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## Original article

## Predicting appendicular lean and fat mass with bioelectrical impedance analysis in older adults with physical function decline – The PROVIDE study

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## SUMMARY

**Background & aims:** No generalizable formulas exist that are derived from bioelectrical impedance analysis (BIA) for predicting appendicular lean mass (ALM) and fat mass (AFM) in sarcopenic older adults. Since precision of regional body composition (BC) data in multicentre trials is essential, this study aimed to: 1) develop and cross-validate soft tissue BIA equations with GE Lunar and Hologic DXA systems as their reference 2) to compare our new ALM equation to two previously published models and 3) to assess the agreement between BIA- and DXA-derived soft tissue ratios as indicators of limb tissue quality.

**Methods:** Two-hundred and ninety-one participants with functional limitations (SPPB-score 4–9; sarcopenia class I or II, measured by BIA) were recruited from 18 study centres in six European countries. BIA equations, using DXA-derived ALM and AFM as the dependent variable, and age, gender, weight, impedance index and reactance as independent variables, were developed using a stepwise multiple linear regression approach.

**Results:** Cross-validation gave rise to 4 equations using the whole sample:

$ALM_{LUNAR} (kg) = 1.821 + (0.168 * height^2 / resistance) + (0.132 * weight) + (0.017 * reactance) - (1.931 * sex)$   
[R<sup>2</sup> = 0.86 and SEE = 1.37 kg]

$AFM_{LUNAR} (kg) = -6.553 - (0.093 * height^2 / resistance) + (0.272 * weight) + (4.295 * sex)$   
[R<sup>2</sup> = 0.70 and SEE = 1.53 kg]

$ALM_{HOLOGIC} (kg) = 4.957 + (0.196 * height^2 / resistance) + (0.060 * weight) - (2.554 * sex)$   
[R<sup>2</sup> = 0.90 and SEE = 1.28 kg]

$AFM_{HOLOGIC} (kg) = -4.716 - (0.142 * height^2 / resistance) + (0.316 * weight) + (4.453 * sex) - (0.040 * reactance)$   
[R<sup>2</sup> = 0.73 and SEE = 1.54 kg]

Both previously published models significantly overestimated ALM in our sample with biases of –0.36 kg to –1.05 kg.

For the ratio of ALM to AFM, a strong correlation (r = 0.82, P < 0.0001) was found between the mean estimate from BIA and the DXA models without significant difference (estimated bias of 0.02 and 95% LOA –0.62, 0.65).

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**Conclusion:** We propose new BIA equations allowing the estimation of appendicular lean and fat mass. Our equations allow to accurately estimate the appendicular lean/fat ratio which might provide information regarding limb tissue quality, in clinical settings. Furthermore, these BIA equations can be applied to characterize sarcopenia with Hologic and Lunar reference values for BC. Previously published BIA-based models tend to overestimate ALM in sarcopenic older adults. Users of both GE Lunar and Hologic may now benefit from these equations in field research.

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## 1. Introduction

One of the most widely used indexes to define sarcopenia is the amount of appendicular lean mass as determined by dual-energy X-ray absorptiometry (DXA). In this context, criteria for sarcopenia based on the amount of lean mass lower than expected for a given fat mass using residuals from regression models and the ratio between appendicular lean mass (ALM) and appendicular fat mass (AFM) have been proposed [1,2]. Whole body DXA provides a precise analysis of regional body composition (BC) at the multi-component molecular-level [3,4]. Although the method's underlying physical basis is the same for all DXA devices, significant differences exist between devices from different manufacturers, and even between different models from the same manufacturer [5]. In the past, it has repeatedly been shown that individual results from Hologic and GE Lunar systems cannot be directly compared [6,7]. Although the relative accuracy between both systems has improved over the last decade with the progression of the technology from pencil-beam to fan-beam X-ray geometry, standardization of BC assessment is still strongly needed. Additionally, NHANES public reference values for BC [8,9] are applicable only to a single model of Hologic DXA systems, complicating their applicability for GE Lunar systems. In attempts to accommodate for these problems in clinical settings and epidemiological studies, regional conversion formulas have been proposed recently and NHANES reference curves applicable to DXA whole body scans acquired on GE Lunar systems have been presented [10,11].

Because radiological devices cannot be used on a regular basis, bioelectrical impedance analysis (BIA) is often regarded as the alternative of choice for the estimation of BC in older adults, given its portability, safety, non-invasiveness and cost-effectiveness. To date, two equations are available for predicting ALM in Caucasian older adults taking DXA as the reference [12,13]. However, as both equations were developed in subjects without physical function decline on a single type of DXA device (Hologic QDR), differences between subject characteristics and manufacturers might possibly affect the accuracy of the BIA equations. Moreover, no AFM prediction equations are available for older persons and the only option is to measure subcutaneous adiposity by skinfolds thicknesses or ultrasound. Nevertheless, the relationship between subcutaneous and total adiposity decreases with aging making predictions less accurate [14]. BIA equations specifically generated from a sample of older subjects with physical function decline would consequently predict ALM and AFM better than equations derived from an age-matched healthy sample population.

Since precision of regional BC data in multicentre trials is essential, this study aimed to: 1) develop and cross-validate soft tissue BIA equations for appendicular lean and fat mass in older adults with physical function decline using both Hologic DXA and GE Lunar systems as their reference 2) to compare our new ALM equation to two other BIA-derived prediction models developed by Kyle et al. [12] and by Sergi et al. [13] and 3) to assess the agreement between BIA- and DXA-derived soft tissue ratios as indicators of limb tissue quality.

