DOI *https://dx.doi.org/10.1016/j.clnu.2019.02.016*

Phase angle and bioelectrical impedance vector analysis in the evaluation of body composition in athletes

Elisabetta Marini[1], Francesco Campa[2], Roberto Buffa[1], Silvia Stagi[1], Catarina N. Matias[3], Stefania Toselli[2], Luís B. Sardinha[3], Analiza M. Silva[3]

[1] Department of Life and Environmental Sciences, Neuroscience and Anthropology Section, University of Cagliari, Cagliari, Italy;
[2] Department of Biomedical and Neuromotor Science, University of Bologna, Bologna, Italy;
[3] Exercise and Health Laboratory, CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Portugal.

Running title: Body composition assessment

Keywords: BIVA; body fluid; extracellular water; intracellular water; fat mass.

Corresponding author:

Elisabetta Marini - Department of Life and Environmental Sciences, Neuroscience and Anthropology Section, University of Cagliari, Cittadella di Monserrato, Cagliari, Italy. tel. +39 0706756607. e-mail: emarini@unica.it. Alternative Corresponding Author: Francesco Campa - Department of Biomedical and Neuromotor Sciences, University of Bologna. Via Selmi, 3, 40121 Bologna, Italy. Tel.: +39-3450031080. E-mail: francesco.campa3@unibo.it

Competing interests statement: The Authors declare no competing financial or non-financial interests.

Abstract

Aims: To analyze the association of classic and specific bioelectrical impedance vector analysis (BIVA) and phase angle with reference techniques for the assessment of body composition in athletes.

Methods: 202 athletes of both sexes (men: 21.5 ± 5.0; women: 20.7 ± 5.1) engaged in different sports were evaluated during the in-season period. Bioelectrical resistance (R, ohm) and reactance (Xc, ohm) were obtained with a phase-sensitive 50 kHz bioelectrical impedance analysis device. The classic and specific BIVA procedures, which respectively correct bioelectrical values for body height (R/H and Xc/H, ohm/m) and body geometry (Rsp and Xcsp, ohm cm), were applied. Dual energy X-ray absorptiometry was used as the reference method to assess fat-mass (FM), fat-free mass (FFM) and %FM. Deuterium dilution and bromide dilution where used as the criterion method for total body water (TBW) and extracellular water (ECW), respectively. Intracellular water (ICW) was calculated as TBW minus ECW.

Results: Specific bioelectrical values (Rsp, Xcsp, Zsp) were positively correlated with FM and %FM (%FM; Zsp men: $r=0.569$, $p<0.001$; Zsp women: $r=0.773$, $p<0.001$). Classic values (R/H, Xc/H, Z/H) were negatively correlated with FM and FFM, but were correlated with %FM only in men (Z/H men $r=-0.214$, $p=0.013$; Z/H women $r=0.218$, $p=0.097$). As to body fluid, classic BIVA showed strong associations (Z/H men: $r=-0.880$, $p<0.001$; Z/H women: $r=-0.829$, $p<0.001$) with TBW, whereas Zsp was not correlated. Phase angle was negatively correlated with ECW/ICW ratio in both sexes (men: $r=-0.493$, $p<0.001$; women: $r=-0.408$, $p<0.001$) and positively with ICW (men: $r=0.327$, $p<0.001$; women: $r=0.243$, $p=0.080$).

Conclusions: Specific BIVA turns out to be more accurate for the analysis of %FM in athletes, while it does not correctly evaluate TBW, for which classic BIVA appears to be a suitable approach. Phase angles, and hence both BIVA approaches, can detect ECW/ICW changes.

INTRODUCTION

The analysis and monitoring of body composition is fundamental in sport, because of its relevance to athletes' health and performance, and to team success. Such analysis can be performed in different contexts and with different approaches, i.e. in cross-sectional studies aimed to characterise sporting group samples, in longitudinal researches finalised to define short-term or long-term changes, or in applications aimed to detect and monitor muscle injuries (1). Variations of body composition can interest diversely athletes practicing different sport, because of their different exercise type and requirements for body physique and composition. In general, lean mass is considered a predictor of muscular fitness (2,3). Furthermore, while overhydration is quite uncommon in athletes, physiological dehydration processes can be induced by physical activity, leading to hypotonic, isotonic, or hypertonic dehydration (4).

Several techniques can be used to assess body composition in athletes. At the molecular level, though the four-compartment model is considered the reference method for body composition assessment (5), dual energy X-ray absorptiometry (DXA), a three-compartment model, has been recognized as a precise and accurate technique for determining fat (FM) and FFM (6). Still, DXA is an expensive method to be used in the field setting as a minimum space to accommodate the DXA machine, a potential radiation shielding, and specialized technicians to perform and analyze the exams are required (7). Considering the main FFM component, total-body water (TBW), its amount and the content of the extracellular water compartment (ECW) can be accurately assessed through dilution techniques, specifically using deuterium and bromide dilution, respectively (8). However, these analytic procedures are time-consuming, costly and laborious, thus compromising their routine use in a clinical or field setting (9). Therefore, simple methods to determine water compartments, easily applied during training and competition, are required.

Bioelectrical impedance analysis (BIA) is a fast, safe and non-invasive method to obtain quantitative estimates of body composition. ~~and its use is very common in sports, especially utilizing the single-frequency devices, i.e. at 50 kH.~~ Multifrequency BIA and, specifically, bioimpedance spectroscopy is preferable for fluid volume measurements, though for general body composition assessment BIA at 50 kHz is more widely used (10,11). Bioelectrical impedance (Z, ohm) is composed by resistance (R, ohm) and reactance (Xc, ohm) [$Z=(R^2+Xc^2)^{0.5}$)]. 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 tissues. Xc, which is detectable by phase sensitive devices only, is related to the capacitance properties of the cell membrane and to variations that can occur depending on its integrity, function and composition (12). Phase angle (PA) [PA=arctn Xc/R 180/π] is determined by the time delay occurring when the electric current passes the cell membrane (13,14).

Bioelectrical impedance can be applied using prediction equations (15). However, the dependency on population-specific equations and hydration status is considered the major weakness of conventional bioelectrical-impedance analysis (16). Alternatively, the analysis can be performed using raw data, namely phase angles, or bioelectrical impedance vectors, i.e. phase angle and vector length jointly, as in the bioelectrical impedance vector analysis approaches (BIVA; (17–19)).

Bioelectrical impedance vector analysis, both classic (14,17) and specific BIVA (20), is based on the analysis of impedance vectors (at 50 kHz), projected on a RXc graph in relation to tolerance ellipses, or for intergroup comparisons (confidence ellipses). The two BIVA approaches differ each other in that classic BIVA analyses bioelectrical values standardized for subject's height (which represents the conductor's length), whereas in specific BIVA R and Xc values are corrected also for cross-sectional areas, in order to reduce the effect of body dimensions. According to classic BIVA (17), variations of bioelectrical vectors along the major axis of tolerance ellipses indicate changes in total body water (TBW) (dehydration towards the upper pole, fluid overload towards the lower pole). The minor axis refers to variations of absolute amount of body cell mass, FM, and

FFM (left side: more mass; right side: less mass) and to variations of extracellular/intracellular water ratio (ECW/ICW) (low values in the left side). Within classic tolerance ellipses, the left upper side would correspond to athletic individuals, whereas the left lower side to obese ones. In specific BIVA (18,19), the major axis relates to %FM variation (higher values toward the upper pole), while the minor axis gives the same information as in classic BIVA (more mass and lower ECW/ICW ratio on the left side). In fact, the minor axis is mainly related to variations of phase angle, which is unaffected by the correction.

PA allows the interpretation of total body water and body cell mass (14,21). However, the analysis of PA only, without considering the information furnished by vector length, can lead to interpretation errors. In fact, groups of individuals characterized by quite identical PA, but different vector lengths, may show different body fluids or %FM (22). The vectorial approach appears to be more efficient, as it considers both influential variables, phase angle and vector length.

PA, classic and specific BIVA have been applied in different groups, particularly obese, athletic subjects, and in the elderly, and in the clinical setting (13,14,16,20,23). A growing body of literature on BIVA in sport and exercise research and practice is also noticeable (see the review by Castizo-Olier et al.(1) and more recently (24,25)), and *specific* BIVA has been proposed as a promising approach in this field (1).

Athought largely used, reliability studies of phase angle, classic or specific BIVA in the assessment of body composition (18,19,21,26), or of hydration (21,26–29) through reference techniques are very scarse in the general population and totally lacking in athletes (1).

Therefore, the aim of this research was to evaluate the accuracy of phase angle, classic and specific BIVA in body composition assessment of athletes, focusing the analysis on absolute values of body mass (FM, FFM, TBW, ECW, ICW), and on values independent from body dimensions (%FM, ECW/ICW). At this purpose, DXA ~~will be~~ was used as a reference for FM, FFM and %FM, and dilution techniques for TBW and ECW.

SUBJECTS AND METHODS

Subjects

This was a cross-sectional, observational study on 202 athletes (139 men and 63 women) over 16 years of age (men: 21.5 ± 5.0; women: 20.7 ± 5.1). The sample included athletes involved in a total of 11 sports (Athletics, Basketball, Handball, Judo, Karate, Pentathlon, Rugby, Soccer, Swimming, Triathlon, Volleyball; suppl. table 1). The results of a medical screening indicated that all subjects were in good health. The following inclusion criteria were used: 1) 10 or more hours of training per week, 2) negative test outcomes for performance-enhancing drugs, and 3) not taking any medications. All subjects and their parents or guardians were informed about the possible risks of the investigation before giving written informed consent to participate. All procedures were approved by the ethics committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association (30).

