Ultrasound-Based Detection of Low Muscle Mass for Diagnosis of Sarcopenia in Older Adults

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Ultrasound-based detection of low muscle mass for diagnosis of sarcopenia in older adults

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ABSTRACT

Objective: To establish muscle-specific cut-off values for ultrasound-based detection of low muscle mass and to assess its prevalence in a population of frail older subjects when applying the cut-points of different muscles and those of different sarcopenic indices.

Design: Cross-sectional study.

Setting: Geriatric outpatient clinic and clinical research laboratory.

Methods: Forty-four older adults (30 women, mean age: 82 yrs) and sixty young subjects (30 women, mean age: 26 yrs) participated. Body composition and thickness of four lower limb muscles (rectus femoris, vastus lateralis, tibialis anterior, medial gastrocnemius) were respectively assessed by bioelectrical impedance analysis (BIA) and ultrasonography.

Main Outcome Measurements: Site-specific cut-points for ultrasound-based assessment of low muscle mass (muscle thickness values 2 SDs below the sex-specific means of our sample of young subjects) and comparative prevalence rates of low muscle mass.

Results: The following site-specific cut-points for muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastus lateralis: 17 mm in men and 15 mm in women; tibialis anterior: 23 mm in men and 22 mm in women; medial gastrocnemius: 13 mm in both men and women. The prevalence of low muscle mass in older adults was highly dependent on the muscle being investigated: it varied from 86% for thigh muscles to 30% for leg muscles. Moreover, the prevalence of low muscle mass was highly dependent on the applied diagnostic criterion and on the adopted cut-off value (it ranged from 2% to 75% for different BIA-derived criteria).

Conclusions: We suggest that muscle ultrasonography provides rehabilitation physicians with a practical and accurate tool for identifying individuals with low muscle mass. However, the usability of cut-off values established in our group of Caucasian healthy young subjects to identify low...
muscle mass in older persons of different ethnic groups remains to be demonstrated in future studies.

ABSTRACT WORD COUNTS: 300 words
INTRODUCTION

Primary sarcopenia, the age-related loss of skeletal muscle mass and function[1,2], is associated with disability and frailty that represent major socioeconomic as well as medical problems. In rehabilitation patients, primary sarcopenia can be further exacerbated by the disuse- or drug-related loss of muscle mass or function. Therefore, elderly rehabilitation patients could benefit from the assessments of skeletal muscle mass and function for the detection of sarcopenia.

A major development in sarcopenia research has been the convergence in its operational definition. Several consensus groups have recently published operational criteria for the diagnosis of sarcopenia (incorporating the evaluation of muscle mass with the assessment of strength and/or physical performance), including the “European Working Group on Sarcopenia in Older People” (EGWSOP) [3], the “International Working Group on Sarcopenia” (IWGS) [4] and the “Foundation for the National Institutes of Health Sarcopenia Project” [5]. All three consensus groups included the appendicular skeletal muscle mass (ASMM) assessment, as realized with dual-energy X-ray absorptiometry (DXA), into the operational definition of sarcopenia. However, different indices of ASMM (such as ASMM normalized to height or to body mass index) and different cut-off points were considered. Other sarcopenic indices, which are commonly used in research as well as in clinical routine, are based on the assessment of the total body skeletal muscle mass (TSMM, normalized to body weight or to height), as realized with bioelectrical impedance analysis (BIA) [6,7]. However, the use of different diagnostic criteria may lead to different conclusions, as evidenced by several investigations recently performed in community-dwelling older adults [8-15]. In addition, although the use of DXA- or BIA-derived sarcopenic indices may be practical for clinical purposes, they do not seem very accurate [1]. This is essentially due to the fact that sarcopenia is not a uniform condition as it affects postural muscles more than non-postural ones [1,2,16-18]. Therefore, site-specific assessment of loss of muscle mass may be required for its early and
accurate detection. Consistently, recent studies showed that thigh sarcopenia can be detected by ultrasound-based assessment of muscle thickness before it appears at the whole body level [19,20]. However, as highlighted by Abe et al. [19], there are no published site-specific cut-points for ultrasonographic assessment of low muscle mass in older adults. Therefore, the aims of this study were: i) to establish muscle-specific cut-off values for ultrasound-based detection of low muscle mass; ii) to assess the prevalence of low muscle mass in a population of frail older subjects when applying the ultrasonographic cut-points of different lower limb muscles; iii) to assess the prevalence of low muscle mass when applying different sarcopenic indices derived from ultrasound, BIA, and anthropometry.

METHODS

Subjects

Forty-four older adults (30 women and 14 men, mean age ± SD: 82 ± 7 yrs; body mass index: 25 ± 5 kg/m²) and sixty young subjects (30 women and 30 men, age: 26 ± 3 yrs; body mass index: 22 ± 3 kg/m²) volunteered to participate in the study (convenience sample). The young subjects were habitually physically active, and none participated in competitive sports. The older group was composed by institution-dwelling subjects with one or more of Fried’s frailty criteria [21]. Side dominance was assessed with the “Waterloo Handedness and Footedness Questionnaires - Revised” [22]. One older and six young subjects were left-side dominant. Each participant received a detailed explanation of the study and gave written informed consent prior to participation. The study conformed to the ethical principles enunciated in the Declaration of Helsinki and was approved by the local Ethics Committee.

Assessments
The following measurements were taken in young subjects in order to obtain normative muscle mass data that could be used for establishing cut-off points (for the detection of low muscle mass): anthropometric measurements (height and weight), TSMM and ASMM using BIA, thickness of four lower limb muscles using ultrasonography. The same measurements were also taken in older subjects while calf circumference, walking speed and handgrip strength were additionally measured in this group.

**Anthropometric measurements**

Measurements of height and weight were made in overnight fasted subjects (in light clothing and barefoot or with socks) on the same day as all the other tests. Standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was determined to the nearest 0.1 kg using a calibrated balance beam scale. Calf circumference (dominant side) was measured to the nearest 0.1 cm while the subjects were seated with their leg hanging loosely. The measurement tape was wrapped around the calf and the highest value was retained. A cut-off point of <31 cm [23] was adopted to identify low muscle mass.

**Physical performance**

Subjects were asked to walk over a 14-m walkway at a self-selected usual speed and their walking speed was evaluated. A stopwatch was used to time the subjects as they walked over the central 10 m of the walkway. The initial 2 m and final 2 m were not considered to allow for acceleration and anticipatory deceleration. The distance covered was divided by the time taken to complete the 10-m walk. Subjects completed three trials and the mean walking speed of the three trials was retained. A cut-off point of <0.8 m/s [3] was adopted to identify subjects with low physical performance.
**Muscle strength**

Handgrip strength was measured on the dominant side using a handheld device (Jamar Plus Digital Dynamometer, Patterson Medical, Warrenville, IL, USA). The subjects were sitting comfortably with the shoulder adducted, the elbow flexed at 90° and both the forearm and the wrist in a neutral position. They were instructed to perform a maximal voluntary isometric contraction by contracting their muscles as forcefully as possible for 4-5 s. The test was repeated three times with 30 s of recovery in between: if the peak forces of the three trials were within 5% of each other, the highest value was retained. Otherwise, additional trials were performed until the 5% criterion was achieved. Cut-off points of <30 kg for men and <20 kg for women [3] were adopted to identify subjects with low handgrip strength.

