE, the respective Lf/Tm values all significantly differed between each other ($P < 0.0001$) (*Figure* 2).

Notably, comparison of the Lf/Tm ratio of young men with that of young women and of the elderly men and elderly women revealed no significant differences between sexes (*Figure* 3A,D). Also, no significant correlation was found either between Lf/Tm and body height (*Figure* 3B,E), or Lf/Tm and body mass (*Figure* 3C,F).

The obtained Z-score of the USI values was then used to achieve a stratification of the elderly populations according to the muscle sarcopenic status. Following this approach, moderately active, inactive, and mobility impaired elderly populations were classified either as non-sarcopenic ($0.00 < Z\text{-}score \leq 1.00$), pre-sarcopenic ($1.00 < Z\text{-}score \leq 2.00$), moderately sarcopenic ($2.00 < Z\text{-}score \leq 3.00$), sarcopenic ($3.00 < Z\text{-}score \leq 4.00$), and severely sarcopenic ($Z\text{-}score > 4$) in terms of muscle mass (*Table 2 and Figure* 4). According to this analysis, the prevalence of non-sarcopenic and pre-sarcopenic elderly progressively decreases from moderately active, to inactive, to mobility impaired. Applying these definitions to the 219 elderly participants of this study, 52 may be considered non-sarcopenic, 52 pre- sarcopenic, 33 moderately sarcopenic, 61 sarcopenic, and 21 as severely sarcopenic (*Figure* 5A–C) (*Table* 2).

Comparison of DXA SMI and corresponding USI data obtained in the subsample of male MAE ($n = 52$) and SE ($n = 24$) individuals showed a significant negative linear correlation ($r = 0.61$, $P < 0.0001$), between SMI and USI (*Figure* 6). *Figure* 6 also shows the cut-off value of SMI for sarcopenia in male individuals (7.26 kg/m^2)[6] and USI cut-off value for moderate sarcopenia (4.76, arbitrary units, a.u.). As may be observed, the two cut-off values meet the USI-SMI regression line. In this graph, the cut-off values for each level of stratification of muscle sarcopenia obtained by USI are also shown.

Discussion

The main finding of the present study is the identification of a novel, non-invasive ultrasound-based biomarker of muscle sarcopenia, termed the 'ultrasound sarcopenia index' (USI). This new biomarker is based on a change in muscle geometric proportions due to a greater decrease in muscle thickness than in fascicle length and enables us to obtain an objective diagnosis of muscle atrophy, deemed essential for the classification of sarcopenia as defined by the revised EWSOP guidelines.[4] The fundamental advantage of using a ratio (fascicle length to muscle thickness) rather than absolute measures of muscle dimensions, such as muscle cross-

Table 1 Values of fascicle length (lf), muscle thickness (tm), and the calculated ultrasound sarcopenia index (USI) for the young controls (YC), moderately active elderly (MEA), sedentary elderly (SE), and mobility impaired elderly (MIE) populations

Population	Lf (mm)	Tm (mm)	USI (Lf/Tm)	Z-score
Young controls (YC) ($n = 60$)	82.1 (13.3)	22.5 (3.5)	3.70 (0.53)	-
Moderately active elderly (MAE) ($n = 67$)	87.2 (13.0)	19.7 (3.5)	4.50 (0.72)	1.52 (1.37)
Sedentary elderly (SE) ($n = 109$)	82.1 (16.3)	16.6 (2.9)	5.05 (1.11)	2.51 (2.11)
Mobility impaired elderly (MIE) ($n = 43$)	77.7 (23.2)	12.5 (3.5)	6.31 (1.38)	4.97 (2.63)