On each subject, all the measurements were obtained in the same morning. Subjects came to the laboratory after an overnight fast (12 h fast), refraining from vigorous exercise at least 15 h, no caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before (figure 1).

"Figure 1 about here"

Anthropometry

All anthropometric data were collected by an ISAK accreditation technician according to a standardized protocol (31). Body weight was measured with a scale, without shoes and wearing minimal clothes, to the nearest 0.01 kg; height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). Body Mass Index (BMI) was calculated as the ratio of body mass to height squared (kg/m^2). Girths were measured by using an anthropometric tape (Lufkin W606PM; Apex Tool Group, Sparks, MD, USA). ~~Skinfold thicknesses were measured by use of a Slim Guide calliper (Creative Health Products, Ann Arbor, MI, USA).~~ The intra-observer technical error of measurement (TEM) and the coefficient of variation (CV) were calculated in a subsample of ten subjects (height: TEM=0.06 cm, CV=0.04; weight: TEM=0.04kg, CV=0.07; arm circumference: TEM=0.09 cm, CV=0.3; waist circumference: TEM=0.3 cm, CV=0.4; calf circumference: TEM=0.06 cm, CV=0.2).

Dual-energy X-ray absorptiometry

Athletes underwent a whole-body DXA scan according to the procedures recommended by the manufacturer on a Hologic Explorer-W fan-beam densitometer (Hologic, Waltham, MA, USA). The equipment measures the attenution of X-rayween 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. According to the protocol described by the manufacturer, a step phantom with six fields of acrylic and aluminum of varying thicknesses and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components. For athletes who were taller than the scan area, we used a validated procedure that consisted of the sum of a head and a trunk plus limbs scans (32). The same technician positioned the participants, performed the scan, and executed the analysis (QDR for Windows software version 12.4; Hologic, Waltham, MA, USA) according to the operator’s manual by using the standard analysis protocol. The DXA measurements included whole-body measurements of absolute FM (kg), percentage FM (%FM) and FFM (kg).

Body fluids

Following the collection of a baseline urine sample, each participant was given an oral dose of 0.1 g of 99.9% H_2O per kg of body weight (SigmaeAldrich; St. Louis, MO) for the determination of TBW by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). Subjects were encouraged to void their bladder prior to the 4-h equilibration period and subsequent sample collection, due to inadequate mixing of pre-existing urine in the bladder (33). Urine samples were prepared for 1 $H/^2H$ analyses using the equilibration technique by Prosser and Scrimgeour (34). Our laboratory has reported a CV in ten subjects for TBW of 0.3%. ECW was assessed from a baseline saliva sample using the sodium bromide (NaBr) dilution method after the subject consumed 0.030 g of 99.0% NaBr (SigmaeAldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water. ICW was calculated as the difference between TBW and ECW.

Bioelectrical impedance

The impedance measurements were performed with BIA (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made on an isolated cot from electrical conductors, the subjects were in the supine position with a leg opening of 45° compared to the median line of the body and the upper limbs, distant 30° from the trunk. After cleaning the skin with alcohol, two electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the right hand back and two electrodes on the neck of the corresponding foot (29). Bioelectrical impedance vector analysis was carried out using the classic and specific BIVA methods, i.e. normalizing resistance (R) and reactance (Xc) parameters for stature (H) in meters (classic BIVA;(17)), or multiplying R and Xc by a correction factor (A/L), where A is the estimated cross-sectional area (or 0.45 × arm area + 0.10 × waist area + 0.45 × calf area) and L the length of the 'conductor' (1.1 × height) (specific BIVA; (18,19)). The length of the vector was calculated as the hypotenuses of individual impedance values. Bioelectrical phase angle (PA) was calculated as the arc-tangent of $Xc/R \times 180°/\pi$. Prior to each test, the analyzer was checked with the calibration deemed successful if R value is 383 Ω and Xc equal to 46 Ω. The test-retest CV in 10 participants in our laboratory for R and Xc was 0.3% and 0.9%, respectively. Italo Spanish bioelectrical specific values (35) were used as a reference. Italo Spanish bioelectrical classic values (unpublished data) were: R/H (men: 284.9 ± 33.6; women: 391.2 ± 41.1); Xc/H (men: 38.0 ± 5.0; women: 44.0 ± 5.8).

Statistical Analysis

Descriptive statistics including means ± standard deviations were calculated for all outcome variables. Normality was evaluated using Shapiro-Wilk test. Since the data showed a normal distribution, the association between bioelectrical impedance and body composition values was investigated using Pearson's correlation analysis. Multiple regression analyses were performed to understand the associations between FM, %FM, FFM, TBW, ICW, and ECW and bioelectrical values. Model adjustments included age and sport practiced. If more than one variable was a predictor in the model, a variance inflation factor (VIF) for each independent variable was calculated to evaluate multicollinearity, and values below 5 were considered not to have multicollinearity issues. The sample distribution of %FM, TBW and ECW/ICW was divided into quartiles and the bioelectrical values of cases below the first quartile (Q1) were compared with those above the third quartile (Q3) by means of Hotelling's T^2 test.

Data were analyzed with IBM SPSS Statistics version 24.0 (IBM, Chicago, IL). Bioelectric variables were calculated using the specific BIVA software (www.specificbiva.unica.it). For all tests, statistical significance was set at $p < 0.05$.

RESULTS

Athletes of both sexes showed a condition of normal weight, with low mean values of BMI and low average values of %FM, as expected in a sample of young sportive subjects (Table 1).

"Table 1 about here"

Anthropometric and body composition measurements showed significant differences between sexes. Consistently with the known pattern of sexual dimorphism in adults, men showed higher values of all anthropometric measurements, FFM, TBW, ECW, ICW, while women showed higher bioelectrical values (with the only exception of *specific* reactance), and higher FM, %FM, and ECW/ICW (table 1).

Both men and women showed significantly higher stature ($p<0.001$), significantly larger circumferences ($p<0.001$ for waist and upper arm circumference; $p<0.05$ for calf circumference, only in men), but a similar BMI with respect to the Italo-Spanish reference population. Classic bioelectrical values (R/H and Xc/H) were significantly lower in Portuguese athletes of both sexes than in the reference population ($p<0.001$), whereas specific values were not significantly different in the two populations, with the exception of Rsp which was higher in the Italo-Spanish group ($p<0.05$). Phase angle was similar in men and significantly higher in Portuguese females ($p<0.001$).

Table 2 shows the correlation matrix between bioelectrical impedance and body composition variables. Following adjustment for covariates, including age and sport practiced, bioelectrical values remained significantly associated with body composition variables. In fact, in the multicollinearity diagnosis we found no VIF above 5, which is the rule of thumb used in regression models to assess if the β is affected.

"Table 2 about here"

In classic BIVA, the correlation between TBW, ECW, ICW and R/H, Xc/H, Z/H was highly and negatively significant in both sexes (table 2, figure 2a), and the mean vectors of groups with lower and higher amounts of body water (below Q1 vs. above Q3 of the TBW) were significantly different (figure 3a,g). The association between FFM or FM and R/H, Xc/H, Z/H was negative in both sexes (table 2), while the correlation with %FM was inconsistent in the two sexes (Z/H negatively correlated in men and positively in women) and reached the significance level only in men (table 2, figure 2c). To be noted that the classic mean vectors of one or both opposite quartiles were located in the left lower region of the tolerance ellipses, towards the region of obesity (figure 3c,i).

"Figure 2 and figure 3 about here"

In specific BIVA, the correlation between FM or %FM and bioelectrical values (Rsp, Xcsp, Zsp) was positive and highly significant in both sexes (table 2, figure 2d), while the association with FFM rarely reached the significance level. The mean vectors of groups with different percentages of body fat (below Q1 vs. above Q3 of the %FM) were significantly separated (figure 3d,l). The mean vectors of opposite quartiles were located within the 50% tolerance ellipses and the group with higher %FM (above $Q3_{\%FM}$) toward the pole of higher %FM, as expected. The association of specific bioelectrical values with TBW, ICW or ECW, instead, was not significant, with the only exception of the positive correlation between Xcsp and ICW ~~or~~ and TBW in men (table 2, figure 2b, figure 3b,h).

PA, and hence both classic and specific BIVA, detected ECW/ICW differences in both sexes, with lower PA values in subjects with higher ECW/ICW ratio (table 2, figure 3e,f,m,n, figure 4). It was also positively associated with ICW and TBW in men and negatively associated with %FM in women (table 2).

"Figure 4 about here"

DISCUSSION

The present study, for the first time, analysed the association of PA, classic and specific BIVA with DXA and dilution techniques, for body composition assessment in athletes. Data showed that classic BIVA correctly detect differences of TBW, but was weak in the assessment of %FM. On the contrary, specific BIVA detected changes of %FM, but not those of TBW. The relation with FM and FFM was different in classic and *specific* BIVA: classic bioelectrical values were negatively related to body compartments (particularly to FFM), while specific bioelectrical values showed a positive correlation (particularly with FM). ~~Both classic and specific BIVA were negatively correlated with FM and FFM.~~ Also, the ~~while their~~ relation with water compartments was different: R/H and Xc/H were negatively related to ICW and ECW, while in specific BIVA only Xcsp was positively related to ICW and only in men. PA, which is the same in classic and specific BIVA, was sensitive to ECW/ICW ratio and ICW. These results were unaffected by age and sports practiced. Although the sexual dimorphism, the association between bioelectrical and body composition variables was quite similar in the two sexes. The only exception was the stronger relation between classic values with %FM or FM in men (with an opposite direction in the two sexes in the case of FM%), and the stronger relation between *specific* values with FM observed in women.

