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## 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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4 **Ultrasound-based detection of low muscle mass for diagnosis of**  
5 **sarcopenia in older adults**

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10 **WORD COUNT:** 4041words

11 **ABSTRACT**

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

15 **Design:**Cross-sectional study.

16 **Setting:**Geriatric outpatient clinic and clinical research laboratory.

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

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

24 **Results:**The following site-specific cut-points for muscle thickness were identified: rectus femoris:  
25 20 mm in men and 16 mm in women; vastuslateralis: 17 mm in men and 15 mm in women; tibialis  
26 anterior: 23 mm in men and 22 mm in women; medial gastrocnemius: 13 mm in both men and  
27 women. The prevalence of low muscle mass in older adults was highly dependent on the muscle  
28 being investigated: it varied from 86% for thigh muscles to 30% for leg muscles. Moreover, the  
29 prevalence of low muscle mass was highly dependent on the applied diagnostic criterion and on  
30 the adopted cut-off value (it ranged from 2% to 75% for different BIA-derived criteria).

31 **Conclusions:**We suggest that muscle ultrasonography provides rehabilitation physicians with a  
32 practical and accurate tool for identifying individuals with low muscle mass. However, the usability  
33 of cut-off values established in our group of Caucasian healthy young subjects to identify low

34 muscle mass in older persons of different ethnic groups remains to be demonstrated in future  
35 studies.

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38 **ABSTRACT WORD COUNTS:** 300words

39 **INTRODUCTION**

40 Primary sarcopenia, the age-related loss of skeletal muscle mass and function[1,2], is associated  
41 with disability and frailty that represent major socioeconomic as well as medical problems. In  
42 rehabilitation patients, primary sarcopenia can be further exacerbated by the disuse- or drug-  
43 related loss of muscle mass or function. Therefore, elderly rehabilitation patients could benefit  
44 from the assessments of skeletal muscle mass and function for the detection of sarcopenia.  
45 A major development in sarcopenia research has been the convergence in its operational  
46 definition. Several consensus groups have recently published operational criteria for the diagnosis  
47 of sarcopenia (incorporating the evaluation of muscle mass with the assessment of strength and/or  
48 physical performance), including the “European Working Group on Sarcopenia in Older People”  
49 (EGWSOP) [3], the “International Working Group on Sarcopenia” (IWGS) [4] and the “Foundation  
50 for the National Institutes of Health Sarcopenia Project” [5]. All three consensus groups included  
51 the appendicular skeletal muscle mass (ASMM) assessment, as realized with dual-energy X-ray  
52 absorptiometry (DXA), into the operational definition of sarcopenia. However, different indices of  
53 ASMM (such as ASMM normalized to height or to body mass index) and different cut-off points  
54 were considered. Other sarcopenic indices, which are commonly used in research as well as in  
55 clinical routine, are based on the assessment of the total body skeletal muscle mass (TSMM,  
56 normalized to body weight or to height), as realized with bioelectrical impedance analysis (BIA)  
57 [6,7]. However, the use of different diagnostic criteria may lead to different conclusions, as  
58 evidenced by several investigations recently performed in community-dwelling older adults [8-15].  
59 In addition, although the use of DXA- or BIA-derived sarcopenic indices may be practical for clinical  
60 purposes, they do not seem very accurate [1]. This is essentially due to the fact that sarcopenia is  
61 not a uniform condition as it affects postural muscles more than non-postural ones [1,2,16-18].  
62 Therefore, site-specific assessment of loss of muscle mass may be required for its early and

63 accurate detection. Consistently, recent studies showed that thigh sarcopenia can be detected by  
64 ultrasound-based assessment of muscle thickness before it appears at the whole body level  
65 [19,20]. However, as highlighted by Abe et al. [19], there are no published site-specific cut-points  
66 for ultrasonographic assessment of low muscle mass in older adults. Therefore, the aims of this  
67 study were: i) to establish muscle-specific cut-off values for ultrasound-based detection of low  
68 muscle mass; ii) to assess the prevalence of low muscle mass in a population of frail older subjects  
69 when applying the ultrasonographic cut-points of different lower limb muscles; iii) to assess the  
70 prevalence of low muscle mass when applying different sarcopenic indices derived from  
71 ultrasound, BIA, and anthropometry.

72

## 73 **METHODS**

### 74 **Subjects**

75 Forty-four older adults (30 women and 14 men, mean age  $\pm$  SD:  $82 \pm 7$  yrs; body mass index:  $25 \pm$   
76  $5 \text{ kg/m}^2$ ) and sixty young subjects (30 women and 30 men, age:  $26 \pm 3$  yrs; body mass index:  $22 \pm$   
77  $3 \text{ kg/m}^2$ ) volunteered to participate in the study (convenience sample). The young subjects were  
78 habitually physically active, and none participated in competitive sports. The older group was  
79 composed by institution-dwelling subjects with one or more of Fried's frailty criteria [21]. Side  
80 dominance was assessed with the "Waterloo Handedness and Footedness Questionnaires -  
81 Revised" [22]. One older and six young subjects were left-side dominant. Each participant received  
82 a detailed explanation of the study and gave written informed consent prior to participation. The  
83 study conformed to the ethical principles enunciated in the Declaration of Helsinki and was  
84 approved by the local Ethics Committee.

85

### 86 **Assessments**

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

93

#### 94 ***Anthropometric measurements***

95 Measurements of height and weight were made in overnight fasted subjects (in light clothing and  
96 barefoot or with socks) on the same day as all the other tests. Standing height was measured to  
97 the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was determined to the nearest  
98 0.1 kg using a calibrated balance beam scale. Calf circumference (dominant side) was measured to  
99 the nearest 0.1 cm while the subjects were seated with their leg hanging loosely. The  
100 measurement tape was wrapped around the calf and the highest value was retained. A cut-off  
101 point of <31 cm [23] was adopted to identify low muscle mass.

102

#### 103 ***Physical performance***

104 Subjects were asked to walk over a 14-m walkway at a self-selected usual speed and their walking  
105 speed was evaluated. A stopwatch was used to time the subjects as they walked over the central  
106 10 m of the walkway. The initial 2 m and final 2 m were not considered to allow for acceleration  
107 and anticipatory deceleration. The distance covered was divided by the time taken to complete  
108 the 10-m walk. Subjects completed three trials and the mean walking speed of the three trials was  
109 retained. A cut-off point of <0.8 m/s [3] was adopted to identify subjects with low physical  
110 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 both the forearm and the wrist in a  
116 neutral position. They were instructed to perform a maximal voluntary 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 body and appendicular skeletal muscle mass***

124 BIA was performed in the morning after an overnight fast, with the subjects lying in the supine  
125 position with both upper and lower limbs slightly 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] and ASMM according to Sergi's equation [25]. The  
130 validity of the BIA device used in this study has 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<sup>2</sup>) [7] to calculate the skeletal muscle index (SMI). ASMM was normalized to the height (and

135 expressed in  $\text{kg}/\text{m}^2$ ) [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 of low 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 five values (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).

