



## Original article

# Assessing appendicular skeletal muscle mass with bioelectrical impedance analysis in free-living Caucasian older adults



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## SUMMARY

**Background & aims:** Aging is characterized by a loss of appendicular skeletal muscle mass (ASMM) leading to physical disability and death. Bioelectrical impedance analysis (BIA) is reliable in estimating ASMM but no prediction equations are available for elderly Caucasian subjects. The aim of the study was to develop and validate an equation derived from bioelectrical impedance analysis (BIA) to predict appendicular skeletal muscle mass (ASMM) in healthy Caucasian elderly subjects, taking dual X-ray absorptiometry (DXA) as the reference method, and comparing the reliability of the new equation with another BIA-based model developed by Kyle et al. (Kyle UG, Genton L, Hans D, Pichard C, 2003).

**Methods:** With a cross-sectional design, 296 free-living, healthy Caucasian subjects (117 men, 179 women) over 60 years of age were enrolled. Lean mass of limbs was measured with DXA to ascertain ASMM (ASMM<sub>DXA</sub>). Whole-body tetrapolar BIA was performed to measure resistance (Rz), resistance normalized for stature (RI), and reactance (Xc). The BIA multiple regression equation for predicting ASMM was developed using a double cross-validation technique. The predicted ASMM values were compared with ASMM<sub>Kyle</sub>, i.e. ASMM estimates derived from the model developed by Kyle et al. (Kyle et al., 2003).

**Results:** Cross-validation resulted in a unique equation using the whole sample: ASMM (kg) =  $-3.964 + (0.227 \cdot RI) + (0.095 \cdot \text{weight}) + (1.384 \cdot \text{sex}) + (0.064 \cdot Xc)$  [ $R^2 = 0.92$  and  $SEE = 1.14$  kg]. In our sample, ASMM<sub>Kyle</sub> differed significantly from the ASMM<sub>DXA</sub> ( $p < 0.0001$ ), with a mean error of  $-0.97 \pm 1.34$  kg ( $5.1 \pm 6.9\%$ ). Unlike the present BIA prediction equation, the Kyle et al. model showed a correlation between the bias and the mean of ASMM<sub>DXA</sub> and ASMM<sub>Kyle</sub> ( $r = -0.406$ ,  $p < 0.001$ ).

**Conclusion:** The new BIA equation provides a valid estimate of ASMM in older Caucasian adults.

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## 1. Introduction

Aging is associated with sarcopenia, a syndrome characterized by a progressive loss of skeletal muscle mass (SMM), muscle strength and functionality, leading to a higher risk of physical disability, poor quality of life and death [1]. This process can be

schematically divided into three increasingly serious stages: pre-sarcopenia, sarcopenia and severe sarcopenia. The element common to these three stages is a decrease in skeletal muscle mass, which is the only marker of sarcopenia detectable in the pre-clinical phase [2].

In assessing muscle mass, the appendicular portion of the SMM (ASMM) is particularly important. It accounts for 73–75% of the total SMM [3], and a decrease in ASMM is associated with disability [4] because it is involved primarily in physical activities [5].

Assessing muscle mass (and ASMM in particular) is challenging, the choice of method depending on the circumstances. Radiological methods such as computerized tomography and magnetic resonance imaging are precise but costly and only available at

Abbreviations: ASMM, appendicular skeletal muscle mass; BIA, bioelectrical impedance analysis; DXA, dual X-ray absorptiometry; RI, resistive index; Rz, resistance; SMM, skeletal muscle mass; Xc, reactance.

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specialized facilities. Measuring muscle metabolites, including creatinine and 3-methylhistidine, demands dietary control and analytical laboratory equipment. Anthropometry is a simple field method that is not very accurate because it is based on dubious assumptions. Another option is dual X-ray absorptiometry (DXA), which is an acceptable reference technique for assessing whole body and appendicular body composition [6,7]. But DXA cannot be used routinely because the method is costly, it is not portable, and it entails exposure to radiation. A safe, non-invasive, portable and reliable alternative is bioelectrical impedance analysis, a method that can be used both in clinical settings and in the field for epidemiological studies.

Although numerous papers have reported on the use of BIA to predict fat-free mass [8] or SMM [9], to our knowledge, the only BIA-derived equations specifically for predicting ASMM in elderly people and taking DXA for reference are the one developed by Kim et al. [10] and that of Yoshida et al. [11]. Nevertheless, been developed in Asian subjects, these equations cannot be used in Caucasian people due to the well-known anthropometric differences. No ASMM prediction equations are available for elderly Caucasian subjects, and the only option is to use the Kyle et al. equation [12], which was obtained in a sample of adults of all ages (20–94 years). The assumptions behind BIA are that the body (limbs and trunk) can be considered as a single conductive cylinder, and the relationship between the main cross-sectional areas remains the same. This model should change with aging, however, because older people experience a gradual reduction in the cross-sectional area of their limbs and a concomitant increase in that of their trunk [13,14]. BIA equations specifically generated from a sample of elderly subjects would consequently predict ASMM better than equations derived from a sample population of all ages. The aim of this study was thus to develop and validate a BIA equation for predicting ASMM in healthy elderly Caucasian subjects, taking DXA for reference. We also compared the reliability of our new equation and the one developed by Kyle in a sample of elderly people.