## 2. Methods

This study is based in the baseline data of the PROVIDE-study, which is described in detail elsewhere [15].

### 2.1. Participants

Briefly, 380 older persons with functional limitations (Short Physical Performance Battery (SPPB)-score 4–9 as described by Guralnik et al. [16]) and sarcopenia class I or II (based on skeletal muscle mass index according to Janssen et al. [17] estimated by BIA) were recruited from 18 study centres in six European countries for the PROVIDE nutritional intervention study. Participants were eligible to participate if they were older than 65 years of age and if they had body mass index (BMI) between 20 and 30. Participants were excluded if they presented chronic diseases such as cancer, organ failure, cardiovascular disease, acute inflammation (CRP  $\geq 10$  mg/L) or cognitive impairment (Mini Mental State Examination  $<25$ ). Out of the 380 recruited participants 291 subjects having both an evaluable DXA and BIA measurement on baseline have been included in this analysis (see Table 1).

All participants provided written informed consent. The study protocol was approved by the local ethical committees at each location and registered under the Dutch trials register with the identifier: NTR2329 (<http://www.trialregister.nl/trialreg>). The study procedures were in accordance to the World Medical Association's Declaration of Helsinki.

### 2.2. Anthropometry

Body mass was measured in minimal clothing on a digital scale (SECA 877 Hamburg, Germany) to the closest 0.05 kg. Stature was recorded to the nearest 1 mm using a mobile stadiometer (SECA 217 Hamburg, Germany) according to standard procedures based on the International Society for Advancement of Kinanthropometry [18]. *Dual energy X-ray absorptiometry.*

All participants were scanned using a fan beam whole body dual energy X-ray absorptiometry device (Hologic [Bedford, Massachusetts, USA] or GE Medical Systems Lunar, [Madison, Wisconsin, USA], depending on the standard equipment of the study centre). Daily calibration of the densitometers was performed in accordance with the instructions provided by the manufacturer. The scanning procedure was standardised across all study centres using a uniform protocol. All raw DXA data were centrally analysed by the same experienced investigator. Apex system software version 4.0.2 was used to analyse scans obtained from Hologic devices and enCORE™ software version 14.10.022 for those from GE Healthcare Lunar devices. The removal of image pixels in the case of an arthroplasty is automatically performed by enCORE™ software and Apex software. Each image was visually checked and pixels were manually adjusted when needed. Manual adjustment is not available for Apex software.

The regions of interest were delineated using a standardized segmentation protocol and are described elsewhere [3]. The body

**Table 1**

General characteristics of the participants for whole sample and for the groups according to DXA device system.

	Whole sample (n = 291)	Hologic (n = 104)	GE lunar (n = 187)
Women (%)	70.1	62.5	74.3 <sup>a</sup>
Arthroplasty (%)	21.6	22.1	21.4
Sarcopenia class I (%)	84.2	85.6	83.4
Sarcopenia class II (%)	15.8	14.4	16.6
Age	77.6 ± 6.9 (65–99)	78.6 ± 7.2 (66–96)	77.0 ± 6.8 (65–99)
Height (cm)	162.6 ± 9.1 (129–187)	162.9 ± 9.4 (143–186)	162.4 ± 9.0 (129–187)
Weight (kg)	68.8 ± 11.2 (40–99)	68.1 ± 12.0 (47–98)	69.2 ± 10.8 (40–99)
BMI (kg/m <sup>2</sup> )	25.9 ± 2.7 (20.0–30.7)	25.6 ± 3.0 (20.0–30.7)	26.1 ± 2.5 (20.4–30.3)
SPPB-score	7.2 ± 1.7 (4–9)	6.9 ± 1.8 (4–9)	7.4 ± 1.6 (4–9) <sup>b</sup>
LM (kg)	40.2 ± 7.2 (20.5–62.4)	40.6 ± 7.9 (26.1–57.9)	39.9 ± 6.8 (20.5–62.4)
FM (kg)	25.6 ± 6.0 (9–46)	24.3 ± 5.9 (9–36)	26.4 ± 6.0 (10–46) <sup>c</sup>
FM (%)	37.2 ± 5.9 (17.6–54.6)	35.7 ± 6.1 (18.7–47.8)	38.4 (34.4–42.1) <sup>c</sup>
ALM (kg)	17.3 ± 3.8 (7.9–29.3)	16.5 ± 3.9 (9.9–25.5)	17.8 ± 3.6 (7.9–29.3) <sup>c</sup>
ALMI <sub>∑</sub> (kg/m <sup>2</sup> )	7.3 ± 0.8 (5.5–9.3)	7.1 ± 0.7 (5.5–8.7)	7.5 ± 0.8 (5.5–9.3) <sup>c</sup>
ALMI <sub>♀</sub> (kg/m <sup>2</sup> )	6.1 ± 0.8 (4.2–8.5)	5.6 ± 0.6 (4.2–7.6)	6.4 ± 0.7 (4.8–8.5) <sup>c</sup>
AFM (kg)	11.3 ± 2.8 (4.4–20.1)	11.2 ± 2.9 (4.4–17.8)	11.4 ± 2.8 (5.2–20.1)
ALM/AFM-ratio	1.62 ± 0.55 (0.76–3.35)	1.57 ± 0.57 (0.76–3.27)	1.66 ± 0.53 (0.76–3.35) <sup>c</sup>
Resistance (Ω)	607.4 ± 83.5 (420–872)	598.1 ± 90.1 (431–847)	612.5 ± 79.4 (420–872)
Reactance (Ω)	49.5 ± 13.8 (20–137)	47.2 ± 10.2 (20–78)	50.7 ± 15.3 (21–137)
Impedance index (cm <sup>2</sup> /Ω)	44.7 ± 9.4 (20.6–78.0)	45.8 ± 10.2 (28.8–70.7)	44.1 ± 9.0 (20.6–78.0)

Data are presented as frequency or mean ± SD (minimum and maximum); BMI = body mass index; SPPB = short physical performance battery; LM = lean mass; FM = fat mass; ALM = appendicular lean mass; AFM = appendicular fat mass; ALMI = appendicular lean mass index; significantly different from Hologic at  $P < 0.05$ .

