



## Original article

# New bioelectrical impedance vector references and phase angle centile curves in 4,367 adults: The need for an urgent update after 30 years



Francesco Campa<sup>a,1</sup>, Giuseppe Coratella<sup>b,\*</sup>, Giuseppe Cerullo<sup>a</sup>, Silvia Stagi<sup>c</sup>, Samuele Paoli<sup>d</sup>, Sofia Marini<sup>e</sup>, Alessia Grigoletto<sup>e</sup>, Alessia Moroni<sup>f</sup>, Cristian Petri<sup>g</sup>, Angela Andreoli<sup>h</sup>, Chiara Ceolin<sup>j</sup>, Raffaella Degan<sup>f</sup>, Pascal Izzicupo<sup>i</sup>, Giuseppe Sergi<sup>j</sup>, Gabriele Mascherini<sup>k</sup>, Margherita Micheletti Cremasco<sup>f</sup>, Elisabetta Marini<sup>c</sup>, Stefania Toselli<sup>e</sup>, Tatiana Moro<sup>a</sup>, Antonio Paoli<sup>a</sup>

<sup>a</sup> Department of Biomedical Sciences, University of Padua, Padua, Italy

<sup>b</sup> Department of Biomedical Sciences for Health, Università Degli Studi di Milano, Milano, Italy

<sup>c</sup> Department of Life and Environmental Sciences, University of Cagliari, Cittadella Universitaria, Monserrato, Cagliari, Italy

<sup>d</sup> Department of Statistical Sciences, University of Padua, Padua, Italy

<sup>e</sup> Department for Life Quality Studies, University of Bologna, Rimini, Italy

<sup>f</sup> Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy

<sup>g</sup> Department of Sports and Computer Science, Section of Physical Education and Sports, Universidad Pablo de Olavide, Seville, Spain

<sup>h</sup> Department of Systems Medicine, University of Tor Vergata, Rome, Italy

<sup>i</sup> Department of Medicine and Aging Sciences, "G. D'Annunzio" University of Chieti-Pescara, Chieti, Italy

<sup>j</sup> Department of Medicine, Geriatrics Division, University of Padua, Padua, Italy

<sup>k</sup> Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

## ARTICLE INFO

## Article history:

Received 27 April 2023

Accepted 27 July 2023

## Keywords:

BIA

BIVA

Body composition

Health

Sport

Nutrition

## SUMMARY

**Background & aims:** The bioelectrical impedance vector analysis (BIVA) represents a qualitative analysis of body composition. The vector, defined by resistance (R) and reactance (Xc) standardized by stature, can be evaluated compared to the 50%, 75%, and 95% tolerance ellipses representative of the reference populations. The tolerance ellipses for healthy adults have been provided in 1995 and were developed by mixing underage, adult, and elderly subjects, possibly misrepresenting the actual adult population. The current multicentric, cross-sectional study aimed to provide new tolerance ellipses specific for the general adult population and as a secondary aim to present centile curves for the bioelectrical phase angle.

**Methods:** R, Xc, and phase angle were measured in 2137 and 2230 males and females using phase-sensitive foot-to-hand analyzers at 50 kHz. A minimum of 35 subjects were included for each sex and age category from 18 to 65 years.

**Results:** The new mean vectors showed a leftward shift on the R–Xc graph with respect to the former reference values (males:  $F = 75.3$ ;  $p < 0.001$ ; females:  $F = 36.6$ ,  $p < 0.001$ ). The results provided new 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th percentile curves for phase angle, identifying time point phases of decrement (males:  $-0.03^\circ$  per year at 33.0–51.0 years and  $-0.05^\circ$  per year after 51 years; females:  $-0.03^\circ$  per year from 37.2 to 57.9 years).

**Conclusions:** Compared to the original references, the new data are characterized by a different distribution within the R-Xc graph with a higher phase angle. Thirty years after the BIVA invention, the current study presents new tolerance ellipses and phase angle reference values for the adult population.

© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

\* Corresponding author. Department of Biomedical Sciences for Health, Università degli Studi di Milano, Via Giuseppe Colombo 71, 20133, Milano, Italy.

E-mail address: [giuseppe.coratella@unimi.it](mailto:giuseppe.coratella@unimi.it) (G. Coratella).

<sup>1</sup> Francesco Campa and Giuseppe Coratella have equally contributed to the work.

## 1. Introduction

The bioelectrical impedance analysis (BIA) has been proposed as a low-cost, portable, and time-efficient method for assessing body composition [1,2]. Based on the electrical properties of each biological structure, pioneering studies have first used BIA to examine the correlation of the impedance with the blood flow conductive volume [3] and then with the total body water [4,5]. At the end of the 1980's Lukaski [6] and other authors [7–13] started to transfer the theoretical basics of BIA to the assessment of different body composition components. In detail, the bioelectrical impedance is geometrically composed by resistance (R) and reactance (Xc) [impedance =  $(R^2 + Xc^2) * 0.5$ ]. The bioelectrical R represents the opposition offered by the body to the flow of an alternating electrical current and is inversely related to the water and electrolyte content of the body [4]. The bioelectrical Xc, is related to the capacitance properties of the cell membrane and to variations that can occur depending on its integrity [4]. Additionally, the capacitance causes the administered current to lag behind the voltage, and it creates a phase shift that is represented by the bioelectrical phase angle [4]. Regardless of the technology used for assessing the bioelectrical features, the term “phase-sensitive devices” is commonly used in the biomedical literature to describe those devices that provide not only the impedance value, but also show its factors (R and Xc) and phase angle [14,15].

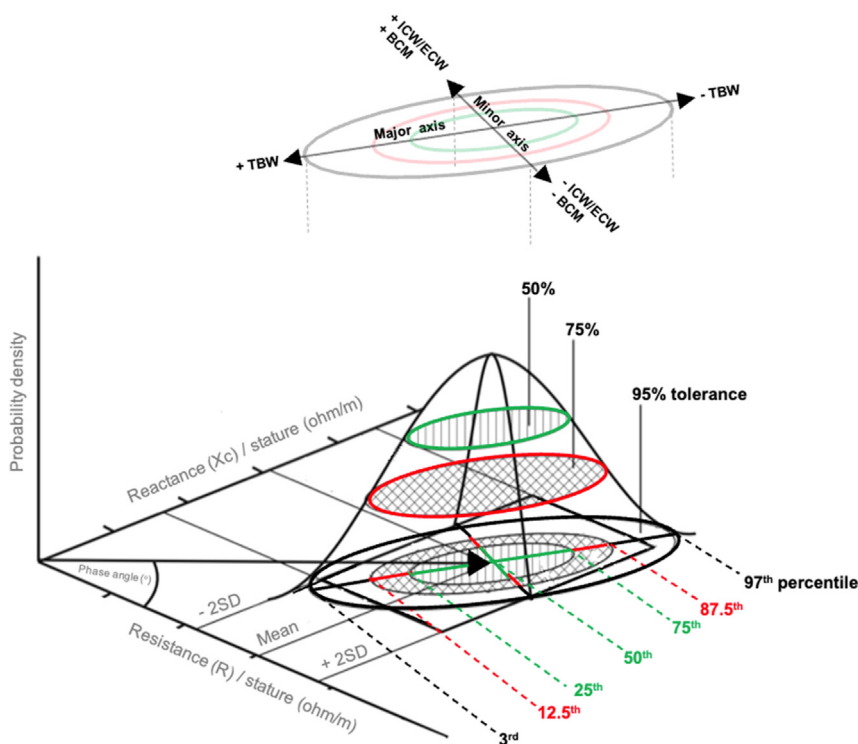
Since then, the BIA has been used to estimate body fluids and other body mass components through predictive equations [14]. However, the predictive equations show high accuracy only when the subjects' characteristics comply with the characteristics of the population for which the predictive equations have been developed [14,16]. Indeed, since the very first predictive equations were based on different pooled populations, following studies have created specific predictive equations for given populations [14,16]. Notwithstanding, some devices do not allow a customized use of the predictive equations and predict body composition regardless of the subject's characteristics [1]. While progressions have been made in developing population-targeted predictive equations, it was immediately clear that the optimal use of the BIA to predict body composition would have required several years to develop a wide range of population-specific predictive equations, differentiating them for each body mass component [17]. Not to mention that even the optimal predictive equations contain a minimum standard error of estimation, and that the proliferation of regression equations hampers the comparability of the results.

