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 massand to assess itsprevalence 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, vastuslateralis, 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 followingsite-specific cut-points for muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastuslateralis: 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: 300words

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 musclemass 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 sarcopenicindices 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 massmay 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 massin 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)toassess the prevalence of low muscle massin a population of frail older subjects when applying theultrasonographiccut-points of differentlower limb muscles; iii) toassess 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 ± 7yrs; body mass index: 25 ± 5kg/m^2) and sixty young subjects (30 women and 30 men, age: 26 ± 3yrs; body mass index: 22 ± 3kg/m^2) 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 consentprior 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 takenin 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

Subjectswere 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.

111

112 ***Muscle strength***

113 Handgrip strength was measured on the dominant side using a handheld device(Jamar Plus Digital
114 Dynamometer, Patterson Medical, Warrenville, IL, USA). The subjects were sitting comfortably
115 with the shoulder adducted, the elbow flexed at 90° and boththe forearm and the wrist in a
116 neutral position. They were instructed to perform a maximalvoluntary isometric contraction by
117 contracting their muscles as forcefully as possible for 4-5 s. The test was repeated three times with
118 30 s of recovery in between: if the peak forces of the three trials were within 5% of each other, the
119 highest value was retained. Otherwise, additional trials were performed until the 5% criterion was
120 achieved. Cut-off points of <30 kg for men and <20 kg for women [3] were adopted to identify
121 subjects with low handgrip strength.

122

123 ***Total bodyand appendicularskeletal muscle mass***

124 BIA was performed in the morning after an overnight fast, with the subjects lying in the supine
125 position with both upperand lower limbsslightly abducted from the body. Source and sensor
126 electrodes were placed on the dorsum of both hand and foot of the right side of the body. Whole-
127 body reactance and resistance to an applied current (frequency: 50 kHz: amplitude: 0.4 mA) were
128 measured with a tetrapolar device (BIA 101 ASE, Akern, Florence, Italy) and used to estimate
129 TSMM according to Janssen's equation [24] andASMM according to Sergi's equation [25].The
130 validity of the BIA device used in this studyhas previously been demonstrated by Janssen et al. [24]
131 and Sergi et al. [25]. The same Authors also demonstrated the validity of the predictive equations
132 for TSMM [24] and ASMM[25].
133 TSMM was normalized to the body weight (and expressed in %) [6]or to the height (and expressed
134 in kg/m^2)[7] to calculate the skeletal muscle index (SMI). ASMM was normalized to the height (and

135 expressed in kg/m^2) [26] or to the body mass index [5,27] to calculate the appendicular skeletal
136 muscle index (ASMI).Ten cut-off values for ASMM, SMI and ASMI were adopted for the detection
137 oflow muscle mass(Table 1): five out of ten values(cut-off values # I – III – V – VII – IX in Table 1)
138 were derived from previous studies[5-7,26,27], while the other fivevalues(cut-off values # II – IV –
139 VI – VIII – X in Table 1) were established based on normative data of muscle mass obtained in our
140 sample of young subjects (values 2 SDs below the sex-specific means of our sample of young
141 subjects were considered).
142
143 Insert Table 1
144
145 ***Muscle thickness***
146 Ultrasound B-mode images of the following lower limb muscles of the dominant side were
147 acquired during a single experimental session: rectus femoris, vastuslateralis, tibialis anterior, and
148 medial gastrocnemius. These muscles were specifically selected as sarcopenia preferentially
149 affects lower limb muscles [1,2,16-18].
150 The same experienced sonographer (MAM) performed all the assessments and acquired all the
151 images. Three consecutive static scans were acquired in the longitudinal plane of each muscle.
152 After each scan, the subject was allowed to move and the transducer was repositioned. To
153 increase the repeatability of the acquisitions and to ensure the optimal representation of the
154 muscle, we adopted the following criteria: *i)*tibialis anterior: we maximized the representation of
155 the bone boundary and of the muscle fascicles; *ii)* rectus femoris: we optimized the representation
156 of the superficial and deep aponeuroses; *iii)*vastuslateralis and medial gastrocnemius: we
157 optimized the representation of the superficial and deep aponeuroses and of the muscle fascicles.