Previous reliability studies on body composition assessment in the general population, using DXA as a reference, have shown quite similar results. Indeed, specific BIVA has demonstrated to evaluate FM, FFM, and %FM accurately in US adults (18) and in Italian elderly (19). Further, both *specific* vector length and phase angle have shown to be able to detect skeletal muscle mass differences (20). The same studies have also shown that classic BIVA can recognize different quantities of absolute mass, but does not perform accurately in evaluating %FM and in the recognition of the obesity and athletic regions within the RXc graph (18,19), as in the present research. Furthermore, Wells et al. (26) recently tested classic BIVA in a sample of healthy children against the criterion 4-component model and recognized inconsistencies in body composition outcomes, particularly for FFM. Accordingly, the recent review on the applications of BIVA in sport sciences (1) has shown that the majority of the studies using classic BIVA did not observe bioelectrical vectors falling in the region of the tolerance ellipses expected for athletes. As suggested by Castizo-Olier et al. (1), this could indicate the need of reference values for each population or sport. However, as discussed with more detail elsewhere (1,20), these unexpected results of classic BIVA could be due to the solely effect of body geometry—cross-sectional areas in particular—on bioelectrical parameters. In fact, according to the Ohm’s law, resistance is directly proportional to the conductor’s length and inversely proportional to its cross-section. Indeed, our sample of athletes, characterized by shorter classic vectors (significantly lower values of R/H and Xc/H) with respect to the reference sample of Italo-Spanish young adults (35) is also characterized by significantly higher circumferences. The correction for cross-sections applied in specific BIVA reduces the differences related to body size and shape, increasing the sensitivity of bioelectrical values to tissues' properties and body composition, such as %FM. In fact, the vectors of Portuguese athletes are located toward the obesity region of classic tolerance ellipses of the Italian-Spanish young adults (35), while they are centrally located within the specific tolerance ellipses of same reference population.

Classic BIVA is commonly used to monitor hydration changes, with fluid overload indicated by shorter vectors, i.e. falling towards the lower pole of the classic tolerance ellipses. The technique has been clinically validated for the evaluation of TBW (29,36–38), and used for detecting body fluids changes in athletes (39). Further, Wells et al. (26) showed that~~, in a sample of children,~~ BIVA outcomes behaved as expected on the basis of theoretical assumptions in the case of FFM hydration, using the 4-component model as a reference. The vector migration has also shown to be consistent with fluid loss determined using dilution techniques (28,29). However, Heavens et al. (28) noticed that the area of normal hydration on the tolerance ellipses is wider than expected on the basis of dilution techniques.

The classic vector length, mainly determined by R/H values, can be also considered indicative of extracellular water (negative relation), being ECW strongly correlated with TBW(40), while Xc/H, which is related to body cell mass, should be positively associated with ICW (17). Instead, we have observed a negative relation between Xc/H and ICW. However, it should be noted that ICW, as well as ECW, is also positively correlated with TBW. Further, nor R/H or Xc/H are expected to give information on fluid distribution between compartments and tissue hydration, especially if considered separately. ~~Such information~~ Fluid distribution is more related to the ECW/ICW ratio, which is not dependent on body dimensions (and hence on absolute values of ICW, ECW, TBW), and mainly detected by PA. In fact, PA has demonstrated to be related to water distribution between the extra- and intra-cellular spaces using dilution as reference technique: the higher PA, the greater proportion of ICW compared to ECW, i.e. the lower ECW/ICW ratio (or ECW/TBW) (21,27). PA is identical in classic and specific BIVA and, accordingly, the two techniques have demonstrated a similar accuracy in detecting ECW/ICW in US adults, based on the comparison with bioelectrical impedance spectroscopy(18).

Body composition and body fluids monitoring is a relevant topic in sports. In fact, an elevated body fat mass can negatively affect the quality of movement and performance in athletes (25,41), while hypo-hydration and fluid accumulation may compromise physical and cognitive performance, and eventually health (42); especially in certain sports (43,44). Futhermore, ICW variations are related to changes in performance (45–47). However, it should be stressed that different physiological adaptations and dehydration processes, diversely affecting the extra cellular and intracellular spaces, can be induced by physical exercise and their relations with biolectrical changes should be better explored (48). ~~Furthermore~~Moreover, as also suggested by Wells et al. (26), further work is needed to improve the understanding of PA meaning at the physiological level~~, especially in younger age groups~~.

This research has the main point of strength of being the first study performed in athletes analysing the association of PA, classic and specific BIVA with DXA and dilution techniques in the assessment of body composition and body fluids.

Despite the encouraging results obtained in this study, some limitations are present and should be considered. In fact, our results are applicable to BIA equipment using the 50 kHz frequency and to a similar population. ~~Even if single-frequency devices are among the most used equipment, similar studies should be conducted using multifrequency equipments.~~ Indeed, even if multifrequency equipments are widely used with acceptable accuracy at the group level to assess and track FFM(49–51), BIVA was originally developed and proposed using single-frequency devices. Moreover, a recently published research (52) showed that BIS values at 50 kHz are not directly comparable to those obtained by single-frequency devices. Thus, further analysis using multifrequency equipments are required and could give useful information. Additional studies should focus on health and disease populations, different age groups, ethnicity, and body regions to better define the suitability of BIVA approaches for body composition assessment.

CONCLUSIONS

The present study shows that specific BIVA is more accurate than classic BIVA in the %FM assessment in athletes, whereas the classic method is able to analyze body fluids with a higher accuracy. PA (and hence both classic and specific BIVA) was sensitive to ECW/ICW ratio. Physicians and sports coaches should consider using both BIVA approaches (classic and specific) to obtain reliable body composition evaluations in athletes. More research is needed to analyse the sensitivity of BIVA to each type of dehydration and to body water compartments. Further, validation studies are also necessary with regard to the variations of body composition and hydration that occur during the competitive season and in pre- to post-exercise.

FIGURE LEGENDS

Figure 1. Timeline of stations performed by the athletes involved in the study.

Figure 2. Correlation between classic or specific impedance vectors with total body water or fat-mass% in men. a: Z/H vs. TBW; b: Zsp vs. TBW; c: Z/H vs. %FM; d: Zsp vs %FM. Z: impedance; H: height; sp: specific; TBW: total body water; %FM: percentage of fat mass.

Figure 3. Classic and specific mean vectors of quartiles (below Q1 vs. above Q3) with different total body water, fat-mass%, and extracellular/intracellular water ratio in men.

circles: below Q1; triangles: above Q3; a: classic BIVA and TBW (men); b: specific BIVA and TBW (men); c: classic BIVA and %FM (men); d: specific BIVA and %FM (men); e: classic BIVA and ECW/ICW (men); f: specific BIVA and ECW/ICW (men); g: classic BIVA and TBW (women); h: specific BIVA and TBW (women); i: classic BIVA and %FM (women); l: specific BIVA and %FM (women); m: classic BIVA and ECW/ICW (women); n: specific BIVA and ECW/ICW (women); TBW: total body water; %FM: percentage of fat mass; ECW/ICW: extracellular/intracellular water ratio.

Figure 4. Correlation between phase angle and extracellular/intracellular water ratio in men.

ACKNOWLEDGMENTS

The authors thank the athletes for participating in the study. This work was supported by the Portuguese Foundation for Science and Technology (Grants: PTDC/DES/69495/2006 and PTDC/DES/098963/2008). Silvia Stagi acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme, European Social Fund 2014-2020 - Axis III Education and training, Thematic goal 10, Priority of investment 10ii, Specific goal 10.5., Action partnership agreement 10.5.12). The study sponsor had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript and in the decision to submit the manuscript for publication.