**Total body and appendicular skeletal muscle mass**

BIA was performed in the morning after an overnight fast, with the subjects lying in the supine position with both upper and lower limbs slightly abducted from the body. Source and sensor electrodes were placed on the dorsum of both hand and foot of the right side of the body. Whole-body reactance and resistance to an applied current (frequency: 50 kHz; amplitude: 0.4 mA) were measured with a tetrapolar device (BIA 101 ASE, Akern, Florence, Italy) and used to estimate TSMM according to Janssen’s equation [24] and ASMM according to Sergi’s equation [25].

The validity of the BIA device used in this study has previously been demonstrated by Janssen et al. [24] and Sergi et al. [25]. The same Authors also demonstrated the validity of the predictive equations for TSMM [24] and ASMM [25].

TSMM was normalized to the body weight (and expressed in %) [6] or to the height (and expressed in kg/m²) [7] to calculate the skeletal muscle index (SMI). ASMM was normalized to the height (and
expressed in kg/m$^2$) [26] or to the body mass index [5,27] to calculate the appendicular skeletal muscle index (ASMI). Ten cut-off values for ASMM, SMI and ASMI were adopted for the detection of low muscle mass (Table 1): five out of ten values (cut-off values # I – III – V – VII – IX in Table 1) were derived from previous studies [5-7,26,27], while the other five values (cut-off values # II – IV – VI – VIII – X in Table 1) were established based on normative data of muscle mass obtained in our sample of young subjects (values 2 SDs below the sex-specific means of our sample of young subjects were considered).

Insert Table 1

Muscle thickness

Ultrasound B-mode images of the following lower limb muscles of the dominant side were acquired during a single experimental session: rectus femoris, vastus lateralis, tibialis anterior, and medial gastrocnemius. These muscles were specifically selected as sarcopenia preferentially affects lower limb muscles [1,2,16-18].

The same experienced sonographer (MAM) performed all the assessments and acquired all the images. Three consecutive static scans were acquired in the longitudinal plane of each muscle. After each scan, the subject was allowed to move and the transducer was repositioned. To increase the repeatability of the acquisitions and to ensure the optimal representation of the muscle, we adopted the following criteria: i) tibialis anterior: we maximized the representation of the bone boundary and of the muscle fascicles; ii) rectus femoris: we optimized the representation of the superficial and deep aponeuroses; iii) vastus lateralis and medial gastrocnemius: we optimized the representation of the superficial and deep aponeuroses and of the muscle fascicles.
Images of the medial gastrocnemius were acquired with the subjects in the prone position, whereas for all the other muscles subjects were positioned supine. In all measurements, the lower limb joints were extended and the subjects were asked to completely relax their muscles. A suitable amount of ultrasound coupling gel was used to ensure optimal image quality and to minimize the transducer pressure on the skin. All scans were performed by placing the transducer in correspondence of the largest muscle diameter at the following anatomical sites, according to previous studies [28,29]: the rectus femoris was measured half-way along the line from the anterior-superior iliac spine to the superior border of the patella; the vastuslateralishalf-way along the line from the anterior-superior iliac spine to the superolateral border of the patella; the tibialis anterior at one-quarter of the distance from the inferior border of the patella to the lateral malleolus; the medial gastrocnemius from the mid-sagittal line of the muscle, midway between the proximal and distal tendon insertions.

All images were acquired using a ClarUs ultrasound device (Telemed, Vilnius, Lithuania) equipped with a linear-array transducer (code L12-5L40N) with a variable-frequency band (5-12 MHz). Gain was set at 50% of the range, dynamic image compression was turned off, and time gain compensation was maintained in the same (neutral) position for all depths. All system-setting parameters were kept constant throughout the study and for each subject, except depth (initially set at 30mm) that was modified during the examination (range: 30-60 mm) to visualize the entire muscle thickness. Pictures were stored as DICOM files and transferred to a computer for processing.

Muscle thickness was measured as the distance between the superficial and deep aponeuroses by using ImageJ (National Institutes of Health, Bethesda, MD, USA). All three images acquired for each muscle were analyzed. As shown in the representative example of Figure 1, the operator measured the muscle thickness in three points, equally spaced along the image. The operator
placed the measurement points on each aponeuroses trying to trace a segment which was orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each point pairs was considered as the muscle thickness. Cut-off values (and 2SD range values) for the thickness of the four muscles (identified as values 2 SDs below the sex-specific means of our sample of young subjects) are reported in Table 1.

**Statistical analysis**

Since the Shapiro–Wilk test for normal distribution of the data failed, the Fisher’s exact test was used for comparisons between proportions and the Mann-Whitney U test was used for comparisons between the two groups of subjects (young vs older). Intrasession and intrarater reliability of the thickness measurement was determined by the intraclasscorrelaton coefficient (ICC3,1) and coefficient of variation using the three scans acquired for each muscle. We obtained the following ICC and CV values: 0.98 and 3.2% for rectus femoris, 0.99 and 3.3% for vastuslateralis, 0.98 and 1.5% for tibialis anterior, 0.97 and 3.7% for medial gastrocnemius. Muscle thickness T-score values were calculated for older subjects using the following formula: \[(\text{individual value} - \text{mean value of the young subjects of the corresponding gender group})/\text{SD of the young subjects of the corresponding gender group}\]. In each of the older subjects, the T-scores calculated for the four muscles were then averaged to obtain: i) a lower limb T-score (i.e., the mean T-score of the four muscles), ii) a thigh T-score (i.e., the mean T-score of rectus femoris and vastuslateralis muscles), iii) a leg T-score (i.e., the mean T-score of tibialis anterior and medial gastrocnemius muscles). Accordingly, the following definitions of low muscle masswere...
The prevalences of these different ultrasound-based definitions of low muscle mass were then compared. Moreover, the prevalence of low muscle mass obtained by using a single ultrasound-derived criterion was compared with the prevalences obtained by using the BIA-derived criteria and the calf-circumference criterion (based on the cut-off values reported in Table 1 and numbered from I to XI).

In each of the older subjects, the diagnosis of sarcopenia was established based on the “EWGSOP” criteria [3]: pre-sarcopenia was defined as the presence of low muscle mass (i.e., low mass of the thigh muscles), sarcopenia was defined as the presence of both low mass of the thigh muscles and poor muscle function (low walking speed or low handgrip strength), severe sarcopenia was defined as the presence of low mass of the thigh muscles, low walking speed and low handgrip strength.

Data were expressed as mean ± SD. The threshold for statistical significance was set to P = .05. All statistical tests were performed with Statistica 6 (Statsoft Inc., Tulsa, OK, USA) software package, with the exception of sensitivity-specificity analyses that were performed with GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) and reliability analysis for thickness measurements that was performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package.