## 2. Materials and methods

### 2.1. Subjects

This cross-sectional study was conducted at the Padova University – Geriatrics Department on a sample of Caucasian subjects over 60 years of age recruited on a voluntary basis among the elderly people attending a twice-weekly mild fitness program at public gyms in Padova. Their healthy condition was established by trained medical personnel, based on their clinical history, a clinical examination and biochemical tests. Individuals with skeletal deformities that might affect their height (i.e. kyphosis, scoliosis), or significant cardiovascular or lung diseases, uncontrolled metabolic disease (diabetes, anemia or thyroid disease), electrolyte abnormalities, cancer or inflammatory conditions in the last 5 years were ruled out. Any use of drugs (corticosteroids, hormones, etc.) that might interfere with body composition was also a reason for exclusion.

Among 304 screened subjects, 8 were excluded because of presence of non-inclusion criteria (3 participants with kyphosis, 2 with cancer in the previous 5 years, 2 with uncontrolled insulin-dependent diabetes, and 1 taking steroids). Therefore, the final sample consisted of 296 subjects.

This study was designed in accordance with the Helsinki Declaration and approved by the local Ethical Committee (IRB approval #491/2011). All participants were fully informed about the nature, purpose, procedures and risks of the study, and gave their written informed consent.

### 2.2. Methods

Each subject underwent all the following measures on the same day.

- Anthropometric measurements: body weight was measured to the nearest 0.1 kg using a standard scale (Seca, Hamburg, Germany) with subjects wearing light clothing and no shoes; barefoot standing height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Magnimeter, Raven Equipment Ltd, Dunmow, Essex, UK). BMI was calculated as the weight in kilograms divided by the height in meters squared.
- Multi-dimensional assessment:
  - functional status was assessed using Activity of Daily Living (ADL) [15] and Instrumental ADL (IADL) [16] scales;
  - physical performance was assessed with the Short Physical Performance Battery (SPPB) [17] including gait speed, five timed chair stands, and the tandem test. Performance was scored from 0 to 12, higher scores indicating a better lower body function;
  - health status was assessed with the Cumulative Illness Rating Scale (CIRS) [18], which classifies comorbidities among 13 organ systems and grades each condition from 1 (no problem) to 5 (severely incapacitating or life-threatening conditions). The comorbidity index (CIRS-CI) is given by the number of conditions graded as  $\geq 3$ .
- Dual x-ray absorptiometry: fat-free mass (FFM), lean mass (LM, i.e. FFM less bone mineral mass) and fat mass (FM) were assessed by means of a whole body scan using a fan-beam densitometer (Hologic QDR Discovery A, Hologic Italy). Appendicular skeletal muscle mass (ASMM<sub>DXA</sub>) was calculated as the sum of the lean mass of the limbs, as described by Heymsfield et al. [19]. ASMM was normalized in relation to the subject's height (ASMM/height in meters squared) to obtain the ASMM index (ASMMI<sub>DXA</sub>, kg/m<sup>2</sup>). The scanner was calibrated daily using a standard calibration block supplied by the manufacturer. All metal items were removed before densitometry. Subjects wearing only underwear were placed supine with their arms at their sides, slightly away from their trunk and correctly centered on the scanning field. The scan took about 180 s and the radiation dose per individual was 0.01 mGy (1.0 mrad). To our knowledge, there is no information available on the precision of the QDR Discovery A for measuring body composition, but for the QDR 4500A (the previous Hologic model) the coefficients of variation are 1.1% for total mass, 1.97% for FM and 1.46% for LM [20].
- BIA: whole-body tetrapolar BIA (BIA 101 Anniversary AKERN/RJL Systems; Florence, Italy) was performed using an alternating sinusoidal electric current of 400  $\mu$ A at a single operating frequency of 50 kHz. The device was calibrated every morning using the standard control circuit supplied by the manufacturer with a known impedance (resistance = 380  $\Omega$ ; reactance = 47  $\Omega$ ). The device's precision was 1% for resistance (Rz), and 5% for reactance (Xc). BIA was performed with subjects supine with their limbs slightly away from their body, after an overnight fast, and bladder voiding. To avoid inter-observer errors, all BIA measurements were taken by the same trained investigator. Active electrodes (BIATRODES<sup>®</sup> Akern Srl; Florence, Italy) were placed on the right side on conventional metacarpal and metatarsal lines, recording electrodes in standard positions at the right wrist and ankle [21]. All resistance measurements were normalized for stature (height in centimeters squared/Rz) to obtain the resistive index (RI). The repeatability and accuracy of the resistance and reactance measurements enabled the smallest changes to be recorded to a resolution of 0.1  $\Omega$ .

### 2.3. Statistical analysis

All analyses were performed using IBM SPSS Statistics, version 20 (SPSS Inc., Chicago). The Shapiro–Wilk test showed that the continuous variables (age, height, weight, BMI, DXA and BIA parameters) were normally distributed, so they were analyzed using parametric tests and the results were expressed as means  $\pm$  standard deviations. CIRS-CI and SPPB scores were analyzed using non-parametric tests and expressed as medians and inter-quartile ranges (IQR).