<sup>a</sup> Chi-squared test.

<sup>b</sup> independent t-test.

<sup>c</sup> ANCOVA controlling for sex and SPPB-score.

components of interest were: total fat mass (FM), total lean mass (LM), ALM (sum of the lean mass in the limbs), AFM (sum of the fat mass in the limbs), and the ratio of ALM to AFM.

### 2.3. Bioelectrical impedance analysis

Bioelectrical impedance analysis was performed with participants lying supine with their limbs slightly away from their body, after overnight fasting and bladder voiding. 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. At each location the same whole-body tetrapolar BIA device (BIA 101 Akern, Florence, Italy) operating at a weak alternating electrical current of 500 μA–1 mA and a single frequency of 50 kHz was used to measure the voltage drop across body tissues. The voltage drop occurring in the body is the product of the current and the impedance (Z), the latter being the vector sum of resistance (R: restriction of current flow) and reactance (X<sub>c</sub>: capacitance of cell membranes and tissue interfaces). All resistance measurements were normalized for stature (height in centimetres squared/R) to obtain the impedance index (I).

### 2.4. Statistical analysis

Data were analyzed by using IBM<sup>®</sup> SPSS<sup>®</sup> Statistics version 22 (2014, SPSS Inc., New York, USA) or Medcalc version 12 (Medcalc Software, Mariakerke, Belgium). Group data are expressed as mean ± SD. Normality of all data was confirmed using D'Agostino-Pearson tests. Comparison of continuous variables included a Chi-squared test, independent samples t-tests and analysis of covariance (ANCOVA).

DXA system-based generation samples (for elaboration of the prediction equations) were created at random using a (software driven) data-splitting approach assigning approximately two-thirds (Hologic n = 69 and GE Lunar n = 124) of the total sample to the respective validation groups. The remaining one-third of the

subjects (Hologic n = 35 and GE Lunar n = 62) was assigned to the cross-validation groups.

Preliminary equations, using DXA-derived appendicular lean and fat mass as the dependent variable, and age, gender, weight, impedance index and reactance as independent variables, were developed using a stepwise multiple linear regression approach. In the equations only significant regressors of appendicular soft tissue masses were considered. Model performance fit was assessed using multiple correlations (R<sup>2</sup>) and standard errors of the estimate (SEE). For each of the appendicular soft tissue components, the model with the lowest standard error of the estimate was used in the cross-validation analysis. The individual and body composition data from the cross-validation samples were then imputed into the developed equations to assess their accuracy. The statistics used for cross-validation included mean difference, limits of agreement and root mean squared error (RMS<sub>error</sub> =  $\sqrt{\frac{1}{n} \sum (\bar{x} - \hat{x})^2}$ , with  $\bar{x}$  = observed value and  $\hat{x}$  = predicted value). In a second phase final equations were calculated based on the total sample (Hologic n = 104 and GE Lunar n = 187). In order to determine the relative contribution of the predictor variables that went into the models, R<sup>2</sup> cumulative and beta-values (β) were calculated.

Additionally the agreement between ALM<sub>HOLOGIC</sub> estimated in our sample (i.e. ALM<sub>PROVIDE</sub>), ALM<sub>SERGI</sub> and ALM<sub>KYLE</sub> was assessed using Passing and Bablok regression analysis. The equations developed by Kyle et al. [12] and Sergi et al. [13] respectively yield:

$$\begin{aligned} \text{ALM}_{\text{KYLE}} \quad (\text{kg}) &= -4.211 + \left(0.267 * \frac{\text{height}^2}{\text{resistance}}\right) \\ &+ (0.095 * \text{weight}) + (1.909 * \text{sex}) + (0.058 * \text{reactance}) - (0.012 * \text{age}) \\ \text{ALM}_{\text{SERGI}} \quad (\text{kg}) &= -3.964 + \left(0.227 * \frac{\text{height}^2}{\text{resistance}}\right) \\ &+ (0.095 * \text{weight}) + (1.384 * \text{sex}) + (0.064 * \text{reactance}) \text{ where} \\ &\text{men} = 1 \text{ and women} = 0. \end{aligned}$$

Finally, the agreement between the ALM/AFM-ratios estimated by DXA and by BIA was evaluated using Bland and Altman analysis. The ALM/AFM-ratios by DXA were calculated separately for Hologic and for GE Lunar. The ALM/AFM-ratios by BIA

were calculated by using the appropriate formula's according to DXA manufacturer and compared to their corresponding DXA reference.

### 3. Results

#### 3.1. Participant's characteristics

The general characteristics of the participants are shown in Table 1. The sample as a whole included 87 men and 204 women, aged between 65 and 99 years. Ninety percent of the participants lived independently, 4% received home-care and 6% were institutionalised. The mean SPPB-score was  $7.2 \pm 1.7$ , the mean Mini Nutritional Assessment score was  $13.1 \pm 1.3$  and the mean Mini Mental State Examination score was  $28.4 \pm 1.6$ . One fifth of the participants had undergone joint replacement surgery: 23 participants had a unilateral hip arthroplasty, 16 had bilateral hip replacement and 10 knee joint arthroplasty.