For all the reasons above, in 1994 Piccoli and colleagues tried to develop an alternative approach to estimate the variation in body fluids using the BIA [17]. The authors used the whole-body R and Xc values derived from a 50-kHz signal, normalized for the subject's stature and plotted on the R-Xc graph, and yields a vector that has a length and a direction [17]. With the bioelectrical impedance vector analysis (BIVA), the length of the vector is inversely related to the total body water [17]. Additionally the vector direction, defined as the phase angle, was initially interpreted as the amount of body cell mass [17], and subsequently as an indicator of the fluid distribution among the intra and extracellular spaces [18–20]. Such a method permits a qualitative analysis, thus addressing the previous limitations concerning the regression error of estimations, the technical error in the reference methods, the limitations of the bioelectrical volume model (that is, the anisotropy of tissues and length of the cylinder), and the biological variability (that is, the inter-individual body composition differences) that propagate [21]. Purposely, Piccoli and colleagues provided graphical elliptical probability areas (50%, 75%, and 95% tolerance ellipses) for comparing individual vectors with normative values for the healthy general population.

These references were initially developed on a sample of 85 male and female subjects [17] and subsequently updated on 354 males and 372 females [22]. Using the center of the major (vertical) axis of the tolerance ellipses as a reference, the vectors ending outside the upper region of the 50% tolerance ellipse are interpreted as a person with less body fluid content than the mean with thresholds at 75% and 95% for more extreme conditions, and vice versa for shorter vectors. Referring to the center of the minor (horizontal) axis, vectors outside of the left region of the 50% tolerance ellipse indicate greater intra/extracellular water ratio, and vice versa for the right pole. The current BIVA paradigm is schematically depicted in Fig. 1. For a proper interpretation of Fig. 1, a seven-point scale is obtained considering the 3rd, 12.5th, 25th, 50th, 75th, 87.5th, and 97th percentiles for both axes. Additionally, the center and the extremities of the axes represent the mean values with standard deviations, respectively.

The BIVA has become popular over the years as a procedure to classify the people's body composition with respect to the reference population. For example, considering the center of the ellipses as the mean bivariate value for the bioelectrical proprieties of a certain population, shorter vectors identify subjects with more fluids, as in the case of obesity or inflammation status, whereas longer vectors represent subjects with less total body water, as in the case of lean individual or dehydration status. In addition, vectors displayed on the left side of the ellipses generally result in subjects with higher muscle mass and in contrast rightward vectors commonly occur in sarcopenic people [17,23]. It appears obvious that the sample constituting the reference population is a key aspect of this qualitative analysis. Indeed, further studies have validated tolerance ellipses for different groups such as pediatric [24–26], elderly [27,28] and several sport-specific populations [15]. That said, possible methodological limitations can be found in the reference values provided by Piccoli et al. [22]. Indeed, the original investigation used a mixed sample made by participants aged 15–85 years, considering underage, adult, and older people as a single population. Additionally, the authors did not specify the subsamples for each age category, so it is impossible to weigh how much each age category influenced the normative values and the tolerance ellipses. Notwithstanding, that work continues to be a milestone for both scientists and practitioners interested in BIVA, resulting in possible mismatching when interpreting the subjects' body composition. For example, subsequent studies showed that elderly or pathological people resulted within the reference 50% tolerance ellipse, which appeared as anomalous given that those ellipses should reflect target values, especially in the case of the 50% tolerance ellipse. More in detail, several studies showed elderly people [29–40] positioned on the left side of the major axis within the 50% where healthy adults are expected [17,41], and other studies within the 75% and 95% tolerance ellipses [40,42] where the athletic population is expected [43,44]. In line with this anomaly, some studies showed male and female sarcopenic [40,45,46] or malnourished [35,37,47] subjects within the 50% tolerance ellipse, at the very least again something unexpected. It appears therefore that either all the populations investigated in these studies represent a uniqueness in the body composition literature, or the tolerance ellipses provided by Piccoli et al. [22] cannot be intended as a reference for the healthy adult population.

Therefore, the purpose of the present study was to provide new tolerance ellipses for the general adult population, considering only adults and subsampling the population in age categories with similar sample size. As a secondary aim, given that the phase angle has been raising attention in the literature as an independent qualitative method to interpret the bioelectrical values [23,48], we aimed to provide centile curves and describe its trend during adulthood.



**Fig. 1.** The R-Xc graph with the probability outcomes of mean and standard deviations (SD), percentiles, and tolerance ellipses. The interpretation considering the placement of the vector along the major and minor axis is projected above. TBW: total body water; ICW/ECW: intra/extracellular ratio; BCM: body cell mass.

## 2. Methods

### 2.1. Participants

A total of 4367 participants aged from 18 to 65 years, 2137 males (BMI = 25.1 ± 3.1 kg/m<sup>2</sup>) and 2230 females (BMI = 23.9 ± 3.2 kg/m<sup>2</sup>) were involved in this study. The detailed anthropometric characteristics of the participants are reported in [Supplementary Table 1](#). Subjects reporting pulmonary disease, severe cardiovascular or uncontrolled metabolic diseases (diabetes, anemia, or thyroid disease), electrolyte abnormalities, cancer, inflammatory conditions, and the use of any implanted electrical devices were excluded from the study. The recruitment occurred through advertisements located in Universities, medical and sports centers across the Italian territory starting from January 2020.

### 2.2. Procedures

The present investigation was conceived as a multicenter, cross-sectional study, involving 10 Departments from eight Italian Universities in the data collection. The anthropometric assessments were taken in agreement with international criteria [49]. All the bioelectrical impedance analyses were performed by using foot-to-hand phase sensitive impedance analyzers (BIA 101, 101 anniversary, or BIVA PRO, Akern, Florence, Italy) at a single frequency of 50 kHz. The measurements were made on isolated cots from electrical conductors, with the participants supine with a leg opening of 45° compared to the median line of the body and the upper limbs, abducted 30° from the trunk [50]. After cleaning the skin with isopropilic alcohol, four low-intrinsic impedance adhesive electrodes (Biatrodes Akern Srl, Florence, Italy) were placed in accordance with the International guidelines [50]. Experienced operators performed the procedures. The participants were instructed to avoid any food or beverage for the previous 4 h, as

well as intensive exercise or alcohol intake for the previous 12 h before the test. No differences were detected between the analyzers used in the different centers (intraclass correlation coefficient = 99.8%). Prior to each test session, the accuracy of the analyzers was verified using a reference circuit with acceptance for R measurements of 383 ohm (Ω) and Xc values of 46 Ω; the test-retest coefficient of variation (CV% = standard deviation/mean × 100%) on duplicate measurements of R and Xc was 0.3% and 0.9%, respectively. The bioelectrical phase angle was calculated as the arctangent of Xc/R × 180/π.