RESULTS

Muscle mass and thickness: comparisons between young and older subjects

Table 2 lists the values of BIA-derived muscle mass for the two groups of subjects stratified by gender. As expected, TSMM and ASMM were higher in young compared to older subjects, while
the SMI (TSMM normalized to height) in men and the ASMI (ASMM normalized to height) in both men and women were comparable between young and older subjects. Figures 2-3 show representative examples of ultrasound images acquired from young and older subjects: muscle thickness was higher in the four muscles of the young subjects compared to older subjects. Similar to these examples, analysis of the group data (Table 2) showed significantly higher muscle thickness values in young compared to older subjects for all muscles (with the exception of the tibialis anterior muscle in men). The thickness values of the four muscles obtained in young subjects were used to establish the cut-off values reported in Table 1.

Detection of low muscle mass: comparisons among cut-off values

As shown in Figure 4A, the prevalence of low muscle mass obtained by using the thigh T-score (86%) was significantly (P=.01) higher than that obtained by using the lower limb T-score (61%), and the latter was significantly (P=.005) higher than that obtained by using the leg T-score (30%). Moreover, the prevalence of low muscle mass obtained by using the rectus femoris T-score (86%) was comparable (P=.18) to that obtained by using the vastus lateralis T-score (73%). A significant (P=.0006) difference was observed between the prevalence of low muscle mass obtained by using the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%).

Briefly, the prevalence of low muscle mass is highly dependent on the muscle being investigated: proximal muscles of the lower limb seem more valid for the detection of low muscle mass than distal muscles. Therefore, we compared the thigh T-score with the other criteria used to detect low muscle mass. As shown in Figure 4B, the prevalence of low muscle mass ranged from 2% to 75% for
Different BIA-derived criteria; it was 52% for the calf-circumference criterion and 86% for the thigh T-score criterion.

Briefly, the prevalence of low muscle mass is highly dependent on the applied diagnostic criterion and on the adopted cut-off value.

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**Diagnosis of sarcopenia**

Of the 44 older subjects, 38 (86%) presented low muscle mass (i.e., low mass of the thigh muscles), 23 (52%) presented low calf circumference (according to cut-off values # XI in Table 1) and 33 (75%) presented low ASMI (according to cut-off values # X in Table 1). Moreover, 38 older subjects (86%) presented low muscle strength (average handgrip strength of the whole group: 16.9 ± 7.3 kg; average handgrip strength of the subjects presenting low muscle strength: 15.1 ± 5.7 kg) and 32 (73%) presented low physical performance (average walking speed of the whole group: 0.62 ± 0.24 m/s; average walking speed of the subjects presenting low walking speed: 0.50 ± 0.15 m/s).

The combination of thigh muscle thickness, strength and performance measurements enabled to classify 6 out of 44 older subjects (14%) as non-sarcopenic, 2 (5%) as pre-sarcopenic, 9 (20%) as sarcopenic (7 out of 9 subjects presented low mass of the thigh muscles and low handgrip strength, while 2 out of 9 subjects presented low muscle mass and low walking speed), and 27 (61%) as severely sarcopenic.

Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia, identified on the basis of low calf circumference (according to cut-off values # XI in Table 1) and poor muscle function, were 0.60 and 1.0, respectively.
Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia, identified on the basis of low ASMI (according to cut-off values # X in Table 1) and poor muscle function, were 0.74 and 0.17, respectively.

Briefly, the diagnosis of sarcopenia is highly dependent on the applied diagnostic criterion.

DISCUSSION

In the present study, 60 young subjects were evaluated with ultrasonography and BIA to establish muscle-specific and population-specific cut-off values for sarcopenic indices which were then applied to a sample of 44 frail older subjects to determine comparative prevalence rates of low muscle mass. This is the first study to report site-specific cut-points for ultrasound-based detection of low muscle mass. These cut-points were established based on normative values of muscle thickness gained from our sample of young subjects that were comparable to those previously observed in healthy young populations (Table 3: left column). Likewise, the muscle thickness values we measured in older subjects were similar to those previously reported in community-dwelling and/or frail elderly individuals (Table 3: right column). Therefore, the high prevalence of low muscle mass (86%) we observed in older subjects and the inter-muscle differences (86% of subjects showed low thickness of the thigh muscles, while only 52% and 16% of subjects showed reduction in medial gastrocnemius and tibialis anterior thickness, respectively) did not result from the application of biased cut-off values (e.g., too large for thigh muscles, thus implying false-positive results, and too stringent for leg muscles, thus implying the overlook of true-positive results). Consistently, such inter-muscle variability in the susceptibility to age-related muscle loss is in line with previous evidence gained from magnetic resonance imaging-[18], computed tomography-[16], and DXA-[17] based measurements showing that age-related muscle loss is greater in lower limb (postural) muscles than in upper limb (non-postural) muscles.
To our knowledge, this study is the first to show that proximal muscles of the lower limb are preferentially affected by thickness loss than distal muscles and that the medial gastrocnemius is more affected by thickness loss than the tibialis anterior. The latter result is in agreement with previous studies showing that the age-related decline in plantar-flexor strength is greater compared to dorsiflexor strength (although the loss of muscle mass alone cannot account for the reduction in muscle strength) [35]. Given the known differences in muscle composition between the tibialis anterior and the other three muscles considered here (the former presents a higher percentage of slow fibers compared to the latter) [36,37], it may be hypothesized that the higher the percentage of insulin-sensitive slow fibers, the lower the susceptibility to age-related loss of muscle mass. Therefore, it may be suggested that in the tibialis anterior of our population of frail older subjects the permissive effect of insulin on protein synthesis [38,39] was greater compared to other less-insulin sensitive muscles and could explain, at least partly, the lower tibialis anterior susceptibility to age-related muscle loss. However, not only muscular, but also neural mechanisms, such as site-specific losses of motor units [40], probably underlie the observed site-specific age-related loss of muscle mass.

In the present study, we found that the prevalence of low muscle mass was highly dependent not only on the muscle being investigated, but also on the applied diagnostic criterion and the adopted cut-points. These findings are in line with previous studies showing that different definitions of sarcopenia have good negative, but poor positive agreement [8,9,10-15]. The low agreement level is mainly determined by different sensitivities for the detection of low muscle mass that characterize the different skeletal muscle mass indices. Given the present and previous [19-20] demonstrations of high sensitivity of the ultrasound-based assessment of low muscle mass, we recommend the inclusion of muscle thickness analysis in future studies investigating the
predictive validity of different operational definitions of sarcopenia for important clinical outcomes such as mortality, disability and functional recovery following rehabilitation.