Table 1 also shows the characteristics of the participants according to DXA device used. The female/male ratio and the SPPB score were significantly higher ( $P < 0.05$ ) in the GE Lunar group compared to the Hologic group. After controlling for sex and SPPB-score, FM, FM%, ALM and the ALM/AFM ratio were significantly higher ( $P < 0.05$ ) in the GE Lunar group.

#### 3.2. Derivation and cross-validation of preliminary BIA equations

Regression models estimating DXA-derived appendicular soft tissue masses using anthropometric and BIA variables were derived for Hologic and GE Lunar groups separately. Validation and cross-validation samples were not significantly different ( $P > 0.05$ ) with regard to independent and dependent variables (data not shown). The best predictive preliminary equations obtained in the validation samples are given in Table 2. In both DXA groups the BIA-based multiple linear regression models predicted the DXA-derived appendicular soft tissue masses with good accuracy ( $P < 0.001$ ). Each equation contained two to four variables with  $R^2$ -values ranging from 0.70 to 0.90. All BIA equations with Hologic as the criterion method included impedance index, weight and gender. The equations with GE Lunar as their reference all included weight and gender, but not reactance.

Cross-validation revealed no significant differences ( $P > 0.05$ ) between DXA-observed and BIA-predicted appendicular soft tissue masses, with mean biases lower than 100 g for ALM and of about 250 g for AFM (Table 2). The limits of agreement for ALM ranged from 2.5 kg to 2.8 kg for Hologic and GE Lunar respectively. For AFM the limits of agreement ranged from 3.3 kg to 3.8 kg for GE Lunar and Hologic respectively. In general  $RMS_{\text{errors}}$  were lower for ALM compared with AFM in both DXA models.

#### 3.3. Derivation of the final BIA equations

Four BIA equations using the whole sample were developed (Table 3). Compared to the BIA equations using Lunar as reference standard, the BIA equations based on Hologic showed higher coefficients of determination and lower SEE's. The main contributor to the ALM equations was the impedance index, which explained 77.2% and 85.2% of the variance respectively. The variables that contributed the most to the prediction of AFM were weight and sex explaining together 68.2% and 69.3% of the variance.

#### 3.4. Comparison with existing ASMM<sub>HOLOGIC</sub> models

Despite a high correlation ( $r = 0.87$ ,  $P < 0.0001$ ),  $ALM_{\text{KYLE}}$  significantly ( $P < 0.0001$ ) overestimated  $ALM_{\text{PROVIDE}}$  (Fig. 1). Passing-Bablok regression indicated a systematic (constant and proportional) difference between predicted  $ALM_{\text{PROVIDE}}$  and  $ALM_{\text{KYLE}}$ . The estimated bias was  $-1.05$  kg (95%CI  $-1.40$  to  $-0.69$  kg) and 95% limits of agreement were  $(-4.61$  kg,  $2.52$  kg).

$ALM_{\text{PROVIDE}}$  and  $ALM_{\text{SERGI}}$  showed a high correlation ( $r = 0.88$ ,  $P < 0.0001$ ) with a significant difference ( $P = 0.047$ ). Passing-Bablok regression indicated a systematic (constant and proportional) difference between  $ALM_{\text{PROVIDE}}$  and  $ALM_{\text{SERGI}}$  (Fig. 1). The estimated bias was  $-0.36$  kg (95%CI  $-0.72$  to  $0.00$  kg) and 95% limits of agreement were  $(-3.95$  kg,  $3.23$  kg).

#### 3.5. Agreement between appendicular soft tissue ratios determined by BIA versus DXA

For the ratio of ALM to AFM, a strong correlation ( $r = 0.82$ ,  $P < 0.0001$ ) was found between the mean estimate from DXA and BIA without significant difference (estimated bias of 0.02 and 95% limits of agreement  $-0.62$ ,  $0.65$ ) (Fig. 2). Table 4 shows the results of the comparisons for each DXA system separately.

## 4. Discussion

We propose new BIA equations for predicting appendicular soft tissue masses in older Caucasians with physical function decline. Accurate estimation of ALM and AFM in ageing adults is particularly important because the age-related decrease in muscle mass and concomitant increase in adipose tissue mass may lead to sarcopenia. From a biomechanical point of view, it has been shown that appendicular soft tissues may influence the magnitude of forces transmitted through the body [19]. In this context, the proportion of ALM to AFM may represent an important aspect of impulsive impact control. From a public health perspective, it is not inconceivable that excess AFM, especially in deep limb compartments (intra- and perimuscular), may have a negative influence on metabolism by enhancing inflammation and insulin resistance [20].

**Table 2**  
Preliminary BIA prediction equations and cross-validation statistics for appendicular soft tissue masses in sarcopenic older persons.