### 2.3. Statistical analysis

Statistical analysis was conducted using R (version 3.4.1), BIVA software [51], and Lambda Mu and Sigma (LMS) method (LMS chart-maker Pro version 2.4, 2008). The mean ± standard deviation was calculated for each variable. Normal distribution of data was evaluated using the Shapiro–Wilk test. The two-sample Hotelling’s T<sup>2</sup> test was used to compare the differences in the mean bioelectrical impedance vector between the reference values provided by Piccoli et al. [22] and those calculated in the current study. The Mahalanobis distance (D<sup>2</sup>), which represents a multivariate measure of effect and a multivariate measure of distance, was calculated to determine the magnitude of difference between the mean group vectors. D<sup>2</sup> was interpreted according to the following Stevens’s [52] guidelines: 0.25–0.49: small; 0.5–0.99: medium; ≥1: large. Thereafter, the 50%, 75%, and 95% tolerance ellipses and smoothed age and sex-specific percentiles (3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th) for phase angle were generated. To better understand the vector distribution of the participants on the new and the former ellipses, they were grouped into six age categories (18 to <20, 20 to <30, 30 to <40, 40 to <50, 50 to <60, and 60–65 years) for representing each decade during the adulthood, as done in previous studies where body composition references were

provided [27,53,54]. The LMS method was used to graphically provide the annual rate of change of phase angle, with three reference curves representing the median (M), the coefficient of variation (S), and the power to remove skewness from the data (L) by age and was implemented in the Generalized Additive Model for Location, Scale, and Shape (GAMLSS) package included in R software. In the LMS method, GAMLSS parameters and the parameters of Box–Cox power exponential distribution were used for model fitting to data. These reference curves were fitted to the original data and the best fit was used to construct smoothed percentile curves. After the application of the BoxCox power transformation, the data at each age were normally distributed and the points on each percentile curve were defined in terms of the formula:  $M = (1 + LSz) / L$  where L, M, and S are values of the fitted curves at each age, and z indicates the z-score for the required percentile. For both sexes, simple linear regressions of the dependent variable (phase angle) vs. the explanatory variable (age) were empirically investigated and tested for changes in the response variables' slope (Davies test) and for the existence of time points (Pscore test). To identify the time point(s) where a change in the slope of phase angle is observed, we performed a segmented regression analysis using the “segmented” package (v 1.0.0), selecting the model with the lower Bayesian information criterion value. Delta method and sandwich estimator for the standard errors were used to compute 95% confidence interval (CI) of the time point estimates. The slope coefficient estimates and the related 95% CIs were reported, and significant slopes were detected using p-value set at <0.05.

### 3. Results

The bivariate comparison between the mean bioelectrical impedance vectors of the new and the former reference ellipses [22] showed a significant difference for both males ( $T^2 = 506.9$ ,  $F = 253.3$ ,  $p < 0.001$ ,  $D^2 = 1.29$ ) and females ( $T^2 = 418.9$ ,  $F = 209.4$ ,  $p < 0.001$ ,  $D^2 = 1.15$ ), resulting in a leftward shift of the new 50%, 75%, and 95% tolerance ellipses on the R-Xc plan (Fig. 2, panel B and D). The new 50%, 75%, and 95% tolerance ellipses are presented in Fig. 2 (panel A and C).

The mean vectors were empirically calculated for six age categories and plotted against the former [22] and the new tolerance ellipses (Fig. 3). The age categories up to 49 years were initially positioned at the extremity of the 50% tolerance ellipse while are now aligned with the major axis of the ellipses. Additionally, the age categories from 50 years were initially positioned within the 50% tolerance ellipse on the left side of the major axis, while are now in the right side with respect to the major axis of the R-Xc graph.

The reference centile curves for the phase angle are shown in Fig. 4 for male (upper panel) and female (lower panel) participants. The mean values are reported in Supplementary Table 2.

Figure 5 shows the annual rate of the changes in phase angle for males (upper panel) and females (lower panel). The vertical dotted lines represent the time point(s) where significant changes occur in the phase angle trend. When phase angle was modeled against age in male participants, we identified two time points of change estimated at 33.0 years (95% CI: from 32.5 to 33.6 years) and 51.0 years (95% CI: from 50.4 to 51.6 years), with a mean decrement of  $-0.029^\circ/\text{y}$  (95% CI: from  $-0.040$  to  $-0.018^\circ/\text{y}$ ,  $p < 0.001$ ) between 33.0 and 51.0 years and  $-0.049^\circ/\text{y}$  (95% CI: from  $-0.065$  to  $-0.034^\circ/\text{y}$ ,  $p < 0.001$ ) after 51 years. Two time points were identified for female participants, showing a mean phase angle decrease of  $-0.026^\circ/\text{y}$  (95% CI: from  $-0.034$  to  $-0.018^\circ/\text{y}$ ,  $p < 0.001$ ) from 37.2 years (95% CI: from 36.7 to 37.8 years) to 57.9 years (95% CI: from 57.3 to 58.5 years). After the second time point the phase

angle plateaued (slope:  $-0.005^\circ/\text{y}$ , 95% CI:  $-0.037$  to  $-0.025^\circ/\text{y}$ ,  $p = 0.781$ ) in the female participants.

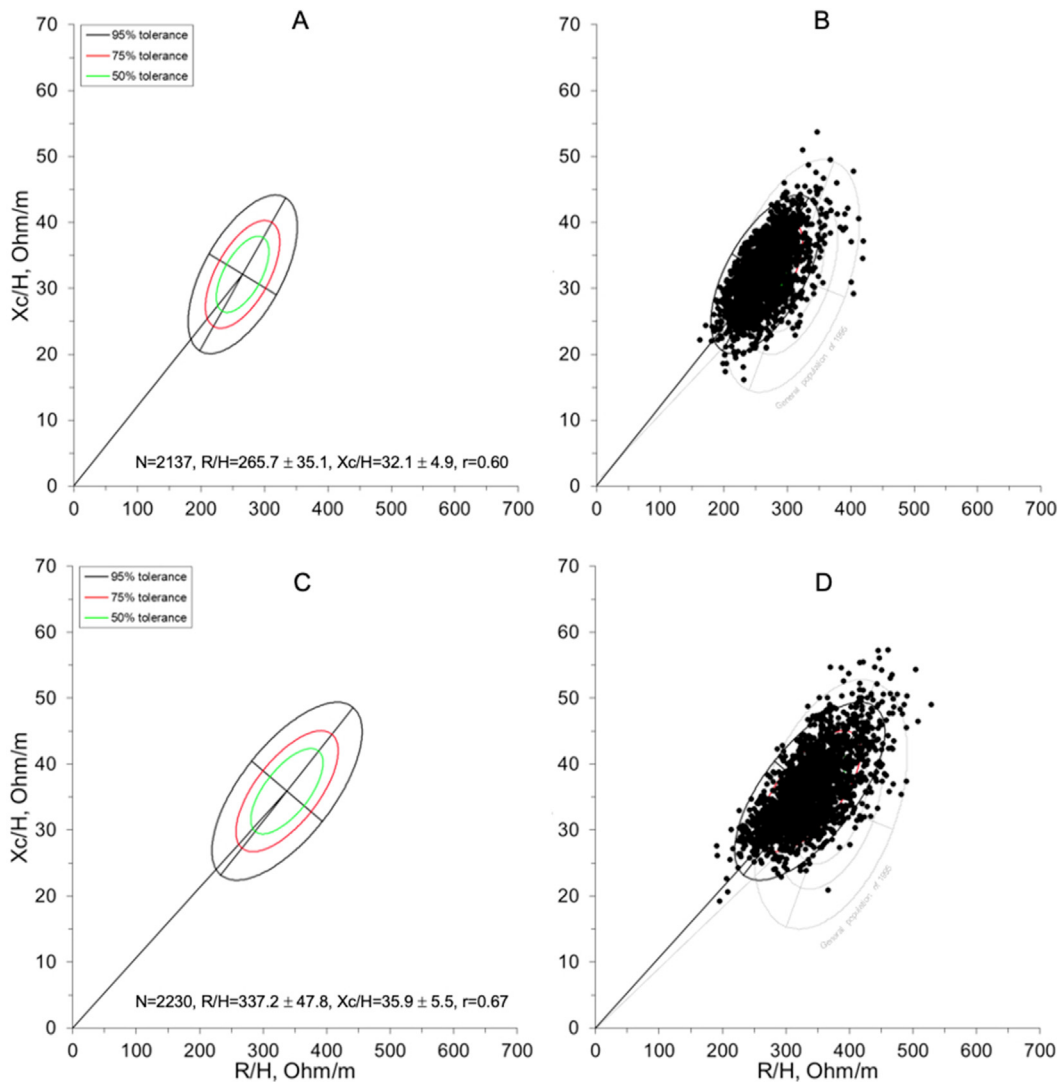
### 4. Discussion

The primary aim of the present study was to provide new 50%, 75%, and 95% reference tolerance ellipses of the general adult male and female population for assessing body composition using BIVA. A secondary aim was to present centile curves for the bioelectrical phase angle, identifying the transition/change time points across the adult's lifespan. The results showed that the new tolerance ellipses have been moved leftward compared to the previous references provided by Piccoli et al. [22]. Therefore, whatever the position on the R-Xc graph of a given population or subject, the vector now shows a rightward shift than in the previous ellipses. Additionally, the phase angle undergoes decrements across the lifespan that are clearly visible at two time points for males (33 and 51 years old) and from 37 to 57 years old in females. The current study provides new and updated reference values for conducting qualitative analysis using BIVA or phase angle.