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Table 1. Participants' characteristics, including the correlation between bioelectrical variables and the comparison between sexes

	Men (n=139)	Women (n=63)		
Variable	Mean ± SD	Mean ± SD	t-Student	*p*
Age (y)	21.5 ± 5.0	20.7 ± 5.1	1.0	0.296
Height (cm)	183.3 ± 9.1	171.1 ± 8.2	9.2	0.000
Weight (kg)	77.2 ± 11.4	63.7 ± 8.9	8.3	0.000
Upper arm crf (cm)	32.3 ± 3.2	28.6 ± 2.6	8.3	0.000
Waist crf (cm)	81.3 ± 6.4	76.5 ± 5.7	5.1	0.000
Calf crf (cm)	37.6 ± 2.4	36.1 ± 2.8	3.6	0.000
BMI (kg/m^2)	22.9 ± 2.6	21.8 ± 2.1	3.1	0.002
R (ohm)	467.9 ± 51.4	566.1 ± 67.4	-11.4	0.000
Xc (ohm)	63.1 ± 8.0	67.6 ± 10.5	-3.4	0.001
Z (ohm)	471.8 ± 51.6	567.2 ± 67.7	-11.2	0.000
PA (degrees)	7.7 ± 0.8	6.8 ± 0.8	7.1	0.000
R/H (ohm/m)	255.8 ± 30.6	331.5 ± 41.2	-14.6	0.000
Xc/H (ohm/m)	34.6 ± 5.1	39.6 ± 6.4	-6.1	0.000
Z/H (ohm/m)	258.2 ± 30.8	334.3 ± 41.3	-14.5	0.000
Rsp (ohm*cm)	324.3 ± 31.2	368.3 ± 46.1	-8.0	0.000
Xcsp (ohm*cm)	43.9 ± 6.2	44.0 ± 7.1	-0.1	0.924
Zsp (ohm*cm)	327.3 ± 31.5	370.9 ± 45.9	-8.2	0.000
FM (kg)	10.8 ± 4.3	15.4 ± 4.4	-6.9	0.000
FM (%)	13.9 ± 3.9	24.1 ± 4.8	-16.0	0.000
FFM (kg)	65.7 ± 8.6	47.9 ± 6.2	14.7	0.000
TBW (kg)	49.5 ± 7.5	35.8 ± 5.3	12.1	0.000
ECW (kg)	19.2 ± 3.1	14.6 ± 1.9	10.2	0.000
ICW (kg)	30.4 ± 5.7	21.2 ± 3.8	10.5	0.000
ECW/ICW (kg)	0.6 ± 0.1	0.7 ± 0.1	-3.4	0.001
r R-Xc	0.577	0.687		
r R/H-Xc/H	0.669	0.729		
r Rsp-Xcsp	0.636	0.716		

BMI, body mass index; R, resistance; Xc, reactance; PA, phase angle; Z, vector length; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Z/H, vector length standardized for height; Rsp, resistance standardized for height and transverse areas; Xcsp, reactance standardized for height and transverse areas; Zsp, vector length standardized for height and transverse areas; FM, fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; r R-Xc, correlation between R-Xc; r R/H-Xc/H, correlation between R/H-Xc/H; r Rsp-Xcsp, correlation between Rsp-Xcsp.

Table 2. Correlation between bioelectrical and body composition variables

Men

	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	PA
FM	-0.312***	-0.356***	-0.316***	-0.406***	-0.398***	-0.443***	0.602***	0.340***	0.588***	-0.085
%FM	-0.144	-0.228**	-0.147	-0.160	-0.215*	-0.214*	0.589***	0.313***	0.569***	-0.105
FFM	-0.539***	-0.462***	-0.542***	-0.781***	-0.625***	-0.778***	0.173*	0.127	0.204*	0.010
TBW	-0.731***	-0.484***	-0.732***	-0.883***	-0.586***	-0.880***	0.068	0.186*	0.099	0.184*
ECW	-0.484***	-0.565***	-0.490***	-0.701***	-0.694***	-0.702***	-0.028	-0.156	-0.019	-0.165
ICW	-0.705***	-0.339***	-0.703***	-0.792***	-0.405***	-0.783***	0.104	0.326***	0.140	0.327***
ECW/ICW	0.295*	-0.170	0.288**	0.207*	-0.204*	0.187*	-0.122	-0.472***	-0.153	-0.493***

Women

	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	PA
FM	0.059	-0.128	0.055	-0.126	-0.256*	-0.127	0.734***	0.414***	0.737***	-0.232
%FM	0.281*	0.001	0.277*	0.222	-0.033	0.218	0.774***	0.407***	0.773***	-0.295*
FFM	-0.475***	-0.333***	-0.475***	-0.734***	-0.525***	-0.731***	0.029	0.055	0.026	0.052
TBW	-0.598***	-0.368***	-0.597***	-0.829***	-0.549***	-0.829***	-0.171	-0.018	-0.156	0.146
ECW	-0.543***	-0.489***	-0.545***	-0.781***	-0.667***	-0.788***	-0.033	0.086	-0.043	0.083
ICW	-0.547***	-0.258	-0.545***	-0.746***	-0.419***	-0.743***	-0.219	0.018	-0.193	0.243
ECW/ICW	0.214	0.127	0.209	0.244	0.085	0.229	0.256	-0.117	0.215	-0.408***

r values are reported in the table; R, resistance; Xc, reactance; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; PA, phase angle; FM, fat mass; %FM, percentage of fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Z, vector length; Zsp, vector length multiplicated for coefficient; Z/H, vector length standardized for height.

Phase angle and bioelectrical impedance vector analysis in the evaluation of body composition in athletes

Elisabetta Marini[1], Francesco Campa[2], Roberto Buffa[1], Silvia Stagi[1], Catarina N. Matias[3], Stefania Toselli[2], Luís B. Sardinha[3], Analiza M. Silva[3]

[1] Department of Life and Environmental Sciences, Neuroscience and Anthropology Section, University of Cagliari, Cagliari, Italy;
[2] Department of Biomedical and Neuromotor Science, University of Bologna, Bologna, Italy;
[3] Exercise and Health Laboratory, CIPER, Faculdade de Motricidade Humana, Universidade de Lisboa, Portugal.

Running title: Body composition assessment

Keywords: BIVA; body fluid; extracellular water; intracellular water; fat mass.

Corresponding author:

Elisabetta Marini - Department of Life and Environmental Sciences, Neuroscience and Anthropology Section, University of Cagliari, Cittadella di Monserrato, Cagliari, Italy. tel. +39 0706756607. e-mail: emarini@unica.it. Alternative Corresponding Author: Francesco Campa - Department of Biomedical and Neuromotor Sciences, University of Bologna. Via Selmi, 3, 40121 Bologna, Italy. Tel.: +39-3450031080. E-mail: francesco.campa3@unibo.it

Competing interests statement: The Authors declare no competing financial or non-financial interests.

Abstract

Aims: To analyze the association of classic and specific bioelectrical impedance vector analysis (BIVA) and phase angle with reference techniques for the assessment of body composition in athletes.

Methods: 202 athletes of both sexes (men: 21.5 ± 5.0; women: 20.7 ± 5.1) engaged in different sports were evaluated during the in-season period. Bioelectrical resistance (R, ohm) and reactance (Xc, ohm) were obtained with a phase-sensitive 50 kHz bioelectrical impedance analysis device. The classic and specific BIVA procedures, which respectively correct bioelectrical values for body height (R/H and Xc/H, ohm/m) and body geometry (Rsp and Xcsp, ohm cm), were applied. Dual energy X-ray absorptiometry was used as the reference method to assess fat-mass (FM), fat-free mass (FFM) and %FM. Deuterium dilution and bromide dilution where used as the criterion method for total body water (TBW) and extracellular water (ECW), respectively. Intracellular water (ICW) was calculated as TBW minus ECW.

Results: Specific bioelectrical values (Rsp, Xcsp, Zsp) were positively correlated with FM and %FM (%FM; Zsp men: $r=0.569$, $p<0.001$; Zsp women: $r=0.773$, $p<0.001$). Classic values (R/H, Xc/H, Z/H) were negatively correlated with FM and FFM, but were correlated with %FM only in men (Z/H men $r=-0.214$, $p=0.013$; Z/H women $r=0.218$, $p=0.097$). As to body fluid, classic BIVA showed strong associations (Z/H men: $r=-0.880$, $p<0.001$; Z/H women: $r=-0.829$, $p<0.001$) with TBW, whereas Zsp was not correlated. Phase angle was negatively correlated with ECW/ICW ratio in both sexes (men: $r=-0.493$, $p<0.001$; women: $r=-0.408$, $p<0.001$) and positively with ICW (men: $r=0.327$, $p<0.001$; women: $r=0.243$, $p=0.080$).

Conclusions: Specific BIVA turns out to be more accurate for the analysis of %FM in athletes, while it does not correctly evaluate TBW, for which classic BIVA appears to be a suitable approach. Phase angles, and hence both BIVA approaches, can detect ECW/ICW changes.

INTRODUCTION

The analysis and monitoring of body composition is fundamental in sport, because of its relevance to athletes' health and performance, and to team success. Such analysis can be performed in different contexts and with different approaches, i.e. in cross-sectional studies aimed to characterise sporting group samples, in longitudinal researches finalised to define short-term or long-term changes, or in applications aimed to detect and monitor muscle injuries (1). Variations of body composition can interest diversely athletes practicing different sport, because of their different exercise type and requirements for body physique and composition. In general, lean mass is considered a predictor of muscular fitness (2,3). Furthermore, while overhydration is quite uncommon in athletes, physiological dehydration processes can be induced by physical activity, leading to hypotonic, isotonic, or hypertonic dehydration (4).

Several techniques can be used to assess body composition in athletes. At the molecular level, though the four-compartment model is considered the reference method for body composition assessment (5), dual energy X-ray absorptiometry (DXA), a three-compartment model, has been recognized as a precise and accurate technique for determining fat (FM) and FFM (6). Still, DXA is an expensive method to be used in the field setting as a minimum space to accommodate the DXA machine, a potential radiation shielding, and specialized technicians to perform and analyze the exams are required (7). Considering the main FFM component, total-body water (TBW), its amount and the content of the extracellular water compartment (ECW) can be accurately assessed through dilution techniques, specifically using deuterium and bromide dilution, respectively (8). However, these analytic procedures are time-consuming, costly and laborious, thus compromising their routine use in a clinical or field setting (9). Therefore, simple methods to determine water compartments, easily applied during training and competition, are required.

