Evaluation of appendicular lean mass using bio impedance in persons aged 80+: a new equation based on the BUTTERFLY-study

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Abbreviations list

ALM  Appendicular Lean Mass

ALMI  Appendicular Lean Mass Index

BC  Body Composition

BIA  Bioelectrical Impedance Analysis

BMC  Bone Mineral Content

BUTTERFLY  BrUssels sTudy on The Early pRedictors of FraiLtY

CT  Computerized Tomography
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<th>Term</th>
<th>Definition</th>
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<td>DXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
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<td>47</td>
<td>EWGSOP</td>
<td>European Working Group on Sarcopenia in Older People</td>
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<td>FFM</td>
<td>Fat Free Mass</td>
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<td>49</td>
<td>FM</td>
<td>Fat Mass</td>
</tr>
<tr>
<td>50</td>
<td>I</td>
<td>Impedance</td>
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<td>51</td>
<td>IWGS</td>
<td>International Working Group on Sarcopenia</td>
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<td>LST</td>
<td>Lean Soft Tissue</td>
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<td>53</td>
<td>MMSE</td>
<td>Mini Mental State Examination</td>
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<tr>
<td>54</td>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>55</td>
<td>R</td>
<td>Resistance</td>
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<td>56</td>
<td>SMM</td>
<td>Skeletal Muscle Mass</td>
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<td>UZ Brussel</td>
<td>Universitary Hospital Brussels</td>
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<td>VUB</td>
<td>Vrije Universiteit Brussel</td>
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Abstract

Background: To date, the accuracy of bio-impedance (BIA) to assess body composition & sarcopenia in persons aged 80 and over remains unclear.

Objective: We aimed to evaluate the agreement between dual energy X-ray absorptiometry (DXA) and BIA equations to determine lean mass, as well as their suitability to identify sarcopenia.

Design: 174 community dwelling well-functioning persons (83 women, 91 men) aged 80 and over were included. Appendicular lean mass (ALM) was predicted using BIA-based equations available in literature, and compared to DXA outcomes. Through cross-validation and stepwise multiple linear regression, an ALM-formula was generated suitable for this population.

Results: Literature-based BIA equations systematically overestimated ALM. The new prediction formula that we propose for the 80+ is:

\[ ALM = 0.827 + (0.19 \times \text{Impedance Index}) + (2.101 \times \text{Sex}) + (0.079 \times \text{Weight}); \quad R^2 = 0.888; \]

\[ \text{SEE} = 1.450 \text{kg} \]

Sarcopenia classification based on our new BIA equation for ALM showed better agreement with DXA \((k \geq 0.454)\) compared to literature-based BIA equations \((k < 0.368)\).

Conclusions: Despite the high correlation between both methods, literature-based BIA equations consistently overestimate ALM compared to DXA in persons aged 80 and over. We proposed a new equation for ALM, reaching higher agreement with DXA and thus improving the accuracy of BIA for this specific age group.

Keywords: body composition, sarcopenia, bioelectrical impedance analysis, dual X-ray absorptiometry, aged 80 and over
Introduction

Body Composition (BC) data provide valuable information and are often used to represent the changes in muscular function associated with ageing (1). One of the most important conditions occurring in an ageing population is sarcopenia, which was initially described by Rosenberg in 1989 (2). Baumgartner was the first to report the prevalence of this phenomenon (3). He defined sarcopenia solely by decreased appendicular skeletal muscle mass, measured by dual energy X-ray absorptiometry (DXA). Nowadays, sarcopenia is a widely studied phenomenon caused by i.a. inflammation, nutritional deficiencies and chronic diseases. This age-related syndrome is known to be highly associated with functional decline, disability and frailty, which highlights the importance of research on this condition (4, 5).

Several working groups defined different consensus based diagnoses of sarcopenia. The European Working Group on Sarcopenia in Older People (EWGSOP) recommended using the presence of both low muscle mass and low muscle function (strength or performance) as diagnostic criteria for sarcopenia whereas the International Working Group on Sarcopenia (IWGS) proposed to use the loss of muscle mass alone or in conjunction with increased fat mass (5-7). Besides the age-related loss of muscle mass, other changes in BC which may be partly responsible for shifts in muscle weakness (such as increasing intramuscular fat) are considered (8). After all, ageing is linked with the redistribution of fat mass to ectopic locations, such as skeletal muscles and liver (9, 10). This awakens the interest in the introduction of fat mass as an alternative definition of sarcopenia, which has been suggested by several researchers (11-13).