Another major determinant of the low level of agreement among different definitions of sarcopenia is the population variability in body size/composition. In fact, the cut-off values for detection of low muscle mass established in a specific ethnic group cannot be applied to other groups. Consistently, we found that the prevalence of low muscle mass differed when considering the BIA-derived cut-points (TSMM normalized to body weight or height, absolute ASMM, and ASMM normalized to height or body mass index) established in our population vs. previously-reported cut-points. As the currently-adopted scaling factors (i.e., body weight, height, body mass index) seem unable to normalize muscle mass (and thickness) for body size/composition, future studies are required on this issue.

There are several limitations to this study. First, we did not assess the thickness of upper limb muscles to further highlight the inter-muscle variability in the susceptibility to age-related mass loss that was observed in lower limb muscles. Second, the usability of ultrasound-based indices of low muscle mass is limited by the skillfulness of the physician to perform musculoskeletal ultrasound and to accurately measure muscle thickness. Automatic tracking of aponeurosis and measurement of muscle thickness can compensate, at least partly, this limitation. Although these tools are not readily available as part of the measurement packages offered on commercially available scanners, it is likely they will be embedded in high-end scanners in a close future.

Finally, the usability of cut-off values established in our group of Caucasian healthy young subjects to identify low muscle mass in older persons of different ethnic groups remains to be demonstrated in future studies. Similar to the approach currently adopted in osteoporosis research and clinical practice, the availability of population-specific cut-off values and the use of
our T-score based criterion could enable the comparison between different studies and the
accurate identification of low muscle mass also in non-Caucasian older subjects.

CONCLUSIONS

This study reports site-specific cut-points for ultrasound-based detection of low muscle mass. To
simplify these cut-points for potential future applications, the following thresholds of muscle
thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastus lateralis: 17
mm in men and 15 mm in women; tibialis anterior: 23 mm in men and 22 mm in women; medial
gastrocnemius: 13 mm in both men and women.

Moreover, we found that the prevalence of low muscle mass was highly dependent on the muscle
being investigated (proximal muscles of the lower limb were more affected than distal muscles and
the medial gastrocnemius was more affected than the tibialis anterior), as well as on the applied
diagnostic criterion and the adopted cut-points (BIA-derived criteria and relative cut-points
underestimated the prevalence of low muscle mass in comparison to the ultrasound-based
determination of muscle thickness). We suggest that muscle ultrasonography provides
rehabilitation physicians with a practical and accurate tool for identifying individuals with (pre-
sarcopenia at increased risk for functional impairment, disability, negative outcomes following
surgery or rehabilitation.

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FIGURE CAPTIONS

Figure 1.
Example of medial gastrocnemius thickness measurement for a representative ultrasound scan. The operator measured the muscle thickness in three points, equally spaced along the image. The operator placed the measurement points on each aponeuroses trying to trace a segment which was orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each point pairs was considered as the muscle thickness.

Figure 2.
Examples of ultrasound scans of rectus femoris and vastuslateralis muscles from representative young (A, C) and older (B, D) subjects. Vertical dotted lines indicate the three thickness measurements considered in each image.

Figure 3.
Examples of ultrasound scans of tibialis anterior and medial gastrocnemius muscles from representative young (A, C) and older (B, D) subjects. Vertical dotted lines indicate the three thickness measurements considered in each image.

Figure 4.
A) Prevalence of low muscle mass obtained in the group of 44 older subjects by using different T-scores: lower limb T-score, thigh T-score, leg T-score, muscle-specific T-scores (RF: rectus femoris; VL: vastuslateralis; TA: tibialis anterior; MG: medial gastrocnemius).
B) Prevalence of low muscle mass obtained in the group of 44 older subjects by using bioelectrical impedance analysis-derived cut-off values (gray columns), calf-circumference cut-off (white column), ultrasound-derived thigh muscle cut-off values (dark column).
Ultrasound-based detection of low muscle mass for diagnosis of sarcopenia in older adults
ABSTRACT

Objective: To establish muscle-specific cut-off values for ultrasound-based detection of low muscle mass and to assess the prevalence of low muscle mass obtained in a population of frail older subjects when applying the cut-points of different muscles and those of different sarcopenic indices.

Design: Cross-sectional study.

Setting: Geriatric outpatient clinic and clinical research laboratory.

Methods: Forty-four older adults (30 women, mean age: 82 yrs) and sixty young subjects (30 women, mean age: 26 yrs) participated. Body composition and thickness of four lower limb muscles (rectus femoris, vastus lateralis, tibialis anterior, and medial gastrocnemius) were respectively assessed by bioelectrical impedance analysis (BIA) and ultrasonography in both populations.

Main Outcome Measurements: Site-specific cut-points for ultrasound-based assessment of low muscle mass (muscle thickness values 2 SDs below the sex-specific means of our sample of young subjects) and comparative prevalence rates of low muscle mass.

Results: The following site-specific cut-points for muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastus lateralis: 17 mm in men and 15 mm in women; tibialis anterior: 23 mm in men and 22 mm in women; medial gastrocnemius: 13 mm in both men and women. The prevalence of low muscle mass in older adults was highly dependent on the muscle being investigated: it varied from 86% for thigh muscles to 30% for leg muscles. Moreover, the prevalence of low muscle mass was highly dependent on the applied diagnostic criterion and on the adopted cut-off value (it ranged from 2% to 75% for different BIA-derived criteria).

Conclusions: BIA-derived criteria and relative cut-points underestimated the prevalence of low muscle mass in comparison to the ultrasound-based assessment of muscle thickness. It is therefore
recommended to adopt the ultrasonographic quantification of muscle thickness and the herein
provided cut-points for identifying individuals with sarcopenia.

We suggest that muscle ultrasonography provides rehabilitation physicians with a practical and
accurate tool for identifying individuals with low muscle mass. However, the usability of cut-off
values established in our group of Caucasian healthy young subjects to identify low muscle mass in
older persons of different ethnic groups remains to be demonstrated in future studies.

ABSTRACT WORD COUNTS: 297300 words
INTRODUCTION

Primary sarcopenia, the age-related loss of skeletal muscle mass and function[1,2], is associated with disability and frailty that represent major socioeconomic as well as medical problems. In rehabilitation patients, primary sarcopenia can be further exacerbated by the disuse- or drug-related loss of muscle mass or function. Therefore, elderly rehabilitation patients could benefit from the assessments of skeletal muscle mass and function for the detection of sarcopenia.

A major development in sarcopenia research has been the convergence in its operational definition. Several consensus groups have recently published operational criteria for the diagnosis of sarcopenia (incorporating the evaluation of muscle mass with the assessment of strength and/or physical performance), including the “European Working Group on Sarcopenia in Older People” (EGWSOP) [3], the “International Working Group on Sarcopenia” (IWGS) [4] and the “Foundation for the National Institutes of Health Sarcopenia Project” [5]. All three consensus groups included the appendicular skeletal muscle mass (ASMM) assessment, as realized with dual-energy X-ray absorptiometry (DXA), into the operational definition of sarcopenia. However, different indices of ASMM (such as ASMM normalized to height or to body mass index) and different cut-off points were considered. Other sarcopenic indices, which are commonly used in research as well as in clinical routine, are based on the assessment of the total body skeletal muscle mass (TSMM, normalized to body weight or to height), as realized with bioelectrical impedance analysis (BIA) [6,7]. However, the use of different diagnostic criteria may lead to different conclusions, as evidenced by several investigations recently performed in community-dwelling older adults [8-15].