The reported Z-scores are calculated using the YC as reference population. Values are mean ± SD, in brackets.
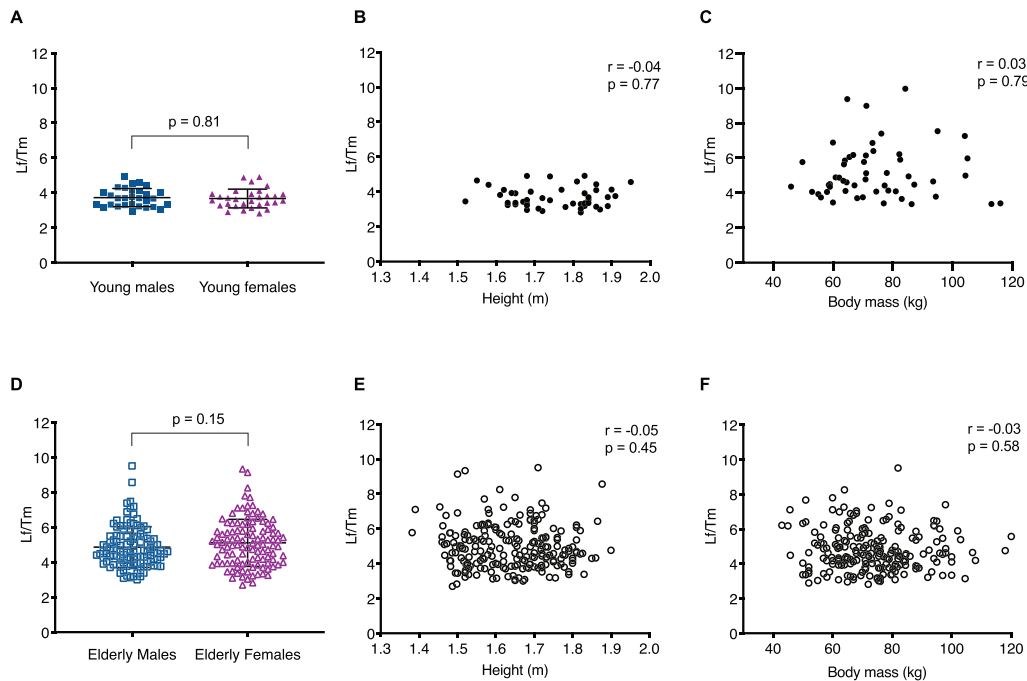
Figure 2 A) Lf/Tm values in young men and women, (B) LF/Tm versus height in young men and women, (C) Lf/Tm versus body mass in young men and women, (D) Lf/Tm values in elderly men and women, (E) Lf/Tm values versus height in elderly men and women, (F) Lf/Tm values versus body mass in elderly men and women.
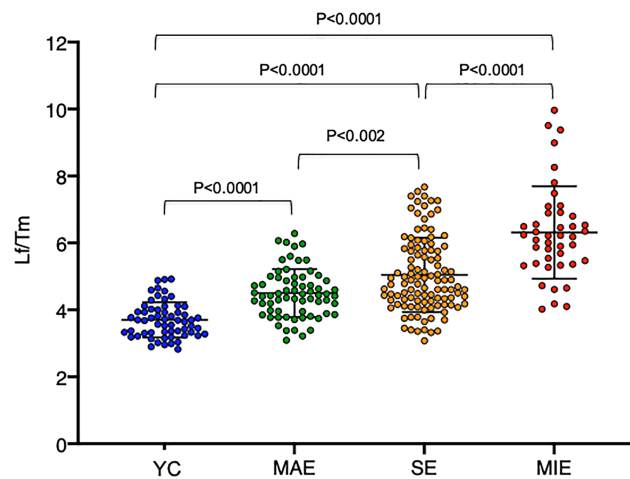
Figure 3 Individual USI values (Lf/Tm) of young controls (YC), moderately active elderly (MAE), sedentary elderly (SE) and of mobility impaired elderly (MIE). Values are mean ± SD.

sectional area or muscle mass/volume, is that the USI is independent of sex, body mass, and height. This is because of allometric scaling, by which individuals with different body mass and height will have different muscle sizes and architectural values (muscle cross-sectional area, thickness, and fascicle length). Instead, the use of a ratio allows one to eliminate these differences, as confirmed by our findings that USI values of young and elderly men and women are practically identical.