Generally accepted methods for the assessment of muscle mass are Magnetic Resonance Imaging (MRI) and Computerized Tomography (CT). Given their reliability and preciseness, they are considered as gold standards (7, 14). However, they come with a high cost and a low
accessibility (15). The use of DXA in order to measure BC, in terms of lean soft tissue, fat mass and bone mineral content of young and older subjects, is widely accepted (16, 17). Despite the fact that some disadvantages have been recognized in literature, it is often used as criterion method (17, 18). However, the DXA instrument is expensive and non-portable, which does not facilitate its use in clinical practice (19). Bioelectrical Impedance Analysis (BIA) may be considered as an interesting option, offering an inexpensive, portable alternative for DXA enabling rapid and accurate estimates of lean mass via prediction formulas. BIA, however, tends to slightly overestimate lean mass (20). Both measurement methods, BIA (21) and DXA (22), are currently being used in the assessment of sarcopenia in the population of older adults.

Several prediction formulas have been developed to determine ALM using BIA (23-25). The prediction formulas found in literature focus on older adults aged 60 or 65 years and over (23, 25) or on a younger cohort (24). BC, however, changes significantly over time, even in the oldest old (26). For this specific age group, no prediction formulas or gender-specific cut-offs for the classification of sarcopenia exist.

The overall aim of this study is to determine the prevalence of sarcopenia in a well-functioning community dwelling population aged 80 and over, by using BIA. We will compare DXA-based and BIA-based ALM data. Subsequently, a new prediction formula for ALM will be created. Next, we will analyze the prevalence of sarcopenia in this population in terms of the EWGSOP cut-offs, by comparing the DXA-measured and BIA-predicted appendicular lean mass index (ALMI). Both existing and newly suggested prediction formulas for ALM are analyzed. Finally, cut-offs for the classification of sarcopenia based on this cohort are evaluated.
1. Materials and Methods

2.1 Study design

Body composition data were collected in the BUTTERFLY study (BrUssels sTudy on The Early pRedictors of FraiLtY), a longitudinal observational cohort study in the oldest old, originating from the Vrije Universiteit Brussel (Belgium). This study was approved by the ethical committee of UZ Brussel (B.U.N. 143201421976). Informed consent was obtained from all participants and the privacy rights of human subjects was observed at all times. For this article, the baseline data were used and cross-sectionally analyzed. The STROBE checklist for cohort, case-control, and cross-sectional studies was used as a reporting guideline (27).

2.2 Setting and Participants

Between February 2015 and April 2017, community dwelling well-functioning adults (male or female) aged 80 years and over were recruited for participation in the BUTTERFLY study. Volunteers were recruited through advertisements on site at the hospital, at the university, via health insurance companies, general practitioners and pharmacies. They were invited at the University Hospital in Jette, Belgium, for an extensive test battery. People underwent standard medical tests (blood analysis, lung and cardiac function control, depression history, assessment of comorbidities etc.), physical tests (body composition, muscle strength and endurance tests, walking speed etc.) and psychosocial tests (questionnaires for social behavior, relationships, cognitive functioning etc.) in order to determine medical, physical and psychosocial capacities.

Participants were allowed to take part in the study if they were aged 80 years or older and if they were able to walk, lived independently at home and if they were mentally fit (MMSE>23/30). Volunteers were excluded if they were recently diagnosed with cancer or if they underwent surgery or any radiotherapy or chemotherapy during the past six months. Also,
in case of a planned surgery, radiotherapy or chemotherapy in the near future, participation in the study was not allowed.

2.3 Variables and measurement methods

**Anthropometry**

Anthropometry included the measurement of weight, height, waist and hip circumference. Weight was measured using a SECA scale (model 877, type 3) to the closest of 0.1 kg. Height was determined using a measuring rod to the nearest of 0.1 cm, which was incorporated in the SECA balance. Waist and hip circumferences were obtained using a flexible steel measuring tape (Lufkin, W606PM). After complete expiration, waist circumference was measured above the upper most lateral boarder of the ilium. Hip circumference was taken around the widest portion of the buttocks. Both circumferences were measured up to the nearest of 0.1 cm.

**Dual Energy X-Ray Absorptiometry**

Body composition of the participants was measured using a fan beam whole body DXA device (Hologic 4500 QDR upgraded to Discovery [Bedford, Massachusetts, USA]). The DXA scan machine is able to distinguish fat mass (FM), bone mineral content (BMC) and lean soft tissue (LST) on the basis of tissue density using two X-ray beams with differing energy levels (28, 29). The DXA instrument was calibrated daily using the spine phantom provided by the manufacturer. Additionally, a step phantom calibration was performed on a weekly basis. For standardization purposes of the scans, the files from the original DXA machine were transferred to a computer where they were analyzed using Apex system software version 4.0.2. The scans were blinded and independently processed by two different researchers. The segmentation protocol as described by Scafoglieri et al. was used to uniform measurements (30).
Bioelectrical Impedance Analysis (BIA)

Body composition of the participants was also measured using BIA, a method based on the principle that various human tissues have different conductive and resistive properties at different frequencies of an administered alternating electrical current (31). BIA measures resistance (R) and reactance (Xc) parameters through which appendicular lean mass (ALM), skeletal muscle mass (SMM) and fat-free mass (FFM) can be estimated using different prediction formulas. We used the 50 kHz frequency of the Single-Frequency Bodystat® QuadScan 4000 with long electrodes (ME400). All equation formulas applied in this study were developed using single-frequency BIA, which corresponds to our measurement method.