DXA was considered the reference method for measuring ASMM. The BIA prediction equation ( $ASMM_{BIA}$ ) was developed by means of a double cross-validation technique [22]. The subjects were randomly split into two samples (Groups 1 and 2). A stepwise multiple regression analysis was used to derive a BIA prediction equation for each group ( $BIA_1$  and  $BIA_2$ ), entering in the model the variables significantly associated with  $ASMM_{DXA}$ , i.e. RI, weight, sex (female = 0; male = 1), and Xc. Then ASMM in Group 1 was estimated using the  $BIA_2$  equation ( $ASMM_2$ ) and, vice versa, the ASMM in Group 2 was estimated using the  $BIA_1$  equation ( $ASMM_1$ ). In each group, the precision of each equation in estimating ASMM was assessed by means of paired *t*-test and intra-class correlation (*r* coefficient). The precision was also established by testing the degree of agreement between the measured and predicted ASMM using Bland–Altman analysis [23], and a simple Pearson correlation was used to test the relationship between the bias and the mean of the measured and predicted ASMM.

The independent-samples *t*-test was used for intra-group and cross-group comparisons of the residuals to see if the two groups could be pooled and a single equation developed for the whole sample. For further confirmation, an analysis of covariance was performed, entering the dichotomous variable “group” in the prediction model for  $ASMM_{DXA}$ .

The ASMM estimated for our sample was also compared with the values predicted using the equation developed by Kyle et al. [12], i.e.  $ASMM_{Kyle} = -4.211 + (0.267*RI) + (0.095*weight) + (-0.012*age) + (0.058*reactance) + (1.909*sex)$ , where men = 1 and women = 0.

Then the precision of the new equation ( $BIA_{WS}$ ) and the Kyle equation ( $BIA_{Kyle}$ ) were analyzed using the above-described methods applied to each of our two groups and to the entire sample. Statistical significance was set at  $p < 0.05$  for all tests.

## 3. Results

### 3.1. Subjects' characteristics

Our participants' characteristics are shown in Table 1. The sample as a whole included 117 men and 179 women, aged from 60 to 85 years, who were all fully independent in ADL and IADL. The median CIRS-CI score was 1.31 (IQR 1.23–1.46), the median SPPB score 11 (IQR 10–12), and 5% of the subjects had a SPPB  $\leq 8$ . On average, the subjects had a BMI of  $27.0 \pm 3.4$  kg/m<sup>2</sup> and an  $ASMM_{DXA}$  of  $18.6 \pm 4.1$  kg.

Table 1 also shows the characteristics of the subjects randomly divided into the two groups. The male/female ratio was much the same, and age, body composition, anthropometric characteristics and bioelectrical parameters also did not differ significantly between the two groups.

### 3.2. Derivation and cross-validation of the BIA regression equations

The equations developed separately for the two groups are shown in Table 2. The  $BIA_1$  equation was:  $ASMM_1$  (kg) =  $-0.374 + (0.263*RI) + (0.079*weight) + (0.840*sex) + (0.080*Xc)$ . The  $R^2$  and SEE values for this regression equation were

0.93 and 1.13 kg, respectively. The main contributor to the multiple regression model was RI, which explained 90% of the variance (Table 2). The  $BIA_2$  equation was:  $ASMM_2$  (kg) =  $-2.798 + (0.197*RI) + (0.106*weight) + (1.824*sex) + (0.054*Xc)$ . The  $R^2$  and SEE values for this regression equation were 0.92 and 1.53 kg, respectively. Here again, the main contributor to the model was RI, which explained 86.7% of the variance (Table 2).

Body weight contributed little to the predicted ASMM values (1.6% and 3.3% in the  $BIA_1$  and  $BIA_2$  equations, respectively).

Table 3 shows the results of our validation of the  $BIA_1$  equation in Group 2 and of the  $BIA_2$  equation in Group 1. In both groups, the  $ASMM_{BIA}$  did not differ significantly from the  $ASMM_{DXA}$ . The  $BIA_1$  equation resulted in a predicted ASMM of  $18.3 \pm 3.7$  kg for Group 2 ( $r = 0.955$ , SEE 1.2 kg), and the mean difference between the measured and predicted ASMM in this group was  $0.21 \pm 6.66\%$ . The  $BIA_2$  equation generated a predicted ASMM of  $18.9 \pm 4.0$  kg in Group 1 ( $r = 0.962$ , SEE 1.1 kg), and the mean difference between measured and predicted ASMM in this group was  $0.97 \pm 6.17\%$ . Bland–Altman analysis showed a good agreement between the measured and predicted ASMM for both equations (Fig. 1). The bias and the mean between measured and predicted ASMM did not correlate with one another for either of the equations.

### 3.3. Derivation of the BIA regression equation

In both groups, the ASMM estimated with the  $BIA_1$  and  $BIA_2$  equations showed the same intra-class correlation coefficient ( $r = 0.997$ ,  $p < 0.0001$ ). The residuals of  $ASMM_{DXA} - ASMM_2$  in Group 1 did not differ significantly from those of  $ASMM_{DXA} - ASMM_1$  in Group 2 ( $-0.10 \pm 1.15$  kg and  $0.13 \pm 1.18$  kg, respectively,  $p = 0.091$ ), and neither differed significantly from zero ( $p = 0.295$  in Group 1,  $p = 0.180$  in Group 2). A visual inspection of the Bland–Altman plots also showed a good agreement between the two equations (Fig. 1). Analysis of covariance confirmed that there were no significant differences between the two equations ( $p = 0.28$ ). A single equation ( $BIA_{WS}$ ) using all 296 participants was therefore developed (Table 4) to estimate the ASMM from the whole sample ( $ASMM_{WS}$ ). The resulting  $BIA_{WS}$  equation was:  $ASMM_{WS}$  (kg) =  $-3.964 + (0.227*RI) + (0.095*weight) + (1.384*sex) + (0.064*Xc)$ . The  $R^2$  and SEE values of this regression equation were 0.92 and 1.14 kg, respectively. The main contributor to the multiple regression model was RI, which explained 88.3% of the variance (Table 4).