	Validation			Cross-validation			
	Preliminary equation	$R^2$	SEE	P	Mean difference ( $-1.96SD$ , $+1.96SD$ )	$RMS_{\text{error}}$	P
$ALM_{\text{HOLOGIC}}$ (kg)	$3.431 + (0.179 \cdot I) + (0.091 \cdot W) - (2.126 \cdot S)$	0.896	1.322	<0.0001	0.046 ( $-2.467$ , $2.562$ )	1.265	0.828
$AFM_{\text{HOLOGIC}}$ (kg)	$-3.556 - (0.132 \cdot I) + (0.292 \cdot W) + (4.379 \cdot S) - (0.040 \cdot X_C)$	0.758	1.283	<0.0001	$-0.224$ ( $-4.074$ , $3.627$ )	1.949	0.505
$ALM_{\text{LUNAR}}$ (kg)	$3.929 + (0.151 \cdot I) + (0.130 \cdot W) - (2.356 \cdot S)$	0.832	1.391	<0.0001	0.092 ( $-2.697$ , $2.881$ )	1.257	0.609
$AFM_{\text{LUNAR}}$ (kg)	$-8.376 + (0.230 \cdot W) + (5.307 \cdot S)$	0.704	1.536	<0.0001	0.264 ( $-3.028$ , $3.556$ )	1.493	0.216

ALM = appendicular lean mass, AFM = appendicular fat mass, I = impedance index ( $\text{cm}^2/\Omega$ ), W = weight (kg), S = sex (men = 0, women = 1),  $X_C$  = reactance ( $\Omega$ ), SEE = standard error of the estimate,  $RMS_{\text{error}}$  = root mean squared error.

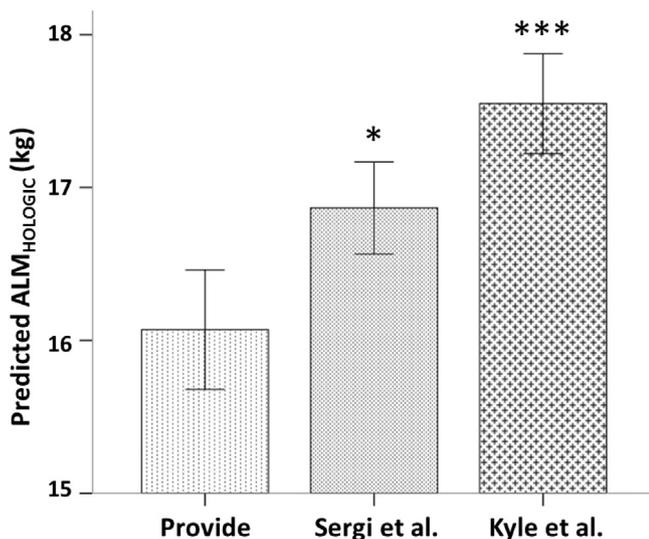
**Table 3**

Multiple linear regression model and explanatory value of the predictor variables included in the appendicular soft tissue BIA equations.

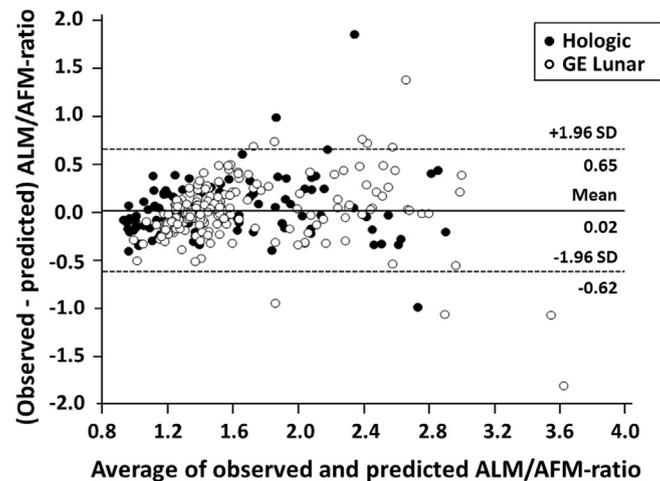
	Unstandardized $\beta$ -value	Standardized $\beta$ -value	P-value	R <sup>2</sup> cumulative	SEE
<b>ALM<sub>HOLOGIC</sub></b>					
Intercept	4.957				
Impedance index	0.196	0.513	<0.0001	0.852	1.503
Sex	-2.554	-0.319	<0.0001	0.887	1.323
Weight	0.060	0.187	0.005	0.895	1.278
<b>AFM<sub>HOLOGIC</sub></b>					
Intercept	-4.716				
Weight	0.316	1.306	<0.0001	0.211	2.596
Sex	4.453	0.745	<0.0001	0.693	1.627
Impedance index	0.142	-0.497	0.001	0.718	1.568
Reactance	-0.040	-0.140	0.025	0.732	1.54
<b>ALM<sub>LUNAR</sub></b>					
Intercept	1.821				
Impedance index	0.168	0.415	<0.0001	0.772	1.746
Weight	0.132	0.391	<0.0001	0.829	1.517
Sex	-1.931	-0.232	<0.0001	0.856	1.393
Reactance	0.017	0.072	0.014	0.860	1.374
<b>AFM<sub>LUNAR</sub></b>					
Intercept	-6.553				
Weight	0.272	1.055	<0.0001	0.213	2.481
Sex	4.295	0.676	<0.0001	0.682	1.582
Impedance index	0.093	-0.300	<0.0001	0.704	1.531

ALM = appendicular lean mass, AFM = appendicular fat mass, SEE = standard error of the estimate.

The impedance index ( $\text{height}^2/\text{resistance}$ ) was the strongest independent predictor of ALM as measured by Hologic and GE Lunar, explaining 85% and 77% of the variability respectively. This observation is in agreement with others [12,13]. Even though the predictive value of the impedance index was slightly higher in the Hologic-derived equations compared to the Lunar-derived ones, the error (SEE) for predicting ALM was similar in both DXA systems (9.1% for Hologic vs. 9.8% Lunar). Furthermore, our results indicate that weight and sex are significant independent predictors of ALM<sub>HOLOGIC</sub>, but not reactance. The latter observation might be



**Fig. 1.** Appendicular lean mass (ALM) predicted by our formula (Provide) compared to the formula's developed by Sergi et al. [13] and Kyle et al. [12], \* $P < 0.05$ , \*\*\* $P < 0.0001$ , error bars represent 95% confidence interval of the mean.