Bioelectrical impedance analysis (BIA) is a fast, safe and non-invasive method to obtain quantitative estimates of body composition. Multifrequency BIA and, specifically, bioimpedance spectroscopy is preferable for fluid volume measurements, though for general body composition assessment BIA at 50 kHz is more widely used (10,11). Bioelectrical impedance (Z, ohm) is composed by resistance (R, ohm) and reactance (Xc, ohm) [$Z=(R^2+Xc^2)^{0.5}$)]. 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 tissues. Xc, which is detectable by phase sensitive devices only, is related to the capacitance properties of the cell membrane and to variations that can occur depending on its integrity, function and composition (12). Phase angle (PA) [$PA=\arctan Xc/R \; 180/\pi$] is determined by the time delay occurring when the electric current passes the cell membrane (13,14).

Bioelectrical impedance can be applied using prediction equations (15). However, the dependency on population-specific equations and hydration status is considered the major weakness of conventional bioelectrical-impedance analysis (16). Alternatively, the analysis can be performed using raw data, namely phase angles, or bioelectrical impedance vectors, i.e. phase angle and vector length jointly, as in the bioelectrical impedance vector analysis approaches (BIVA; (17–19)).

Bioelectrical impedance vector analysis, both classic (14,17) and specific BIVA (20), is based on the analysis of impedance vectors (at 50 kHz), projected on a RXc graph in relation to tolerance ellipses, or for intergroup comparisons (confidence ellipses). The two BIVA approaches differ each other in that classic BIVA analyses bioelectrical values standardized for subject's height (which represents the conductor’s length), whereas in specific BIVA R and Xc values are corrected also for cross-sectional areas, in order to reduce the effect of body dimensions. According to classic BIVA (17), variations of bioelectrical vectors along the major axis of tolerance ellipses indicate changes in total body water (TBW) (dehydration towards the upper pole, fluid overload towards the lower pole). The minor axis refers to variations of absolute amount of body cell mass, FM, and FFM (left side: more mass; right side: less mass) and to variations of extracellular/intracellular water ratio (ECW/ICW) (low values in the left side). Within classic tolerance ellipses, the left upper side would correspond to athletic individuals, whereas the left lower side to obese ones. In specific BIVA (18,19), the major axis relates to %FM variation (higher values toward the upper pole), while the minor axis gives the same information as in classic BIVA (more mass and lower ECW/ICW ratio on the left side). In fact, the minor axis is mainly related to variations of phase angle, which is unaffected by the correction.

PA allows the interpretation of total body water and body cell mass (14,21). However, the analysis of PA only, without considering the information furnished by vector length, can lead to interpretation errors. In fact, groups of individuals characterized by quite identical PA, but different vector lengths, may show different body fluids or %FM (22). The vectorial approach appears to be more efficient, as it considers both influential variables, phase angle and vector length.

PA, classic and specific BIVA have been applied in different groups, particularly obese, athletic subjects, and in the elderly, and in the clinical setting (13,14,16,20,23). A growing body of literature on BIVA in sport and exercise research and practice is also noticeable (see the review by Castizo-Olier et al.(1) and more recently (24,25)), and *specific* BIVA has been proposed as a promising approach in this field (1).

Athought largely used, reliability studies of phase angle, classic or specific BIVA in the assessment of body composition (18,19,21,26), or of hydration (21,26–29) through reference techniques are very scarse in the general population and totally lacking in athletes (1).

Therefore, the aim of this research was to evaluate the accuracy of phase angle, classic and specific BIVA in body composition assessment of athletes, focusing the analysis on absolute values of body mass (FM, FFM, TBW, ECW, ICW), and on values independent from body dimensions (%FM, ECW/ICW). At this purpose, DXA was used as a reference for FM, FFM and %FM, and dilution techniques for TBW and ECW.

SUBJECTS AND METHODS

Subjects

This was a cross-sectional, observational study on 202 athletes (139 men and 63 women) over 16 years of age (men: 21.5 ± 5.0; women: 20.7 ± 5.1). The sample included athletes involved in a total of 11 sports (Athletics, Basketball, Handball, Judo, Karate, Pentathlon, Rugby, Soccer, Swimming, Triathlon, Volleyball; suppl. table 1). The results of a medical screening indicated that all subjects were in good health. The following inclusion criteria were used: 1) 10 or more hours of training per week, 2) negative test outcomes for performance-enhancing drugs, and 3) not taking any medications. All subjects and their parents or guardians were informed about the possible risks of the investigation before giving written informed consent to participate. All procedures were approved by the ethics committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association (30).

On each subject, all the measurements were obtained in the same morning. Subjects came to the laboratory after an overnight fast (12 h fast), refraining from vigorous exercise at least 15 h, no caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before (figure 1).

"Figure 1 about here"

Anthropometry

All anthropometric data were collected by an ISAK accreditation technician according to a standardized protocol (31). Body weight was measured with a scale, without shoes and wearing minimal clothes, to the nearest 0.01 kg; height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). Body Mass Index (BMI) was calculated as the ratio of body mass to height squared (kg/m^2). Girths were measured by using an anthropometric tape (Lufkin W606PM; Apex Tool Group, Sparks, MD, USA). The intra-observer technical error of measurement (TEM) and the coefficient of variation (CV) were calculated in a subsample of ten subjects (height: TEM=0.06 cm, CV=0.04; weight: TEM=0.04kg, CV=0.07; arm circumference: TEM=0.09 cm, CV=0.3; waist circumference: TEM=0.3 cm, CV=0.4; calf circumference: TEM=0.06 cm, CV=0.2).

Dual-energy X-ray absorptiometry

Athletes underwent a whole-body DXA scan according to the procedures recommended by the manufacturer on a Hologic Explorer-W fan-beam densitometer (Hologic, Waltham, MA, USA). The equipment measures the attenution of X-rayween 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. According to the protocol described by the manufacturer, a step phantom with six fields of acrylic and aluminum of varying thicknesses and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components. For athletes who were taller than the scan area, we used a validated procedure that consisted of the sum of a head and a trunk plus limbs scans (32). The same technician positioned the participants, performed the scan, and executed the analysis (QDR for Windows software version 12.4; Hologic, Waltham, MA, USA) according to the operator's manual by using the standard analysis protocol. The DXA measurements included whole-body measurements of absolute FM (kg), percentage FM (%FM) and FFM (kg).

Body fluids

Following the collection of a baseline urine sample, each participant was given an oral dose of 0.1 g of 99.9% H_2O per kg of body weight (SigmaeAldrich; St. Louis, MO) for the determination of TBW by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa

Scientific, UK). Subjects were encouraged to void their bladder prior to the 4-h equilibration period and subsequent sample collection, due to inadequate mixing of pre-existing urine in the bladder (33). Urine samples were prepared for 1 H/^{2}H analyses using the equilibration technique by Prosser and Scrimgeour (34). Our laboratory has reported a CV in ten subjects for TBW of 0.3%. ECW was assessed from a baseline saliva sample using the sodium bromide (NaBr) dilution method after the subject consumed 0.030 g of 99.0% NaBr (SigmaeAldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water. ICW was calculated as the difference between TBW and ECW.

Bioelectrical impedance

The impedance measurements were performed with BIA (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made on an isolated cot from electrical conductors, the subjects were in the supine position with a leg opening of 45° compared to the median line of the body and the upper limbs, distant 30° from the trunk. After cleaning the skin with alcohol, two electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the right hand back and two electrodes on the neck of the corresponding foot (29). Bioelectrical impedance vector analysis was carried out using the classic and specific BIVA methods, i.e. normalizing resistance (R) and reactance (Xc) parameters for stature (H) in meters (classic BIVA;(17)), or multiplying R and Xc by a correction factor (A/L), where A is the estimated cross-sectional area (or 0.45 × arm area + 0.10 × waist area + 0.45 × calf area) and L the length of the 'conductor' (1.1 × height) (specific BIVA; (18,19)). The length of the vector was calculated as the hypotenuses of individual impedance values. Bioelectrical phase angle (PA) was calculated as the arc-tangent of Xc/R × 180°/π. Prior to each test, the analyzer was checked with the calibration deemed successful if R value is 383 Ω and Xc equal to 46 Ω. The test-retest CV in 10 participants in our laboratory for R and Xc was 0.3% and 0.9%, respectively. Italo Spanish bioelectrical specific values (35) were used as a reference. Italo Spanish bioelectrical classic values (unpublished data) were: R/H (men: 284.9 ± 33.6; women: 391.2 ± 41.1); Xc/H (men: 38.0 ± 5.0; women: 44.0 ± 5.8).

Statistical Analysis

Descriptive statistics including means ± standard deviations were calculated for all outcome variables. Normality was evaluated using Shapiro-Wilk test. Since the data showed a normal distribution, the association between bioelectrical impedance and body composition values was investigated using Pearson’s correlation analysis. Multiple regression analyses were performed to understand the associations between FM, %FM, FFM, TBW, ICW, and ECW and bioelectrical values. Model adjustments included age and sport practiced. If more than one variable was a predictor in the model, a variance inflation factor (VIF) for each independent variable was calculated to evaluate multicollinearity, and values below 5 were considered not to have multicollinearity issues. The sample distribution of %FM, TBW and ECW/ICW was divided into quartiles and the bioelectrical values of cases below the first quartile (Q1) were compared with those above the third quartile (Q3) by means of Hotelling's T^2 test.