158 Images of the medial gastrocnemius were acquired with the subjects in the prone position,
159 whereas for all the other muscles subjects were positioned supine. In all measurements, the lower
160 limb joints were extended and the subjects were asked to completely relax their muscles. A
161 suitable amount of ultrasound coupling gel was used to ensure optimal image quality and to
162 minimize the transducer pressure on the skin. All scans were performed by placing the transducer
163 in correspondence of the largest muscle diameter at the following anatomical sites, according to
164 previous studies [28,29]: the rectus femoris was measured half-way along the line from the
165 anterior-superior iliac spine to the superior border of the patella; the vastuslateralishalf-way along
166 the line from the anterior-superior iliac spine to the superolateral border of the patella; the tibialis
167 anterior at one-quarter of the distance from the inferior border of the patella to the lateral
168 malleolus; the medial gastrocnemius from the mid-sagittal line of the muscle, midway between
169 the proximal and distal tendon insertions.
170 All images were acquired using a ClarUs ultrasound device (Telemed, Vilnius, Lithuania) equipped
171 with a linear-array transducer (code L12-5L40N) with a variable-frequency band (5-12 MHz).Gain
172 was set at 50% of the range, dynamic image compression was turned off, and time gain
173 compensation was maintained in the same (neutral) position for all depths. All system-setting
174 parameters were kept constant throughout the study and for each subject, except depth (initially
175 set at 30mm) that was modified during the examination (range: 30-60 mm) to visualize the entire
176 muscle thickness. Pictures were stored as DICOM files and transferred to a computer for
177 processing.
178 Muscle thickness was measured as the distance between the superficial and deep aponeurosesby
179 using ImageJ (National Institutes of Health, Bethesda, MD, USA). All three images acquired for
180 each muscle were analyzed.As shown in the representative example of Figure 1, the operator
181 measured the muscle thickness in three points, equally spaced along the image. The operator

182 placed the measurement points on each aponeuroses trying to trace a segment which was
183 orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each
184 point pairs was considered as the muscle thickness.
185 Cut-off values (and 2SD range values) for the thickness of the four muscles (identified as values 2
186 SDs below the sex-specific means of our sample of young subjects) are reported in Table 1.
187
188 Insert Figure 1
189
190 ***Statistical analysis***
191 Since the Shapiro–Wilk test for normal distribution of the data failed,the Fisher's exact test was
192 used for comparisons between proportions and the Mann-Whitney U test was used for
193 comparisons between the two groups of subjects (young vs older).
194 Intrasession and intrarater reliability of the thickness measurement was determined by the
195 intraclasscorrelaton coefficient (ICC3,1) and coefficient of variation using the three scans acquired
196 for each muscle. We obtained the following ICC and CV values: 0.98 and 3.2% for rectus femoris,
197 0.99 and 3.3% for vastuslateralis, 0.98 and 1.5% for tibialis anterior, 0.97 and 3.7% for medial
198 gastrocnemius.
199 Muscle thickness T-score values were calculated for older subjects using the following
200 formula:[(individual value - mean value of the young subjects of the corresponding gender
201 group)/SD of the young subjects of the corresponding gender group]. In each of the older subjects,
202 the T-scores calculated for the four muscles were then averaged to obtain: i)a lower limb T-score
203 (i.e., the mean T-score of the four muscles), ii)a thigh T-score (i.e., the mean T-score of rectus
204 femoris and vastuslateralis muscles), iii)a leg T-score (i.e., the mean T-score of tibialis anterior and
205 medial gastrocnemius muscles). Accordingly, the following definitions of low muscle masswere

considered: low mass of the lower limb muscles (i.e., lower limb T-score < -2), low mass of thethigh muscles (i.e., thigh T-score < -2), low mass ofthe 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 obtainedby 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 speedand low handgrip strength.

Data were expressed as mean $\pm$ 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.

Figures2-3 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 valuesin 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.

Insert Table 2 and Figures2-3

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

As shown in Figure 4A, the prevalence of low muscle massobtained 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 massobtained by using the rectus femoris T-score (86%) was comparable (P=.18)to that obtained by using the vastuslateralis T-score (73%). A significant (P=.0006) difference was observed between the prevalence of low muscle massobtained by using the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%).

Briefly, the prevalence of low muscle massis highly dependent on the muscle being investigated: proximal muscles of the lower limb seem more valid for the detection of low muscle massthan distal muscles.

Therefore, we compared the thigh T-score with the other criteria used todetectlow muscle mass.As shown in Figure 4B, the prevalence of low muscle massranged from 2% to 75% for

254 different BIA-derived criteria; it was 52% for the calf-circumference criterionand 86% for the thigh
255 T-score criterion.
256 Briefly, the prevalence of low muscle massis highly dependent on the applied diagnostic criterion
257 and on the adopted cut-off value.
258
259 Insert Figure 4
260
261 ***Diagnosis of sarcopenia***
262 Of the 44 older subjects, 38 (86%) presented low muscle mass (i.e., low mass of the thigh
263 muscles), 23 (52%) presented low calf circumference (according to cut-off values # XI in Table 1)
264 and 33 (75%) presented low ASMI (according to cut-off values # X in Table 1).
265 Moreover, 38 older subjects (86%) presented low muscle strength (average handgrip strength of
266 the whole group: 16.9 ± 7.3 kg; average handgrip strength of the subjects presenting low muscle
267 strength: 15.1 ± 5.7 kg) and 32 (73%) presented low physical performance (average walking speed
268 of the whole group: 0.62 ± 0.24 m/s; average walking speed of the subjects presenting low walking
269 speed: 0.50 ± 0.15 m/s).
270 The combination of thigh muscle thickness, strength and performance measurementsenabled to
271 classify 6 out of 44 older subjects (14%) as non-sarcopenic, 2 (5%) as pre-sarcopenic, 9 (20%) as
272 sarcopenic (7 out of 9 subjects presented low mass of the thigh muscles and low handgrip
273 strength, while 2 out of 9 subjects presented low muscle mass and low walking speed), and 27
274 (61%) as severely sarcopenic.
275 Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia,
276 identified on the basis of low calf circumference (according to cut-off values # XI in Table 1) and
277 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 withultrasonographyand 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 detectionof 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 valueswemeasured 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 subjectsshowed reduction in medial gastrocnemius andtibialis 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 lossis 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 lossthan distal muscles and that the medial gastrocnemius is more affected by thickness lossthan 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 compositionbetween 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 thatthe 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 subjectsthe 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 masswas 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 massthat 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 massestablished in a specific ethnic group cannot be applied to other groups.Consistently, we found that the prevalence of low muscle massdifferedwhen 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 massis limited by the skillfulnessof 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 themeasurement packages offered on commercially available scanners, it is likely they will be embedded in high-end scanners ina 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 lowmuscle massalso in non-Caucasian older subjects.