Participants were positioned in supine position on an examination table with their arms slightly separated from the body and their legs spread. They had to stay in this position for 5 minutes before the BIA measurement could be performed (during these 5 minutes the participants underwent the DXA measurement). The four contact points on the skin were degreased before placing the electrodes on the right hand and foot. Electrodes were placed at the metacarpal-phalangeal joints and the metatarsal-phalangeal joints on the dorsal surface of both right hand and foot.

Regression formulas

For comparison between the obtained DXA data and BIA data, three BIA equation formulas for ALM were used (23-25). All formulas were validated against DXA Hologic. A new regression formula for ALM will be proposed for this specific age group, based on data from our study population.
Table 1: Summary of prediction formulas for ALM

<table>
<thead>
<tr>
<th>Author</th>
<th>Regression Formula</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyle et al. 2003</td>
<td>ALM = -4,211+(0,267<em>H²/R)+(0,095</em>W)+(1,909<em>sex)-(0,012</em>age)+(0,058*Xc)</td>
<td>0,95</td>
<td>&lt; 0,001</td>
</tr>
<tr>
<td>Sergi et al. 2015</td>
<td>ALM = -3,964+(0,227<em>H²/R)+(0,095</em>W)+(1,384<em>sex)+(0,064</em>Xc)</td>
<td>0,94</td>
<td>&lt; 0,001</td>
</tr>
<tr>
<td>Scafoglieri et al. 2017</td>
<td>ALM = 4,957+(0,196<em>H²/R)+(0,06</em>W)-(2,554*sex)</td>
<td>0,90</td>
<td>&lt; 0,001</td>
</tr>
</tbody>
</table>

DXA = dual energy X-ray absorptiometry; H = height (cm); R = resistance (Ω); W = weight (kg); Xc = reactance (Ω); sex = men: 1, women: 0, r=correlation

2.4 Sarcopenia

EWGSOP composed an algorithm for sarcopenia case findings in older individuals in 2010 (7). This algorithm consists of three consecutive measurements: gait speed, grip strength and muscle mass, each with their own cut-off values. These cut-offs are used for the classification of sarcopenia (7). Since the focus of this study is specifically aimed at appendicular muscle mass, only those cut-offs defined by EWGSOP were used for the classification of sarcopenia. Data from three BIA equation formulas for ALM (Kyle et al., Sergi et al. and Scafoglieri et al.) were each compared to the EWGSOP cut-offs (3, 13, 32). Therefore, our ALM values were subjected to a transformation consisting of a correction for body height (ALM/height²), since cut-off values were presented as appendicular lean mass corrected for height.

2.5 Statistical methods

Statistical analysis was performed using SPSS version 24.0 (2016, SPSS Inc. New York, USA). The Kolmogorov-Smirnov Goodness of Fit test was used to determine the normal distribution of the population. Descriptive statistics of the study population are presented as mean ± standard deviation (SD). Low muscle mass in the characteristics is defined according to Delmonico’s cut-offs (32). The values of the BIA equations for the prediction of ALM were compared with
the data obtained by DXA measurements. Since ALM was measured using DXA as a criterion reference, paired samples t-tests and Pearson’s correlation coefficients were used in order to establish differences and correlations between measurement methods (33). To determine the 95% interval of the differences between the two measurement methods, limits of agreements were calculated. To visualize the level of agreement between methods, Bland and Altman plots were created. In order to create a new prediction equation for the 80+, first an at random allocation was performed to divide the cohort into two groups: 70% of the sample for validation and 30% for cross-validation (both groups contained an equal proportion of males/females and sarcopenic/non-sarcopenic people). Then, a preliminary equation for ALM was calculated using stepwise multiple linear regression in the 70% group. Independent variables were age, sex, weight, impedance index (height in cm²/resistance), reactance and waist-hip ratio. Evaluation of the equation was based on multiple correlations (R²) and standard errors of the estimate (SEE). Cross-validation was performed in the 30% group, including mean differences, correlations and RMS_error as statistics. Ultimately, one final equation was calculated for ALM based on the total sample, using stepwise multiple linear regression. Agreement for the classification of sarcopenia by BIA and DXA measurements was performed by using a Cohen’s kappa. For interpreting these results, the guidelines provided by Landis & Koch were used (34). To determine gender-specific cut-offs for this age group, we performed a Receiver-Operating-Characteristics (ROC) analysis. For a sensitivity of minimum 95%, which was set a priori in order to minimize the number of false negatives, we aimed for a specificity of minimum 85% to ensure its clinical usefulness.