Data were analyzed with IBM SPSS Statistics version 24.0 (IBM, Chicago, IL). Bioelectric variables were calculated using the specific BIVA software (www.specificbiva.unica.it). For all tests, statistical significance was set at $p < 0.05$.

RESULTS

Athletes of both sexes showed a condition of normal weight, with low mean values of BMI and low average values of %FM, as expected in a sample of young sportive subjects (Table 1).

“Table 1 about here”

Anthropometric and body composition measurements showed significant differences between sexes. Consistently with the known pattern of sexual dimorphism in adults, men showed higher values of all anthropometric measurements, FFM, TBW, ECW, ICW, while women showed higher bioelectrical values (with the only exception of *specific* reactance), and higher FM, %FM, and ECW/ICW (table 1).

Both men and women showed significantly higher stature ($p<0.001$), significantly larger circumferences ($p<0.001$ for waist and upper arm circumference; $p<0.05$ for calf circumference, only in men), but a similar BMI with respect to the Italo-Spanish reference population. Classic bioelectrical values (R/H and Xc/H) were significantly lower in Portuguese athletes of both sexes than in the reference population ($p<0.001$), whereas specific values were not significantly different in the two populations, with the exception of Rsp which was higher in the Italo-Spanish group ($p<0.05$). Phase angle was similar in men and significantly higher in Portuguese females ($p<0.001$).

Table 2 shows the correlation matrix between bioelectrical impedance and body composition variables. Following adjustment for covariates, including age and sport practiced, bioelectrical values remained significantly associated with body composition variables. In fact, in the multicollinearity diagnosis we found no VIF above 5, which is the rule of thumb used in regression models to assess if the β is affected.

“Table 2 about here”

In classic BIVA, the correlation between TBW, ECW, ICW and R/H, Xc/H, Z/H was highly and negatively significant in both sexes (table 2, figure 2a), and the mean vectors of groups with lower and higher amounts of body water (below Q1 vs. above Q3 of the TBW) were significantly different (figure 3a,g). The association between FFM or FM and R/H, Xc/H, Z/H was negative in both sexes (table 2), while the correlation with %FM was inconsistent in the two sexes (Z/H negatively correlated in men and positively in women) and reached the significance level only in men (table 2, figure 2c). To be noted that the classic mean vectors of one or both opposite quartiles were located in the left lower region of the tolerance ellipses, towards the region of obesity (figure 3c,i).

“Figure 2 and figure 3 about here”

In specific BIVA, the correlation between FM or %FM and bioelectrical values (Rsp, Xcsp, Zsp) was positive and highly significant in both sexes (table 2, figure 2d), while the association with FFM rarely reached the significance level. The mean vectors of groups with different percentages of body fat (below Q1 vs. above Q3 of the %FM) were significantly separated (figure 3d,l). The mean vectors of opposite quartiles were located within the 50% tolerance ellipses and the group with higher %FM (above $Q3_{\%FM}$) toward the pole of higher %FM, as expected. The association of specific bioelectrical values with TBW, ICW or ECW, instead, was not significant, with the only exception of the positive correlation between Xcsp and ICW and TBW in men (table 2, figure 2b, figure 3b,h).

PA, and hence both classic and specific BIVA, detected ECW/ICW differences in both sexes, with lower PA values in subjects with higher ECW/ICW ratio (table 2, figure 3e,f,m,n, figure 4). It was also positively associated with ICW and TBW in men and negatively associated with %FM in women (table 2).

“Figure 4 about here”

DISCUSSION

The present study, for the first time, analysed the association of PA, classic and specific BIVA with DXA and dilution techniques, for body composition assessment in athletes. Data showed that classic BIVA correctly detect differences of TBW, but was weak in the assessment of %FM. On the contrary, specific BIVA detected changes of %FM, but not those of TBW. The relation with FM and FFM was different in classic and *specific* BIVA: classic bioelectrical values were negatively related to body compartments (particularly to FFM), while specific bioelectrical values showed a positive correlation (particularly with FM). Also, the relation with water compartments was different: R/H and Xc/H were negatively related to ICW and ECW, while in specific BIVA only Xcsp was positively related to ICW and only in men. PA, which is the same in classic and specific BIVA, was sensitive to ECW/ICW ratio and ICW. These results were unaffected by age and sports practiced. Although the sexual dimorphism, the association between bioelectrical and body composition variables was quite similar in the two sexes. The only exception was the stronger relation between classic values with %FM or FM in men (with an opposite direction in the two sexes in the case of FM%), and the stronger relation between *specific* values with FM observed in women.

Previous reliability studies on body composition assessment in the general population, using DXA as a reference, have shown quite similar results. Indeed, specific BIVA has demonstrated to evaluate FM, FFM, and %FM accurately in US adults (18) and in Italian elderly (19). Further, both *specific* vector length and phase angle have shown to be able to detect skeletal muscle mass differences (20). The same studies have also shown that classic BIVA can recognize different quantities of absolute mass, but does not perform accurately in evaluating %FM and in the recognition of the obesity and athletic regions within the RXc graph (18,19), as in the present research. Furthermore, Wells et al. (26) recently tested classic BIVA in a sample of healthy children against the criterion 4-component model and recognized inconsistencies in body composition outcomes, particularly for FFM. Accordingly, the recent review on the applications of BIVA in sport sciences (1) has shown that the majority of the studies using classic BIVA did not observe bioelectrical vectors falling in the region of the tolerance ellipses expected for athletes. As suggested by Castizo-Olier et al. (1), this could indicate the need of reference values for each population or sport. However, as discussed with more detail elsewhere (1,20), these unexpected results of classic BIVA could be due to the solely effect of body geometry—cross-sectional areas in particular—on bioelectrical parameters. In fact, according to the Ohm's law, resistance is directly proportional to the conductor's length and inversely proportional to its cross-section. Indeed, our sample of athletes, characterized by shorter classic vectors (significantly lower values of R/H and Xc/H) with respect to the reference sample of Italo-Spanish young adults (35) is also characterized by significantly higher circumferences. The correction for cross-sections applied in specific BIVA reduces the differences related to body size and shape, increasing the sensitivity of bioelectrical values to tissues' properties and body composition, such as %FM. In fact, the vectors of Portuguese athletes are located toward the obesity region of classic tolerance ellipses of the Italian-Spanish young adults (35), while they are centrally located within the specific tolerance ellipses of same reference population.

Classic BIVA is commonly used to monitor hydration changes, with fluid overload indicated by shorter vectors, i.e. falling towards the lower pole of the classic tolerance ellipses. The technique has been clinically validated for the evaluation of TBW (29,36–38), and used for detecting body fluids changes in athletes (39). Further, Wells et al. (26) showed that BIVA outcomes behaved as expected on the basis of theoretical assumptions in the case of FFM hydration, using the 4-component model as a reference. The vector migration has also shown to be consistent with fluid loss determined using dilution techniques (28,29). However, Heavens et al. (28) noticed that the area of normal hydration on the tolerance ellipses is wider than expected on the basis of dilution techniques.

The classic vector length, mainly determined by R/H values, can be also considered indicative of extracellular water (negative relation), being ECW strongly correlated with TBW(40), while Xc/H, which is related to body cell mass, should be positively associated with ICW (17). Instead, we have observed a negative relation between Xc/H and ICW. However, it should be noted that ICW, as well as ECW, is also positively correlated with TBW. Further, nor R/H or Xc/H are expected to give information on fluid distribution between compartments and tissue hydration, especially if considered separately. Fluid distribution is more related to the ECW/ICW ratio, which is not dependent on body dimensions (and hence on absolute values of ICW, ECW, TBW), and mainly detected by PA. In fact, PA has demonstrated to be related to water distribution between the extra- and intra-cellular spaces using dilution as reference technique: the higher PA, the greater proportion of ICW compared to ECW, i.e. the lower ECW/ICW ratio (or ECW/TBW) (21,27). PA is identical in classic and specific BIVA and, accordingly, the two techniques have demonstrated a similar accuracy in detecting ECW/ICW in US adults, based on the comparison with bioelectrical impedance spectroscopy(18).

Body composition and body fluids monitoring is a relevant topic in sports. In fact, an elevated body fat mass can negatively affect the quality of movement and performance in athletes (25,41), while hypo-hydration and fluid accumulation may compromise physical and cognitive performance, and eventually health (42); especially in certain sports (43,44). Futhermore, ICW variations are related to changes in performance (45–47). However, it should be stressed that different physiological adaptations and dehydration processes, diversely affecting the extra cellular and intracellular spaces, can be induced by physical exercise and their relations with biolectrical changes should be better explored (48). Moreover, as also suggested by Wells et al. (26), further work is needed to improve the understanding of PA meaning at the physiological level.

This research has the main point of strength of being the first study performed in athletes analysing the association of PA, classic and specific BIVA with DXA and dilution techniques in the assessment of body composition and body fluids.

Despite the encouraging results obtained in this study, some limitations are present and should be considered. In fact, our results are applicable to BIA equipment using the 50 kHz frequency and to a similar population. Indeed, even if multifrequency equipments are widely used with acceptable accuracy at the group level to assess and track FFM(49–51), BIVA was originally developed and proposed using single-frequency devices. Moreover, a recently published research (52) showed that BIS values at 50 kHz are not directly comparable to those obtained by single-frequency devices. Thus, further analysis using multifrequency equipments are required and could give useful information. Additional studies should focus on health and disease populations, different age groups, ethnicity, and body regions to better define the suitability of BIVA approaches for body composition assessment.