In addition, although the use of DXA- or BIA-derived sarcopenic indices may be practical for clinical purposes, they do not seem very accurate [1]. This is essentially due to the fact that sarcopenia is not a uniform condition as it affects postural muscles more than non-postural ones [1,2,16-18]. Therefore, site-specific assessment of loss of muscle mass may be required for its early and
accurate detection. Consistently, recent studies showed that thigh sarcopenia can be detected by ultrasound-based assessment of muscle thickness before it appears at the whole body level [19,20]. However, as highlighted by Abe et al. [19], there are no published site-specific cut-points for ultrasonographic assessment of low muscle mass in older adults. Therefore, the aims of this study were: i) to establish muscle-specific cut-off values for ultrasound-based detection of low muscle mass; ii) to assess the prevalence of low muscle mass in a population of frail older subjects when applying the ultrasonographic cut-points of different lower limb muscles; iii) to assess the prevalence of low muscle mass when applying different sarcopenic indices derived from ultrasound, BIA, and anthropometry.

METHODS

Subjects

Forty-four older adults (30 women and 14 men, mean age ± SD: 82 ± 7 yrs; body mass index: 25 ± 5 kg/m²) and sixty young subjects (30 women and 30 men, age: 26 ± 3 yrs; body mass index: 22 ± 3 kg/m²) volunteered to participate in the study (convenience sample). The young subjects were habitually physically active, and none participated in competitive sports. The older group was composed by institution-dwelling subjects with one or more of Fried’s frailty criteria [21]. Side dominance was assessed with the “Waterloo Handedness and Footedness Questionnaires - Revised” [22]. One older and six young subjects were left-side dominant. Each participant received a detailed explanation of the study and gave written informed consent prior to participation. The study conformed to the ethical principles enunciated in the Declaration of Helsinki and was approved by the local Ethics Committee.

Assessments
The following measurements were taken in young subjects in order to obtain normative muscle mass data that could be used for establishing cut-off points (for the detection of low muscle mass): anthropometric measurements (height and weight), TSMM and ASMM using BIA, thickness of four lower limb muscles using ultrasonography. The same measurements were also taken in older subjects while calf circumference, walking speed and handgrip strength were additionally measured in this group.

**Anthropometric measurements**

Measurements of height and weight were made in overnight fasted subjects (in light clothing and barefoot or with socks) on the same day as all the other tests. Standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was determined to the nearest 0.1 kg using a calibrated balance beam scale. Calf circumference (dominant side) was measured to the nearest 0.1 cm while the subjects were seated with their leg hanging loosely. The measurement tape was wrapped around the calf and the highest value was retained. A cut-off point of <31 cm [23] was adopted to identify low muscle mass.

**Physical performance**

Subjects were asked to walk over a 14-m walkway at a self-selected usual speed and their walking speed was evaluated. A stopwatch was used to time the subjects as they walked over the central 10 m of the walkway. The initial 2 m and final 2 m were not considered to allow for acceleration and anticipatory deceleration. The distance covered was divided by the time taken to complete the 10-m walk. Subjects completed three trials and the mean walking speed of the three trials was retained. A cut-off point of <0.8 m/s [3] was adopted to identify subjects with low physical performance.
Muscle strength

Handgrip strength was measured on the dominant side using a handheld device (Jamar Plus Digital Dynamometer, Patterson Medical, Warrenville, IL, USA). The subjects were sitting comfortably with the shoulder adducted, the elbow flexed at 90° and both the forearm and the wrist in a neutral position. They were instructed to perform a maximal voluntary isometric contraction by contracting their muscles as forcefully as possible for 4–5 s. The test was repeated three times with 30 s of recovery in between: if the peak forces of the three trials were within 5% of each other, the highest value was retained. Otherwise, additional trials were performed until the 5% criterion was achieved. Cut-off points of <30 kg for men and <20 kg for women [3] were adopted to identify subjects with low handgrip strength.

Total body and appendicular skeletal muscle mass

BIA was performed in the morning after an overnight fast, with the subjects lying in the supine position with both upper and lower limbs slightly abducted from the body. Source and sensor electrodes were placed on the dorsum of both hand and foot of the right side of the body. Whole-body reactance and resistance to an applied current (frequency: 50 kHz; amplitude: 0.4 mA) were measured with a tetrapolar device (BIA 101 ASE, Akern, Florence, Italy) and used to estimate TSMM according to Janssen’s equation [24] and ASMM according to Sergi’s equation [25]. The validity of the BIA device used in this study has previously been demonstrated by Janssen et al. [24] and Sergi et al. [25]. The same Authors also demonstrated the validity of the predictive equations for TSMM [24] and ASMM[25].

TSMM was normalized to the body weight (and expressed in %) [6] or to the height (and expressed in kg/m²) [7] to calculate the skeletal muscle index (SMI). ASMM was normalized to the height (and
expressed in kg/m$^2$) [26] or to the body mass index [5,27] to calculate the appendicular skeletal muscle index (ASMI). Ten cut-off values for ASMM, SMI and ASMI were adopted for the detection of low muscle mass (Table 1): five out of ten values (cut-off values # I – III – V – VII – IX in Table 1) were derived from previous studies [5-7,26,27], while the other five values (cut-off values # II – IV – VI – VIII – X in Table 1) were established based on normative data of muscle mass obtained in our sample of young subjects (values 2 SDs below the sex-specific means of our sample of young subjects were considered).

Muscle thickness

Ultrasound B-mode images of the following lower limb muscles of the dominant side were acquired during a single experimental session: rectus femoris, vastus lateralis, tibialis anterior, and medial gastrocnemius. These muscles were specifically selected as sarcopenia preferentially affects lower limb muscles [1,2,16-18].

The same experienced sonographer (MAM) performed all the assessments and acquired all the images. Three consecutive static scans were acquired in the longitudinal plane of each muscle. After each scan, the subject was allowed to move and the transducer was repositioned. To increase the repeatability of the acquisitions and to ensure the optimal representation of the muscle, we adopted the following criteria: i) tibialis anterior: we maximized the representation of the bone boundary and of the muscle fascicles; ii) rectus femoris: we optimized the representation of the superficial and deep aponeuroses; iii) vastus lateralis and medial gastrocnemius: we optimized the representation of the superficial and deep aponeuroses and of the muscle fascicles.
Images of the medial gastrocnemius were acquired with the subjects in the prone position, whereas for all the other muscles subjects were positioned supine. In all measurements, the lower limb joints were extended and the subjects were asked to completely relax their muscles. A suitable amount of ultrasound coupling gel was used to ensure optimal image quality and to minimize the transducer pressure on the skin. All scans were performed by placing the transducer in correspondence of the largest muscle diameter at the following anatomical sites, according to previous studies [28,29]: the rectus femoris was measured half-way along the line from the anterior-superior iliac spine to the superior border of the patella; the vastuslateralishalf-way along the line from the anterior-superior iliac spine to the superolateral border of the patella; the tibialis anterior at one-quarter of the distance from the inferior border of the patella to the lateral malleolus; the medial gastrocnemius from the mid-sagittal line of the muscle, midway between the proximal and distal tendon insertions.