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Insert Table 1

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### ***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.

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.

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188

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Insert Figure 1

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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 intraclass correlation 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:  $[(\text{individual value} - \text{mean value of the young subjects of the corresponding gender}$   
201  $\text{group}) / \text{SD of the young subjects of the corresponding gender group}]$ . 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 mass were

206 considered: low mass of the lower limb muscles (i.e., lower limb T-score < -2), low mass of  
207 the thigh muscles (i.e., thigh T-score < -2), low mass of the leg muscles (i.e., leg T-score < -2),  
208 muscle-specific low mass (i.e., muscle thickness lower than the cut-off values reported in Table 1).  
209 The prevalences of these different ultrasound-based definitions of low muscle mass were then  
210 compared. Moreover, the prevalence of low muscle mass obtained by using a single ultrasound-  
211 derived criterion was compared with the prevalences obtained by using the BIA-derived criteria  
212 and the calf-circumference criterion (based on the cut-off values reported in Table 1 and  
213 numbered from I to XI).

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

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

225

## 226 **RESULTS**

### 227 ***Muscle mass and thickness: comparisons between young and older subjects***

228 Table 2 lists the values of BIA-derived muscle mass for the two groups of subjects stratified by  
229 gender. As expected, TSMM and ASMM were higher in young compared to older subjects, while

230 the SMI (TSMM normalized to height) in men and the ASMI (ASMM normalized to height) in both  
231 men and women were comparable between young and older subjects.  
232 Figures 2-3 show representative examples of ultrasound images acquired from young and older  
233 subjects: muscle thickness was higher in the four muscles of the young subjects compared to older  
234 subjects. Similar to these examples, analysis of the group data (Table 2) showed significantly  
235 higher muscle thickness values in young compared to older subjects for all muscles (with the  
236 exception of the tibialis anterior muscle in men). The thickness values of the four muscles  
237 obtained in young subjects were used to establish the cut-off values reported in Table 1.

238

239 Insert Table 2 and Figures 2-3

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

242 As shown in Figure 4A, the prevalence of low muscle mass obtained by using the thigh T-score  
243 (86%) was significantly ( $P=.01$ ) higher than that obtained by using the lower limb T-score (61%),  
244 and the latter was significantly ( $P=.005$ ) higher than that obtained by using the leg T-score (30%).  
245 Moreover, the prevalence of low muscle mass obtained by using the rectus femoris T-score (86%)  
246 was comparable ( $P=.18$ ) to that obtained by using the vastus lateralis T-score (73%). A significant  
247 ( $P=.0006$ ) difference was observed between the prevalence of low muscle mass obtained by using  
248 the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%).

249 Briefly, the prevalence of low muscle mass is highly dependent on the muscle being investigated:  
250 proximal muscles of the lower limb seem more valid for the detection of low muscle mass than  
251 distal muscles.

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

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

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

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259

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Insert Figure 4

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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:  $16.9 \pm 7.3$  kg; average handgrip strength of the subjects presenting low muscle  
267 strength:  $15.1 \pm 5.7$  kg) and 32 (73%) presented low physical performance (average walking speed  
268 of the whole group:  $0.62 \pm 0.24$  m/s; average walking speed of the subjects presenting low walking  
269 speed:  $0.50 \pm 0.15$  m/s).

270 The combination of thigh muscle thickness, strength and performance measurements enabled 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.

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

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

282

## 283 **DISCUSSION**

284 In the present study, 60 young subjects were evaluated with ultrasonography and BIA to establish  
285 muscle-specific and population-specific cut-off values for sarcopenic indices which were then  
286 applied to a sample of 44 frail older subjects to determine comparative prevalence rates of low  
287 muscle mass. This is the first study to report site-specific cut-points for ultrasound-based  
288 detection of low muscle mass. These cut-points were established based on normative values of  
289 muscle thickness gained from our sample of young subjects that were comparable to those  
290 previously observed in healthy young populations (Table 3: left column). Likewise, the muscle  
291 thickness values we measured in older subjects were similar to those previously reported in  
292 community-dwelling and/or frail elderly individuals (Table 3: right column). Therefore, the high  
293 prevalence of low muscle mass (86%) we observed in older subjects and the inter-muscle  
294 differences (86% of subjects showed low thickness of the thigh muscles, while only 52% and 16%  
295 of subjects showed reduction in medial gastrocnemius and tibialis anterior thickness,  
296 respectively) did not result from the application of biased cut-off values (e.g., too large for thigh  
297 muscles, thus implying false-positive results, and too stringent for leg muscles, thus implying the  
298 overlook of true-positive results). Consistently, such inter-muscle variability in the susceptibility to  
299 age-related muscle loss is in line with previous evidence gained from magnetic resonance imaging-  
300 [18], computed tomography-[16], and DXA-[17] based measurements showing that age-related  
301 muscle loss is greater in lower limb (postural) muscles than in upper limb (non-postural) muscles.



302 To our knowledge, this study is the first to show that proximal muscles of the lower limb are  
303 preferentially affected by thickness loss than distal muscles and that the medial gastrocnemius is  
304 more affected by thickness loss than the tibialis anterior. The latter result is in agreement with  
305 previous studies showing that the age-related decline in plantar-flexor strength is greater  
306 compared to dorsiflexor strength (although the loss of muscle mass alone cannot account for the  
307 reduction in muscle strength) [35]. Given the known differences in muscle composition between  
308 the tibialis anterior and the other three muscles considered here (the former presents a higher  
309 percentage of slow fibers compared to the latter) [36,37], it may be hypothesized that the higher  
310 the percentage of insulin-sensitive slow fibers, the lower the susceptibility to age-related loss of  
311 muscle mass. Therefore, it may be suggested that in the tibialis anterior of our population of frail  
312 older subjects the permissive effect of insulin on protein synthesis [38,39] was greater compared  
313 to other less-insulin sensitive muscles and could explain, at least partly, the lower tibialis anterior  
314 susceptibility to age-related muscle loss. However, not only muscular, but also neural mechanisms,  
315 such as site-specific losses of motor units [40], probably underlie the observed site-specific age-  
316 related loss of muscle mass.

317 In the present study, we found that the prevalence of low muscle mass was highly dependent not  
318 only on the muscle being investigated, but also on the applied diagnostic criterion and the  
319 adopted cut-points. These findings are in line with previous studies showing that different  
320 definitions of sarcopenia have good negative, but poor positive agreement [8,9,10-15]. The low  
321 agreement level is mainly determined by different sensitivities for the detection of low muscle  
322 mass that characterize the different skeletal muscle mass indices. Given the present and previous  
323 [19-20] demonstrations of high sensitivity of the ultrasound-based assessment of low muscle  
324 mass, we recommend the inclusion of muscle thickness analysis in future studies investigating the

325 predictive validity of different operational definitions of sarcopenia for important clinical  
326 outcomes such as mortality, disability and functional recovery following rehabilitation.