2. Results
2.1 Participants

So far, 189 older adults participated in the BUTTERFLY-study. However, not all of them provided all the data needed for the analysis of body composition. Five participants with a pacemaker were excluded from the analysis, as this is a contraindication for performing the BIA-assessment. Ten participants were excluded, since no DXA-scan was performed. One hundred and seventy-four participants (83 women and 91 men, age range 80-95) were finally considered for analysis. Characteristics of the study population as well as the DXA and BIA outcomes are summarized in Table 2. Mean values regarding BMI were 26.4 ± 3.9 kg/m² for women and 27.0 ± 3.2 kg/m² for men. When considering the waist-hip ratio of our population, a mean of 0.90 for women and 0.98 for men was found, which can be considered as obese and overweight, respectively (35). In total, 19.5% of the participants had undergone a total joint replacement (mainly hip or knee arthroplasty), but this explained only 2.4% of the variance in ALM. Low or normal muscle mass was determined according to Delmonico’s DXA-based cut-offs for the diagnosis of sarcopenia (Men: <7.25 kg/m², women <5.67 kg/m²), assigning almost 44% of the total population with low muscle mass (32).
## Table 2: Characteristics of the study population

<table>
<thead>
<tr>
<th>General Characteristics</th>
<th>Total (n=174)</th>
<th>Men (n=91)</th>
<th>Women (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>83.3 ± 3.0</td>
<td>83.3 ± 2.9</td>
<td>83.3 ± 3.0</td>
</tr>
<tr>
<td><strong>MMSE (score/30)</strong></td>
<td>27.9 ± 2.0</td>
<td>28.3 ± 1.7</td>
<td>27.3 ± 2.1*</td>
</tr>
<tr>
<td><strong>Handgrip strength (kPa)</strong></td>
<td>57.5 ± 17.1</td>
<td>67.5 ± 16.5</td>
<td>46.6 ± 9.2*</td>
</tr>
<tr>
<td><strong>Gait speed (m/s)</strong></td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1.0 ± 0.2†</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td>3.5 ± 2.1</td>
<td>3.1 ± 2.1</td>
<td>4.0 ± 2.1†</td>
</tr>
<tr>
<td><strong>Arthroplasty (%)</strong></td>
<td>20%</td>
<td>15%</td>
<td>24%</td>
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</table>

### Anthropometrics

<table>
<thead>
<tr>
<th></th>
<th>Total (n=174)</th>
<th>Men (n=91)</th>
<th>Women (n=83)</th>
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</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td>163.7 ± 8.7</td>
<td>169.2 ± 7.0</td>
<td>157.7 ± 6.0*</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>71.9 ± 12.5</td>
<td>77.5 ± 11.4</td>
<td>65.8 ± 10.6*</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26.8 ± 3.5</td>
<td>27.0 ± 3.2</td>
<td>26.4 ± 3.9</td>
</tr>
<tr>
<td><strong>Waist-Hip ratio</strong></td>
<td>0.94 ± 0.09</td>
<td>0.98 ± 0.07</td>
<td>0.90 ± 0.09*</td>
</tr>
</tbody>
</table>

### Dual Energy X-ray Absorptiometry

<table>
<thead>
<tr>
<th></th>
<th>Total (n=174)</th>
<th>Men (n=91)</th>
<th>Women (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lean Mass (kg)</strong></td>
<td>43.9 ± 8.7</td>
<td>50.2 ± 6.4</td>
<td>37.0 ± 4.5*</td>
</tr>
<tr>
<td><strong>Appendicular Lean Mass (kg)</strong></td>
<td>18.4 ± 4.3</td>
<td>21.5 ± 3.2</td>
<td>14.9 ± 2.2*</td>
</tr>
<tr>
<td><strong>Low muscle mass (n)</strong></td>
<td>76</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

### Bioelectrical Impedance Analysis

<table>
<thead>
<tr>
<th></th>
<th>Total (n=174)</th>
<th>Men (n=91)</th>
<th>Women (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistance (Ω)</strong></td>
<td>488.6 ± 76.5</td>
<td>442.2 ± 52.9</td>
<td>539.5 ± 65.3*</td>
</tr>
<tr>
<td><strong>Reactance (Ω)</strong></td>
<td>42.9 ± 9.1</td>
<td>40.4 ± 5.8</td>
<td>45.5 ± 11.1*</td>
</tr>
<tr>
<td><strong>Impedance Index (cm²/Ω)</strong></td>
<td>56.8 ± 13.0</td>
<td>65.8 ± 9.8</td>
<td>47.0 ± 8.0*</td>
</tr>
</tbody>
</table>