All images were acquired using a ClarUs ultrasound device (Telemed, Vilnius, Lithuania) equipped with a linear-array transducer (code L12-5L40N) with a variable-frequency band (5-12 MHz). Gain was set at 50% of the range, dynamic image compression was turned off, and time gain compensation was maintained in the same (neutral) position for all depths. All system-setting parameters were kept constant throughout the study and for each subject, except depth (initially set at 30mm) that was modified during the examination (range: 30-60 mm) to visualize the entire muscle thickness. Pictures were stored as DICOM files and transferred to a computer for processing.

Muscle thickness was measured as the distance between the superficial and deep aponeuroses by using ImageJ (National Institutes of Health, Bethesda, MD, USA). All three images acquired for each muscle were analyzed. As shown in the representative example of Figure 1, the operator measured the muscle thickness in three points, equally spaced along the image. The operator
placed the measurement points on each aponeuroses trying to trace a segment which was orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each point pairs was considered as the muscle thickness. Cut-off values (and 2SD range values) for the thickness of the four muscles (identified as values 2 SDs below the sex-specific means of our sample of young subjects) are reported in Table 1.

Statistical analysis

Since the Shapiro–Wilk test for normal distribution of the data failed, the Fisher’s exact test was used for comparisons between proportions and the Mann-Whitney U test was used for comparisons between the two groups of subjects (young vs older).

Intrasession and intrarater reliability of the thickness measurement was determined by the intraclasscorrelation coefficient (ICC3,1) and coefficient of variation using the three scans acquired for each muscle. We obtained the following ICC and CV values: 0.98 and 3.2% for rectus femoris, 0.99 and 3.3% for vastuslateralis, 0.98 and 1.5% for tibialis anterior, 0.97 and 3.7% for medial gastrocnemius.

Muscle thickness T-score values were calculated for older subjects using the following formula: \[ \frac{(\text{individual value} - \text{mean value of the young subjects of the corresponding gender group})}{\text{SD of the young subjects of the corresponding gender group}} \]. In each of the older subjects, the T-scores calculated for the four muscles were then averaged to obtain: i) a lower limb T-score (i.e., the mean T-score of the four muscles), ii) a thigh T-score (i.e., the mean T-score of rectus femoris and vastuslateralis muscles), iii) a leg T-score (i.e., the mean T-score of tibialis anterior and medial gastrocnemius muscles). Accordingly, the following definitions of low muscle mass were
considered: low mass of the lower limb muscles (i.e., lower limb T-score < -2), low mass of the thigh muscles (i.e., thigh T-score < -2), low mass of the leg muscles (i.e., leg T-score < -2), muscle-specific low mass (i.e., muscle thickness lower than the cut-off values reported in Table 1).

The prevalences of these different ultrasound-based definitions of low muscle mass were then compared. Moreover, the prevalence of low muscle mass obtained by using a single ultrasound-derived criterion was compared with the prevalences obtained by using the BIA-derived criteria and the calf-circumference criterion (based on the cut-off values reported in Table 1 and numbered from I to XI).

In each of the older subjects, the diagnosis of sarcopenia was established based on the “EWGSOP” criteria [3]: pre-sarcopenia was defined as the presence of low muscle mass (i.e., low mass of the thigh muscles), sarcopenia was defined as the presence of both low mass of the thigh muscles and poor muscle function (low walking speed or low handgrip strength), severe sarcopenia was defined as the presence of low mass of the thigh muscles, low walking speed and low handgrip strength.

Data were expressed as mean ± SD. The threshold for statistical significance was set to $P = 0.05$. All statistical tests were performed with Statistica 6 (Statsoft Inc., Tulsa, OK, USA) software package, with the exception of sensitivity-specificity analyses that were performed with GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) and reliability analysis for thickness measurements that was performed with SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software package.

RESULTS

Muscle mass and thickness: comparisons between young and older subjects

Table 2 lists the values of BIA-derived muscle mass for the two groups of subjects stratified by gender. As expected, TSMM and ASMM were higher in young compared to older subjects, while
the SMI (TSMM normalized to height) in men and the ASMI (ASMM normalized to height) in both men and women were comparable between young and older subjects. Figures 2-3 show representative examples of ultrasound images acquired from young and older subjects: muscle thickness was higher in the four muscles of the young subjects compared to older subjects. Similar to these examples, analysis of the group data (Table 2) showed significantly higher muscle thickness values in young compared to older subjects for all muscles (with the exception of the tibialis anterior muscle in men). The thickness values of the four muscles obtained in young subjects were used to establish the cut-off values reported in Table 1.

Detection of low muscle mass: comparisons among cut-off values

As shown in Figure 4A, the prevalence of low muscle mass obtained by using the thigh T-score (86%) was significantly (P=0.01) higher than that obtained by using the lower limb T-score (61%), and the latter was significantly (P=0.005) higher than that obtained by using the leg T-score (30%). Moreover, the prevalence of low muscle mass obtained by using the rectus femoris T-score (86%) was comparable (P=0.18) to that obtained by using the vastus lateralis T-score (73%). A significant (P=0.0006) difference was observed between the prevalence of low muscle mass obtained by using the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%). Briefly, the prevalence of low muscle mass is highly dependent on the muscle being investigated: proximal muscles of the lower limb seem more valid for the detection of low muscle mass than distal muscles. Therefore, we compared the thigh T-score with the other criteria used to detect low muscle mass. As shown in Figure 4B, the prevalence of low muscle mass ranged from 2% to 75% for
different BIA-derived criteria; it was 52% for the calf-circumference criterion and 86% for the thigh T-score criterion.

Briefly, the prevalence of low muscle mass is highly dependent on the applied diagnostic criterion and on the adopted cut-off value.

**Diagnosis of sarcopenia**

Of the 44 older subjects, 38 (86%) presented low muscle mass (i.e., low mass of the thigh muscles), 23 (52%) presented low calf circumference (according to cut-off values # XI in Table 1) and 33 (75%) presented low ASMI (according to cut-off values # X in Table 1).

Moreover, 38 older subjects (86%) presented low muscle strength (average handgrip strength of the whole group: 16.9 ± 7.3 kg; average handgrip strength of the subjects presenting low muscle strength: 15.1 ± 5.7 kg) and 32 (73%) presented low physical performance (average walking speed of the whole group: 0.62 ± 0.24 m/s; average walking speed of the subjects presenting low walking speed: 0.50 ± 0.15 m/s).