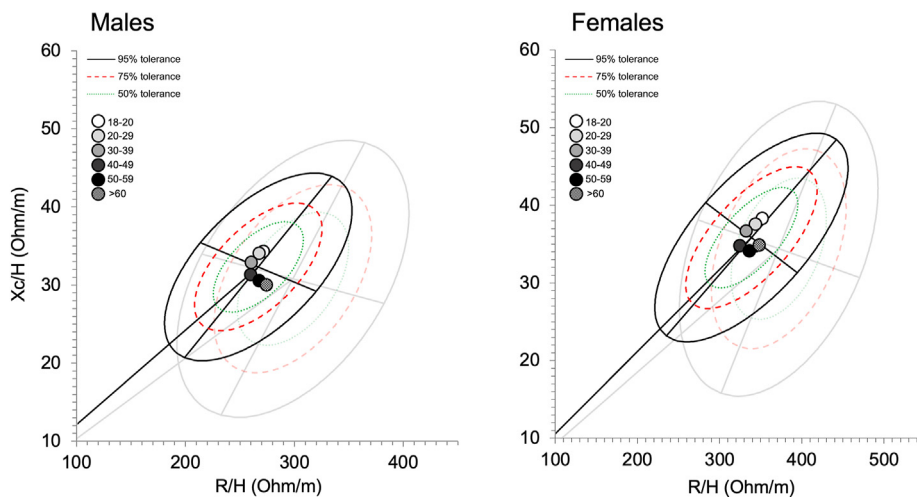
The qualitative analysis using the BIVA consists of evaluating the vector position within the R-Xc graph with respect to 50%, 75%, and 95% tolerance ellipses (Fig. 1). The center of the ellipses indicates the mean of the population's bivariate values of R/H and Xc/H, as well as the 50th percentiles with respect to the major and minor axes. In practical terms and in accordance with the relationship between the bioelectrical properties and the body composition, the major axis describes the body fluid content, while the minor axis describes the body fluid distribution between the intra and extra-cellular spaces [23,55]. Therefore, when comparing the new vs the former reference tolerance ellipses provided by Piccoli et al. [22], it appears quite clear that the two samples used for indicating the healthy population have different body composition features, depending on a series of reasons. First, while we examined a sample including apparently healthy people aged from 18 to 65 years old, Piccoli et al. [22] developed their tolerance ellipses based on a population spanning from 15 to 85 years old, thus including both underage and elderly people. Both these populations have different characteristics than adults, and especially lower muscle mass and consequently less intracellular water [56], whose amount reflects the direction of the vector. Thus, the tolerance ellipses by Piccoli et al. [22] for the general population appear affected by the characteristics of other populations that should have been considered separately. Second, strictly linked with the previous point, the sub-samples for each age category were not stated [22], thus it is unknown how much each population's characteristics weighted on the position of the ellipses within the R-Xc plan. To address this issue, the new reference tolerance ellipses have been made using consistent sub-sampling for each age category. To summarize, we acknowledge the enormous value of the work made by Piccoli and colleagues to address the issues concerning the quantitative analysis through BIVA. However, the current study provides updated references based on more restrictive criteria, and we believe that the new tolerance ellipses are more specific for the adult population.

The use of reference tolerance ellipses that were not reflecting the characteristics of the general adult population may have led to inappropriate interpretation of BIVA in several studies (Fig. 6). For example, elderly people were placed on the left of the major axis within the 50% [29–34,36,38], 75% [26,38,39,42], and 95% [35,37] tolerance ellipses, where adults or physically active people are expected [41]. In contrast, when plotting them on the new tolerance ellipses, they are now on the right of the major axis, where people below the mean (in terms of total body water and intra/extra cellular water ratio) are expected. Similarly, malnourished





**Fig. 2.** The new tolerance ellipses for the male (panel A) and female (panel C) population;  $r$  = coefficient of correlation between resistance and reactance standardized for the subjects' stature. The individual bioelectrical impedance vectors plotted on the new and the former (in the background) [22] reference tolerance ellipses are shown for the male (panel B) and female (panel D) participants.



**Fig. 3.** The mean vectors for different age categories plotted on the new and the former [22] tolerance ellipses shown in the background.

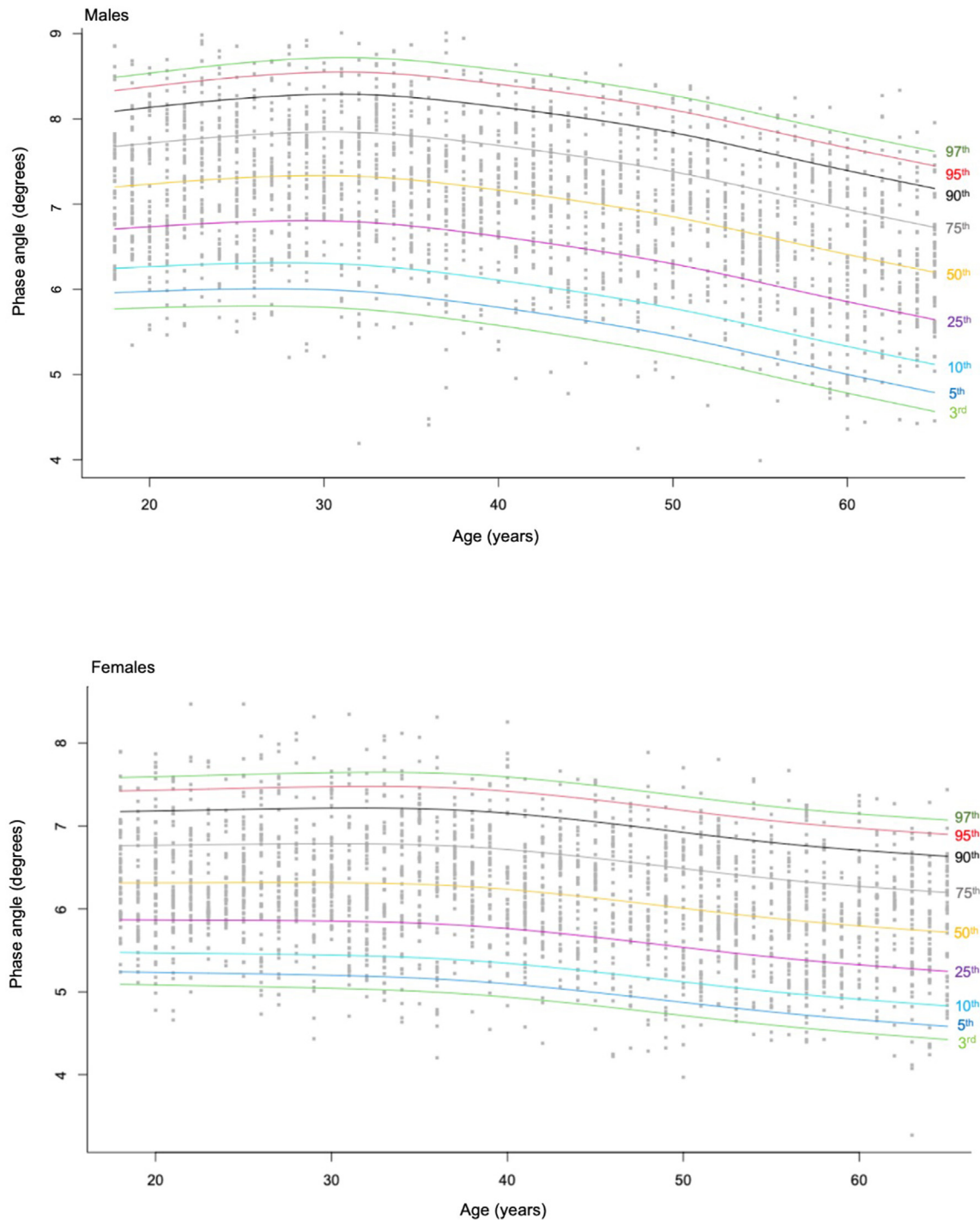


Fig. 4. The reference percentile curves for the phase angle in male (upper panel) and female (lower panel) participants.