Notably, all three elderly populations tested in the study presented USI values significantly higher than those of the young controls. The greatest differences were found for the mobility impaired elderly and sedentary elderly populations, whose mean USI values were respectively 1.71-fold and 1.37-fold higher than those of the young controls. Our data also show that an active lifestyle reduces the USI value of the older individuals but not to the level of young controls. Indeed, the moderately active elderly had mean USI values
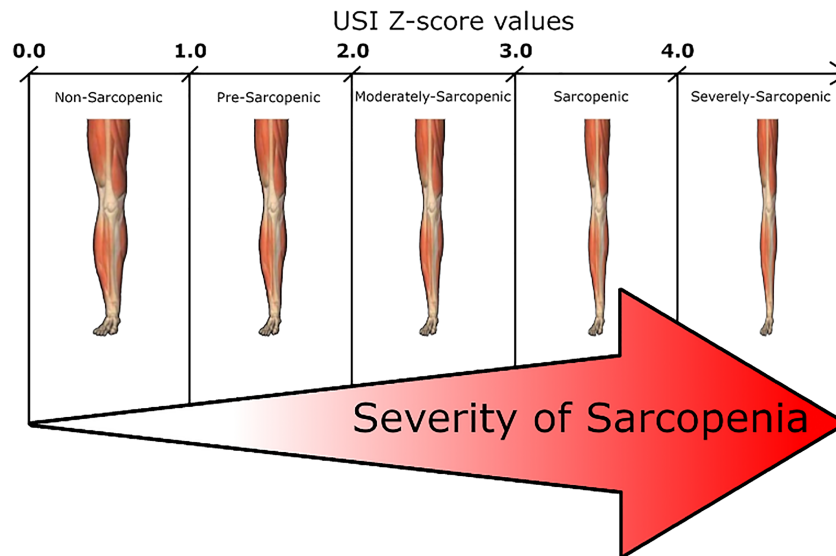
Figure 4 Classification of muscle sarcopenic status according to the USI *Z*-score.
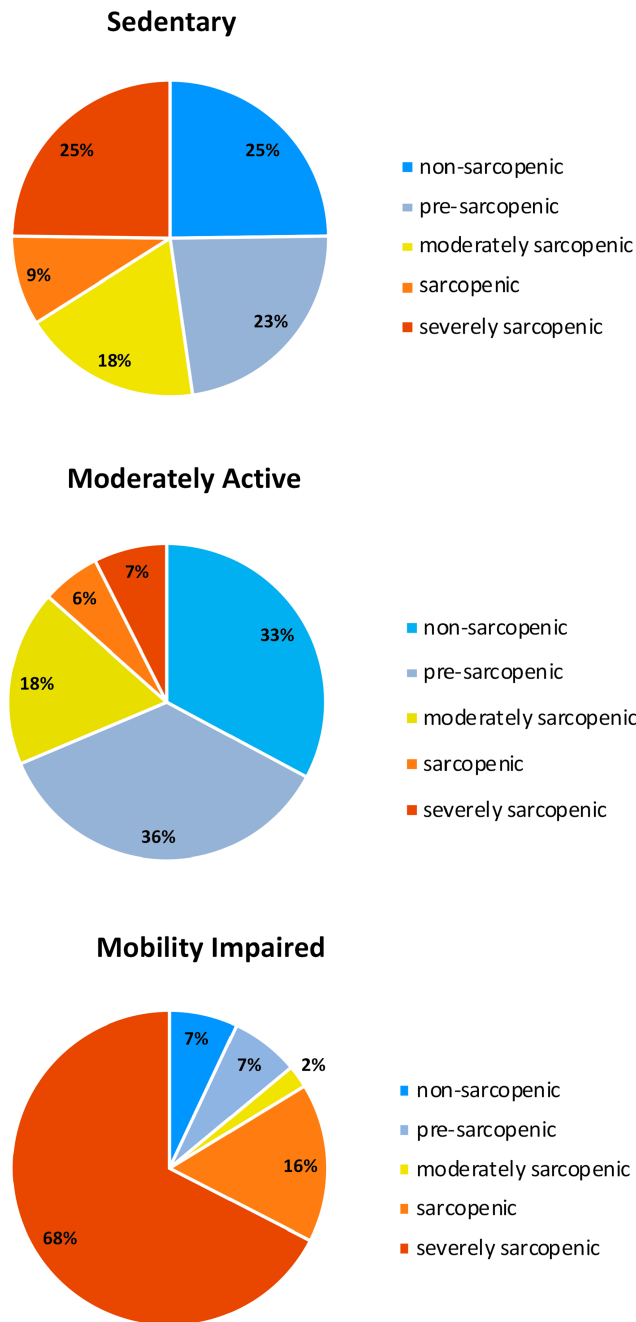
1.22-fold higher than of the young controls. This observation supports the concept that muscle sarcopenia can be significantly mitigated but not fully prevented by an active lifestyle. This is probably due to the fact that although an active lifestyle seems to afford some protection against the age-related muscle denervation and neuromuscular junction damage[36–38] those motor units that are lost cannot be rescued by physical activity.