Mean and standard deviation (SD) are raw data obtained by anthropometrics, DXA scans and BIA values. MMSE=Mini Mental State Examination; kPa=KiloPascal; m/s=meter/second; low muscle mass according to Delmonico et al. (2007); Ω = ohm; *p<0.001; †p<0.05

### 2.2 Comparison of ALM (DXA vs BIA)

ALM was measured using DXA as a criterion reference. BIA equation formulas from different researchers (Kyle et al., Sergi et al. and Scafoglieri et al.) were used to predict ALM. Means as well as standard deviations of ALM are described in Table 3. High correlations were found...
between DXA and BIA equations for ALM; 0.93, 0.92 and 0.93 for the equations proposed by Kyle et al., Sergi et al. and Scafoglieri et al., respectively.

Mean differences between methods (ALM_{DXA} – ALM_{BIA}) were calculated for each formula (Table 3). The smallest mean difference was found for BIA_{Sergi}: 0.88 kg (95%CI [0.64 to 1.13 kg]; p<0.001). The highest mean difference was found using BIA_{Kyle}: 1.94 kg (95%CI [1.67 to 2.22 kg]; p<0.001). According to those data, BIA has the tendency to overestimate ALM. To visualize the level of agreement between methods, Bland and Altman plots were created (figure 1).

Table 3: Summary of statistics

<table>
<thead>
<tr>
<th>Author prediction formula</th>
<th>BIA predicted values (kg)</th>
<th>DXA observed values (kg)</th>
<th>Mean difference (kg) (95% CI)</th>
<th>Limits of agreement (kg)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kyle et al. 2003</td>
<td>20.3 ± 4.9</td>
<td>18.4 ± 4.3</td>
<td>1.94 (1.67; 2.22)</td>
<td>-1.66; 5.54</td>
<td>0.93*</td>
</tr>
<tr>
<td>Sergi et al. 2015</td>
<td>19.3 ± 4.2</td>
<td>18.4 ± 4.3</td>
<td>0.88 (0.64; 1.13)</td>
<td>-2.34; 4.11</td>
<td>0.92*</td>
</tr>
<tr>
<td>Scafoglieri et al. 2017</td>
<td>19.5 ± 3.9</td>
<td>18.4 ± 4.3</td>
<td>1.06 (0.81; 1.30)</td>
<td>-2.11; 4.22</td>
<td>0.93*</td>
</tr>
</tbody>
</table>

Mean and standard deviations (SD) of observed values by DXA and predicted values by BIA are shown. CI = confidence interval; Limits of agreement were calculated as mean difference ± 1.96 times SD; r = Pearson’s Correlation; *p< 0.001
Figure 1: Bland and Altman plots: ALM according to DXA and BIA

Bland and Altman plots to show the agreement between DXA and BIA for the measurement of ALM. The solid line represents the mean, dotted lines illustrate the upper and lower limits of agreement.
2.3 Derivation of a BIA-equation for ALM

**Preliminary equation and cross-validation**

After the random allocation of the cohort into two comparable groups (70% and 30%), a preliminary BIA-equation for ALM was calculated using multiple linear regression models. Independent variables were age (years), sex (0=women, 1=men), weight (kg), impedance index (height in cm²/resistance (Ω)), reactance (Ω) and waist-hip ratio (analysis with waist circumference and hip circumference separately provided the same results). Selected variables for the formula were impedance index, weight and sex.

Cross-validation in the 30% group was successful (table 4), with no significant differences between DXA-derived and BIA-observed ALM (p<0.001). A low mean difference of 143g was established. It should be noted that in this analysis BIA overestimates ALM compared to DXA.
Table 4: Cross-validated prediction formula

<table>
<thead>
<tr>
<th>Preliminary BIA-equations (70% of cohort)</th>
<th>Cross-validation (30% of cohort)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation formula</td>
<td>R²</td>
</tr>
<tr>
<td>ALM: 0.684 + (0.175<em>I) + (0.092</em>W) + (2.279*S)</td>
<td>0.884</td>
</tr>
</tbody>
</table>

ALM = Appendicular Lean Mass, R² = Coefficient of determination, SEE = Standard Error of the Estimate, r = Pearson’s correlation, RMSerror = Root Mean Squared error, I = Impedance index (cm²/Ω), W = Weight (kg), S = Sex (women=0, men=1), Xc = Reactance (Ω); *p<0.001

**Final BIA-equation**

Given the successful cross-validation, a new BIA equation formula for ALM was developed using the whole sample. The impedance index was the most substantial predictor with an R² of 0.836. Cumulative R² (combined with sex and weight) explained up to 89% of the variability (table 5). Table 5 displays the new prediction formula constructed from the complete sample and figure 2 shows the Bland and Altman plot on the agreement between DXA and BIAButterfly.