CONCLUSIONS

This study reports site-specific cut-points for ultrasound-based detectionof low muscle mass. To simplify these cut-points for potential future applications, the following thresholdsof muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastuslateralis: 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 masswas highly dependent on the muscle being investigated (proximal muscles of the lower limb weremore affected than distal muscles and the medial gastrocnemius wasmore 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 massin comparison to the ultrasound-basedassessment 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 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 massobtained 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 massobtained 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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Ultrasound-based detection of low muscle mass for diagnosis of sarcopenia in older adults

WORD COUNT: ~~3978~~4041words

ABSTRACT

Objective: To establish muscle-specific cut-off values for ultrasound-based detection of low muscle massand to assess ~~the~~ itsprevalence ~~of low muscle massobtained~~ 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, vastuslateralis, 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 followingsite-specific cut-points for muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastuslateralis: 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 underestimatedthe prevalence of low muscle massin comparison to the ultrasound-based assessment of muscle thickness. It is therefore~~

~~recommended to adopt the ultrasonographic quantification of muscle thickness and theherein 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: ~~297~~300words

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 musclemass 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 sarcopenicindices 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 massmay 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 massin 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)toassess the prevalence of low muscle massin a population of frail older subjects when applying theultrasonographiccut-points of differentlower limb muscles; iii) toassess 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 ± 7yrs; body mass index: 25 ± 5kg/m^2) and sixty young subjects (30 women and 30 men, age: 26 ± 3yrs; body mass index: 22 ± 3kg/m^2) 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 consentprior 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 takenin 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

Subjectswere 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 boththe forearm and the wrist in a neutral position. They were instructed to perform a maximalvoluntary 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 bodyand appendicularskeletal muscle mass

BIA was performed in the morning after an overnight fast, with the subjects lying in the supine position with both upperand lower limbsslightly 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] andASMM according to Sergi's equation [25].The validity of the BIA device used in this studyhas 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/m^2)[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 oflow 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 fivevalues(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, vastuslateralis, 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)*vastuslateralis 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 aponeurosesby 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.

Insert Figure 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:[(individual value - mean value of the young subjects of the corresponding gender group)/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

considered: low mass of the lower limb muscles (i.e., lower limb T-score < -2), low mass of thethigh muscles (i.e., thigh T-score < -2), low mass ofthe 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 obtainedby 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 speedand 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.

Figures2-3 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 valuesin 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.

Insert Table 2 and Figures2-3

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

As shown in Figure 4A, the prevalence of low muscle massobtained 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 massobtained by using the rectus femoris T-score (86%) was comparable (P=0.18)to that obtained by using the vastuslateralis T-score (73%). A significant (P=0.0006) difference was observed between the prevalence of low muscle massobtained by using the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%).

Briefly, the prevalence of low muscle massis highly dependent on the muscle being investigated: proximal muscles of the lower limb seem more valid for the detection of low muscle massthan distal muscles.

Therefore, we compared the thigh T-score with the other criteria used todetectlow muscle mass.As shown in Figure 4B, the prevalence of low muscle massranged from 2% to 75% for

different BIA-derived criteria; it was 52% for the calf-circumference criterionand 86% for the thigh T-score criterion.

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

Insert Figure 4

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 measurementsenabled 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 presentedlow 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 withultrasonographyand 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 detectionof 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 valueswemeasured 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 subjectsshowed reduction in medial gastrocnemius andtibialis 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 lossis 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 lossthan distal muscles and that the medial gastrocnemius is more affected by thickness lossthan 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 compositionbetween 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 thatthe 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 subjectsthe 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 masswas 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 massthat 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 massestablished in a specific ethnic group cannot be applied to other groups.Consistently, we found that the prevalence of low muscle massdifferedwhen 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 massis limited by the skillfulnessof 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 themeasurement packages offered on commercially available scanners, it is likely they will be embedded in high-end scanners ina 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 lowmuscle massalso in non-Caucasian older subjects.