CONCLUSIONS

The present study shows that specific BIVA is more accurate than classic BIVA in the %FM assessment in athletes, whereas the classic method is able to analyze body fluids with a higher accuracy. PA (and hence both classic and specific BIVA) was sensitive to ECW/ICW ratio. Physicians and sports coaches should consider using both BIVA approaches (classic and specific) to obtain reliable body composition evaluations in athletes. More research is needed to analyse the sensitivity of BIVA to each type of dehydration and to body water compartments. Further, validation studies are also necessary with regard to the variations of body composition and hydration that occur during the competitive season and in pre- to post-exercise.

FIGURE LEGENDS

Figure 1. Timeline of stations performed by the athletes involved in the study.

Figure 2. Correlation between classic or specific impedance vectors with total body water or fat-mass% in men. a: Z/H vs. TBW; b: Zsp vs. TBW; c: Z/H vs. %FM; d: Zsp vs %FM. Z: impedance; H: height; sp: specific; TBW: total body water; %FM: percentage of fat mass.

Figure 3. Classic and specific mean vectors of quartiles (below Q1 vs. above Q3) with different total body water, fat-mass%, and extracellular/intracellular water ratio in men.

Circles: below Q1; triangles: above Q3; a: classic BIVA and TBW (men); b: specific BIVA and TBW (men); c: classic BIVA and %FM (men); d: specific BIVA and %FM (men); e: classic BIVA and ECW/ICW (men); f: specific BIVA and ECW/ICW (men); g: classic BIVA and TBW (women); h: specific BIVA and TBW (women); i: classic BIVA and %FM (women); l: specific BIVA and %FM (women); m: classic BIVA and ECW/ICW (women); n: specific BIVA and ECW/ICW (women); TBW: total body water; %FM: percentage of fat mass; ECW/ICW: extracellular/intracellular water ratio.

Figure 4. Correlation between phase angle and extracellular/intracellular water ratio in men.

ACKNOWLEDGMENTS

The authors thank the athletes for participating in the study. This work was supported by the Portuguese Foundation for Science and Technology (Grants: PTDC/DES/69495/2006 and PTDC/DES/098963/2008). Silvia Stagi acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme, European Social Fund 2014-2020 - Axis III Education and training, Thematic goal 10, Priority of investment 10ii, Specific goal 10.5., Action partnership agreement 10.5.12). The study sponsor had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript and in the decision to submit the manuscript for publication.

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Table 1. Participants' characteristics, including the correlation between bioelectrical variables and the comparison between sexes

	Men (n=139)	Women (n=63)		
Variable	Mean ± SD	Mean ± SD	t-Student	*p*
Age (y)	21.5 ± 5.0	20.7 ± 5.1	1.0	0.296
Height (cm)	183.3 ± 9.1	171.1 ± 8.2	9.2	0.000
Weight (kg)	77.2 ± 11.4	63.7 ± 8.9	8.3	0.000
Upper arm crf (cm)	32.3 ± 3.2	28.6 ± 2.6	8.3	0.000
Waist crf (cm)	81.3 ± 6.4	76.5 ± 5.7	5.1	0.000
Calf crf (cm)	37.6 ± 2.4	36.1 ± 2.8	3.6	0.000
BMI (kg/m^2)	22.9 ± 2.6	21.8 ± 2.1	3.1	0.002
R (ohm)	467.9 ± 51.4	566.1 ± 67.4	-11.4	0.000
Xc (ohm)	63.1 ± 8.0	67.6 ± 10.5	-3.4	0.001
Z (ohm)	471.8 ± 51.6	567.2 ± 67.7	-11.2	0.000
PA (degrees)	7.7 ± 0.8	6.8 ± 0.8	7.1	0.000
R/H (ohm/m)	255.8 ± 30.6	331.5 ± 41.2	-14.6	0.000
Xc/H (ohm/m)	34.6 ± 5.1	39.6 ± 6.4	-6.1	0.000
Z/H (ohm/m)	258.2 ± 30.8	334.3 ± 41.3	-14.5	0.000
Rsp (ohm*cm)	324.3 ± 31.2	368.3 ± 46.1	-8.0	0.000
Xcsp (ohm*cm)	43.9 ± 6.2	44.0 ± 7.1	-0.1	0.924
Zsp (ohm*cm)	327.3 ± 31.5	370.9 ± 45.9	-8.2	0.000
FM (kg)	10.8 ± 4.3	15.4 ± 4.4	-6.9	0.000
FM (%)	13.9 ± 3.9	24.1 ± 4.8	-16.0	0.000
FFM (kg)	65.7 ± 8.6	47.9 ± 6.2	14.7	0.000
TBW (kg)	49.5 ± 7.5	35.8 ± 5.3	12.1	0.000
ECW (kg)	19.2 ± 3.1	14.6 ± 1.9	10.2	0.000
ICW (kg)	30.4 ± 5.7	21.2 ± 3.8	10.5	0.000
ECW/ICW (kg)	0.6 ± 0.1	0.7 ± 0.1	-3.4	0.001
r R-Xc	0.577	0.687		
r R/H-Xc/H	0.669	0.729		
r Rsp-Xcsp	0.636	0.716		

BMI, body mass index; R, resistance; Xc, reactance; PA, phase angle; Z, vector length; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Z/H, vector length standardized for height; Rsp, resistance standardized for height and transverse areas; Xcsp, reactance standardized for height and transverse areas; Zsp, vector length standardized for height and transverse areas; FM, fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; r R-Xc, correlation between R-Xc; r R/H-Xc/H, correlation between R/H-Xc/H; r Rsp-Xcsp, correlation between Rsp-Xcsp.

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Table 2. Correlation between bioelectrical and body composition variables

Men

	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	PA
FM	-0.312***	-0.356***	-0.316***	-0.406***	-0.398***	-0.443***	0.602***	0.340***	0.588***	-0.085
%FM	-0.144	-0.228**	-0.147	-0.160	-0.215*	-0.214*	0.589***	0.313***	0.569***	-0.105
FFM	-0.539***	-0.462***	-0.542***	-0.781***	-0.625***	-0.778***	0.173*	0.127	0.204*	0.010
TBW	-0.731***	-0.484***	-0.732***	-0.883***	-0.586***	-0.880***	0.068	0.186*	0.099	0.184*
ECW	-0.484***	-0.565***	-0.490***	-0.701***	-0.694***	-0.702***	-0.028	-0.156	-0.019	-0.165
ICW	-0.705***	-0.339***	-0.703***	-0.792***	-0.405***	-0.783***	0.104	0.326***	0.140	0.327***
ECW/ICW	0.295*	-0.170	0.288**	0.207*	-0.204*	0.187*	-0.122	-0.472***	-0.153	-0.493***

Women

	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	PA
FM	0.059	-0.128	0.055	-0.126	-0.256*	-0.127	0.734***	0.414***	0.737***	-0.232
%FM	0.281*	0.001	0.277*	0.222	-0.033	0.218	0.774***	0.407***	0.773***	-0.295*
FFM	-0.475***	-0.333***	-0.475***	-0.734***	-0.525***	-0.731***	0.029	0.055	0.026	0.052
TBW	-0.598***	-0.368***	-0.597***	-0.829***	-0.549***	-0.829***	-0.171	-0.018	-0.156	0.146
ECW	-0.543***	-0.489***	-0.545***	-0.781***	-0.667***	-0.788***	-0.033	0.086	-0.043	0.083
ICW	-0.547***	-0.258	-0.545***	-0.746***	-0.419***	-0.743***	-0.219	0.018	-0.193	0.243
ECW/ICW	0.214	0.127	0.209	0.244	0.085	0.229	0.256	-0.117	0.215	-0.408***

r values are reported in the table; R, resistance; Xc, reactance; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; PA, phase angle; FM, fat mass; %FM, percentage of fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Z, vector length; Zsp, vector length multiplicated for coefficient; Z/H, vector length standardized for height.

Figure 1

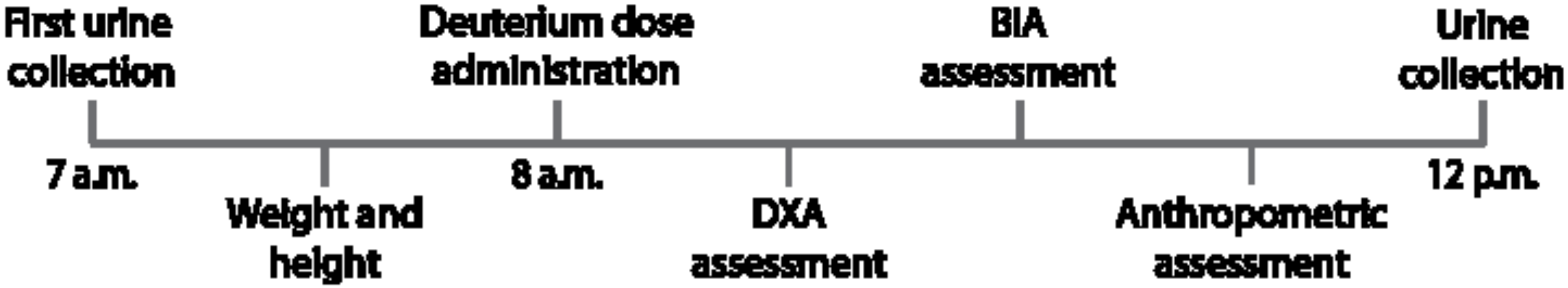
First urine collection
Deuterium dose administration
BIA assessment
Urine collection
7 a.m.
8 a.m.
12 p.m.
Weight and height
DXA assessment
Anthropometric assessment