The stratification of the elderly participants according to their sarcopenic status based on their respective USI *Z*-score seems of considerable clinical value (*Table 2 and Figure* 4). Indeed, through this analysis, it was possible to classify individuals either as non-sarcopenic, pre-sarcopenic, moderately sarcopenic, sarcopenic, and severely sarcopenic in terms of muscle mass. It seems noteworthy that using this stratification, the presence of muscle sarcopenia (moderate sarcopenia) starts with USI values greater than 2 SD of the young control mean USI value. This observation seems in line with the approach used by common techniques of defining muscle sarcopenia employing CT, DXA, and BIA, which classifies as sarcopenic values of muscle mass that are below 2 SD of a young control population mean. Also, the 1 SD steps used in this study for the stratification of muscle sarcopenia seem particularly useful for identifying individuals as pre-sarcopenic and for differentiating between individuals with different degrees of muscle sarcopenia. The correlation between USI and SMI data (*Figure* 6) also seems to support this concept. Indeed, it is noteworthy that, in the available male population, the USI cut-off value for moderate sarcopenia (4.76 a.u.) coincides with the DXA cut-off value of sarcopenia (7.26 kg/m^2). This seems to confirm the validity of the diagnosis of muscle sarcopenia by USI. In addition, by applying the different cut-off values provided by USI, a

stratification of individuals above and below the cut-off value for moderate sarcopenia may be achieved. This affords the classification of individuals either as pre-sarcopenic, non-sarcopenic, sarcopenic, or severely sarcopenic (*Figure* 5A–C) in terms of muscle mass.

The finding that both age and sedentarism lead to an increase in the USI index confirms the hypothesis that muscle sarcopenia is not a harmonic process, as it entails differential alterations in muscle geometric proportions. This inhomogeneous loss of muscle tissue across muscle thickness and length hypothetically suggests that sarcopenia is mostly due to a loss of sarcomeres in parallel (determinants of radial muscle loss and thus muscle thickness) rather than to a loss of sarcomeres in series (determinants of fascicle length and thus muscle length). Because muscle length is constrained by the muscle proximal and medial insertions, not affected by ageing, it seems logical that fewer sarcomere in series (thus fascicle length) than in parallel (thus muscle thickness) should be lost with ageing, which explains the increase in the Lf/Tm ratio.

It could be argued that from a trigonometric point of view, this ratio corresponds to 1/sin (cosecant) of the pennation angle (PA), and that rather than measuring Lf and Tm, one could simply measure PA and calculate the cosecant of sin PA. However, this argument would only hold if the two aponeuroses delimiting the fascicles were absolutely parallel. This is often not the case, particularly in sarcopenic muscle. In fact, although Lf/Tm significantly correlates to 1/sin PA (*Figure* 7), it can be seen that the greater the ratio of Lf/Tm (i.e. the more sarcopenic the muscle is), the more the data deviate from the line of identity (y = x). Hence, for reasons of accuracy, we prefer to use an index based on Lf/Tm rather than based on 1/sin PA.