Table 5: Final BIA-equation formulas for ALM

<table>
<thead>
<tr>
<th>Equation</th>
<th>R²</th>
<th>SEE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALM: 0.827 + (0.19<em>I) + (2.101</em>S) + (0.079*W)</td>
<td>0.888</td>
<td>1.450</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ALM = Appendicular Lean Mass, R² = Coefficient of determination, SEE = Standard Error of the Estimate, I = Impedance index (cm²/Ω), W = Weight (kg), S = Sex (women=0, men=1)

Figure 2: Bland and Altman plot: ALM according to DXA and BIAButterfly
2.4 Prevalence and agreement of sarcopenia

Values for ALM originating from the existing and new BIA prediction formulas were corrected for height, according to the standard reference cut-offs for the diagnosis of sarcopenia proposed by EWGSOP, and based on ALM (7). They were then compared to their matching cut-offs in order to diagnose people as either normal or sarcopenic. Results of these comparisons are presented in table 6.
Table 6: Prevalence of sarcopenia by DXA and BIA

<table>
<thead>
<tr>
<th>EWGSOP Cut-off</th>
<th>Prevalence of Sarcopenia</th>
<th>Prediction formula BIA</th>
<th>Prevalence of Sarcopenia</th>
<th>Cohen's Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total n(%)</td>
<td>Male (n)</td>
<td>Female (n)</td>
<td>Total n(%)</td>
</tr>
<tr>
<td>Baumgartner</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men: &lt; 7,26 kg/m²</td>
<td>59 (34%)</td>
<td>38</td>
<td>21</td>
<td>Kyle et al (2003)</td>
</tr>
<tr>
<td>Women: &lt; 5,5 kg/m²</td>
<td></td>
<td></td>
<td></td>
<td>Sergi et al (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scafoglieri et al (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BUTTERFLY (2017)</td>
</tr>
<tr>
<td>Delmonico</td>
<td></td>
<td></td>
<td></td>
<td>Kyle et al (2003)</td>
</tr>
<tr>
<td>Men: &lt; 7,25 kg/m²</td>
<td>76 (44%)</td>
<td>38</td>
<td>38</td>
<td>Sergi et al (2015)</td>
</tr>
<tr>
<td>Women: &lt; 5,67 kg/m²</td>
<td></td>
<td></td>
<td></td>
<td>Scafoglieri et al (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BUTTERFLY (2017)</td>
</tr>
<tr>
<td>Newman</td>
<td></td>
<td></td>
<td></td>
<td>Kyle et al (2003)</td>
</tr>
<tr>
<td>Men: &lt; 7,23 kg/m²</td>
<td>75 (43%)</td>
<td>37</td>
<td>38</td>
<td>Sergi et al (2015)</td>
</tr>
<tr>
<td>Women: &lt; 5,67 kg/m²</td>
<td></td>
<td></td>
<td></td>
<td>Scafoglieri et al (2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BUTTERFLY (2017)</td>
</tr>
</tbody>
</table>

Prevalence of sarcopenia according to DXA-measurements and BIA-prediction formulas. Agreement is determined with Cohen’s kappa. All kappa’s are p<0.001
When using Baumgartner’s cut-offs, the classification resulted in 59 people (34%) diagnosed as sarcopenic (3). By applying Newman’s and Delmonico’s cut-offs on the other hand, a larger number of subjects (n=76 (44%) and n=75 (43%), respectively) was diagnosed with sarcopenia (table 6) (13, 32).

**BIA existing formulas**

For each gender specific cut-off suggested by EWGSOP, the prevalence of sarcopenia based on the existing equation formulas is presented in table 6. According to BIA\textsubscript{Sergi} and BIA\textsubscript{Scafoglieri}, sarcopenia is more frequent in men. BIA\textsubscript{Kyle} does not result in differences between males and females, but does show an obviously lower prevalence of sarcopenia than the other formulas.

The Cohen’s kappa values never exceeded 0.4, which indicates slight to fair agreement (36). The highest agreement was found when comparing ALMI (Appendicular Lean Mass Index) for DXA with BIA\textsubscript{Sergi} using Baumgartner’s cut-off values (k= 0,37). The lowest agreement was found when comparing ALMI for DXA with BIA\textsubscript{Kyle} using Delmonico’s and Newman’s cut-off values (k= 0,19). All Cohen’s kappa’s were significant. ALMI by BIA\textsubscript{Sergi} classified the highest number of participants as sarcopenic for all three cut-offs. In general, when comparing results from ALMI for DXA with these of BIA, the number of participants diagnosed with sarcopenia was higher for DXA for all three cut-off values.