and sarcopenic people [35,37,40,45–47] were initially positioned within the 50% tolerance ellipse, and are now within the 75% [35,37,45] and out of the 95% tolerance ellipses [40,46]. Since the purpose of the tolerance ellipses should be to provide target zones for specific populations, our study offers new solutions to researchers and practitioners interested in BIVA, having the characteristics of the general adult population as a reference. However, the comparisons with previous studies may suffer from the differences among populations involved in the research. Indeed, the literature has shown bioelectrical differences in samples of

individuals classified by ethnic group [57]. On the other hand, the differences among individuals of European ancestry appear to be not very pronounced [58], and the present references could be used confidently.

The development of new bioelectrical references to qualitatively evaluate body composition using BIVA has called for a necessary presentation of centile curves for the bioelectrical phase angle. The phase angle was associated with body composition [15,23], mortality rate [48,59], nutritional status [60], and physical performance [15,23], hence pointed as a different possibility for a qualitative

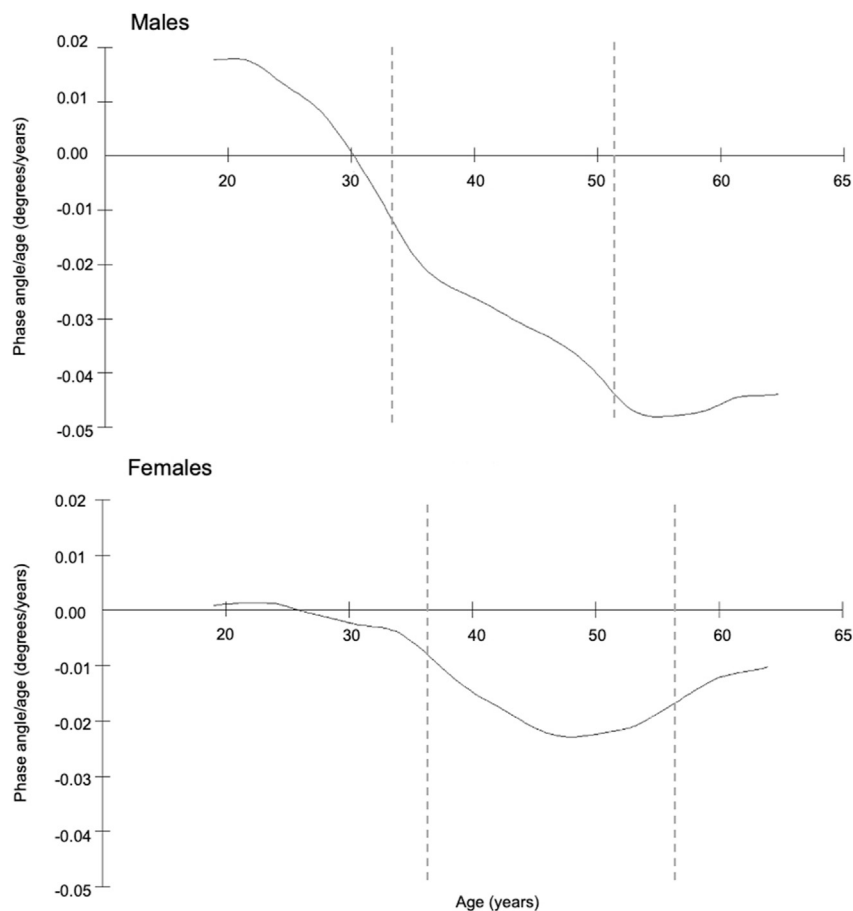


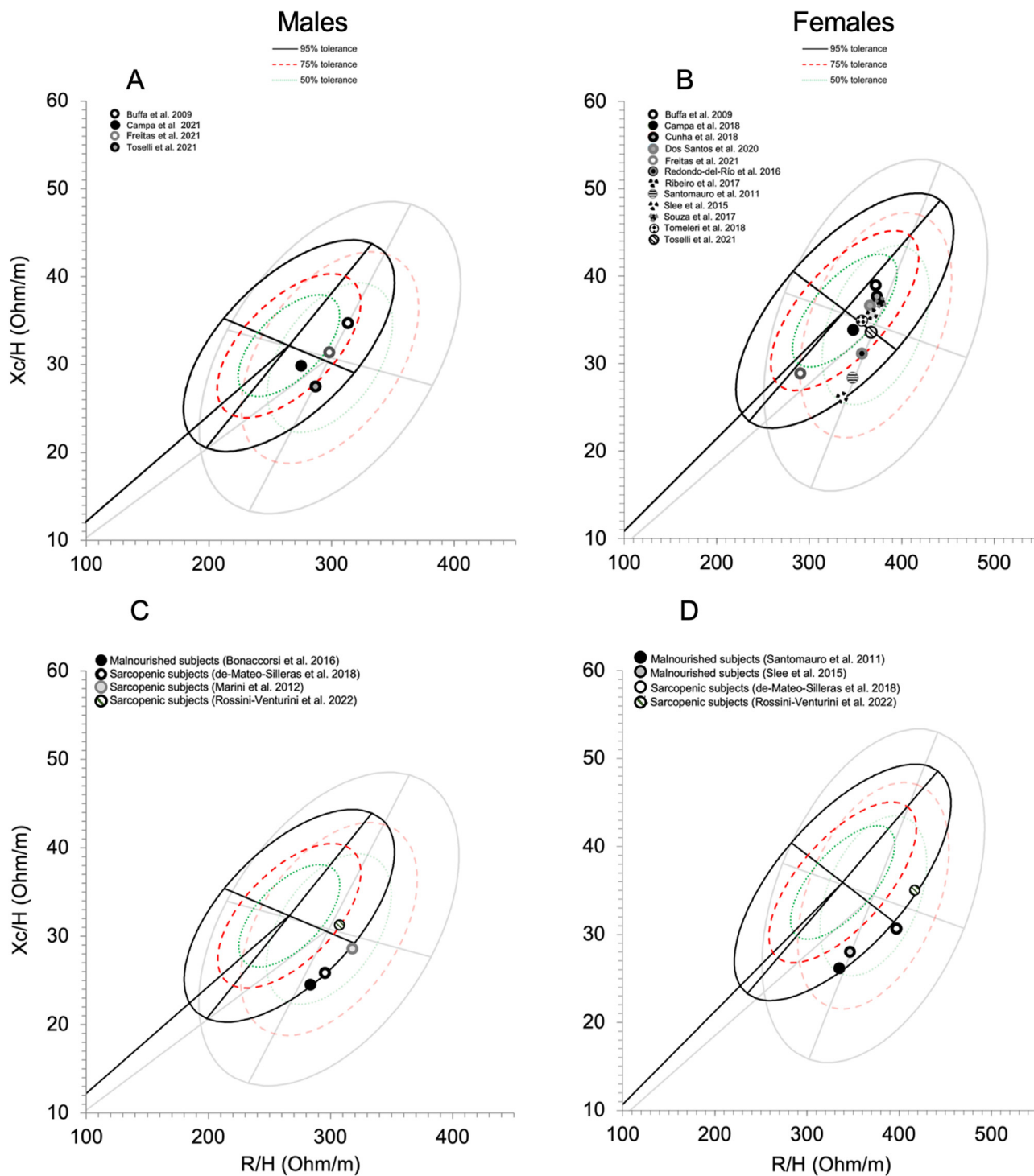
Fig. 5. Annual rate of change in phase angle for male (upper panel) and female (lower panel) participants; dotted lines identify time points of change.