As shown in Table 5, the BIA equation developed on the whole sample resulted in a predicted ASMM of  $18.9 \pm 4.0$  kg for Group 1 ( $r = 0.964$ , SEE 1.1 kg),  $18.4 \pm 3.8$  kg for Group 2 ( $r = 0.958$ , SEE 1.1 kg), and  $18.6 \pm 3.9$  kg for the whole sample ( $r = 0.961$ , SEE 1.1 kg). The predicted ASMM did not differ significantly from the  $ASMM_{DXA}$ , the mean error being  $0.81 \pm 6.02\%$  for Group 1 and  $0.09 \pm 6.45\%$  for Group 2. A visual inspection of the Bland–Altman plots also showed a good agreement between measured and predicted ASMM (Fig. 2).

### 3.4. Comparing the $ASMM_{WS}$ with the $ASMM_{Kyle}$

Table 5 compares the  $ASMM_{DXA}$ ,  $ASMM_{Kyle}$  and  $ASMM_{WS}$  in the two groups, and in the sample as a whole.

The  $BIA_{Kyle}$  equation predicted an ASMM of  $19.9 \pm 4.8$  kg in Group 1 ( $r = 0.963$ , SEE 1.1 kg),  $19.3 \pm 4.5$  kg in Group 2 ( $r = 0.957$ , SEE 1.1 kg), and  $19.6 \pm 4.6$  kg in our whole sample ( $r = 0.960$ , SEE 1.1 kg). The  $ASMM_{Kyle}$  differed significantly from the  $ASMM_{DXA}$  in all three cases ( $p < 0.0001$ ), with a mean error of  $-1.09 \pm 1.34$  kg ( $5.7 \pm 6.7\%$ ) for Group 1,  $-0.85 \pm 1.33$  kg ( $4.2 \pm 7.1\%$ ) for Group 2, and  $-0.97 \pm 1.34$  kg ( $5.1 \pm 6.9\%$ ) for the whole sample. While the

**Table 1**  
General characteristics of the whole sample and of the two groups of subjects expressed as frequencies or means  $\pm$  SD, as appropriate. Minimum and maximum values are given in brackets.

	Whole sample (n = 296)	Group 1 (n = 143)	Group 2 (n = 153)
Women (%)	60.5	59.1	62.1
Age (years)	71.4 $\pm$ 5.4 (60–85)	71.1 $\pm$ 5.4 (60.0–85.0)	71.7 $\pm$ 5.5 (60.0–85.0)
Height (cm)	161.9 $\pm$ 8.7 (144–182)	162.2 $\pm$ 8.6 (144.0–182.0)	161.7 $\pm$ 8.8 (144.0–182.0)
Weight (kg)	71.1 $\pm$ 11.8 (47–99)	71.6 $\pm$ 11.7 (49.0–99.0)	70.7 $\pm$ 12.0 (47.0–99.0)
BMI (kg/m <sup>2</sup> )	27.0 $\pm$ 3.4 (19.3–38.0)	27.1 $\pm$ 3.4 (20.3–38.0)	27.0 $\pm$ 3.5 (19.3–36.7)
FM (kg)	24.6 $\pm$ 5.8 (10.4–43.4)	24.6 $\pm$ 5.8 (10.8–43.4)	24.6 $\pm$ 5.8 (10.4–39.2)
FM (%)	34.7 $\pm$ 6.3 (17.1–48.5)	35.5 $\pm$ 6.5 (18.6–47.6)	34.9 $\pm$ 6.0 (17.1–48.5)
FFM (kg)	46.7 $\pm$ 9.3 (31.3–70.7)	47.1 $\pm$ 9.5 (31.5–70.7)	46.3 $\pm$ 9.1 (31.3–67.2)
ASMM <sub>DXA</sub> (kg)	18.6 $\pm$ 4.1 (11.8–29.3)	18.8 $\pm$ 4.2 (12.2–29.3)	18.5 $\pm$ 4.0 (11.8–27.3)
ASMMI <sub>DXA</sub> (kg/m <sup>2</sup> )	7.0 $\pm$ 1.0 (4.9–10.2)	7.1 $\pm$ 1.0 (4.9–10.2)	7.0 $\pm$ 1.0 (5.0–9.7)
Resistance ( $\Omega$ )	502.1 $\pm$ 67.5 (336–680)	496.5 $\pm$ 70.7 (355.0–680.0)	507.3 $\pm$ 64.1 (336.0–677.0)
Reactance ( $\Omega$ )	48.5 $\pm$ 6.4 (30–75)	48.2 $\pm$ 6.4 (31.0–65.0)	48.7 $\pm$ 6.5 (30.0–75.0)
Phase angle	5.6 $\pm$ 0.6 (4.0–7.7)	5.6 $\pm$ 0.6 (4.0–7.8)	5.5 $\pm$ 0.6 (4.0–7.7)
RI (cm <sup>2</sup> / $\Omega$ )	53.7 $\pm$ 11.7 (34.9–86.5)	54.7 $\pm$ 12.3 (34.9–86.5)	52.9 $\pm$ 11.1 (35.0–84.0)