**BIA BUTTERFLY**

With our new prediction formula, a higher prevalence of sarcopenia was established, regardless the EWGSOP cut-off ( 6). A moderate agreement was established for all cut-offs: Baumgartner k=0,45; Delmonico k=0,51 and Newman k=0,48, implying greater accordance with DXA measurements.

In figure 3, a comparison is made between DXA, the existing prediction formulas and the newly proposed formula for ALM (Butterfly) corrected for height (kg/m²). The cut-offs for sarcopenia according to
Delmonico et al (2007) are represented by the horizontal lines. DXA measured and BIA_Butterfly predicted ALMI show the highest agreement with the suggested cut-offs.

![Figure 3: Appendicular Lean Mass Index according to DXA and ALM prediction formulas](image)

Sex specific bar plots on the mean ALMI observed by DXA and predicted by BIA prediction formulas. ALM was corrected for height (kg/m²). The horizontal lines represent Delmonico’s cut-offs for sarcopenia (Men: < 7.25 kg/m²; Women: < 5.67 kg/m²)

2.5 BIA-based cut-offs for the classification of sarcopenia

A Receiver-Operating-Characteristics (ROC) analysis was performed in order to verify whether gender-specific cut-offs for sarcopenia can be proposed. The AUC for women was 0.77 and for men 0.86. For the women in our cohort, with a sensitivity higher than 95%, we reached a specificity of 40% at the cut-off level of 5.49 kg/m². For the male participants, the same level of sensitivity yielded a specificity of 47% at the cut-off level of 6.98 kg/m².
3. Discussion

The main aim of this study was to analyze the prevalence of sarcopenia in a well-functioning community dwelling population aged 80 years and over, by using bio-electrical impedance analysis. Subsequently, the agreement between DXA measured and BIA predicted ALMI data was calculated for the classification of sarcopenia.

This study focuses on a group of older adults aged 80 years and over, a group that is highly underrepresented in terms of research for body composition. Similar studies were mostly performed considering either geriatric (20), hospitalized (37) or younger population groups (38). Therefore, a comparison with previously reported outcomes might appear to be divergent. When describing our participants (table 2), we defined low muscle mass based on the DXA-based cut-offs suggested by Delmonico et al (2007) (32). We chose to apply this cut-off since it implicates the smallest chance for false negatives, which is an important reasoning for clinical practice.

Prediction formulas

In accordance with previous prediction formulas found in literature, certain parameters were suggested to calculate a final prediction formula through stepwise multiple linear regression: age, sex, weight, impedance index, reactance, and waist-hip ratio (23-25). Impedance index, sex and weight were consistently present in all formulas found in literature, as well as in our newly suggested prediction formula.

Despite the high correlations found for DXA measured and BIA predicted ALM, a systematic overestimation by BIA was found, in accordance with existing literature (20, 37, 39). The smallest mean difference was found for BIA_{Sergi}, 0.88 kg (95%CI [0.64 to 1.13 kg]; p<0.001) which can be considered evident since their formula was composed for healthy Caucasian older adults (23). Since the equation of Kyle was composed for a population aged 20 to 94 it might not come as a surprise that this mean difference was the highest of all three (1.94 kg (95%CI [1.67 to 2.22 kg]; p<0.001)) (24). The equation proposed by
Scafoglieri et al. was developed for a population with functional limitations. Considering the age of their population (77.6 ± 6.9), the rather low mean difference can be explained (25).

**Sarcopenia**

Interestingly, there has been some disagreement in literature on the prevalence of sarcopenia. We expected this well-functioning population to be comparable to other investigated robust populations (23). However, our study population showed remarkably more sarcopenic subjects than in some studies (40-42), but less than in other studies (43). Based on the existing prediction formulas, all Cohen’s kappa’s showed slight to fair agreement (table 6). The highest kappa was found each time when using Baumgartner’s cut-offs. It is striking that the EWGSOP cut-offs are much more divergent for women (max. difference of 0.17kg/m²) than for men (max. difference of 0.03kg/m²). This implies that 10% more women are found sarcopenic according to Delmonico’s and Newman’s cut-offs compared to Baumgartner, by applying the Butterfly ALMI. This is an interesting finding, emphasizing the limitation of applying cut-offs for clinical decision making and the importance of the grey zone around these cut-offs. The strongest agreement for the classification of sarcopenia was found between DXA and BIA_Butterfly. Nevertheless, no substantial agreement was found for this classification. Given the high age of our study population, heterogeneity might partly explain why these kappa’s are not higher. Another explanation might be that EWGSOP cut-offs used to classify into sarcopenic and non-sarcopenic were based on studies using DXA as reference method. BIA-based cut-offs for absolute muscle mass are also made available by EWGSOP, but none for appendicular lean mass (7). This highlights the fact that no BIA-based cut-offs for ALMI have been described for the classification of sarcopenia. Therefore, we performed a Receiver-Operating-Characteristics (ROC) analysis, to verify whether gender-specific cut-offs for sarcopenia can be proposed. Although a fair AUC was found for women (0.77) and for men (0.86), no good cut-offs for clinical practice could be established. Abiding by the sensitivity level which was initially set at 95%, we obtained a low specificity (40% for women, 47% for men). These results are not satisfying in reaching a similar identification of sarcopenia as
 Nevertheless, we should aim for higher agreement when suggesting the use of BIA in clinical practice, to obtain a minimum of false negatives.