approach to the bioelectrical data. The average data of all the participants showed a phase angle equal to 6.9° for males and 6.1° for females. A direct comparison with the work of Piccoli et al. [22] cannot be made because the phase angle values were not provided. However, we had the possibility to compare the two mean vectors, resulting in a large ( $D^2 > 1$ ) leftward shift of the present mean vector compared to what reported by Piccoli et al. [22]. Since the phase angle is the graphical representation of the distance of the vector from the X axis, we may argue that the phase angle values of the population examined by Piccoli et al. [22] should have been lower. A further counterprove is that the people aged >60 years examined here (mean phase angle = 6.2° for males and 5.8° for females) were positioned on the left side, far from the center of the former tolerance ellipses (Fig. 3). Recently, reference centiles for the athletic population [61] have been developed starting with a sample aged 20–30 years old, showing phase angle mean values at the 50th percentile of 7.7° and 6.8° for males and females, respectively. Performing an age-matched comparison, the present data indicate the 50th percentile mean values of 7.3° and 6.2° in the same age category. Such a between-population difference should derive from the greater amount of intracellular water, possibly reflecting greater muscle mass in the athletic population [61]. The centile curves provided here can help practitioners with novel reference values to evaluate the body composition characteristics of a given person with respect to the general adult population.

Another important aspect to be considered is how the phase angle changes across the lifespan. A recent meta-analysis involving more than 250,000 male and female subjects [59]

highlighted that the phase angle increases progressively from the first years of life until 18 years, then stabilizes from 19 until 48 years and progressively decreases thereafter. However, the authors acknowledged that the majority of the studies did not report the characteristics of the devices used for the BIA [59]. Since recent studies showed a lack of agreement between raw bioelectrical parameters measured with different technologies [62,63], the validity of the data resulting from the meta-analysis can be questioned. In the current study we used the foot-to-hand technology at a single frequency of 50 kHz to collect all data, an analysis acknowledged as the most accurate with respect to the gold standard methods [1]. As concerns the male sample, increments in phase angle are visible up to 33 years, when an inversion of the curve started with a decrement in phase angle, that becomes significantly greater at 51 years. In females the phase angle started to decrease at 37 years and plateaued around 58 years. Importantly, We would like to underline that physical activity and particularly resistance training can increase the phase angle, contrasting its decline induced by inactivity or aging [64].

A strength of this study is the large sample size of 2137 males and 2230 females in comparison to the 354 male and 372 female subjects proposed as reference for the general population studied by Piccoli et al. [22]. Apart from what has already been said, these new ellipses arrive 30 years after the first, a period during which even the secular trend [65] could have had an effect on the change in body composition characteristics in the general population. Some limitations of this study should also be acknowledged. Our



**Fig. 6.** Mean vector data from literature [26,29–38,40,42,45–47] plotted on the new and former (in the background) [22] reference ellipses for males (panel A and C) and females (panel B and D). Upper panels (A and B) include data on elderly people. Lower panels (C and D) include data on pathological subjects.

results are only applicable when using phase-sensitive foot-to-hand technologies operating at a single frequency of 50 kHz. Moreover, the proposed references are applicable to the adult population that was examined in the present study and to populations with similar biological and cultural characteristics, for example most people of European geographic ancestry. The newly proposed ellipses could be tested to check their suitability in other adult populations, considering possible genetic differences in body

size and proportions, or socio-cultural peculiarities related to ethnicity. However, more studies are needed to analyze population variability of body composition and to disentangle the effects of biology and culture. Although we believe that the present results give added value to BIVA, in absence of the outcomes of such studies and of possible new population-specific references, scientists and practitioners should be aware that the present ellipses need caution when interpreted in different populations.



## 5. Conclusions

The present study provides new 50%, 75%, and 95% tolerance ellipses for using in BIVA in relation to adult references 30 years after their invention. The current updated references allow researchers and practitioners for a more specific assessment to compare the bioelectrical characteristics with those of the general adult population, considering that the current references derive from people from the Italian territory. A further possibility has been presented for qualitatively using the single-frequency BIA data providing centile curves for the phase angle. It is now possible to refer to age- and sex-specific 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th percentiles, considering that time points of decrement occur at 33 years and 51 years for males and from 37 up to 57 years for females. The qualitative BIVA and phase angle assessments are now possible using reference values for the adult population.

## Ethics approval and consent to participate

All the participants were informed of the study procedures and gave the written consent to participate. All procedures were approved by the Ethics Committee of the University of Bologna (approval code: 0224252) and were conducted in accordance with the Declaration of Helsinki for studies involving human subjects (1964 and further updates).

## Consent for publication

Not applicable.

## Data availability

Data described in the manuscript will be made available upon request to the corresponding author, Giuseppe Coratella (giuseppe.coratella@unimi.it).

## Funding disclosure

The authors received no funding for the present investigation.

## Authors' contributions

All authors made significant contributions in the preparation of the first draft of the manuscript, by participating in the process of interpreting data, and by providing meaningful revision and feedback. All authors participated in the processing of collecting and analyzing data. All authors have read and approved the final manuscript and agree with the order of presentation of the authors.

## Conflicts of interest

The authors declare that they have no competing interests.

## Acknowledgements

The authors are grateful to all people who took part in this study.

## List of abbreviations

BIA	bioelectrical impedance analysis
BIVA	bioelectrical impedance vector analysis
R	resistance
Xc	reactance

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2023.07.025>.