ASMM<sub>DXA</sub>: appendicular skeletal muscle mass measured by DXA; ASMMI: ASMM index; BMI: body mass index; DXA: dual-X rays absorptiometry; FFM: fat-free mass; FM: fat mass; LM: lean mass; RI: resistive index.

bias and the mean between measured and predicted ASMM did not correlate with one another when the BIA<sub>WS</sub> was used, applying the BIA<sub>Kyle</sub> gave rise to a significant correlation for all three groups (Group 1:  $r = -0.425$ ,  $p < 0.001$ ; Group 2:  $r = -0.382$ ,  $p < 0.001$ ; Whole sample:  $r = -0.406$ ,  $p < 0.001$ ).

#### 4. Discussion

The present study led to the development and validation of a BIA equation for predicting ASMM in elderly Caucasian subjects. This equation seems to be more reliable when applied to the elderly than the one previously published in the literature [12], which was derived from a population of adults of all ages.

Estimating older adults' ASMM is particularly important because of the age-related decrease in muscle mass (especially in the limbs), which can lead to sarcopenia. Not only the underweight elderly lose muscle mass in the limbs, this happens in people with a high BMI too [24]. In this latter case (a condition called sarcopenic

obesity), an increase in fat mass and decrease in muscle mass may have a compound effect on health outcomes, leading to disability, morbidity and mortality [25]. That is why subjects with extreme BMI values, who were underweight, overweight or obese, were included in the present study. The subjects enrolled varied widely in terms of BMI (from 19.3 to 38.0 kg/m<sup>2</sup>) and ASMM (11.8–29.3 kg); 25% of them were sarcopenic according to the Rosetta Study criteria (i.e. ASMM index  $\leq 7.26$  kg/m<sup>2</sup> in men and  $\leq 5.45$  kg/m<sup>2</sup> in women) [26], and 5% had sarcopenic obesity.

The BIA-derived ASMM prediction equation emerging from the present study accounted for less variance in the model than the equation developed by Kyle et al. (92% vs 95%, respectively) [12]. This difference is attributable to the latter's inclusion of young and older adults (with those >60 years old representing only an unspecified part of the sample), whereas all the subjects in our sample were over sixty. This interpretation is supported by the fact that Kim et al. [10] and Yoshida et al. [11], who developed a BIA equation for elderly Asian subjects, reported  $R^2$  values (in the first one,

**Table 2**  
Multiple linear regression model for predicting ASMM<sub>DXA</sub> from BIA variables (RI and Xc), body weight and sex in the two groups of subjects.

	Group 1					Group 2						
	Coefficient	SE	p-Value	R <sup>2</sup> partial	R <sup>2</sup> cumulative	SEE	Coefficient	SE	p-Value	R <sup>2</sup> partial	R <sup>2</sup> cumulative	SEE
Constant	-5.374	1.466	<0.0001	–	–	–	-2.798	1.482	<0.061	–	–	–
RI (cm <sup>2</sup> / $\Omega$ )	0.263	0.021	<0.0001	0.900	0.900	1.336	0.197	0.022	<0.0001	0.867	0.867	1.456
Weight (kg)	0.079	0.013	<0.0001	0.015	0.915	1.238	0.106	0.013	<0.0001	0.031	0.898	1.279
Sex (men = 1; women = 0)	0.840	0.362	0.022	0.006	0.921	1.196	1.824	0.367	<0.0001	0.015	0.913	1.183
Xc ( $\Omega$ )	0.080	0.019	<0.0001	0.009	0.930	1.128	0.054	0.018	0.003	0.005	0.918	1.152

ASMM<sub>DXA</sub>: appendicular skeletal muscle mass measured by dual-energy X-ray absorptiometry; BIA: bioelectrical impedance analysis; RI: resistive index; SE: standard error of the coefficient; SEE: standard error of the estimate; Xc: reactance.

**Table 3**  
Comparison of ASMM<sub>DXA</sub> with ASMM<sub>BIA</sub> as predicted by the equations derived from Groups 1 and 2.