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

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

344 Finally, the usability of cut-off values established in our group of Caucasian healthy young subjects  
345 to identify low muscle mass in older persons of different ethnic groups remains to be  
346 demonstrated in future studies. Similar to the approach currently adopted in osteoporosis  
347 research and clinical practice, the availability of population-specific cut-off values and the use of

348 our T-score based criterion could enable the comparison between different studies and the  
349 accurate identification of low muscle mass also in non-Caucasian older subjects.

350

## 351 **CONCLUSIONS**

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

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

366

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371

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474 **FIGURE CAPTIONS**

475 **Figure 1.**

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

481

482 **Figure 2.**

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

486

487 **Figure 3.**

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

491

492 **Figure 4.**

493 A) Prevalence of low muscle mass obtained in the group of 44 older subjects by using different T-  
494 scores: lower limb T-score, thigh T-score, leg T-score, muscle-specific T-scores (RF: rectus femoris;  
495 VL: vastuslateralis; TA: tibialis anterior; MG: medial gastrocnemius).

496 B) Prevalence of low muscle mass obtained in the group of 44 older subjects by using bioelectrical  
497 impedance analysis-derived cut-off values (gray columns), calf-circumference cut-off (white  
498 column), ultrasound-derived thigh muscle cut-off values (dark column).

1 | Submitted to PM&R

~~July 25th~~ September 7th, 2015

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4 | **Ultrasound-based detection of low muscle mass for diagnosis of**  
5 | **sarcopenia in older adults**

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10 | **WORD COUNT:** ~~3978~~4041 words

11 **ABSTRACT**

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

16 **Design:**Cross-sectional study.

17 **Setting:**Geriatric outpatient clinic and clinical research laboratory.

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

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

26 **Results:**The following site-specific cut-points for muscle thickness were identified: rectus femoris:  
27 20 mm in men and 16 mm in women; vastuslateralis: 17 mm in men and 15 mm in women; tibialis  
28 anterior: 23 mm in men and 22 mm in women; medial gastrocnemius: 13 mm in both men and  
29 women. The prevalence of low muscle mass in older adults was highly dependent on the muscle  
30 being investigated: it varied from 86% for thigh muscles to 30% for leg muscles. Moreover, the  
31 prevalence of low muscle mass was highly dependent on the applied diagnostic criterion and on  
32 the adopted cut-off value (it ranged from 2% to 75% for different BIA-derived criteria).

33 **Conclusions:**~~BIA-derived criteria and relative cut-points underestimated the prevalence of low~~  
34 ~~muscle mass in comparison to the ultrasound-based assessment of muscle thickness. It is therefore~~

35 ~~recommended to adopt the ultrasonographic quantification of muscle thickness and the herein~~  
36 ~~provided cut points for identifying individuals with sarcopenia.~~  
37 We suggest that muscle ultrasonography provides rehabilitation physicians with a practical and  
38 accurate tool for identifying individuals with low muscle mass. However, the usability of cut-off  
39 values established in our group of Caucasian healthy young subjects to identify low muscle mass in  
40 older persons of different ethnic groups remains to be demonstrated in future studies.

41

42

43 **ABSTRACT WORD COUNTS:** 297300words

44 **INTRODUCTION**

45 Primary sarcopenia, the age-related loss of skeletal muscle mass and function[1,2], is associated  
46 with disability and frailty that represent major socioeconomic as well as medical problems. In  
47 rehabilitation patients, primary sarcopenia can be further exacerbated by the disuse- or drug-  
48 related loss of muscle mass or function. Therefore, elderly rehabilitation patients could benefit  
49 from the assessments of skeletal muscle mass and function for the detection of sarcopenia.  
50 A major development in sarcopenia research has been the convergence in its operational  
51 definition. Several consensus groups have recently published operational criteria for the diagnosis  
52 of sarcopenia (incorporating the evaluation of muscle mass with the assessment of strength and/or  
53 physical performance), including the “European Working Group on Sarcopenia in Older People”  
54 (EGWSOP) [3], the “International Working Group on Sarcopenia” (IWGS) [4] and the “Foundation  
55 for the National Institutes of Health Sarcopenia Project” [5].All three consensus groups included  
56 the appendicular skeletal muscle mass (ASMM) assessment, as realized with dual-energy X-ray  
57 absorptiometry (DXA), into the operational definition of sarcopenia. However, different indices of  
58 ASMM (such as ASMM normalized to height or to body mass index) and different cut-off points  
59 were considered. Other sarcopenic indices, which are commonly used in research as well as in  
60 clinical routine, are based on the assessment of the total body skeletal muscle mass (TSMM,  
61 normalized to body weight or to height), as realized with bioelectrical impedance analysis (BIA)  
62 [6,7]. However, the use of different diagnostic criteria may lead to different conclusions, as  
63 evidenced by several investigations recently performed in community-dwelling older adults [8-15].  
64 In addition, although the use of DXA- or BIA-derived sarcopenic indices may be practical for clinical  
65 purposes, they do not seem very accurate [1]. This is essentially due to the fact that sarcopenia is  
66 not a uniform condition as it affects postural muscles more than non-postural ones [1,2,16-18].  
67 Therefore, site-specific assessment of loss of muscle mass may be required for its early and

68 accurate detection. Consistently, recent studies showed that thigh sarcopenia can be detected by  
69 ultrasound-based assessment of muscle thickness before it appears at the whole body level  
70 [19,20]. However, as highlighted by Abe et al. [19], there are no published site-specific cut-points  
71 for ultrasonographic assessment of low muscle mass in older adults. Therefore, the aims of this  
72 study were: i) to establish muscle-specific cut-off values for ultrasound-based detection of low  
73 muscle mass; ii) to assess the prevalence of low muscle mass in a population of frail older subjects  
74 when applying the ultrasonographic cut-points of different lower limb muscles; iii) to assess the  
75 prevalence of low muscle mass when applying different sarcopenic indices derived from  
76 ultrasound, BIA, and anthropometry.

77

## 78 **METHODS**

### 79 **Subjects**

80 Forty-four older adults (30 women and 14 men, mean age  $\pm$  SD:  $82 \pm 7$  yrs; body mass index:  $25 \pm$   
81  $5 \text{ kg/m}^2$ ) and sixty young subjects (30 women and 30 men, age:  $26 \pm 3$  yrs; body mass index:  $22 \pm$   
82  $3 \text{ kg/m}^2$ ) volunteered to participate in the study (convenience sample). The young subjects were  
83 habitually physically active, and none participated in competitive sports. The older group was  
84 composed by institution-dwelling subjects with one or more of Fried's frailty criteria [21]. Side  
85 dominance was assessed with the "Waterloo Handedness and Footedness Questionnaires -  
86 Revised" [22]. One older and six young subjects were left-side dominant. Each participant received  
87 a detailed explanation of the study and gave written informed consent prior to participation. The  
88 study conformed to the ethical principles enunciated in the Declaration of Helsinki and was  
89 approved by the local Ethics Committee.