## References

- [1] Campa F, Gobbo LA, Stagi S, Cyrino LT, Toselli S, Marini E, et al. Bioelectrical impedance analysis versus reference methods in the assessment of body composition in athletes. *Eur J Appl Physiol* 2022. <https://doi.org/10.1007/s00421-021-04879-y>.
- [2] Foster KR, Lukaski HC. Whole-body impedance—what does it measure? *Am J Clin Nutr* 1996;64:388S–96S. <https://doi.org/10.1093/ajcn/64.3.388S>.
- [3] Nyboer J. Electrical impedance plethysmography; a physical and physiologic approach to peripheral vascular study. *Circulation* 1950;2:811–21.
- [4] Thomasset MA. [Bioelectric properties of tissue. Impedance measurement in clinical medicine. Significance of curves obtained]. *Lyon Med* 1962;94:107–18.
- [5] Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. *J Appl Physiol* 1969;27:531–4. <https://doi.org/10.1152/jappl.1969.27.4.531>.
- [6] Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr* 1985;41:810–7. <https://doi.org/10.1093/ajcn/41.4.810>.
- [7] Kotler DP, Burastero S, Wang J, Pierson Jr RN. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *Am J Clin Nutr* 1996;64:489S–97S. <https://doi.org/10.1093/ajcn/64.3.489S>.
- [8] Kushner R, Schoeller DA, Fjeld CR, Danford L. Is the Impedance index (ht2/R) significant in predicting total body water? *Am J Clin Nutr* 1992;56:835–9. <https://doi.org/10.1093/ajcn/56.5.835>.
- [9] Van Loan M, Mayclin P. Bioelectrical impedance analysis: is it a reliable estimator of lean body mass and total body water? *Hum Biol* 1987;69:299–309.
- [10] Pichard C, Kyle UG, Gremion G, Gerbase M, Slosman DO. Body composition by x-ray absorptiometry and bioelectrical impedance in female runners. *Med Sci Sports Exerc* 1997;29:1527–34. <https://doi.org/10.1097/00005768-199711000-00021>.
- [11] Houtkooper LB, Lohman TG, Going SB, Howell WH. Why bioelectrical impedance analysis should be used for estimating adiposity. *Am J Clin Nutr* 1996;64:436S–48S. <https://doi.org/10.1093/ajcn/64.3.436S>.
- [12] Sergi G, Bussolotto M, Perini P, Calliari I, Giantin V, Cecon A, et al. Accuracy of bioelectrical impedance analysis in estimation of extracellular space in healthy subjects and in fluid retention states. *Ann Nutr Metab* 1994;38:158–65. <https://doi.org/10.1159/000177806>.
- [13] Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. *Am J Clin Nutr* 1986;44:417–24. <https://doi.org/10.1093/ajcn/44.3.417>.
- [14] Ward LC. Bioelectrical impedance analysis for body composition assessment: reflections on accuracy, clinical utility, and standardisation. *Eur J Clin Nutr* 2019;73:194–9. <https://doi.org/10.1038/s41430-018-0335-3>.
- [15] Campa F, Toselli S, Mazzilli M, Gobbo LA, Coratella G. Assessment of body composition in athletes: a narrative review of available methods with special reference to quantitative and qualitative bioimpedance analysis. *Nutrients* 2021;13. <https://doi.org/10.3390/nu13051620>.
- [16] Coratella G, Campa F, Matias CN, Toselli S, Koury JC, Andreoli A, et al. Generalized bioelectric impedance-based equations underestimate body fluids in athletes. *Scand J Med Sci Sports* 2021. <https://doi.org/10.1111/sms.14033>.
- [17] Piccoli A, Rossi B, Pillon L, Bucciantone G. A new method for monitoring body fluid variation by bioimpedance analysis: the RxC graph. *Kidney Int* 1994;46:534–9.
- [18] Buffa R, Saragat B, Cabras S, Rinaldi AC, Marini E. Accuracy of specific BIVA for the assessment of body composition in the United States population. *PLoS One* 2013;8:e58533. <https://doi.org/10.1371/journal.pone.0058533>.
- [19] Chertow GM, Lowrie EG, Wilmore DW, Gonzalez J, Lew NL, Ling J, et al. Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. *J Am Soc Nephrol* 1995;6:75–81.
- [20] Marini E, Campa F, Buffa R, Stagi S, Matias CN, Toselli S, et al. Phase angle and bioelectrical impedance vector analysis in the evaluation of body composition in athletes. *Clin Nutr* 2020;39:447–54. <https://doi.org/10.1016/j.clnu.2019.02.016>.
- [21] Lukaski HC. Evolution of bioimpedance: a circuitous journey from estimation of physiological function to assessment of body composition and a return to clinical research. *Eur J Clin Nutr* 2013;67(Suppl 1):S2–9. <https://doi.org/10.1038/ejcn.2012.149>.
- [22] Piccoli A, Nigrelli S, Caberlotto A, Bottazzo S, Rossi B, Pillon L, et al. Bivariate normal values of the bioelectrical impedance vector in adult and elderly populations. *Am J Clin Nutr* 1995;61:269–70. <https://doi.org/10.1093/ajcn/61.2.269>.
- [23] Lukaski H, Raymond-Pope CJ. New frontiers of body composition in sport. *Int J Sports Med* 2021. <https://doi.org/10.1055/a-1373-5881>.
- [24] Meleleo D, Bartolomeo N, Cassano L, Nitti A, Susca G, Mastroianni G, et al. Evaluation of body composition with bioimpedance. A comparison between