Sedentary

- non-sarcopenic
- pre-sarcopenic
- moderately sarcopenic
- sarcopenic
- severely sarcopenic

Moderately Active

- non-sarcopenic
- pre-sarcopenic
- moderately sarcopenic
- sarcopenic
- severely sarcopenic

Mobility Impaired

- non-sarcopenic
- pre-sarcopenic
- moderately sarcopenic
- sarcopenic
- severely sarcopenic

Figure 5 Stratification of muscle sarcopenia in (A) moderately active (*n* = 67), (B) sedentary (*n* = 109), and (C) mobility impaired elderly (*n* = 43).

Table 2 Classification of muscle sarcopenic profile according to the USI *Z*-score

| Population	*Z*-score range	USI range
Non-sarcopenic	$0.00 < Z\text{-score} \le 1.00$	$3.70 < \text{USI} \le 4.23$
Pre-sarcopenic	$1.00 < Z\text{-score} \le 2.00$	$4.23 < \text{USI} \le 4.76$
Moderately sarcopenic	$2.00 < Z\text{-score} \le 3.00$	$4.76 < \text{USI} \le 5.29$
Sarcopenic	$3.00 < Z\text{-score} \le 4.00$	$5.29 < \text{USI} \le 5.82$
Severely sarcopenic	$Z\text{-score} > 4.00$	$\text{USI} > 5.82$
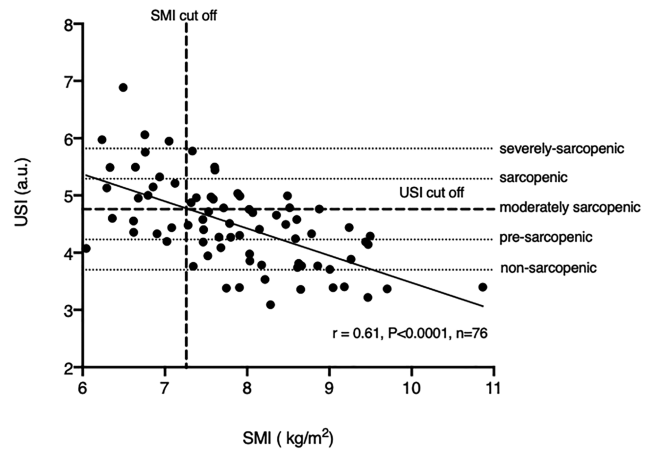
Figure 6 Correlation between DXA skeletal muscle mass index (SMI) values and ultrasound sarcopenia index (USI) values obtained in a sub-sample of male MAE (*n* = 52) and SE individuals (*n* = 24) of this study. The sarcopenia cut-off values for the SMI (7.26 kg/m², (2)) and for the USI (4.76, arbitrary units, a.u.) are shown in the graph, together with the cut-off values of the stratification of sarcopenia by USI.

Although the USI has been specifically developed as a clinical tool for the diagnosis of muscle sarcopenia, we believe that this index could find a useful application also in other clinical conditions involving acute muscle wasting, as in critical illness or neurodegenerative conditions. Indeed, in an ultrasound-based study in critically ill patients, Puthucheary *et al.*[39] have shown that the higher is the number of organs undergoing failure, the faster is the decrease in muscle size.

Because the USI may be sensitive to the potential loss of sarcomeres in parallel and in series, it may prove to be a useful early marker of atrophy in this class of patients, as in any other patients afflicted by muscle wasting due to disuse or disease.