The combination of thigh muscle thickness, strength and performance measurements enabled to classify 6 out of 44 older subjects (14%) as non-sarcopenic, 2 (5%) as pre-sarcopenic, 9 (20%) as sarcopenic (7 out of 9 subjects presented low mass of the thigh muscles and low handgrip strength, while 2 out of 9 subjects presented low muscle mass and low walking speed), and 27 (61%) as severely sarcopenic.

Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia, identified on the basis of low calf circumference (according to cut-off values # XI in Table 1) and poor muscle function, were 0.60 and 1.0, respectively.
Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia, identified on the basis of low ASMI (according to cut-off values # X in Table 1) and poor muscle function, were 0.74 and 0.17, respectively.

Briefly, the diagnosis of sarcopenia is highly dependent on the applied diagnostic criterion.

DISCUSSION
In the present study, 60 young subjects were evaluated with ultrasonography and BIA to establish muscle-specific and population-specific cut-off values for sarcopenic indices which were then applied to a sample of 44 frail older subjects to determine comparative prevalence rates of low muscle mass. This is the first study to report site-specific cut-points for ultrasound-based detection of low muscle mass. These cut-points were established based on normative values of muscle thickness gained from our sample of young subjects that were comparable to those previously observed in healthy young populations (Table 3: left column). Likewise, the muscle thickness values we measured in older subjects were similar to those previously reported in community-dwelling and/or frail elderly individuals (Table 3: right column). Therefore, the high prevalence of low muscle mass (86%) we observed in older subjects and the inter-muscle differences (86% of subjects showed low thickness of the thigh muscles, while only 52% and 16% of subjects showed reduction in medial gastrocnemius and tibialis anterior thickness, respectively) did not result from the application of biased cut-off values (e.g., too large for thigh muscles, thus implying false-positive results, and too stringent for leg muscles, thus implying the overlook of true-positive results). Consistently, such inter-muscle variability in the susceptibility to age-related muscle loss is in line with previous evidence gained from magnetic resonance imaging-[18], computed tomography-[16], and DXA-[17] based measurements showing that age-related muscle loss is greater in lower limb (postural) muscles than in upper limb (non-postural) muscles.
To our knowledge, this study is the first to show that proximal muscles of the lower limb are preferentially affected by thickness loss than distal muscles and that the medial gastrocnemius is more affected by thickness loss than the tibialis anterior. The latter result is in agreement with previous studies showing that the age-related decline in plantar-flexor strength is greater compared to dorsiflexor strength (although the loss of muscle mass alone cannot account for the reduction in muscle strength) [35]. Given the known differences in muscle composition between the tibialis anterior and the other three muscles considered here (the former presents a higher percentage of slow fibers compared to the latter) [36,37], it may be hypothesized that the higher the percentage of insulin-sensitive slow fibers, the lower the susceptibility to age-related loss of muscle mass. Therefore, it may be suggested that in the tibialis anterior of our population of frail older subjects the permissive effect of insulin on protein synthesis [38,39] was greater compared to other less-insulin sensitive muscles and could explain, at least partly, the lower tibialis anterior susceptibility to age-related muscle loss. In fact, insulin is permissive for protein synthesis and suppressive for protein breakdown [38,39]. However, not only muscular, but also neural mechanisms, such as site-specific losses of motor units [40], probably underlie the observed site-specific age-related loss of muscle mass. In the present study, we found that the prevalence of low muscle mass was highly dependent not only on the muscle being investigated, but also on the applied diagnostic criterion and the adopted cut-points. These findings are in line with previous studies showing that different definitions of sarcopenia have good negative, but poor positive agreement [8,9,10-15]. The low agreement level is mainly determined by different sensitivities for the detection of low muscle mass that characterize the different skeletal muscle mass indices. Given the present and previous [19-20] demonstrations of high sensitivity of the ultrasound-based assessment of low muscle mass, we recommend the inclusion of muscle thickness analysis in future studies investigating the
predictive validity of different operational definitions of sarcopenia for important clinical outcomes such as mortality, disability and functional recovery following rehabilitation.

Another major determinant of the low level of agreement among different definitions of sarcopenia is the population variability in body size/composition. In fact, the cut-off values for detection of low muscle mass established in a specific ethnic group cannot be applied to other groups. Consistently, we found that the prevalence of low muscle mass differed when considering the BIA-derived cut-points (TSMM normalized to body weight or height, absolute ASMM, and ASMM normalized to height or body mass index) established in our population vs. previously-reported cut-points. As the currently-adopted scaling factors (i.e., body weight, height, body mass index) seem unable to normalize muscle mass (and thickness) for body size/composition, future studies are required on this issue.

There are several limitations to this study. First, we did not assess the thickness of upper limb muscles to further highlight the inter-muscle variability in the susceptibility to age-related mass loss that was observed in lower limb muscles. Second, the usability of ultrasound-based indices of low muscle mass is limited by the skillfulness of the physician to perform musculoskeletal ultrasound and to accurately measure muscle thickness. Automatic tracking of aponeurosis and measurement of muscle thickness can compensate, at least partly, this limitation. Although these tools are not readily available as part of the measurement packages offered on commercially available scanners, it is likely they will be embedded in high-end scanners in a close future. Finally, the usability of cut-off values established in our group of Caucasian healthy young subjects to identify low muscle mass in older persons of different ethnic groups remains to be demonstrated in future studies. Similar to the approach currently adopted in osteoporosis research and clinical practice, the availability of population-specific cut-off values and the use of
Our T-score based criterion could enable the comparison between different studies and the accurate identification of low muscle mass also in non-Caucasian older subjects.

CONCLUSIONS

This study reports site-specific cut-points for ultrasound-based detection of low muscle mass. To simplify these cut-points for potential future applications, the following thresholds of muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastus lateralis: 17 mm in men and 15 mm in women; tibialis anterior: 23 mm in men and 22 mm in women; medial gastrocnemius: 13 mm in both men and women.

Moreover, we found that the prevalence of low muscle mass was highly dependent on the muscle being investigated (proximal muscles of the lower limb were more affected than distal muscles and the medial gastrocnemius was more affected than the tibialis anterior), as well as on the applied diagnostic criterion and the adopted cut-points (BIA-derived criteria and relative cut-points underestimated the prevalence of low muscle mass in comparison to the ultrasound-based assessment of muscle thickness). We suggest that muscle ultrasonography provides rehabilitation physicians with a practical and accurate tool for identifying individuals with (pre-)sarcopenia at increased risk for functional impairment, disability, negative outcomes following surgery or rehabilitation.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1.
Example of medial gastrocnemius thickness measurement for a representative ultrasound scan. The operator measured the muscle thickness in three points, equally spaced along the image. The operator placed the measurement points on each aponeuroses trying to trace a segment which was orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each point pairs was considered as the muscle thickness.

Figure 2.
Examples of ultrasound scans of rectus femoris and vastuslateralismuscles from representative young (A, C) and older (B, D) subjects. Vertical dotted lines indicate the three thickness measurements considered in each image.