**Fig. 2.** Bland-Altman for the difference between the DXA-observed and BIA-predicted appendicular soft tissue ratio. The solid line represents the mean difference. The dashed lines represent the limits of agreement. ALM = appendicular lean mass, AFM = appendicular fat mass.

somewhat surprising since reactance is related to capacitance properties of the cell membranes including skeletal muscle cells [21]. However, since reactance only marginally contributes to the prediction of ALM and given the fact that it is low in older and ill subjects [22] we suggest that reactance fails to improve the predictive value of ALM in subjects with sarcopenia. This might explain why reactance was not a significant predictor in our ALM<sub>HOLOGIC</sub> equation. This was also found by Pietrobelli et al. [23] who failed to improve the predictive value of their equation by adding phase angle, an indicator of cell membrane integrity.

Although AFM may provide relevant information about limb BC quality, its prediction using BIA is less accurate compared to ALM, with coefficients of determination ranging from 0.70 to 0.73. This might be the result of the smaller weight and the greater inter-individual variability in the fat compartment compared to the lean compartment in limbs of older persons (Fig. 3). The order of entry of the predictor variables for AFM was weight, sex and subsequently impedance index. This makes sense since fat is poorly conductive and thus is excluded from the current flow of BIA [24]. Therefore it is suggested that impedance and/or reactance are more likely to provide clinically useful information about fluid volume and distribution than about fat.

The proposed ALM equations seem to be more reliable when applied to sarcopenic older adults than the ones previously published in the literature [12,13], which were developed in adults without physical function decline. The accuracy and precision of the newly proposed Hologic equation for predicting appendicular lean mass was compared to previously published equations. The existing models of Kyle et al. [12] and of Sergi et al. [13] over-estimated significantly lean-DXA outcome. The functionality of the subjects in Sergi's sample was substantially higher compared to the physical performance in our sample. Only 5% had an SPPB-score  $\leq 8$  in the former compared to 62% in our study. In the present study, all subjects had a low skeletal muscle mass index (i.e.  $\leq 37\%$  men and  $\leq 28\%$  women) measured by BIA [15]. However, according to the definition of sarcopenia proposed by the Health ABC Study (i.e. ALM index  $< 5.67 \text{ kg/m}^2$  for women and  $< 7.23 \text{ kg/m}^2$  for men) 62% of the women and 54% of the men in the Hologic group were sarcopenic, while only 33% and 14% in the Lunar group respectively [1]. This confirms that the methodology used for the assessment of skeletal muscle mass may lead to discrepancies in prevalence rates of sarcopenia in clinical and epidemiological studies [25].

**Table 4**  
Agreement between the observed and the predicted ALM to AFM ratio according to DXA manufacturer system.

	Observed ratio by DXA (Mean $\pm$ SD)	Predicted ratio by BIA (Mean $\pm$ SD)	r (95% CI)	P-value	Mean difference (95% CI)	Limits of agreement (-1.96SD, + 1.96SD)	P-value
Hologic	1.57 $\pm$ 0.57	1.54 $\pm$ 0.53	0.83 (0.76–0.88)	<0.0001	-0.03 (-0.09–0.03)	-0.66, 0.60	0.369
GE Lunar	1.66 $\pm$ 0.53	1.65 $\pm$ 0.53	0.81 (0.76–0.86)	<0.0001	-0.01 (-0.06–0.03)	-0.65, 0.63	0.588

ALM = appendicular lean mass, AFM = appendicular fat mass, CI = confidence interval, SD = standard deviation.

Despite their success in other fields the use of BC ratios has not found extensive application in nutritional research. As a result, no published data are available on the ALM/AFM ratio in older subjects. This makes the interpretation of this ratio difficult in relation to physical function decline. Since its relation with sarcopenia may also vary, further studies should assess the usefulness of this ratio for screening of older persons with physical function decline and sarcopenia. We propose that the ratio can be used to explain the potential mismatch between lean mass and strength because of a progressive deterioration of muscle 'quality' mainly by fat-infiltration around and within muscle fibers. The two most important parameters to clinically assess the ALM/AFM-ratio are resistance and weight as expressed by their standardized  $\beta$ -values. For the clinician it might be of added value to monitor the change in ALM/AFM-ratio (~limb quality) by following-up resistance (~most important contributor to AFM) and weight (most important contributor to AFM) over time. As such, lowering resistance with weight stabilization or stabilizing resistance with lowering weight may be indicative of successful treatment.

#### 4.1. Study limitations

DXA was used as the reference method for appendicular lean mass (i.e. the sum of proteins and water) [26,27]. It has to be emphasized that DXA cannot distinguish skeletal muscle from other lean components such as skin, connective tissue and blood vessels [28]. For quantifying muscle and/or adipose tissue only tissue-system level multi-component models (e.g. MRI, CT) can be considered gold standards. Although it has been shown that lean DXA in the limbs is significantly interrelated to muscle mass in older persons, variability in the distribution of water over different tissue compartments may have affected the accuracy of the prediction [29]. For example, water in the extracellular space and

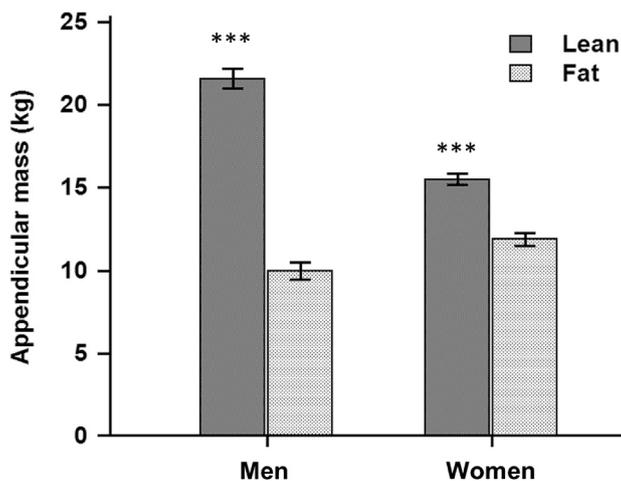
adipose tissue compartment is considered to be intracellular muscle fluid. It is also to be expected that overhydrated vs. underhydrated limbs may significantly differ in their lean/muscle ratio.