The introduction of fat mass or appendicular fat mass might be of added value for the definition of sarcopenia. When looking at the changes in lean and fat mass with ageing, it becomes clear that there is not only a decrease in muscle mass, but also an increase in ectopic and visceral fat, partly emerging in muscle and other organs. Given the importance of fat mass in the assessment of BC by BIA in the context of sarcopenia, and given the previous argumentation on a lack of agreement between the DXA versus BIA based classification of sarcopenia, we want to suggest research on the importance of fat in the identification of sarcopenia in clinical practice. There is an increasing amount of studies on the relation between fat and sarcopenia, in terms of biomarkers or lean/fat proportions (44). Throughout the years, alternative definitions for BMI, such as waist-hip ratio, have been proposed since they are more able to predict visceral fat and possibly associated health risks (45, 46). Furthermore, obesity tends to induce inflammatory processes, which on their turn lead to sarcopenia (47). Combining these findings with the concept of sarcopenic obesity, which was described by Baumgartner et al (48), further research on the relationship between sarcopenia and obesity in our study population is very interesting and clinically relevant (49, 50).

Consequently, we want to follow previous research in suggesting alternative definitions for sarcopenia (12, 13).

Strengths and limitations of the study

To our knowledge, this study is the first to suggest a BIA-based ALM prediction formula for the oldest old, encouraging sarcopenia research in this fast growing age group. Focusing on this group, however, might influence generalizability of our results. We suggest further research of the newly obtained prediction formula for ALM in a wider context. In this study, DXA was used as reference method for determining ALM. However, tissue-system level multicomponent models, such as CT and MRI, are considered the gold standards. (7, 14) Unfortunately, we did not have access to those devices. Since the predetermined criterion
reference is DXA, and given the fact that EWGSOP only suggests DXA-validated cut-offs for the classification of sarcopenia based on appendicular skeletal muscle mass, we focused on the available literature-based prediction formulas for ALM. Following this reasoning, we chose not to use a number of published formulas for total skeletal muscle mass. Nevertheless, this might be important for sarcopenia classification.

4. Conclusion

A very high positive correlation (all $R \geq 0.92$) was found for appendicular lean mass obtained by BIA equations compared to DXA. Despite these correlations, a systematic overestimation of ALM was found. A new BIA prediction formula was suggested for ALM, based on our cohort of well-functioning community dwelling adults aged 80 years and over. For all BIA prediction formulas discussed, an underestimation for the prevalence of sarcopenia was observed by comparing DXA to BIA, which was confirmed by the rather low Cohen’s kappa values found. Since the EWGSOP cut-offs for ALMI were based on DXA, we suggested BIA-based cut-offs to determine the prevalence of sarcopenia. Unfortunately, those cut-offs did not reach a sufficient level of sensitivity and specificity. Further research to realize good sarcopenia classification based on BIA-derived equations, possibly including reasonings around fat mass, is thus necessary.
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Statement of authorship

All authors have made substantial contributions to
- The conception and design of the study, or acquisition of data, or analysis and interpretation of data; AND
- Drafting the article or revising it critically for important intellectual content; AND
- Have given their final approval of the version to be submitted.

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Jansen B: 1, 3
Delaere A: 2, 5
Antoine A: 2, 5
Bautmans I: 1, 3, 4, 5, 6
Scafoglieri A: 1, 3, 4, 5

The Gerontopole Brussels Study group: 1, 3

1. designed research (project conception, development of overall research plan, and study oversight);

2. conducted research (hands-on conduct of the experiments and data collection);

3. provided essential reagents or provided essential materials (contributed by providing constructs, databases, etc, necessary for research);

4. analyzed data or performed statistical analysis;

5. wrote paper (major contribution);

6. had primary responsibility for final content.

Conflict of interest

The authors have no other conflict of interest to declare.
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