Figure 3.
Examples of ultrasound scans of tibialis anterior and medial gastrocnemiusmuscles from representative young (A, C) and older (B, D) subjects. Vertical dotted lines indicate the three thickness measurements considered in each image.

Figure 4.
A) Prevalence of low muscle mass obtained in the group of 44 older subjects by using different T-scores: lower limb T-score, thigh T-score, leg T-score, muscle-specific T-scores (RF: rectus femoris; VL: vastuslateralis; TA: tibialis anterior; MG: medial gastrocnemius).
B) Prevalence of low muscle mass obtained in the group of 44 older subjects by using bioelectrical impedance analysis-derived cut-off values (gray columns), calf-circumference cut-off (white column), ultrasound-derived thigh muscle cut-off values (dark column).
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<th>Women</th>
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<td>I SMI = TSMM/weight (%)</td>
<td>31%</td>
<td>22%</td>
<td>[6]</td>
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<td>II SMI = TSMM/weight (%)</td>
<td>38%</td>
<td>29%</td>
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<td>III SMI = TSMM/height² (kg/m²)</td>
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<td>VII ASMM (kg)</td>
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<td>[27]</td>
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<td>[5,27]</td>
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<tr>
<td>XI Calf circumference (cm)</td>
<td>&lt;31 cm</td>
<td>&lt;31 cm</td>
<td>[23]</td>
</tr>
<tr>
<td><strong>US</strong></td>
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<tr>
<td>XII Muscle thickness</td>
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AM: anthropometric measurement; ASMI: appendicular skeletal muscle index; ASMM: appendicular skeletal muscle mass; BIA: bioelectrical impedance analysis; BMI: body mass index; TSMM: total body skeletal muscle mass; SDs: standard deviations; SMI: skeletal muscle index; US: ultrasonography.
### Table 2 Characteristics of study participants stratified for gender and age

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<th>Variable</th>
<th>MEN</th>
<th>WOMEN</th>
<th>P value</th>
<th>MEN</th>
<th>WOMEN</th>
<th>P value</th>
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<td>Age (years)</td>
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<td>24.8±2.8</td>
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<td>BMI (kg/m²)</td>
<td>23.0±2.9</td>
<td>24.9±5.3</td>
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<td>TSMM (kg)</td>
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<tr>
<td>SMI = TSMM/weight (%)</td>
<td>47.9±4.8</td>
<td>43.4±5.7</td>
<td>&lt;0.01</td>
<td>40.3±5.5</td>
<td>29.7±4.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SMI = TSMM/height² (kg/m³)</td>
<td>10.90±0.74</td>
<td>10.67±1.84</td>
<td>0.46</td>
<td>8.56±0.64</td>
<td>7.48±1.29</td>
<td>&lt;0.0001</td>
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<tr>
<td>ASMI = ASMM/height² (kg/m³)</td>
<td>8.19±0.65</td>
<td>7.55±1.47</td>
<td>0.10</td>
<td>6.55±0.45</td>
<td>6.25±0.99</td>
<td>0.05</td>
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<tr>
<td>ASMI = ASMM/BMI</td>
<td>1.135±0.129</td>
<td>0.828±0.088</td>
<td>&lt;0.0001</td>
<td>0.837±0.110</td>
<td>0.572±0.083</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rectusfemoristhickness (mm)</td>
<td>25.5±2.8</td>
<td>13.6±5.3</td>
<td>&lt;0.0001</td>
<td>20.1±2.1</td>
<td>13.7±2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vastuslateralisthickness (mm)</td>
<td>23.5±3.1</td>
<td>12.5±5.0</td>
<td>&lt;0.0001</td>
<td>19.8±2.3</td>
<td>12.9±5.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tibialisanteriorthickness (mm)</td>
<td>29.5±3.2</td>
<td>27.0±5.5</td>
<td>0.22</td>
<td>25.2±1.5</td>
<td>24.1±2.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Medial gastrocnemius thickness (mm)</td>
<td>19.7±3.1</td>
<td>14.2±3.0</td>
<td>&lt;0.0001</td>
<td>19.1±2.9</td>
<td>12.3±2.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*ASMI: appendicular skeletal muscle index; ASMM: appendicular skeletal muscle mass; BMI: body mass index; TSMM: total body skeletal muscle mass; SMI: skeletal muscle index.*  
*Reported values are means ± SDs.*
Table 3. Muscle thickness (values in mm) comparisons between young and older subjects reported in previous studies

<table>
<thead>
<tr>
<th>Investigated muscle (gender)</th>
<th>Young</th>
<th>Older</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectusfemoris (men &amp; women)</td>
<td>18.1±4.0</td>
<td>13.5±1.9</td>
<td>[30]</td>
</tr>
<tr>
<td>Rectusfemoris (women)</td>
<td>22.9±3.4</td>
<td>16.7±3.7</td>
<td>[31]</td>
</tr>
<tr>
<td>Vastuslateralis (men &amp; women)</td>
<td>22.6±3.8</td>
<td>19.8±2.4</td>
<td>[30]</td>
</tr>
<tr>
<td>Vastuslateralis (men)</td>
<td>21.2±3.7</td>
<td>10.3±3.1</td>
<td>[32]</td>
</tr>
<tr>
<td>Vastuslateralis (men)</td>
<td>25.1±3.1</td>
<td>18.3±3.8</td>
<td>[33]</td>
</tr>
<tr>
<td>Vastuslateralis (women)</td>
<td>21.1±3.8</td>
<td>17.1±3.6</td>
<td>[33]</td>
</tr>
<tr>
<td>Vastuslateralis (women)</td>
<td>22.0±3.2</td>
<td>13.9±4.0</td>
<td>[31]</td>
</tr>
<tr>
<td>Medial gastrocnemius (men)</td>
<td>-</td>
<td>14.7±2.1</td>
<td>[34]</td>
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<tr>
<td>Medial gastrocnemius (men)</td>
<td>22.8±2.6</td>
<td>19.3±2.7</td>
<td>[33]</td>
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<tr>
<td>Medial gastrocnemius (women)</td>
<td>20.2±2.6</td>
<td>17.7±2.3</td>
<td>[33]</td>
</tr>
<tr>
<td>Medial gastrocnemius (women)</td>
<td>16.3±2.3</td>
<td>11.1±2.7</td>
<td>[31]</td>
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</tbody>
</table>

*Reported values are means ± SDs.*
Figure 4

A

Prevalence of low muscle mass (%)

<table>
<thead>
<tr>
<th>Region</th>
<th>T-score</th>
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<tbody>
<tr>
<td>Lower limb</td>
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<tr>
<td>Thigh</td>
<td>T-score</td>
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<tr>
<td>RF</td>
<td>T-score</td>
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<tr>
<td>VL</td>
<td>T-score</td>
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<tr>
<td>Leg</td>
<td>T-score</td>
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<tr>
<td>TA</td>
<td>T-score</td>
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<tr>
<td>MG</td>
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B

Prevalence of low muscle mass (%)

<table>
<thead>
<tr>
<th>Cut-off value</th>
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<tr>
<td>I</td>
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