The newly proposed formulas apply to whole body BIA devices that produce raw data (resistance, reactance) at a single frequency of 50 kHz. This implies that these equations are not validated for segmental BIA devices (foot-to-foot, hand-to-hand) and multi-frequency analyzers (e.g. bioimpedance spectroscopy devices). However, the majority of BIA devices used in clinical settings operate at a fixed frequency of 50 kHz, and thus we expect that our proposed equations can be widely implemented in daily practice. Since BIA equations rely on body tissue resistance, compartmental shifts in body water may equally influence BIA. Unfortunately, no direct measure of extracellular hydration was available. Thus, we do not know whether this factor had any effect on the study outcome. Further, to the knowledge of the authors, studies reporting the interrater technical error of measurement (TEM) of 'raw' BIA parameters are scarce. In a study of adolescents Vicente-Rodriguez et al. [30] showed a significant interrater effect for the impedance components, resistance and reactance at one study site, but no effect was found at another site. Although they found no statistical difference in body composition, it was suggested that their observations required further research. In our study no assessment of interrater reliability of the users of BIA was made. Although the authors recognize its importance in multicentre trials, this was practically and financially unfeasible. This bias, however, was minimized by restricting BC analysis to well-trained clinicians with an expertise in the use of BIA. According to the same investigators the interrater TEM for resistance yielded 16.7 $\Omega$  or 2.8%. Given the fact that resistance is the denominator in the impedance index of both the lean and fat mass equations in our study, the ratio ALM/AFM would hardly change.

Finally, the subjects assessed by Hologic and GE Lunar were similar but not identical. Because of the lower male/female ratio in the GE Lunar group we expected ALM to be significantly lower in this group compared to the Hologic group. However, this was not the case. It is therefore suggested that Hologic and GE Lunar use different hardware and software equations to estimate BC. Consequently BC results obtained by both manufacturers should not be used interchangeably without correcting for manufacturer model.

#### 5. Conclusion

We propose new BIA equations allowing the estimation of appendicular lean and fat mass. Our equations allow to accurately estimate the appendicular lean/fat ratio, which might provide information regarding limb tissue quality in clinical settings. Furthermore, these BIA equations can be applied to characterize sarcopenia with Hologic and Lunar reference values for BC. Previously published BIA-based models tend to overestimate ALM in sarcopenic older adults. Users of both GE Lunar and Hologic may now benefit from these equations in field research. Further research should examine the suitability of the appendicular lean to fat ratio for defining sarcopenia.



**Fig. 3.** Appendicular soft tissue distribution measured by dual energy X-ray absorptiometry in 87 men and 204 women. \*\*\*P < 0.0001, error bars represent 95% confidence interval of the mean.

## Statement of authorship

AS and IB participated in the conception and design of the study. All the authors participated in the interpretation of the data, contributed to writing the manuscript and the final approval of the submitted version. The corresponding author had full access to all the baseline data in the PROVIDE study and had final responsibility for the decision to submit for publication. The members of the Provide study group include Kirsten Brandt, Lorenzo M. Donini, Marcello Maggio, Marion E.T. McMurdo, Chris Seal, Sander L. Wijers, Gian Paolo Ceda, Giuseppe De Vito, Gilbert Donders, Michael Drey, Carolyn Greig, Ulf Holmbäck, Marco Narici, Jamie McPhee, Eleonora Poggiogalle, Dermot Power and Ralf Schultz.

## Conflict of interest

The authors, the Frailty in Ageing research group and the Radiology department of the Vrije Universiteit Brussel have no conflicts of interest to declare. The sponsors of this study had no role in the study conception, data analysis and interpretation, or writing the report. All authors have completed the Unified Competing Interest form and declare that no one has received support for submitting this work.