CONCLUSIONS

This study reports site-specific cut-points for ultrasound-based detectionof low muscle mass. To simplify these cut-points for potential future applications, the following thresholdsof muscle thickness were identified: rectus femoris: 20 mm in men and 16 mm in women; vastuslateralis: 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 masswas highly dependent on the muscle being investigated (proximal muscles of the lower limb weremore affected than distal muscles and the medial gastrocnemius wasmore 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 massin comparison to the ultrasound-basedassessment 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 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 massobtained 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 massobtained 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).

Table 1 Cut-off values used to detect low muscle mass

		Variable		Men	Women	Reference
BIA	**I**	SMI = TSMM/weight (%)		31%	22%	[6]
	II	SMI = TSMM/weight (%)	2 SDs below the sex-specific means of young subjects	38%	29%	
	III	SMI = TSMM/height^2 (kg/m^2)		8.50 kg/m^2	5.75 kg/m^2	[7]
	IV	SMI = TSMM/height^2 (kg/m^2)	2 SDs below the sex-specific means of young subjects	9.42 kg/m^2	7.27 kg/m^2	
	V	ASMI = ASMM/height^2 (kg/m^2)		7.26 kg/m^2	5.45 kg/m^2	[26]
	VI	ASMI = ASMM/height^2 (kg/m^2)	2 SDs below the sex-specific means of young subjects	6.88 kg/m^2	5.65 kg/m^2	
	VII	ASMM (kg)		19.75 kg	15.02 kg	[27]
	VIII	ASMM (kg)	2 SDs below the sex-specific means of young subjects	19.91 kg	14.37 kg	
	IX	ASMI = ASMM/BMI		0.789	0.512	[5,27]
	X	ASMI = ASMM/BMI	2 SDs below the sex-specific means of young subjects	0.878	0.622	
AM	**XI**	Calf circumference (cm)		<31 cm	<31 cm	[23]
US	**XII**	Musclethickness	2 SDs below the sex-specific means of young subjects			
		Rectusfemoris (mm)		19.9 mm	15.9 mm	
		2 SD range (mm)		19.9-31.0	15.9-24.4	
		Vastuslateralis (mm)		17.3 mm	15.2 mm	
		2 SD range (mm)		17.3-29.9	15.2-24.3	
		Tibialisanterior (mm)		23.1 mm	22.2 mm	
		2 SD range (mm)		23.1-35.9	22.2-28.4	
		Medialgastrocnemius (mm)		13.5 mm	13.3 mm	
		2 SD range (mm)		13.5-25.8	13.3-25.2	

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

Variable	MEN			WOMEN		
	Young (n=30)	Older (n=14)	P value	Young (n=30)	Older (n=30)	P value
Age (years)	26.9±3.7	79.2±8.3	<0.0001	24.8±2.8	83.7±6.2	<0.0001
BMI (kg/m^2)	23.0±2.9	24.9±5.3	0.31	21.4±2.7	25.5±4.6	<0.001
TSMM (kg)	34.5±3.6	29.1±6.4	<0.01	23.3±2.4	17.3±3.4	<0.0001
ASMM (kg)	25.9±3.0	20.6±5.2	<0.0001	17.9±1.7	14.4±2.5	<0.0001
SMI = TSMM/weight (%)	47.9±4.8	43.4±4.7	<0.01	40.3±5.5	29.7±4.8	<0.0001
SMI = TSMM/height^2 (kg/m^2)	10.90±0.74	10.67±1.84	0.46	8.56±0.64	7.48±1.29	<0.0001
ASMI = ASMM/height^2 (kg/m^2)	8.19±0.65	7.55±1.47	0.10	6.55±0.45	6.25±0.99	0.05
ASMI = ASMM/BMI	1.135±0.129	0.828±0.088	<0.0001	0.837±0.110	0.572±0.083	<0.0001
Rectusfemoristhickness (mm)	25.5±2.8	13.6±5.3	<0.0001	20.1±2.1	13.7±2.6	<0.0001
Vastuslateralisthickness (mm)	23.5±3.1	12.5±5.0	<0.0001	19.8±2.3	12.9±5.0	<0.0001
Tibialisanteriorthickness (mm)	29.5±3.2	27.0±5.5	0.22	25.2±1.5	24.1±2.8	0.03
Medial gastrocnemius thickness (mm)	19.7±3.1	14.2±3.0	<0.0001	19.1±2.9	12.3±2.8	<0.0001

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

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

Reported values are means ± SDs.