90

### 91 **Assessments**

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

98

### 99 ***Anthropometric measurements***

100 Measurements of height and weight were made in overnight fasted subjects (in light clothing and  
101 barefoot or with socks) on the same day as all the other tests. Standing height was measured to  
102 the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was determined to the nearest  
103 0.1 kg using a calibrated balance beam scale. Calf circumference (dominant side) was measured to  
104 the nearest 0.1 cm while the subjects were seated with their leg hanging loosely. The  
105 measurement tape was wrapped around the calf and the highest value was retained. A cut-off  
106 point of <31 cm [23] was adopted to identify low muscle mass.

107

### 108 ***Physical performance***

109 Subjects were asked to walk over a 14-m walkway at a self-selected usual speed and their walking  
110 speed was evaluated. A stopwatch was used to time the subjects as they walked over the central  
111 10 m of the walkway. The initial 2 m and final 2 m were not considered to allow for acceleration  
112 and anticipatory deceleration. The distance covered was divided by the time taken to complete  
113 the 10-m walk. Subjects completed three trials and the mean walking speed of the three trials was  
114 retained. A cut-off point of <0.8 m/s [3] was adopted to identify subjects with low physical  
115 performance.



116

117 ***Muscle strength***

118 Handgrip strength was measured on the dominant side using a handheld device (Jamar Plus Digital  
119 Dynamometer, Patterson Medical, Warrenville, IL, USA). The subjects were sitting comfortably  
120 with the shoulder adducted, the elbow flexed at 90° and both the forearm and the wrist in a  
121 neutral position. They were instructed to perform a maximal voluntary isometric contraction by  
122 contracting their muscles as forcefully as possible for 4-5 s. The test was repeated three times with  
123 30 s of recovery in between: if the peak forces of the three trials were within 5% of each other, the  
124 highest value was retained. Otherwise, additional trials were performed until the 5% criterion was  
125 achieved. Cut-off points of <30 kg for men and <20 kg for women [3] were adopted to identify  
126 subjects with low handgrip strength.

127

128 ***Total body and appendicular skeletal muscle mass***

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

138 TSMM was normalized to the body weight (and expressed in %) [6] or to the height (and expressed  
139 in kg/m<sup>2</sup>) [7] to calculate the skeletal muscle index (SMI). ASMM was normalized to the height (and

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

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Insert Table 1

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### ***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.

163 Images of the medial gastrocnemius were acquired with the subjects in the prone position,  
164 whereas for all the other muscles subjects were positioned supine. In all measurements, the lower  
165 limb joints were extended and the subjects were asked to completely relax their muscles. A  
166 suitable amount of ultrasound coupling gel was used to ensure optimal image quality and to  
167 minimize the transducer pressure on the skin. All scans were performed by placing the transducer  
168 in correspondence of the largest muscle diameter at the following anatomical sites, according to  
169 previous studies [28,29]: the rectus femoris was measured half-way along the line from the  
170 anterior-superior iliac spine to the superior border of the patella; the vastuslateralishalf-way along  
171 the line from the anterior-superior iliac spine to the superolateral border of the patella; the tibialis  
172 anterior at one-quarter of the distance from the inferior border of the patella to the lateral  
173 malleolus; the medial gastrocnemius from the mid-sagittal line of the muscle, midway between  
174 the proximal and distal tendon insertions.

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

183 Muscle thickness was measured as the distance between the superficial and deep aponeurosesby  
184 using ImageJ (National Institutes of Health, Bethesda, MD, USA). All three images acquired for  
185 each muscle were analyzed.As shown in the representative example of Figure 1, the operator  
186 measured the muscle thickness in three points, equally spaced along the image. The operator

187 placed the measurement points on each aponeuroses trying to trace a segment which was  
188 orthogonal to the centerline between the two aponeuroses. The Euclidean distance between each  
189 point pairs was considered as the muscle thickness.

190 Cut-off values (and 2SD range values) for the thickness of the four muscles (identified as values 2  
191 SDs below the sex-specific means of our sample of young subjects) are reported in Table 1.

192

193

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Insert Figure 1

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194

### 195 ***Statistical analysis***

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

199 Intrasession and intrarater reliability of the thickness measurement was determined by the  
200 intraclass correlation coefficient (ICC<sub>3,1</sub>) and coefficient of variation using the three scans acquired  
201 for each muscle. We obtained the following ICC and CV values: 0.98 and 3.2% for rectus femoris,  
202 0.99 and 3.3% for vastus lateralis, 0.98 and 1.5% for tibialis anterior, 0.97 and 3.7% for medial  
203 gastrocnemius.

204 Muscle thickness T-score values were calculated for older subjects using the following  
205 formula:  $[(\text{individual value} - \text{mean value of the young subjects of the corresponding gender}$   
206  $\text{group}) / \text{SD of the young subjects of the corresponding gender group}]$ . In each of the older subjects,  
207 the T-scores calculated for the four muscles were then averaged to obtain: i) a lower limb T-score  
208 (i.e., the mean T-score of the four muscles), ii) a thigh T-score (i.e., the mean T-score of rectus  
209 femoris and vastus lateralis muscles), iii) a leg T-score (i.e., the mean T-score of tibialis anterior and  
210 medial gastrocnemius muscles). Accordingly, the following definitions of low muscle mass were

211 considered: low mass of the lower limb muscles (i.e., lower limb T-score < -2), low mass of  
212 the thigh muscles (i.e., thigh T-score < -2), low mass of the leg muscles (i.e., leg T-score < -2),  
213 muscle-specific low mass (i.e., muscle thickness lower than the cut-off values reported in Table 1).  
214 The prevalences of these different ultrasound-based definitions of low muscle mass were then  
215 compared. Moreover, the prevalence of low muscle mass obtained by using a single ultrasound-  
216 derived criterion was compared with the prevalences obtained by using the BIA-derived criteria  
217 and the calf-circumference criterion (based on the cut-off values reported in Table 1 and  
218 numbered from I to XI).

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

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

230

## 231 **RESULTS**

### 232 ***Muscle mass and thickness: comparisons between young and older subjects***

233 Table 2 lists the values of BIA-derived muscle mass for the two groups of subjects stratified by  
234 gender. As expected, TSMM and ASMM were higher in young compared to older subjects, while

235 the SMI (TSMM normalized to height) in men and the ASMI (ASMM normalized to height) in both  
236 men and women were comparable between young and older subjects.

237 Figures 2-3 show representative examples of ultrasound images acquired from young and older  
238 subjects: muscle thickness was higher in the four muscles of the young subjects compared to older  
239 subjects. Similar to these examples, analysis of the group data (Table 2) showed significantly  
240 higher muscle thickness values in young compared to older subjects for all muscles (with the  
241 exception of the tibialis anterior muscle in men). The thickness values of the four muscles  
242 obtained in young subjects were used to establish the cut-off values reported in Table 1.