Finally, the USI index presented in this paper is specific to the vastus lateralis (VL) muscle, purposely chosen because it plays a fundamental role in locomotion and is the second largest lower limb muscle of the human body.[40] Indeed, the VL is one of the most investigated muscles in studies on muscle architecture and in most biopsy-based investigations on the molecular mechanisms and protein metabolism of muscle atrophy and hypertrophy. We cannot exclude that other values of USI could be obtained on different muscles of the lower or upper limbs, but the USI values presented in this paper and their use for diagnosing sarcopenia are specific for the VL muscle only. Also, the present USI values are specific to measurements of Tm and Lf of the VL muscle performed at the distal 35% of femur length (with the lower border of the probe coinciding with the 35% position. Other groups prefer acquiring ultrasound images at the mid-thigh region, measured as the distance from tendon to tendon
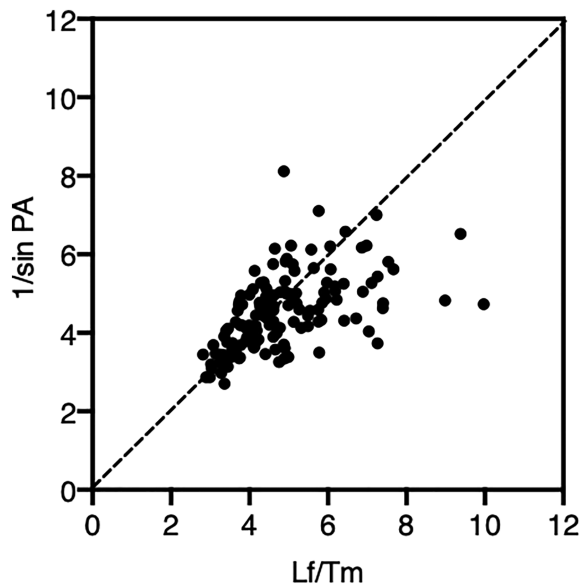
Figure 7 Lf/tm (USI) values plotted against values of 2/sin PA. As can be observed, values fall along the identity line for low values of USI but with increasing values, the data increasingly move away from the identity line.

be affected by ethnicity. In addition, it ought to be pointed out that these USI values have been obtained in young and older populations of non-obese individuals. In obese young and older individuals, pennation angle, and thus muscle thickness, are significantly greater because of the added inert fat mass acting as a chronic mechanical stimulus, partly compensating for muscle sarcopenia, while fascicle length seems unaffected.[43,44] Hence, one would expect the Lf/Tm ratio (USI) to be lower in obese older people compared with normoweight people of the same age group, as obesity seems to mitigate the age-related loss of muscle mass.[43,44]

In conclusion, we introduce for the first time a quick, reliable, and reproducible ultrasound method for diagnosing the loss of muscle mass associated with sarcopenia, called the ultrasound sarcopenic index (USI), based on changes in muscle geometric proportions. These changes provide a useful 'signature of sarcopenia' and allow the stratification of individuals according to the presence and severity of changes in muscle geometry. We are convinced that the USI will be a useful clinical tool for confirming the diagnosis of muscle sarcopenia, of which the assessment of muscle mass is a key component.

Acknowledgements

Conflict of interest

The authors declare that they have no conflict of interest.

insertions.[41,42] However, the measurement at mid-thigh is indeed contiguous with the 35% distal femur length measured as the distance between the inferior border of the greater trochanter and the lateral condyle of the tibial bone. In fact, ultrasound measurements in these two regions overlap with one another. It thus seems probable that the USI values reported in the present study may also apply to the mid-thigh region referred by Perkisas et al.[41,42]; however, this should be confirmed by future studies.