Figure 1

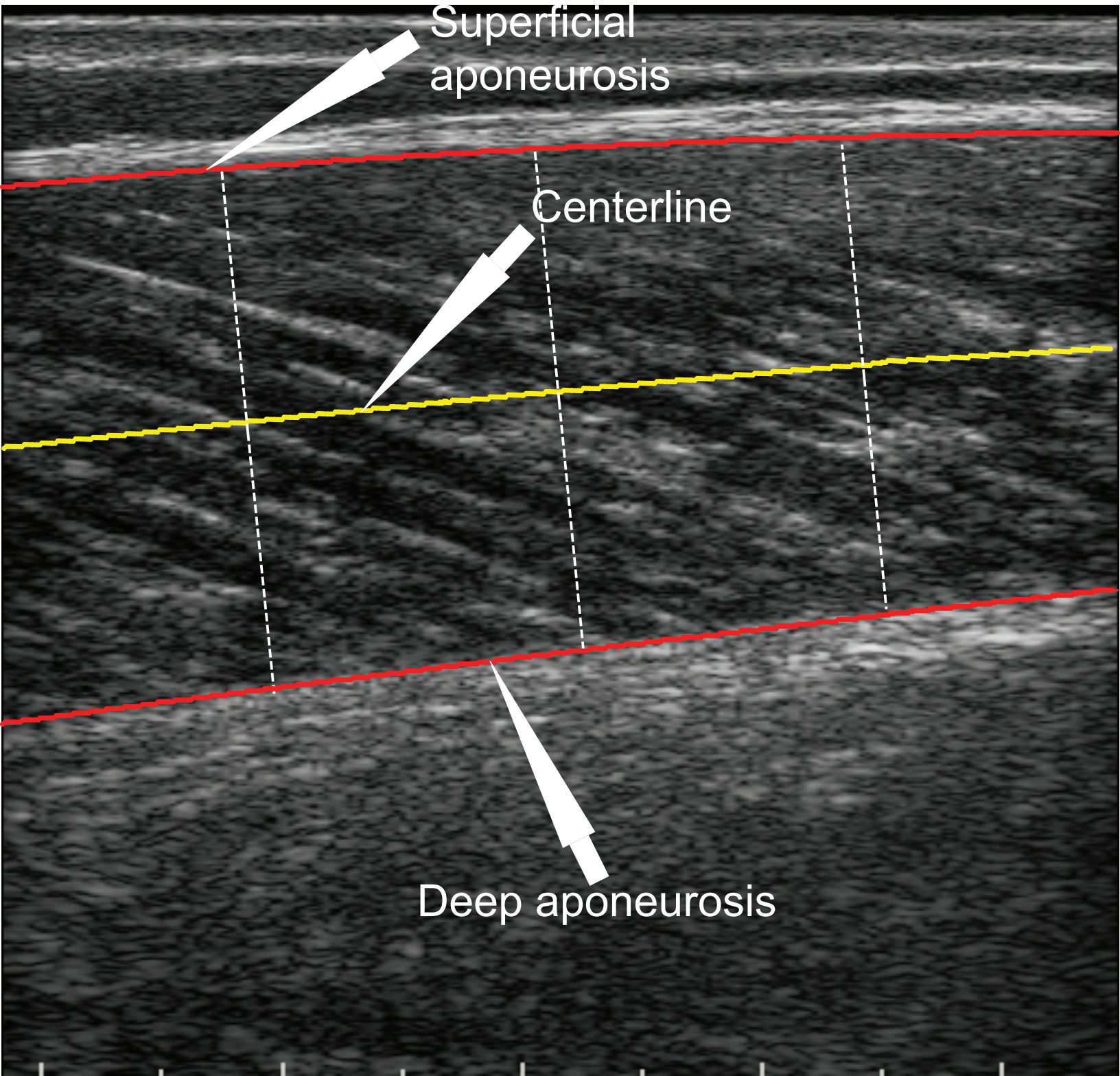


Figure 2

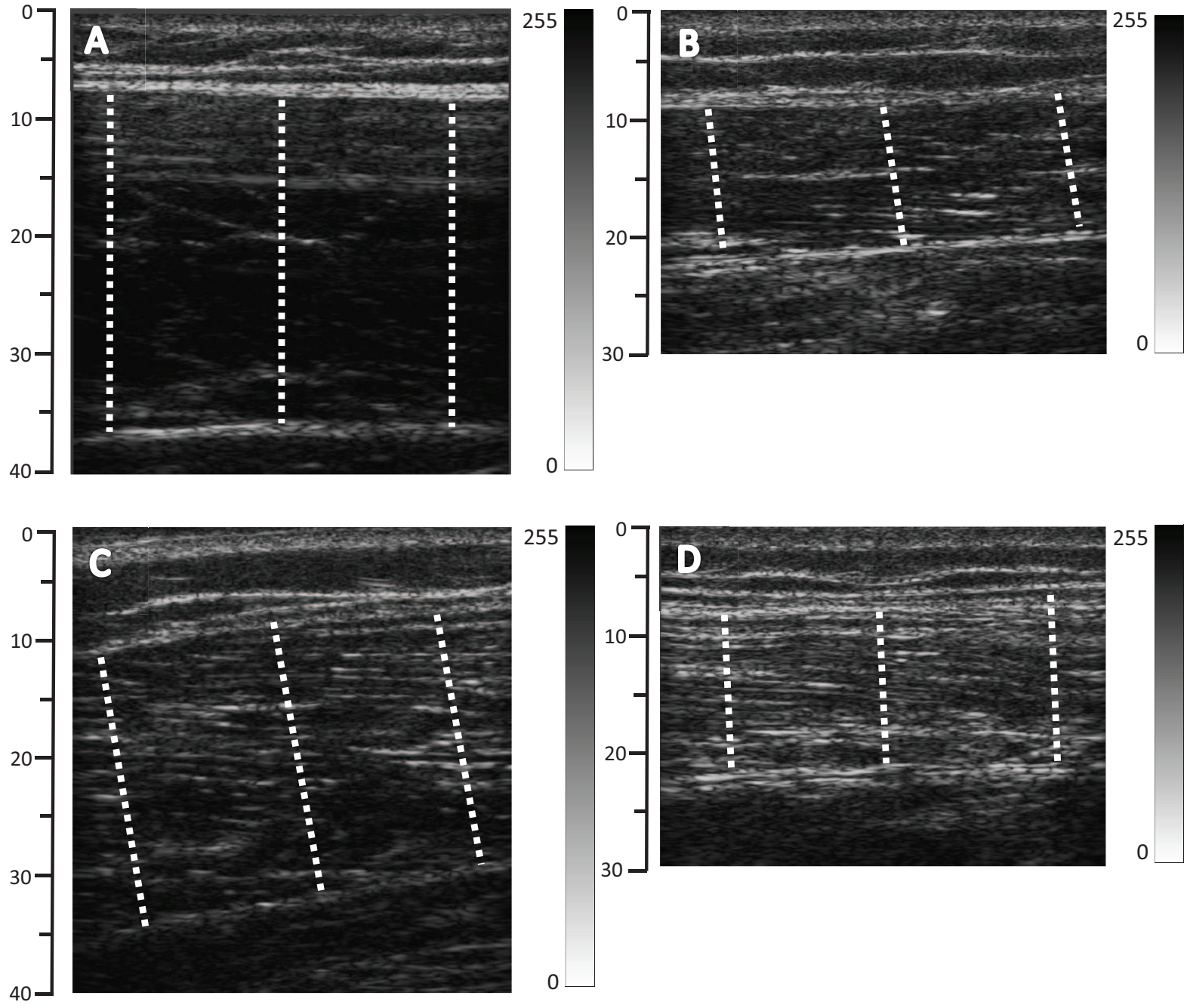


Figure 3

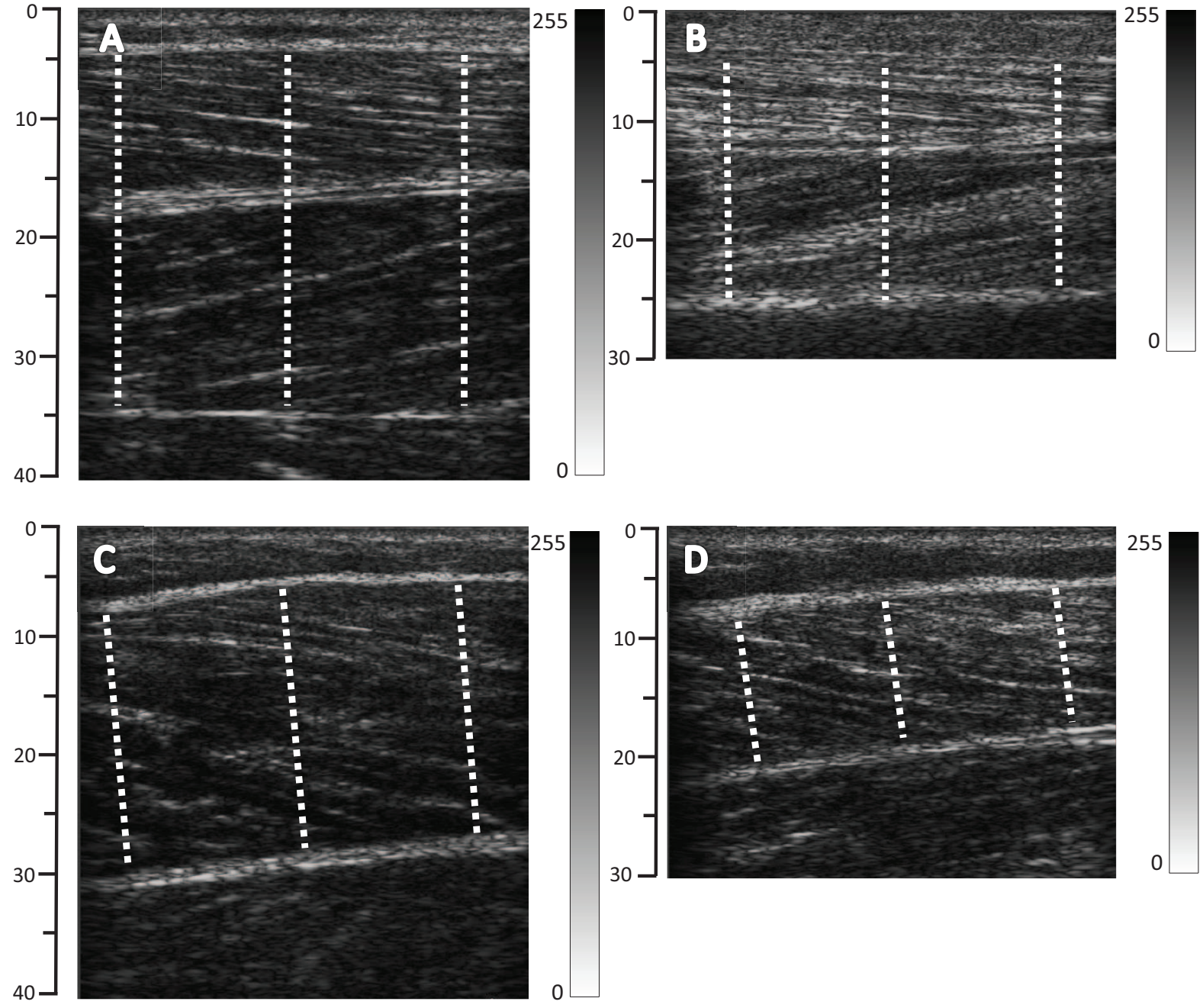
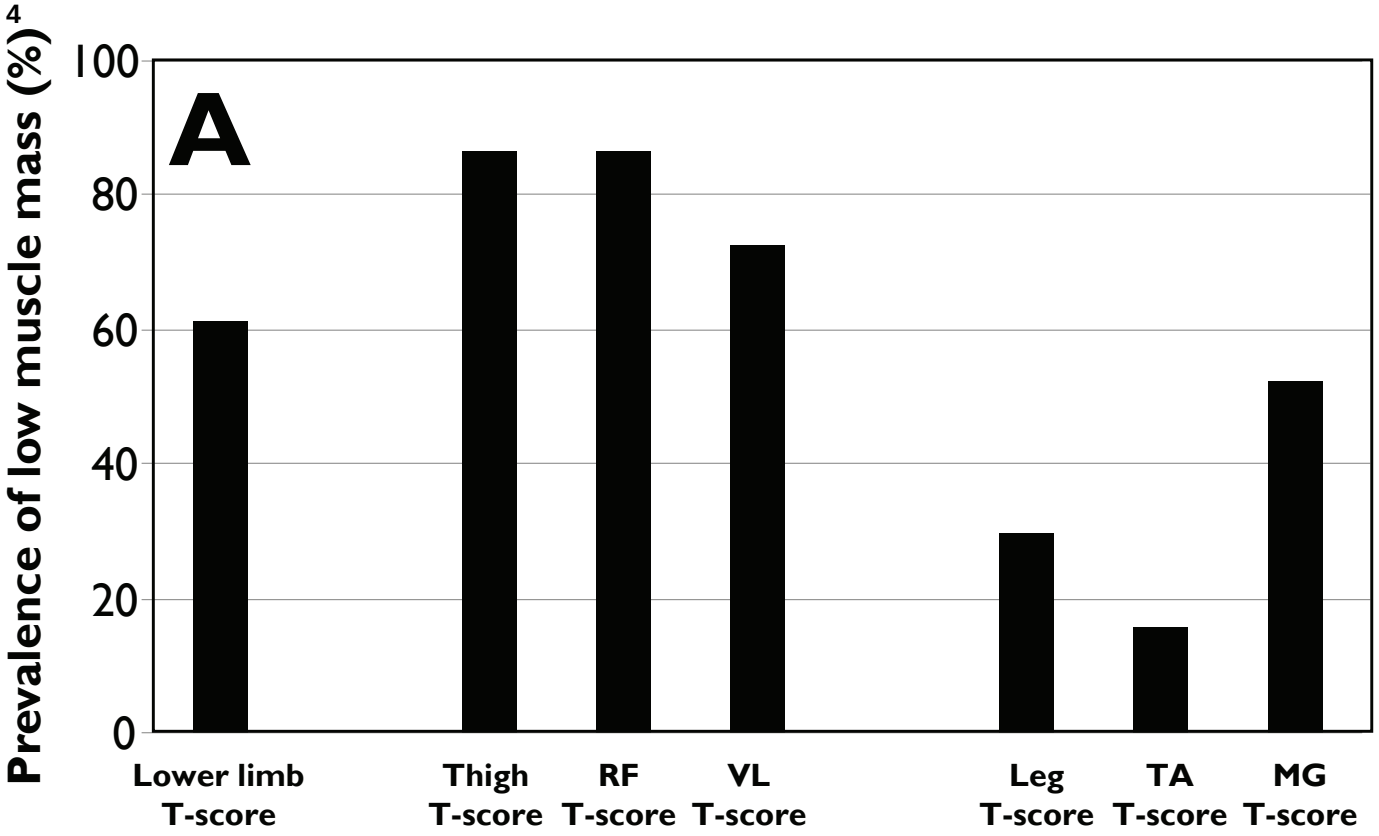
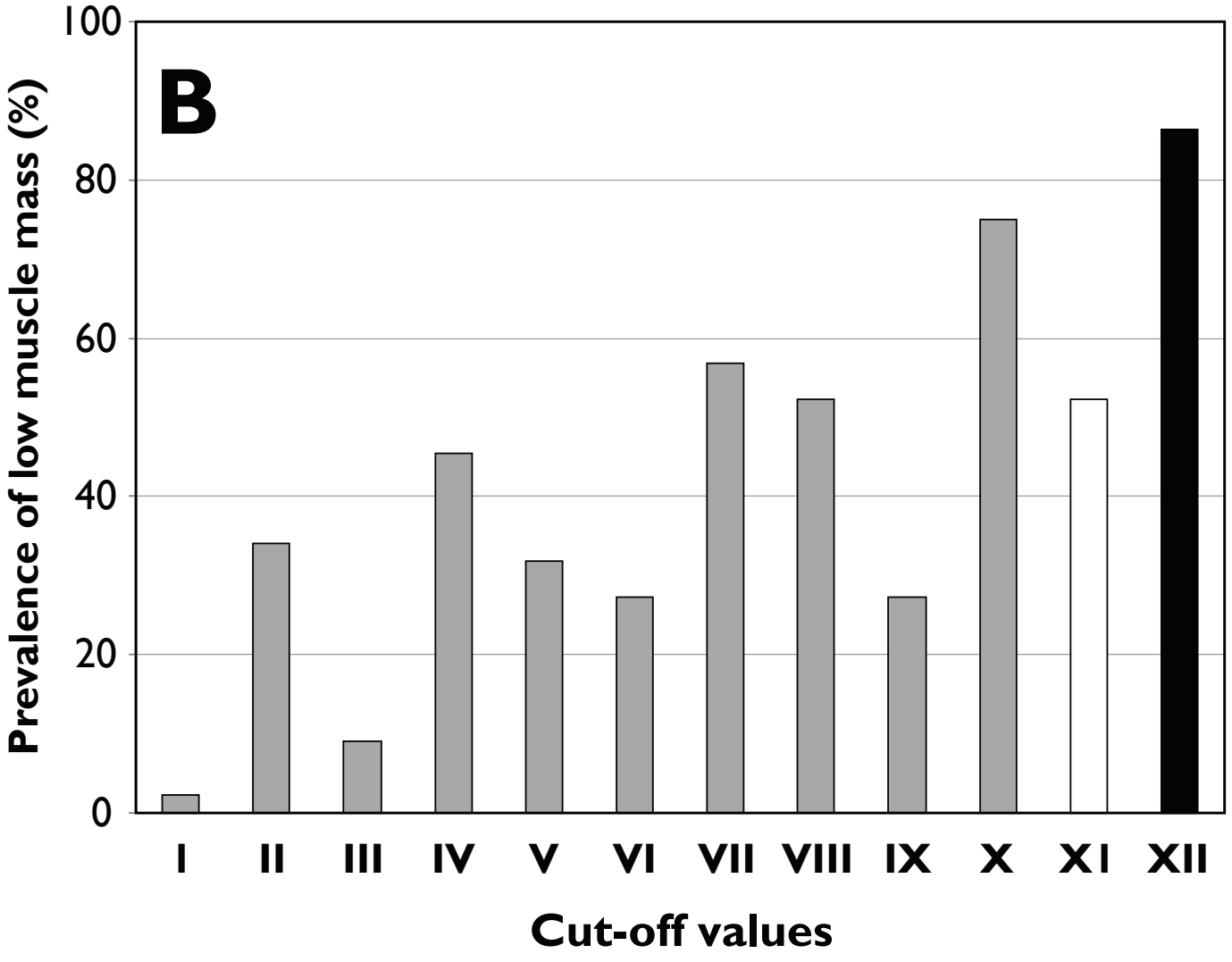


Figure 4


A
Prevalence of low muscle mass (%)
100
80
60
40
20
0
Lower limb T-score
Thigh T-score
RF T-score
VL T-score
Leg T-score
TA T-score
MG T-score


B
Prevalence of low muscle mass (%)
100
80
60
40
20
0
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
Cut-off values

STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1-3
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	5
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
Test methods	7	The reference standard and its rationale.	6-10
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	6-10
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	6-10
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	8-9
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	8
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	10-11
	13	Methods for calculating test reproducibility, if done.	10
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	5
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	6
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	5
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	11-13
	20	Any adverse events from performing the index tests or the reference standard.	-
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	13
	22	How indeterminate results, missing data and outliers of the index tests were handled.	-
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	-
	24	Estimates of test reproducibility, if done.	-
DISCUSSION	25	Discuss the clinical applicability of the study findings.	17