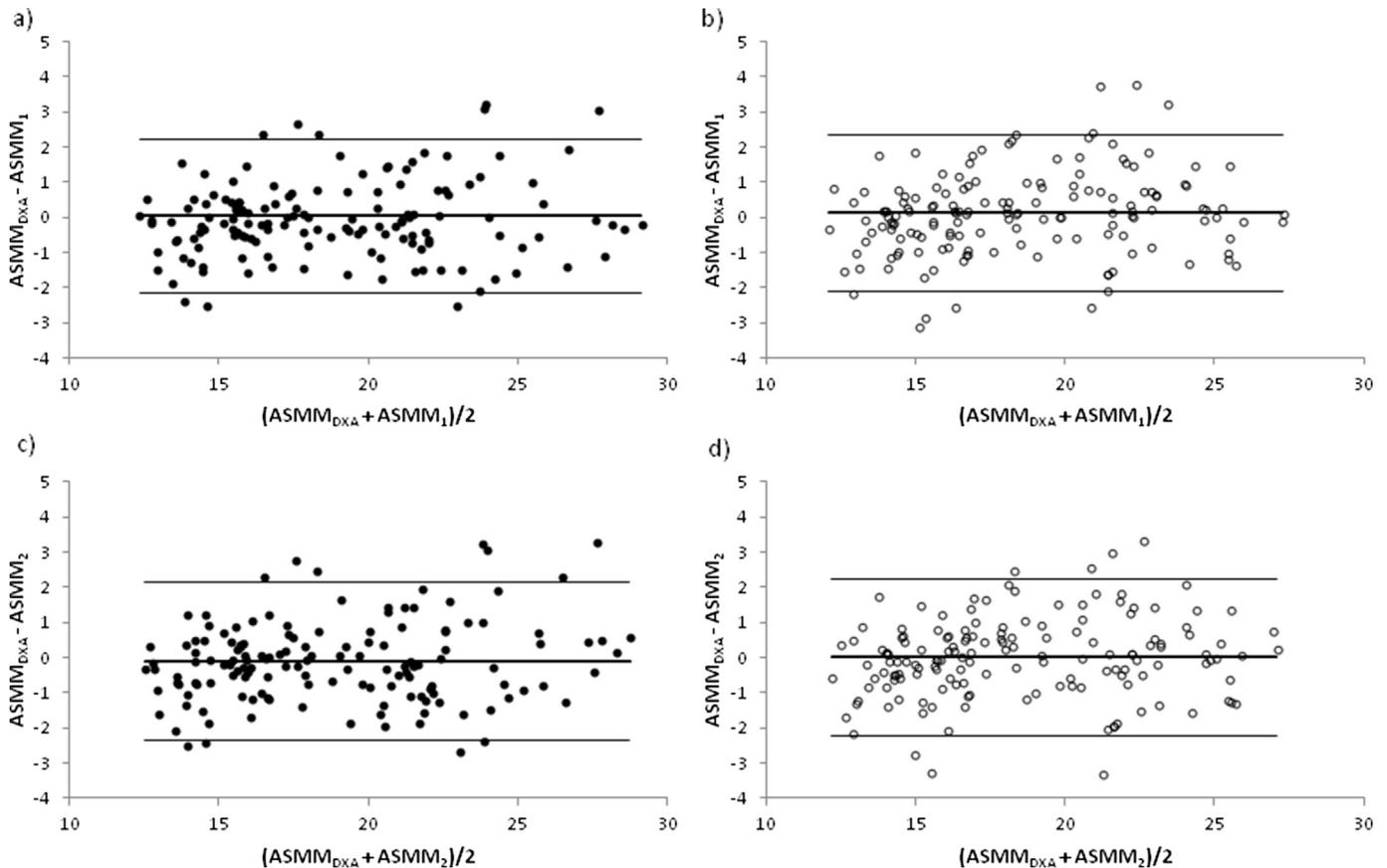
	ASMM <sub>DXA</sub> (kg)	BIA equation	ASMM <sub>BIA</sub> (kg)	p-Value	r <sup>a</sup>	ASMM <sub>DXA-BIA</sub> (kg)	ASMM <sub>DXA-BIA</sub> (%)	Limits of agreement (kg)	r <sup>b</sup>
Group 1	18.8 $\pm$ 4.2	ASMM <sub>1</sub>	18.9 $\pm$ 4.1	0.653	0.964***	-0.04 $\pm$ 1.11	-0.60 $\pm$ 5.96	-2.13, 2.21	0.129
		ASMM <sub>2</sub>	18.9 $\pm$ 4.0	0.295	0.962***	-0.10 $\pm$ 1.15	-0.97 $\pm$ 6.17	-2.35, 2.15	0.162
Group 2	18.5 $\pm$ 4.0	ASMM <sub>1</sub>	18.3 $\pm$ 3.7	0.180	0.955***	0.13 $\pm$ 1.18	0.21 $\pm$ 6.66	-2.10, 2.36	0.196
		ASMM <sub>2</sub>	18.4 $\pm$ 3.8	0.862	0.958***	0.02 $\pm$ 1.14	-0.34 $\pm$ 6.43	-2.21, 2.25	0.142

ASMM: appendicular skeletal muscle mass; ASMM<sub>1</sub>: ASMM predicted by BIA equation derived from Group 1; ASMM<sub>2</sub>: ASMM predicted by BIA equation derived from Group 2; ASMM<sub>BIA</sub>: ASMM predicted by BIA equation; ASMM<sub>DXA</sub>: ASMM measured by dual-energy X-ray absorptiometry; ASMM<sub>DXA-BIA</sub>: ASMM<sub>DXA</sub> less ASMM<sub>BIA</sub> (expressed in kilograms and as a percentage of the ASMM<sub>DXA</sub>).

\*\*\*  $p < 0.001$ .

<sup>a</sup> Intra-class correlation between ASMM<sub>DXA</sub> and ASMM<sub>BIA</sub>.

<sup>b</sup> Simple Pearson correlation between ASMM<sub>(DXA+BIA)/2</sub> and ASMM<sub>DXA-BIA</sub>.