243

244 Insert Table 2 and Figures 2-3

245

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

247 As shown in Figure 4A, the prevalence of low muscle mass obtained by using the thigh T-score  
248 (86%) was significantly ( $P=0.01$ ) higher than that obtained by using the lower limb T-score (61%),  
249 and the latter was significantly ( $P=0.005$ ) higher than that obtained by using the leg T-score (30%).  
250 Moreover, the prevalence of low muscle mass obtained by using the rectus femoris T-score (86%)  
251 was comparable ( $P=0.18$ ) to that obtained by using the vastus lateralis T-score (73%). A significant  
252 ( $P=0.0006$ ) difference was observed between the prevalence of low muscle mass obtained by using  
253 the medial gastrocnemius T-score (52%) versus the tibialis anterior T-score (16%).

254 Briefly, the prevalence of low muscle mass is highly dependent on the muscle being investigated:  
255 proximal muscles of the lower limb seem more valid for the detection of low muscle mass than  
256 distal muscles.

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

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

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

263

264

---

Insert Figure 4

---

265

### 266 ***Diagnosis of sarcopenia***

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

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

275 The combination of thigh muscle thickness, strength and performance measurements enabled to  
276 classify 6 out of 44 older subjects (14%) as non-sarcopenic, 2 (5%) as pre-sarcopenic, 9 (20%) as

277 sarcopenic 7 out of 9 subjects presented low mass of the thigh muscles and low handgrip strength,

278 while 2 out of 9 subjects presented low muscle mass and low walking speed, and 27 (61%) as

279 severely sarcopenic.

280 Sensitivity and specificity for the presence of either pre- or sarcopenia or severe sarcopenia,

281 identified on the basis of low calf circumference (according to cut-off values # XI in Table 1) and

282 poor muscle function, were 0.60 and 1.0, respectively.

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

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

287

## 288 **DISCUSSION**

289 In the present study, 60 young subjects were evaluated with ultrasonography and BIA to establish  
290 muscle-specific and population-specific cut-off values for sarcopenic indices which were then  
291 applied to a sample of 44 frail older subjects to determine comparative prevalence rates of low  
292 muscle mass. This is the first study to report site-specific cut-points for ultrasound-based  
293 detection of low muscle mass. These cut-points were established based on normative values of  
294 muscle thickness gained from our sample of young subjects that were comparable to those  
295 previously observed in healthy young populations (Table 3: left column). Likewise, the muscle  
296 thickness values we measured in older subjects were similar to those previously reported in  
297 community-dwelling and/or frail elderly individuals (Table 3: right column). Therefore, the high  
298 prevalence of low muscle mass (86%) we observed in older subjects and the inter-muscle  
299 differences (86% of subjects showed low thickness of the thigh muscles, while only 52% and 16%  
300 of subjects showed reduction in medial gastrocnemius and tibialis anterior thickness,  
301 respectively) did not result from the application of biased cut-off values (e.g., too large for thigh  
302 muscles, thus implying false-positive results, and too stringent for leg muscles, thus implying the  
303 overlook of true-positive results). Consistently, such inter-muscle variability in the susceptibility to  
304 age-related muscle loss is in line with previous evidence gained from magnetic resonance imaging-  
305 [18], computed tomography-[16], and DXA-[17] based measurements showing that age-related  
306 muscle loss is greater in lower limb (postural) muscles than in upper limb (non-postural) muscles.



307 To our knowledge, this study is the first to show that proximal muscles of the lower limb are  
308 preferentially affected by thickness loss than distal muscles and that the medial gastrocnemius is  
309 more affected by thickness loss than the tibialis anterior. The latter result is in agreement with  
310 previous studies showing that the age-related decline in plantar-flexor strength is greater  
311 compared to dorsiflexor strength (although the loss of muscle mass alone cannot account for the  
312 reduction in muscle strength) [35]. Given the known differences in muscle composition between  
313 the tibialis anterior and the other three muscles considered here (the former presents a higher  
314 percentage of slow fibers compared to the latter) [36,37], it may be hypothesized that the higher  
315 the percentage of insulin-sensitive slow fibers, the lower the susceptibility to age-related loss of  
316 muscle mass. Therefore, it may be suggested that in the tibialis anterior of our population of frail  
317 older subjects the permissive effect of insulin on protein synthesis [38,39] was greater compared  
318 to other less-insulin sensitive muscles and could explain, at least partly, the lower tibialis anterior  
319 susceptibility to age-related muscle loss. ~~In fact, insulin is permissive for protein synthesis and~~  
320 ~~suppressive for protein breakdown [38,39].~~ However, not only muscular, but also neural  
321 mechanisms, such as site-specific losses of motor units [40], probably underlie the observed site-  
322 specific age-related loss of muscle mass.

323 In the present study, we found that the prevalence of low muscle mass was highly dependent not  
324 only on the muscle being investigated, but also on the applied diagnostic criterion and the  
325 adopted cut-points. These findings are in line with previous studies showing that different  
326 definitions of sarcopenia have good negative, but poor positive agreement [8,9,10-15]. The low  
327 agreement level is mainly determined by different sensitivities for the detection of low muscle  
328 mass that characterize the different skeletal muscle mass indices. Given the present and previous  
329 [19-20] demonstrations of high sensitivity of the ultrasound-based assessment of low muscle  
330 mass, we recommend the inclusion of muscle thickness analysis in future studies investigating the

331 predictive validity of different operational definitions of sarcopenia for important clinical  
332 outcomes such as mortality, disability and functional recovery following rehabilitation.

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

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

350 Finally, the usability of cut-off values established in our group of Caucasian healthy young subjects  
351 to identify low muscle mass in older persons of different ethnic groups remains to be  
352 demonstrated in future studies. Similar to the approach currently adopted in osteoporosis  
353 research and clinical practice, the availability of population-specific cut-off values and the use of

354 our T-score based criterion could enable the comparison between different studies and the  
355 accurate identification of low muscle mass also in non-Caucasian older subjects.

356

## 357 **CONCLUSIONS**

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

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

372

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377

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480 **FIGURE CAPTIONS**

481 **Figure 1.**

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

487

488 **Figure 2.**

489 Examples of ultrasound scans of rectus femoris and vastuslateralismuscles from representative  
490 young (A, C) and older (B, D) subjects.

491 Vertical dotted lines indicate the three thickness measurements considered in each image.

492

493 **Figure 3.**

494 Examples of ultrasound scans of tibialis anterior and medial gastrocnemiusmuscles from  
495 representative young (A, C) and older (B, D) subjects.

496 Vertical dotted lines indicate the three thickness measurements considered in each image.

497

498 **Figure 4.**

499 A) Prevalence of low muscle mass obtained in the group of 44 older subjects by using different T-  
500 scores: lower limb T-score, thigh T-score, leg T-score, muscle-specific T-scores (RF: rectus femoris;  
501 VL: vastuslateralis; TA: tibialis anterior; MG: medial gastrocnemius).