It should also be pointed out that the present USI values were obtained in a white Caucasian population. As such, it cannot be assumed that they would necessarily apply to populations of different ethnicity. However, because based on a ratio rather than on absolute values, we expect USI not

References

1. Rosenberg IH. Summary comments. *Am J Clin Nutr* 1989;**50**:1231–1233.
2. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;**393**:2636–2646.
3. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis. *Age Ageing* 2010;**39**: 412–423.
4. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: Revised European consensus on definition and diagnosis. *Age Ageing* 2019;**48**:16–31.
5. Clark BC, Manini TM. Sarcopenia != Dynapenia. *J Gerontol Ser A Biol Sci Med Sci* 2008;**63**:829–834.
6. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, et al. Epidemiology of Sarcopenia among the Elderly in New Mexico. *Am J Epidemiol* 1998;**147**:755–763.
7. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 2000;**89**:81–88.
8. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;**50**: 889–896.
9. Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: A systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age Ageing* 2014;**43**:48–759.
10. Narici MV, Maganaris CN, Reeves ND, Capodaglio P. Effect of aging on human muscle architecture. *J Appl Physiol* 2003;**95**:2229–2234.
11. Lieber RL, Fridén J. Functional and clinical significance of skeletal muscle architecture. *Muscle Nerve* 2000;**23**: 1647–1666.
12. Ticinesi A, Meschi T, Narici MV, Lauretani F, Maggio M. Muscle Ultrasound and Sarcopenia in Older Individuals: A Clinical Perspective. *J Am Med Dir Assoc* 2017;**18**: 290–300.
13. Ticinesi A, Narici MV, Lauretani F, Nouvenne A, Colizzi E, Mantovani M, et al. Assessing sarcopenia with vastus

lateralis muscle ultrasound: an operative protocol. *Aging Clin Exp Res* 2018;**30**: 1437–1443.

14. Vezzoli A, Mrakic-Sposta S, Montorsi M, Porcelli S, Vago P, Cereda F, et al. Moderate Intensity Resistive Training Reduces Oxidative Stress and Improves Muscle Mass and Function in Older Individuals. *Antioxidants* 2019;**8**:431.

15. Jamali AA, Afshar P, Abrams RA, Lieber RL. Skeletal muscle response to tenotomy. *Muscle Nerve* 2000;**23**:851–862.

16. Williams PE, Goldspink G. Longitudinal growth of striated muscle fibres. *J Cell Sci* 1971;**9**:751–767.

17. Tabary JC, Tabary C, Tardieu C, Tardieu G, Goldspink G. Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J Physiol* 1972;**224**:231–244.

18. Narici MV, Maganaris CN. Plasticity of the muscle-tendon complex with disuse and aging. *Exerc Sport Sci Rev* 2007;**35**: 126–134.

19. Morse CI, Thom JM, Birch KM, Narici MV. Changes in triceps surae muscle architecture with sarcopenia. *Acta Physiol Scand* 2005;**183**:291–298.

20. Kubo K, Kanehisa H, Azuma K, Ishizu M, Kuno SY, Okada M, et al. Muscle architectural characteristics in young and elderly men and women. *Int J Sports Med* 2003;**24**:125–130.

21. Reeves ND, Narici MV, Maganaris CN. In vivo human muscle structure and function: adaptations to resistance training in old age. *Exp Physiol* 2004;**89**:675–689.

22. Reeves ND, Maganaris CN, Narici MV. Ultrasonographic assessment of human skeletal muscle size. *Eur J Appl Physiol* 2004;**91**:116–118, Epub 2003 Nov 25.

23. Seynnes OR, Maganaris CN, de Boer MD, di Prampero PE, Narici MV. Early structural adaptations to unloading in the human calf muscles. *Acta Physiol* 2008;**193**:265–274.

24. Jandova T, Narici MV, Steffl M, Bondi D, D'amico M, Pavlu D, et al. Muscle hypertrophy and architectural changes in response to eight-week neuromuscular electrical stimulation training in healthy older people. *Life* 2020;**10**:1–15.

25. Folstein MF, Folstein SE, McHugh PR. 'Minimental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;**12**:189–198.

26. Portney LG, Watkins MP. *Foundations of clinical research Applications to practice*, 2nd ed. Upper Saddle River. - References - Scientific Research Publishing. https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=577760: Prentice Hall Health; 2000. Accessed 18 December 2020.