**Fig. 1.** Bland–Altman plot of the difference between appendicular skeletal muscle mass measured by DXA and predicted by the BIA equation derived from Group 1 and applied to Group 1 (plot a), and Group 2 (plot b), and predicted by the BIA equation derived from Group 2 and applied to Group 1 (plot c), and Group 2 (plot d). Footnotes: The thick line indicates the bias between the measured and predicted ASMM, the thin lines the limits of agreement. ASMM: appendicular skeletal muscle mass (kg); ASMM<sub>1</sub>: ASMM predicted with the BIA equation derived from Group 1; ASMM<sub>2</sub>: ASMM predicted with the BIA equation derived from Group 2; ASMM<sub>DXA</sub>: ASMM measured by DXA.

$R^2 = 0.890$ ; in the latter,  $R^2 = 0.87$  in men and  $0.89$  in women) more similar to ours than to Kyle's [12].

A factor that may account for a higher BIA measurement variability in older adults concerns discrepancies in the assumptions of the bioelectrical model for older as opposed to younger adults. With aging, the decrease in ASMM and a redistribution of adipose tissue from the limbs to the trunk give rise to narrower diameters for the conductive volumes (cylinders) of the limbs [13,14]. Hydration of the fat-free body also varies more in the elderly than in younger age groups, and this difference in hydration is known to interfere with the accuracy of estimates of older people's body composition using prediction equations derived younger samples [27]. It is noteworthy that age is an independent predictor of FFM in Kyle's regression model, unlike the case of the present model.

**Table 4**

Multiple linear regression model for predicting ASMM<sub>DXA</sub> from BIA variables (height<sup>2</sup>/resistance and reactance), body weight and sex in the whole sample.

	Coefficient	SE	<i>p</i> -Value	<i>R</i> <sup>2</sup> partial	<i>R</i> <sup>2</sup> cumulative	SEE
Constant	−3.964	1.037	<0.0001	—	—	—
RI (cm <sup>2</sup> /Ω)	0.227	0.015	<0.0001	0.883	0.883	1.401
Weight (kg)	0.095	0.009	<0.0001	0.023	0.906	1.260
Sex (men = 1; women = 0)	1.384	0.254	<0.0001	0.010	0.916	1.189
Xc (Ω)	0.064	0.013	<0.0001	0.007	0.923	1.143

ASMM<sub>DXA</sub>: appendicular skeletal muscle mass measured by dual-energy X-ray absorptiometry; BIA: bioelectrical impedance analysis; RI: resistive index; SE: standard error of the coefficient; SEE: standard error of the estimate; Xc: reactance.

A key aspect of the present study lies in that it clarifies the importance of the resistive index (RI) as an independent variable in predicting ASMM. Among all the parameters considered in our BIA equation, RI was the single best predictor of ASMM (88%), whereas body weight, reactance, and gender contributed very little (2%, 1% and 1%, respectively) to the variability in the ASMM prediction. Our findings are therefore consistent with previous reports [10–12] that the RI is the dominant independent predictor of ASMM in older adults, irrespective of the above-mentioned factors that might influence the BIA parameters with aging.

Our study hypothesis was that BIA equations developed for the general population may not be entirely reliable in predicting ASMM in older subjects. So we tested the reliability of Kyle's equation [12] in our population. When we compared the results with those obtained with our own, newly-developed equation, the latter proved more reliable [12] for our older adults. Although the standard error of the predicted ASMM was similar for the two equation (1.1 kg), the biases differed considerably. Considering our whole sample, the Kyle equation [12] overestimated the ASMM by 5% on average, and the magnitude of the bias was inversely proportional to the ASMM values, as shown by the inverse correlation between the bias and the mean of the measured and predicted ASMM ( $r = -0.41$ ,  $p < 0.001$ ). Instead, the mean bias of our equation came close to zero, with no correlation between bias and mean ASMM values, meaning that the DXA and BIA were equally consistent throughout the range of ASMM measurements. One reason why Kyle's equation would be less reliable than ours would relate to the differences between the two samples: the sample considered by Kyle et al.

**Table 5**  
Characteristics of the BIA equation derived from the whole sample, and comparison with Kyle's equation.

	ASMM <sub>DXA</sub> (kg)	BIA equation	ASMM <sub>BIA</sub> (kg)	Paired <i>t</i> -test	<i>r</i> <sup>a</sup>	ASMM <sub>DXA-BIA</sub> (kg)	ASMM <sub>DXA-BIA</sub> (%)	Limits of agreement (kg)	<i>r</i> <sup>b</sup>
Group 1	18.8 ± 4.2	ASMM <sub>WS</sub>	18.9 ± 4.0	0.393	0.964***	-0.08 ± 1.12	-0.81 ± 6.02	-2.27, 2.11	0.132
		ASMM <sub>Kyle</sub>	19.9 ± 4.8	<0.0001	0.963***	-1.09 ± 1.34	-5.66 ± 6.75	-3.71, 1.53	-0.425***
Group 2	18.5 ± 4.0	ASMM <sub>WS</sub>	18.4 ± 3.8	0.498	0.958***	0.06 ± 1.14	-0.09 ± 6.45	-2.17, 2.29	0.154
		ASMM <sub>Kyle</sub>	19.3 ± 4.5	<0.0001	0.957***	-0.85 ± 1.33	-4.52 ± 7.11	-3.46, 1.76	-0.382***
Whole sample	18.6 ± 4.1	ASMM <sub>WS</sub>	18.6 ± 3.9	0.922	0.961***	-0.01 ± 1.13	0.44 ± 6.24	-2.22, 2.20	0.139
		ASMM <sub>Kyle</sub>	19.6 ± 4.6	<0.0001	0.960***	-0.97 ± 1.34	-5.07 ± 6.95	-3.60, 1.66	-0.406***