502 B) Prevalence of low muscle mass obtained in the group of 44 older subjects by using bioelectrical  
503 impedance analysis-derived cut-off values (gray columns), calf-circumference cut-off (white  
504 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	
<b>BIA</b>	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 <sup>2</sup> (kg/m <sup>2</sup> )	8.50 kg/m <sup>2</sup>	5.75 kg/m <sup>2</sup>	[7]	
	IV SMI = TSMM/height <sup>2</sup> (kg/m <sup>2</sup> )	2 SDs below the sex-specific means of young subjects	9.42 kg/m <sup>2</sup>	7.27 kg/m <sup>2</sup>	
	V ASMI = ASMM/height <sup>2</sup> (kg/m <sup>2</sup> )	7.26 kg/m <sup>2</sup>	5.45 kg/m <sup>2</sup>	[26]	
	VI ASMI = ASMM/height <sup>2</sup> (kg/m <sup>2</sup> )	2 SDs below the sex-specific means of young subjects	6.88 kg/m <sup>2</sup>	5.65 kg/m <sup>2</sup>	
	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	
<b>AM</b>	XI Calf circumference (cm)	<31 cm	<31 cm	[23]	
<b>US</b>	XII Muscletickness	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 <sup>2</sup> )	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 <sup>2</sup> (kg/m <sup>2</sup> )	10.90±0.74	10.67±1.84	0.46	8.56±0.64	7.48±1.29	<0.0001
ASMI = ASMM/height <sup>2</sup> (kg/m <sup>2</sup> )	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
Tibialisanterioristhickness (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