27. Chen X, Abbey S, Bharmal A, Harris S, Hudson E, Krinner L, et al. Neurovascular structures in human vastus lateralis muscle and the ideal biopsy site. *Scand J Med Sci Sports* 2019;**29**:sms.13369.

28. Melendez MM, Vosswinkel JA, Shapiro MJ, Gelato MC, Mynarcik D, Gavi S, et al. Wall Suction Applied to Needle Muscle Biopsy-A Novel Technique for Increasing Sample Size. *J Surg Res* 2007;**142**:301–303.

29. de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV. Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. *J Physiol* 2007;**583**:1079–1091.

30. de Boer MD, Selby A, Atherton P, Smith K, Seynnes OR, Maganaris CN *et al*. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. 2007.

31. Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Peter Magnusson S, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. *J Physiol* 2004;**534**: 613–623.

32. Suetta C, Andersen JL, Dalgas U, Berget J, Koskinen S, Aagaard P, et al. Resistance training induces qualitative changes in muscle morphology, muscle architecture, and muscle function in elderly postoperative patients. *J Appl Physiol* 2008;**105**: 180–186.

33. Franchi MV, Longo S, Mallinson J, Quinlan JI, Taylor T, Greenhaff PL, et al. Muscle thickness correlates to muscle cross-sectional area in the assessment of strength training-induced hypertrophy. *Scand J Med Sci Sports* 2018;**28**:846–853.

34. Infantolino BW, Challis JH. Short communication: Pennation angle variability in human muscle. *J Appl Biomech* 2014;**30**: 663–667.

35. McPhee JS, Hogrel JY, Maier AB, Seppet E, Seynnes OR, Sipilä S, et al. Physiological and functional evaluation of healthy young and older men and women: Design of the European MyoAge study. *Biogerontology* 2013;**14**:325–337.

36. Mosole S, Rossini K, Kern H, Löfler S, Fruhmann H, Vogelauer M, et al. Reinnervation of Vastus lateralis is increased significantly in seniors (70-years old) with a lifelong history of high-level exercise. *Eur J Transl Myol* 2013;**23**:205–210.

37. Power GA, Dalton BA, Behm DG, Vandervoort AA, Doherty TJ, Rice CL. Motor Unit Number Estimates in Masters Runners. *Med Sci Sports Exerc* 2010;**42**: 1644–1650.

38. Nishimune H, Stanford JA, Mori Y. Role of exercise in maintaining the integrity of the neuromuscular junction. *Muscle Nerve* 2014;**49**:315–324.

39. Puthucheary ZA, Rawal J, McPhail M, Connolly B, Ratnayake G, Chan P, et al. Acute skeletal muscle wasting in critical illness. *JAMA - J Am Med Assoc* 2013;**310**: 1591–1600.

40. Handsfield GG, Meyer CH, Hart JM, Abel MF, Blemker SS. Relationships of 35 lower limb muscles to height and body mass quantified using MRI. *J Biomech* 2014;**47**: 631–638.

41. Perkisas S, Baudry S, Bauer J, Beckwée D, De Cock AM, Hobbelen H, et al. Application of ultrasound for muscle assessment in sarcopenia: towards standardized measurements. *Eur Geriatr Med* 2018;**9**: 739–757.

42. Perkisas S, Bastijns S, Baudry S, Bauer J, Beaudart C, Beckwée D, et al. Application of ultrasound for muscle assessment in sarcopenia: 2020 SARCUS update. *Eur Geriatr Med* 2021;**12**:45–59.

43. Tomlinson DJ, Erskine RM, Winwood K, Morse CI, Onambélé GL. The impact of obesity on skeletal muscle architecture in untrained young vs. old women. *J Anat* 2014;**225**:675–684.

44. Rastelli F, Capodaglio P, Orgiu S, Santovito C, Caramenti M, Cadioli M, et al. Effects of muscle composition and architecture on specific strength in obese older women. *Exp Physiol* 2015;**100**:1159–1167.