ASMM: appendicular skeletal muscle mass;; ASMM<sub>BIA</sub>: ASMM predicted by the BIA equation; ASMM<sub>DXA</sub>: ASMM measured by dual-energy X-ray absorptiometry; ASMM<sub>DXA-BIA</sub>: ASMM<sub>DXA</sub> less ASMM<sub>BIA</sub> (expressed in kilograms and as a percentage of the ASMM<sub>DXA</sub>); ASMM<sub>Kyle</sub>: ASMM predicted by Kyle's equation; ASMM<sub>WS</sub>: ASMM predicted by the BIA equation derived from the whole sample.

\*\*\* *p* < 0.001.

<sup>a</sup> Intra-class correlation between ASMM<sub>DXA</sub> and ASMM<sub>BIA</sub>.

<sup>b</sup> Simple Pearson correlation between ASMM<sub>(DXA+BIA)/2</sub> and ASMM<sub>DXA-BIA</sub>.

consisted of adults of all ages (and the proportion and characteristics of the elderly subjects involved are not mentioned).

Our study has some limitations. One concerns our use of DXA as the reference method, which is less accurate than underwater weighing, neutron activating analysis, total body potassium, or dilution techniques, for instance. But such methods are invasive and not applicable in the clinical setting, especially in the case of older adults. On the other hand, Kyle et al. took DXA for reference too, and this makes our comparison between the two equations technically more appropriate. Another weakness of our study lies in

that any older adults with significant chronic comorbidities were ruled out. The prevalence of sarcopenia would be higher among such subjects and its identification would be more valuable, so further studies should assess the reliability of our BIA equation for the elderly in this group of subjects too.

In conclusion, the present study proposes a new BIA equation for estimating ASMM in older adults with a view to identifying conditions of pre-sarcopenia and sarcopenia more effectively. Our findings seem to confirm that it would be more appropriate to adopt age-specific equations for the purpose of predicting ASMM in the geriatric population, although Kyle's equation can be used with an acceptable reliability.

#### Statement of authorship

G.S., A. C. and E. M. designed research; F. B., N. V., L. B., S. C. and G. B. acquisition of data; M. D. R. and E. P. analyzed data; and G. S. and M. D. R. wrote the paper. E. M. interpreted data. All authors revised the manuscript critically and approved the final article.

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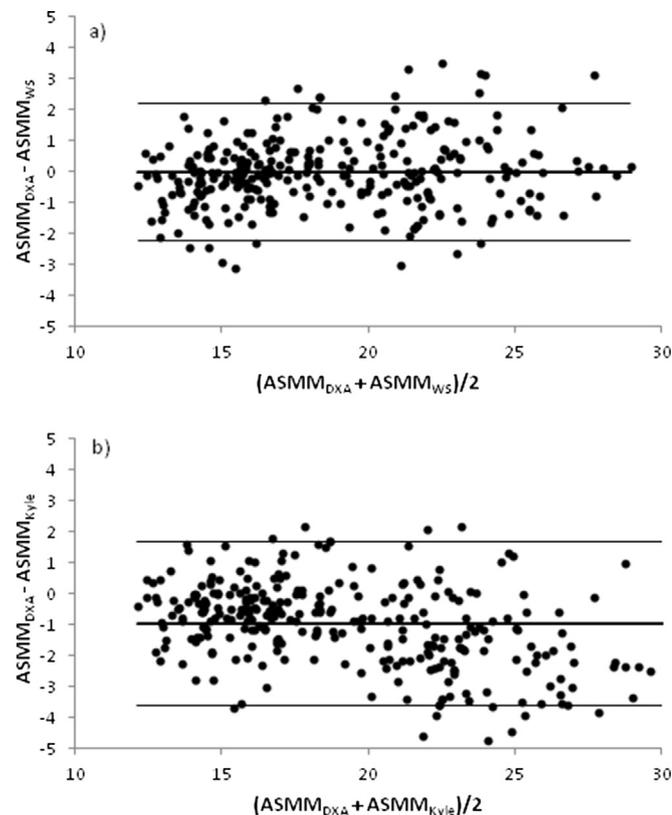
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#### Conflict of interest statement

The authors have no conflicts of interest to declare.

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**Fig. 2.** Bland–Altman plot, for the sample as a whole, of the difference between the appendicular skeletal muscle mass measured by DXA and predicted with the BIA equation derived from the whole sample (plot a) and predicted using Kyle's equation (plot b). Footnotes: The thick line indicates the bias between the measured and predicted ASMM, the thin lines the limits of agreement. ASMM: appendicular skeletal muscle mass (kg); ASMM<sub>DXA</sub>: ASMM measured by DXA; ASMM<sub>Kyle</sub>: ASMM predicted using Kyle's equation; ASMM<sub>WS</sub>: ASMM predicted with the BIA equation derived from the whole sample.

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