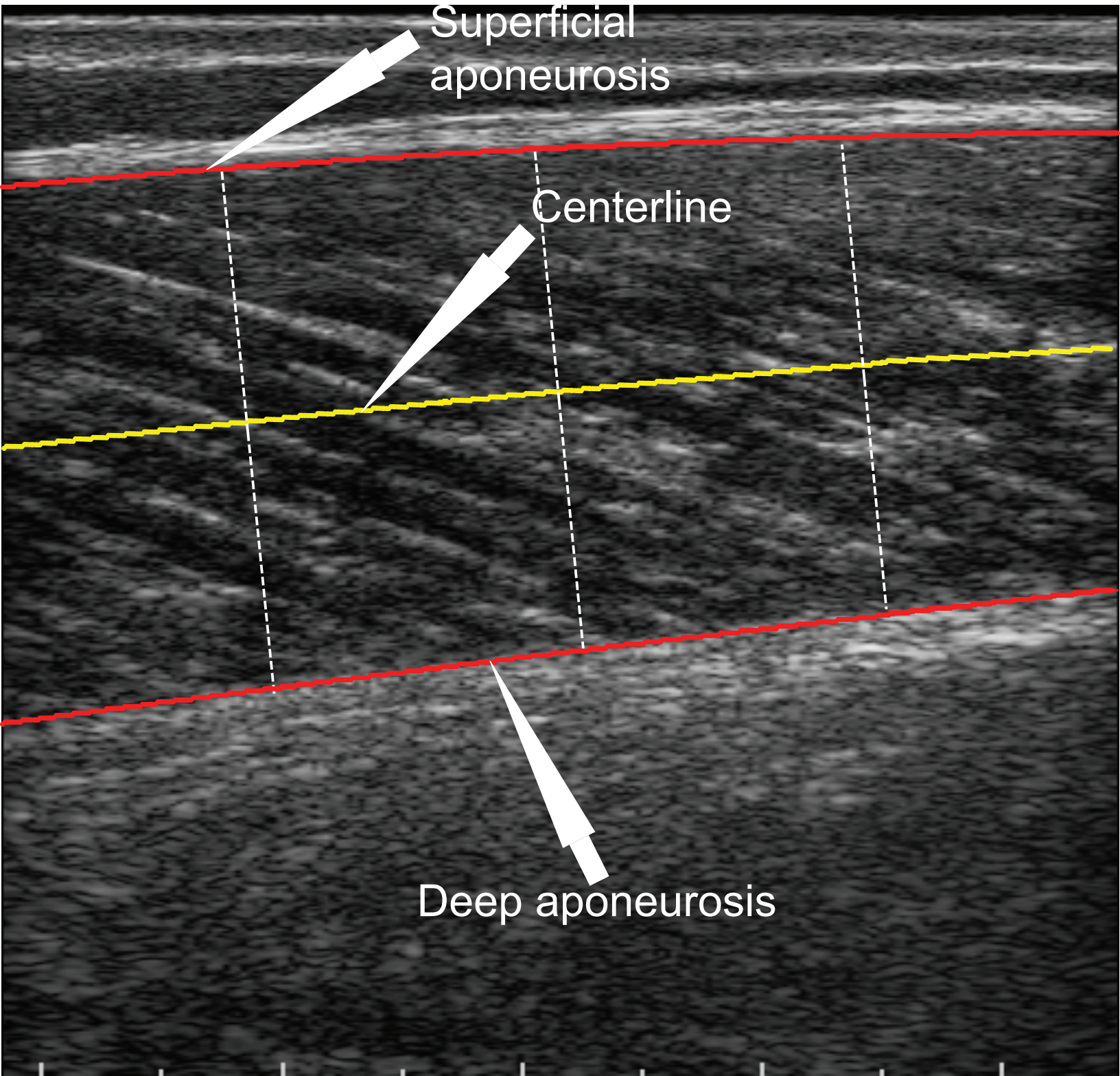


Figure 2

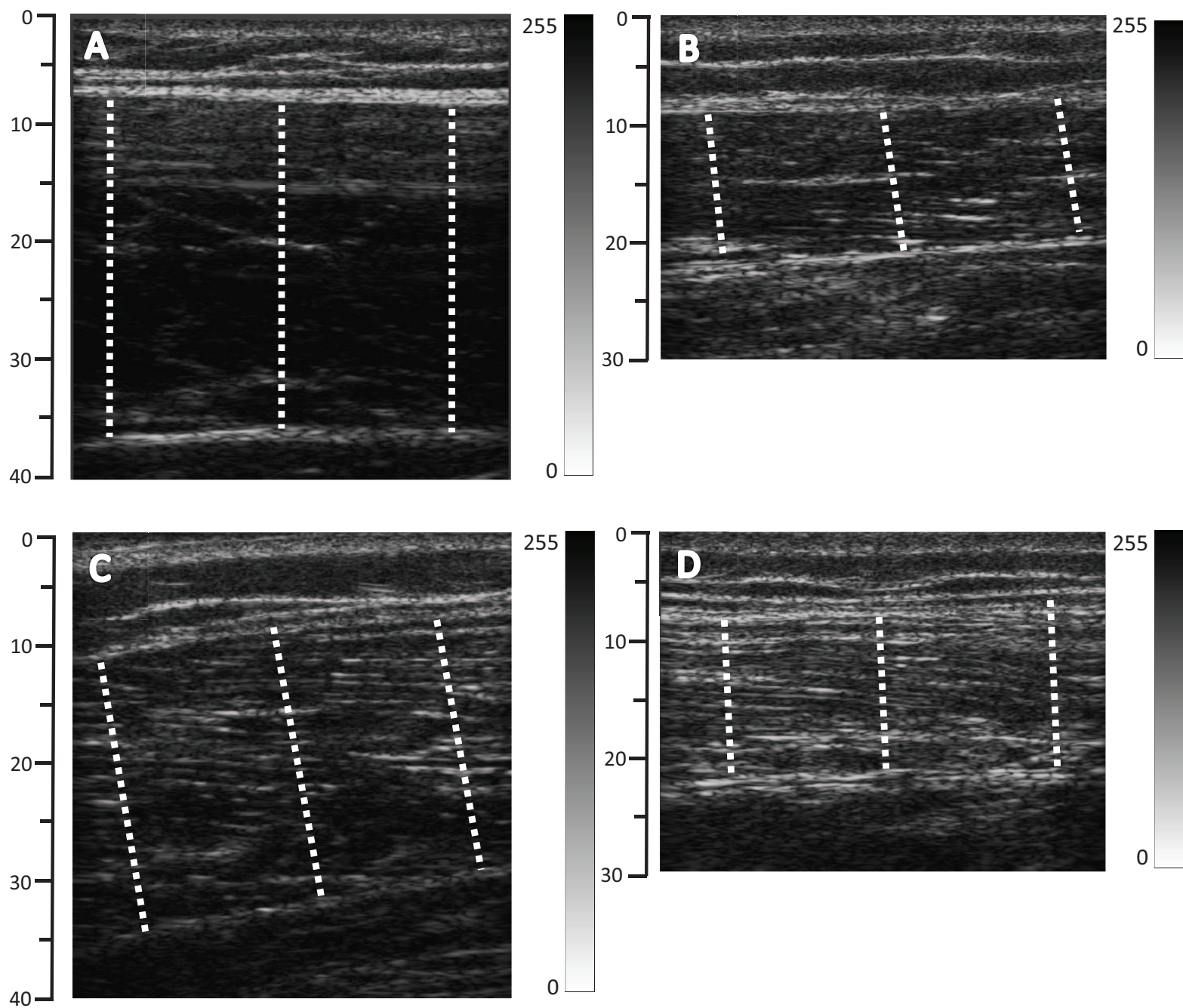


Figure 3

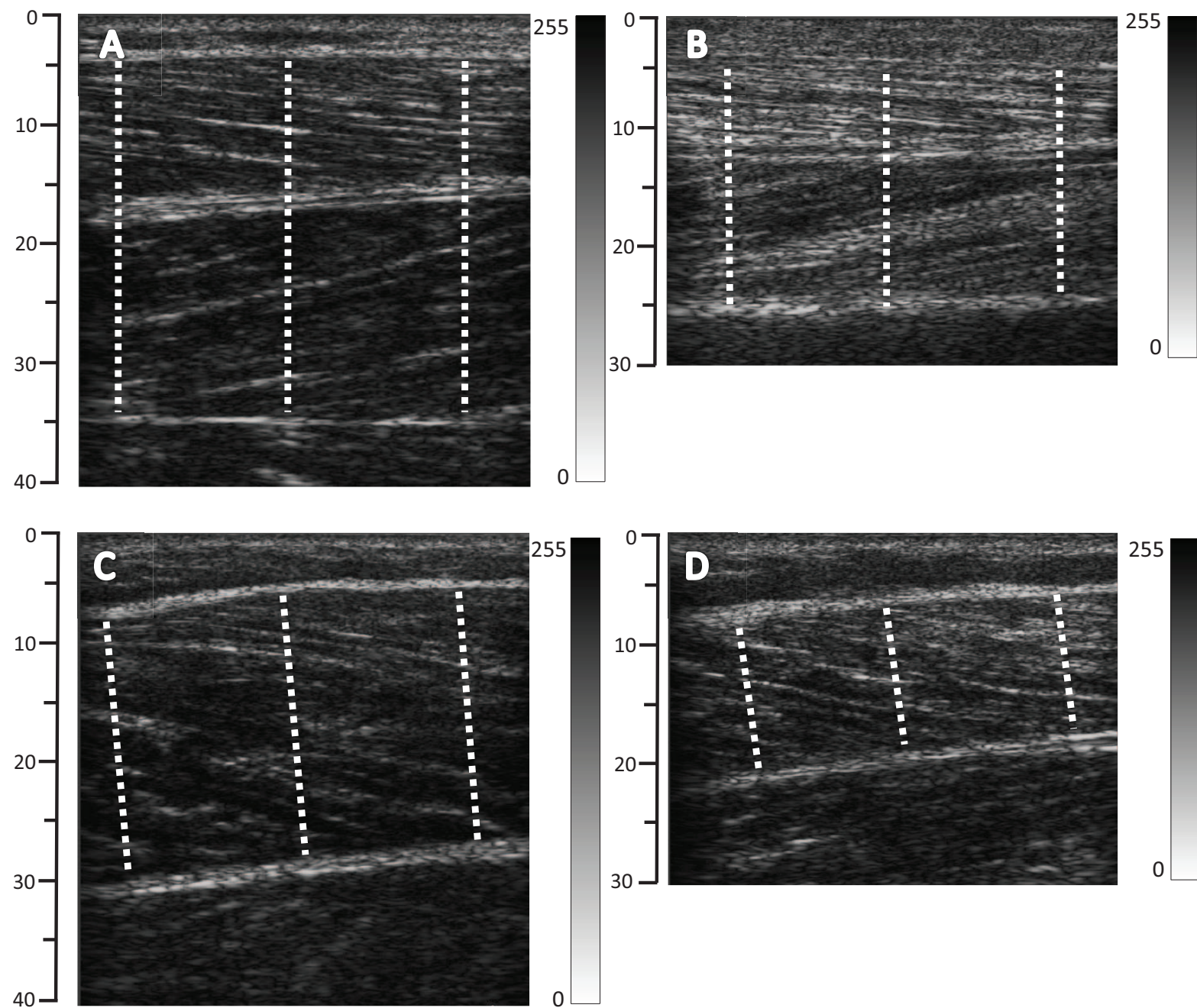
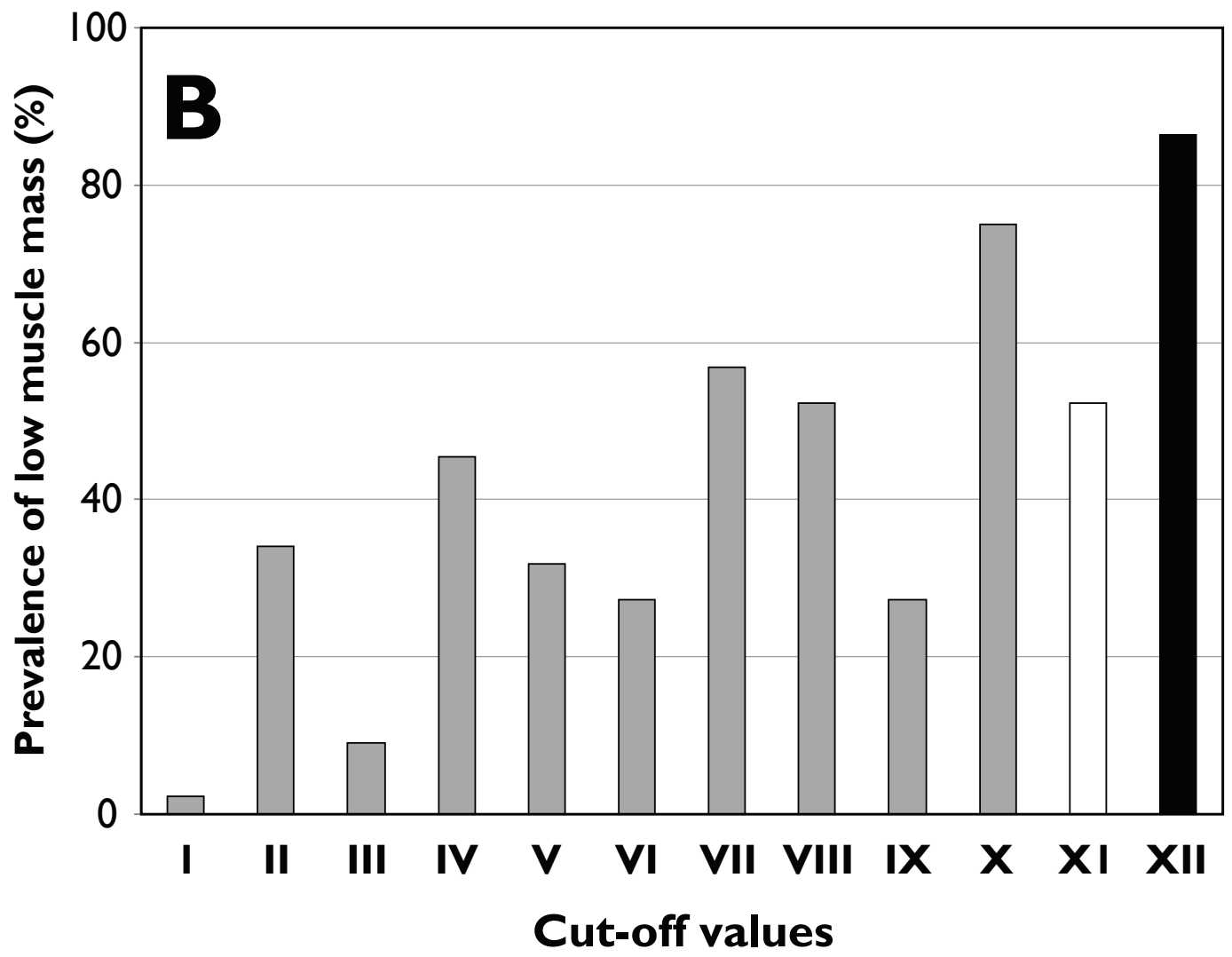
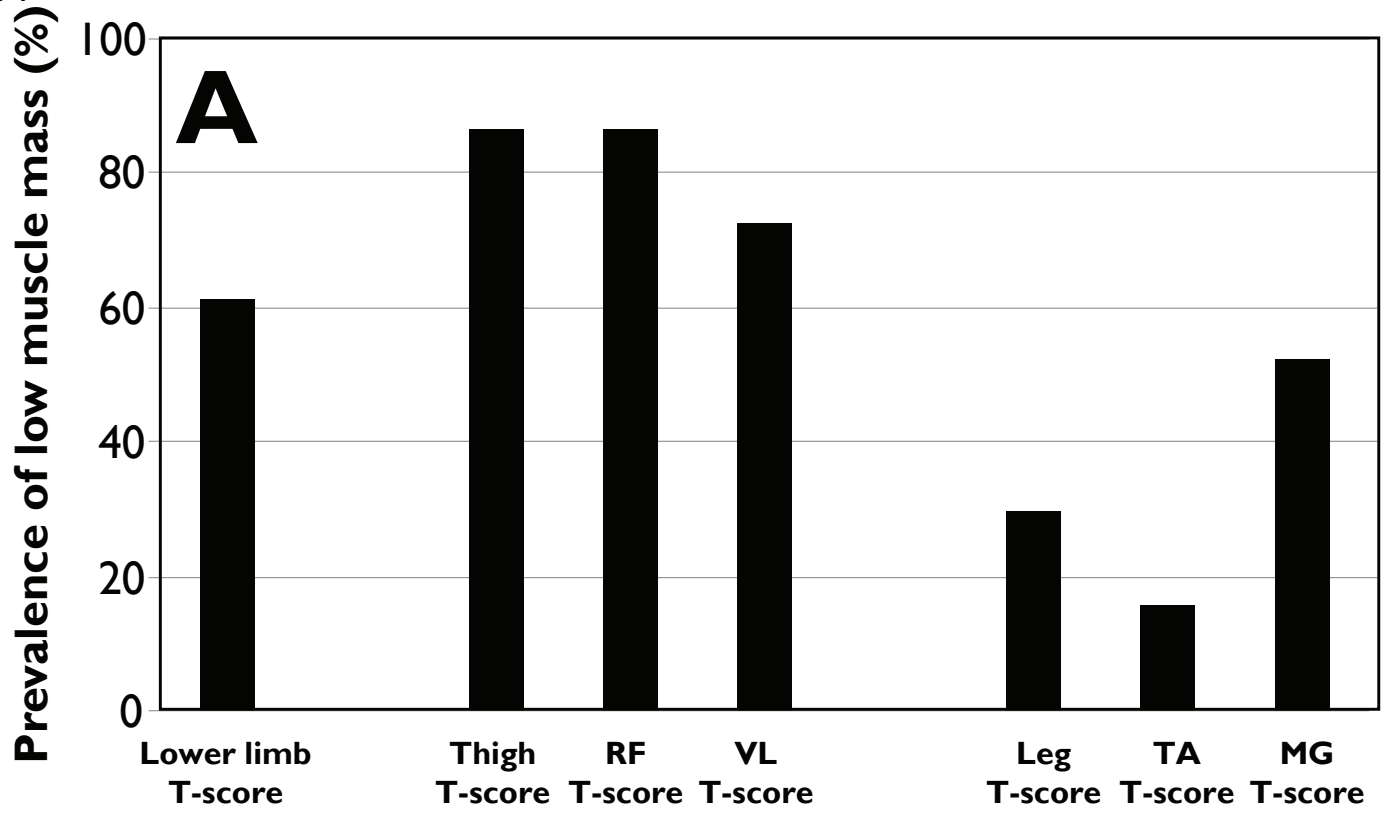




Figure 4



**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			
<i>Participants</i>	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
<i>Test methods</i>	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
<i>Statistical methods</i>	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			
<i>Participants</i>	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
<i>Test results</i>	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.	-
<i>Estimates</i>	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