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## References

- [1] Newman AB, Kupelian V, Visser M, Simonsick E, Goodpaster B, Nevitt M, et al. Health ABC study investigators. Sarcopenia: alternative definitions and associations with lower extremity function. *J Am Geriatr Soc* 2003;51:1602–9.
- [2] Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, Ferrucci L. Sarcopenic obesity: definition, cause and consequences. *Curr Opin Clin Nutr Metab Care* 2008;11:693–700.
- [3] Scafoglieri A, Tresignie J, Provyn S, Marfell-Jones M, Reilly T, Bautmans I, et al. Prediction of segmental lean mass using anthropometric variables in young adults. *J Sports Sci* 2012;30:777–85.
- [4] Hind K, Oldroyd B. In-vivo precision of the GE Lunar iDXA densitometer for the measurement of appendicular and trunk lean and fat mass. *Eur J Clin Nutr* 2013;67:1331–3.
- [5] Covey MK, Berry JK, Hacker ED. Regional body composition: cross-calibration of DXA scanners—QDR4500W and discovery Wi. *Obesity (Silver Spring)* 2010;18:632–7.
- [6] Modlesky CM, Lewis RD, Yetman KA, Rose B, Roszkopf LB, Snow TK, et al. Comparison of body composition and bone mineral measurements from two DXA instruments in young men. *Am J Clin Nutr* 1996;64:669–76.
- [7] Kistorp CN, Svendsen OL. Body composition analysis by dual energy X-ray absorptiometry in female diabetics differ between manufacturers. *Eur J Clin Nutr* 1997;51:449–54.
- [8] Kelly TL, Wilson KE, Heymsfield SB. Dual energy X-Ray absorptiometry body composition reference values from NHANES. *PLoS One* 2009;4:e7038.
- [9] Borrud LG, Flegal KM, Looker AC, Everhart JE, Harris TB, Shepherd JA. Body composition data for individuals 8 years of age and older: U.S. population, 1999–2004. *Vital Health Stat* 2010;1–87. Series 11, Data from the National Survey.
- [10] Shepherd JA, Fan B, Lu Y, Wu XP, Wacker WK, Ergun DL, et al. A multinational study to develop universal standardization of whole-body bone density and composition using GE healthcare lunar and hologic DXA systems. *J Bone Min Res* 2012;27:2208–16.
- [11] Fan B, Shepherd JA, Levine MA, Steinberg D, Wacker W, Barden HS, et al. National health and nutrition examination survey whole-body dual-energy X-ray absorptiometry reference data for GE lunar systems. *J Clin Densitom* 2014;17:344–77.
- [12] Kyle UG, Genton L, Hans D, Pichard C. Validation of a bioelectrical impedance analysis equation to predict appendicular skeletal muscle mass (ASMM). *Clin Nutr* 2003;22:537–43.
- [13] Sergi G, De Rui M, Veronese N, Bolzetta F, Berton L, Carraro S, et al. Assessing appendicular skeletal muscle mass with bioelectrical impedance analysis in free-living Caucasian older adults. *Clin Nutr* 2015;34:667–73.
- [14] Scafoglieri A, Clarys JP, Cattrysse E, Bautmans I. Use of anthropometry for the prediction of regional body tissue distribution in adults: benefits and limitations in clinical practice. *Aging Dis* 2013a;5:373–93.
- [15] Bauer JM, Verlaan S, Bautmans I, Brandt K, Donini LM, Maggio M, et al. Effects of a Vitamin D and leucine-enriched whey protein nutritional supplement on measures of Sarcopenia in older adults, the provide study: a randomized, double-blind, placebo-controlled trial. *J Am Med Dir Assoc* 2015;16:740–7.
- [16] Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;49:M85–94.
- [17] Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc* 2002;50:889–96.
- [18] Marfell-Jones M, Olds T, Stewart AD, Carter JEL. International standards for anthropometric assessment. Potchefstroom, Republic of South Africa: North-West University; 2006.
- [19] Burkhart TA, Arthurs KL, Andrews DM. Reliability of upper and lower extremity anthropometric measurements and the effect on tissue mass predictions. *J Biomech* 2008;41:1604–10.
- [20] Khan IM, Perrard XY, Brunner G, Lui H, Sparks LM, Smith SR, et al. Inter-muscular and perimuscular fat expansion in obesity correlates with skeletal muscle T cell and macrophage infiltration and insulin resistance. *Int J Obes (Lond)* 2015;39:1607–18.
- [21] Barbosa-Silva MC, Barros AJ, Wang J, Heymsfield SB, Pierson Jr RN. Bioelectrical impedance analysis: population reference values for phase angle by age and sex. *Am J Clin Nutr* 2005;82:49–52.
- [22] Slee A, Birc D, Stokoe D. Bioelectrical impedance vector analysis, phase-angle assessment and relationship with malnutrition risk in a cohort of frail older hospital patients in the United Kingdom. *Nutrition* 2015;31:132–7.
- [23] Pietrobelli A, Morini P, Battistini N, Chiumello G, Nuñez C, Heymsfield SB. Appendicular skeletal muscle mass: prediction from multiple frequency segmental bioimpedance analysis. *Eur J Clin Nutr* 1998;52:507–11.
- [24] Foster KR, Lukaski HC. Whole-body impedance—what does it measure? *Am J Clin Nutr* 1996;64:388S–96S.
- [25] Müller MJ, Geisler C, Pourhassan M, Glüer CC, Bosy-Westphal A. Assessment and definition of lean body mass deficiency in the elderly. *Eur J Clin Nutr* 2014;68:1220–7.
- [26] Fosbol MO, Zerahn B. Contemporary methods of body composition measurement. *Clin Physiol Funct Imaging* 2015;35:81–97.
- [27] Toombs RJ, Ducher G, Shepherd JA, De Souza MJ. The impact of recent technological advances on the trueness and precision of DXA to assess body composition. *Obesity (Silver Spring)* 2012;20:30–9.
- [28] Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual energy X-ray absorptiometry body composition model: review of physical concepts. *Am J Physiol* 1996;271:E941–51.
- [29] Scafoglieri A, Deklerck R, Tresignie J, De Mey J, Clarys JP, Bautmans I. Assessment of regional adipose tissue depots: a DXA and CT comparison in cadavers of elderly persons. *Exp Gerontol* 2013b;48:985–91.
- [30] Vicente-Rodriguez G, Rey-Lopez JP, Masana MI, Poortvliet E, Ortega FB, Polito A, et al. Moreno LA on behalf of the HELENA study group. Reliability and intermethod agreement of body fat assessment among two field and two laboratory methods in adolescents. *Obesity* 2012;20:221–8.