- athletic and non-athletic children. *Eur J Sport Sci* 2017;17:710–9. <https://doi.org/10.1080/17461391.2017.1291750>.
- [25] De Palo T, Messina G, Edefonti A, Perfumo F, Pisanello L, Peruzzi L, et al. Normal values of the bioelectrical impedance vector in childhood and puberty. *Nutrition* 2000;16:417–24.
- [26] Redondo-Del-Río MP, Camina-Martín MA, Moya-Gago L, de-la-Cruz-Marcos S, Malafarina V, de-Mateo-Silleras B. Vector bioimpedance detects situations of malnutrition not identified by the indicators commonly used in geriatric nutritional assessment: a pilot study. *Exp Gerontol* 2016;85:108–11. <https://doi.org/10.1016/j.exger.2016.10.002>.
- [27] Saragat B, Buffa R, Mereu E, De Rui M, Coin A, Sergi G, et al. Specific bioelectrical impedance vector reference values for assessing body composition in the Italian elderly. *Exp Gerontol* 2014;50:52–6. <https://doi.org/10.1016/j.exger.2013.11.016>.
- [28] Reljic D, Zarafat D, Jensen B, Herrmann HJ, Neurath MF, Konturek PC, et al. Phase angle and vector analysis from multifrequency segmental bioelectrical impedance analysis: new reference data for older adults. *J Physiol Pharmacol an Off J Polish Physiol Soc* 2020;71. <https://doi.org/10.26402/jpp.2020.4.04>.
- [29] Campa F, Silva AM, Toselli S. Changes in phase angle and handgrip strength induced by suspension training in older women. *Int J Sports Med* 2018. <https://doi.org/10.1055/a-0574-3166>.
- [30] Souza MF, Tomeleri CM, Ribeiro AS, Schoenfeld BJ, Silva AM, Sardinha LB, et al. Effect of resistance training on phase angle in older women: a randomized controlled trial. *Scand J Med Sci Sports* 2017;27:1308–16. <https://doi.org/10.1111/sms.12745>.
- [31] Campa F, Schoenfeld BJ, Marini E, Stagi S, Mauro M, Toselli S. Effects of a 12-week suspension versus traditional resistance training program on body composition, bioimpedance vector patterns, and handgrip strength in older men: a randomized controlled trial. *Nutrients* 2021;13. <https://doi.org/10.3390/nu13072267>.
- [32] Cunha PM, Tomeleri CM, do Nascimento MA, Nunes JP, Antunes M, Nabuco HCG, et al. Improvement of cellular health indicators and muscle quality in older women with different resistance training volumes. *J Sports Sci* 2018;36:2843–8. <https://doi.org/10.1080/02640414.2018.1479103>.
- [33] Ribeiro AS, Schoenfeld BJ, Souza MF, Tomeleri CM, Silva AM, Teixeira DC, et al. Resistance training prescription with different load-management methods improves phase angle in older women. *Eur J Sport Sci* 2017;17:913–21. <https://doi.org/10.1080/17461391.2017.1310932>.
- [34] Tomeleri CM, Ribeiro AS, Cavaglieri CR, Deminice R, Schoenfeld BJ, Schiavoni D, et al. Correlations between resistance training-induced changes on phase angle and biochemical markers in older women. *Scand J Med Sci Sports* 2018;28:2173–82. <https://doi.org/10.1111/sms.13232>.
- [35] 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. <https://doi.org/10.1016/j.nut.2014.06.002>.
- [36] dos Santos L, Ribeiro AS, Gobbo LA, Nunes JP, Cunha PM, Campa F, et al. Effects of resistance training with different pyramid systems on bioimpedance vector patterns, body composition, and cellular health in older women: a randomized controlled trial. *Sustain Times* 2020;12. <https://doi.org/10.3390/su12166658>.
- [37] Santomauro F, Olimpini N, Baggiani L, Comodo N, Mantero S, Bonaccorsi G. Bioelectrical impedance vector analysis and mini nutritional assessment in elderly nursing home residents. *J Nutr Health Aging* 2011;15:163–7. <https://doi.org/10.1007/s12603-010-0104-z>.
- [38] Buffa R, Floris G, Marini E. Assessment of nutritional status in free-living elderly individuals by bioelectrical impedance vector analysis. *Nutrition* 2009;25:3–5. <https://doi.org/10.1016/j.nut.2008.07.014>.
- [39] Toselli S, Campa F, Matias CN, de Alencar Silva BS, Dos Santos VR, Maietta Latessa P, et al. Predictive equation for assessing appendicular lean soft tissue mass using bioelectric impedance analysis in older adults: effect of body fat distribution. *Exp Gerontol* 2021;150:111393. <https://doi.org/10.1016/j.exger.2021.111393>.
- [40] de-Mateo-Silleras B, Camina-Martín MA, de-Frutos-Allas JM, de-la-Cruz-Marcos S, Carreño-Enciso L, Redondo-Del-Río MP. Bioimpedance analysis as an indicator of muscle mass and strength in a group of elderly subjects. *Exp Gerontol* 2018;113:113–9. <https://doi.org/10.1016/j.exger.2018.09.025>.
- [41] Campa F, Matias CN, Teixeira FJ, Reis JF, Valamatos MJ, Coratella G, et al. Comparison of generalized and athletic bioimpedance-based predictive equations for estimating fat-free mass in resistance-trained exercisers. *Nutrition* 2022;102:111694. <https://doi.org/10.1016/j.nut.2022.111694>.
- [42] Freitas SP, Jádice PB, Hetherington-Rauth M, Magalhães JP, Correia IR, Lopes JM, et al. The impact of 2 weeks of detraining on phase angle, BIVA patterns, and muscle strength in trained older adults. *Exp Gerontol* 2021;144:111175. <https://doi.org/10.1016/j.exger.2020.111175>.
- [43] Campa F, Silva AM, Talluri J, Matias CN, Badicu G, Toselli S. Somatotype and bioimpedance vector analysis: a new target zone for male athletes. *Sustainability* 2020;12. <https://doi.org/10.3390/su12114365>.
- [44] Campa F, Matias C, Gatterer H, Toselli S, Koury JC, Andreoli A, et al. Classic bioelectrical impedance vector reference values for assessing body composition in male and female athletes. *Int J Environ Res Publ Health* 2019;16. <https://doi.org/10.3390/ijerph16245066>.
- [45] Rossini-Venturini AC, Abdalla PP, Fassini PG, Dos Santos AP, Tasinafo Junior MF, Alves TC, et al. Association between classic and specific bioimpedance vector analysis and sarcopenia in older adults: a cross-sectional study. *BMC Sport Sci Med Rehabil* 2022;14:170. <https://doi.org/10.1186/s13102-022-00559-2>.
- [46] Marini E, Buffa R, Saragat B, Coin A, Toffanello ED, Berton L, et al. The potential of classic and specific bioelectrical impedance vector analysis for the assessment of sarcopenia and sarcopenic obesity. *Clin Interv Aging* 2012;7:585–91. <https://doi.org/10.2147/CI.A.S38488>.
- [47] Bonaccorsi G, Santomauro F, Lorini C, Indiani L, Pellegrino E, Pasquini G, et al. Risk of malnutrition in a sample of nonagenarians: specific versus classic bioelectrical impedance vector analysis. *Nutrition* 2016;32:368–74. <https://doi.org/10.1016/j.nut.2015.09.011>.
- [48] Norman K, Stobaus N, Pirlich M, Bösby-Westphal A. Bioelectrical phase angle and impedance vector analysis—clinical relevance and applicability of impedance parameters. *Clin Nutr* 2012;31:854–61. <https://doi.org/10.1016/j.clnu.2012.05.008>.
- [49] Lohman T, Roche AF, Martorell R. *Anthropometric standardization reference manual*. Champaign, IL: Human Kinetics Books; 1988.
- [50] Bioelectrical NIH. *Impedance analysis in body composition measurement: national institutes of health technology assessment conference statement, December 12–14, 1994*. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Office of Medical Applications Research; 1994.
- [51] Piccoli A, Pastori G. *BIVA software*. Order A J theory ordered sets its Appl 2002: 1–17. 2002.
- [52] Stevens J. *Applied multivariate statistics for the social sciences*. Lawrence E. Mahwah; 2002.
- [53] Kirk B, Bani Hassan E, Brennan-Olsen S, Vogrin S, Bird S, Zanker J, et al. Body composition reference ranges in community-dwelling adults using dual-energy X-ray absorptiometry: the Australian Body Composition (ABC) Study. *J Cachexia Sarcopenia Muscle* 2021;12:880–90. <https://doi.org/10.1002/jcsm.12712>.
- [54] Ofenheimer A, Breyer-Kohansal R, Hartl S, Burghuber OC, Krach F, Schrott A, et al. Reference values of body composition parameters and visceral adipose tissue (VAT) by DXA in adults aged 18–81 years—results from the LEAD cohort. *Eur J Clin Nutr* 2020;74:1181–91. <https://doi.org/10.1038/s41430-020-0596-5>.
- [55] Lukaski HC, Piccoli A. *Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions*. In: Preedy V, editor. *Handb. Anthr.* London: Springer; 2012. p. 287–305.
- [56] Chumlea WC, Guo SS, Zeller CM, Reo NV, Siervogel RM. Total body water data for white adults 18 to 64 years of age: the Fels Longitudinal Study. *Kidney Int* 1999. <https://doi.org/10.1046/j.1523-1755.1999.00532.x>.
- [57] Ward LC, Heitmann BL, Craig P, Stroud D, Azinge EC, Jebb S, et al. Association between ethnicity, body mass index, and bioelectrical impedance. Implications for the population specificity of prediction equations. *Ann N Y Acad Sci* 2000;904:199–202. <https://doi.org/10.1111/j.1749-6632.2000.tb06449.x>.
- [58] Ibanez ME, Mereu E, Buffa R, Gualdi-Russo E, Zaccagni L, Cossu S, et al. New specific bioelectrical impedance vector reference values for assessing body composition in the Italian-Spanish young adult population. *Am J Hum Biol* 2015;27:871–6. <https://doi.org/10.1002/ajhb.22728>.
- [59] Mattiello R, Amaral MA, Mundstock E, Ziegelmann PK. Reference values for the phase angle of the electrical bioimpedance: systematic review and meta-analysis involving more than 250,000 subjects. *Clin Nutr* 2020;39:1411–7. <https://doi.org/10.1016/j.clnu.2019.07.004>.
- [60] Barrea L, Muscogiuri G, Pugliese G, Laudisio D, de Alteriis G, Graziadio C, et al. Phase angle as an easy diagnostic tool of meta-inflammation for the nutritionist. *Nutrients* 2021;13. <https://doi.org/10.3390/nu13051446>.
- [61] Campa F, Thomas DM, Watts K, Clark N, Baller D, Morin T, et al. Reference percentiles for bioelectrical phase angle in athletes. *Biol* 2022;11. <https://doi.org/10.3390/biology11020264>.
- [62] Silva AM, Matias CN, Nunes CL, Santos DA, Marini E, Lukaski HC, et al. Lack of agreement of in vivo raw bioimpedance measurements obtained from two single and multi-frequency bioelectrical impedance devices. *Eur J Clin Nutr* 2018. <https://doi.org/10.1038/s41430-018-0355-z>.
- [63] Stratton MT, Smith RW, Harty PS, Rodríguez C, Johnson BA, Dellinger JR, et al. Longitudinal agreement of four bioimpedance analyzers for detecting changes in raw bioimpedance during purposeful weight gain with resistance training. *Eur J Clin Nutr* 2021;75:1060–8. <https://doi.org/10.1038/s41430-020-00811-3>.
- [64] Campa F, Colognesi LA, Moro T, Paoli A, Casolo A, Santos L, et al. Effect of resistance training on bioelectrical phase angle in older adults: a systematic review with Meta-analysis of randomized controlled trials. *Rev Endocr Metab Disord* 2022. <https://doi.org/10.1007/s11554-022-09747-4>.
- [65] Sedeaud A, Marc A, Schipman J, Schaal K, Daniau M, Guillaume M, et al. Secular trend: morphology and performance. *J Sports Sci* 2014;32:1146–54. <https://doi.org/10.1080/02640414.2014.889841>.