Figure 2

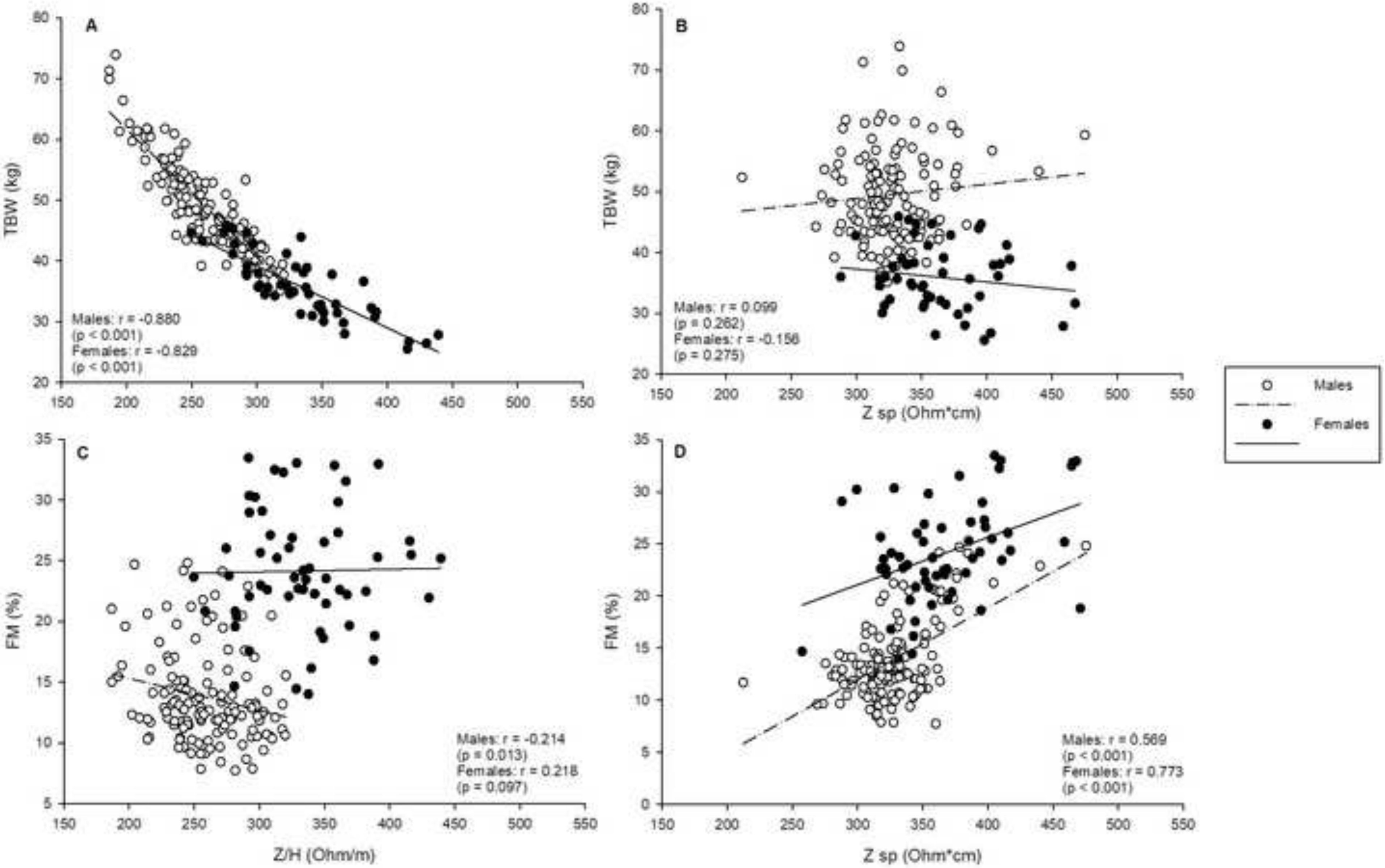
A
TBW (kg)
80
70
60
50
40
30
20
150
200
250
300
350
400
450
500
550
Males: r = -0.880
(p < 0.001)
Females: r = -0.829
(p < 0.001)
B
TBW (kg)
Males: r = 0.099
(p = 0.262)
Females: r = -0.156
(p = 0.275)
Z sp (Ohm*cm)
○ Males
● Females
C
FM (%)
35
30
25
20
15
10
5
Males: r = -0.214
(p = 0.013)
Females: r = 0.218
(p = 0.097)
Z/H (Ohm/m)
D
FM (%)
0
Males: r = 0.569
(p < 0.001)
Females: r = 0.773
(p < 0.001)
Z sp (Ohm*cm)

Figure 3

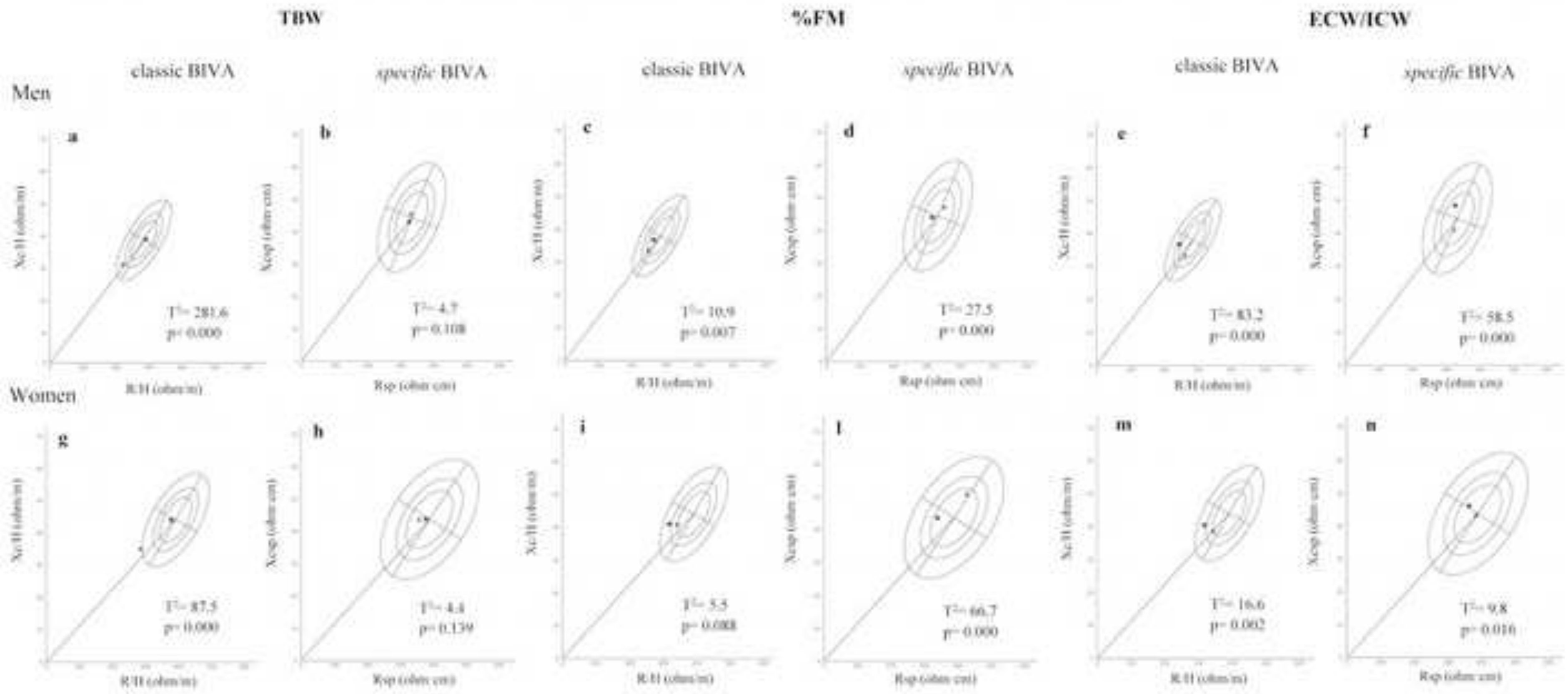
TBW
%FM
ECW/ICW
classic BIVA
specific BIVA
classic BIVA
specific BIVA
classic BIVA
specific BIVA
Men
a
Xc/H (ohm/m)
R/H (ohm/m)
T²= 281.6
p= 0.000
b
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 4.7
p= 0.108
c
Xc/H (ohm/m)
R/H (ohm/m)
T²= 10.9
p= 0.007
d
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 27.5
p= 0.000
e
Xc/H (ohm/m)
R/H (ohm/m)
T²= 83.2
p= 0.000
f
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 58.5
p= 0.000
Women
g
Xc/H (ohm/m)
R/H (ohm/m)
T²= 87.5
p= 0.000
h
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 4.4
p= 0.139
i
Xc/H (ohm/m)
R/H (ohm/m)
T²= 5.5
p= 0.088
l
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 66.7
p= 0.000
m
Xc/H (ohm/m)
R/H (ohm/m)
T²= 16.6
p= 0.002
n
Xcsp (ohm cm)
Rsp (ohm cm)
T²= 9.8
p= 0.016

Figure 4

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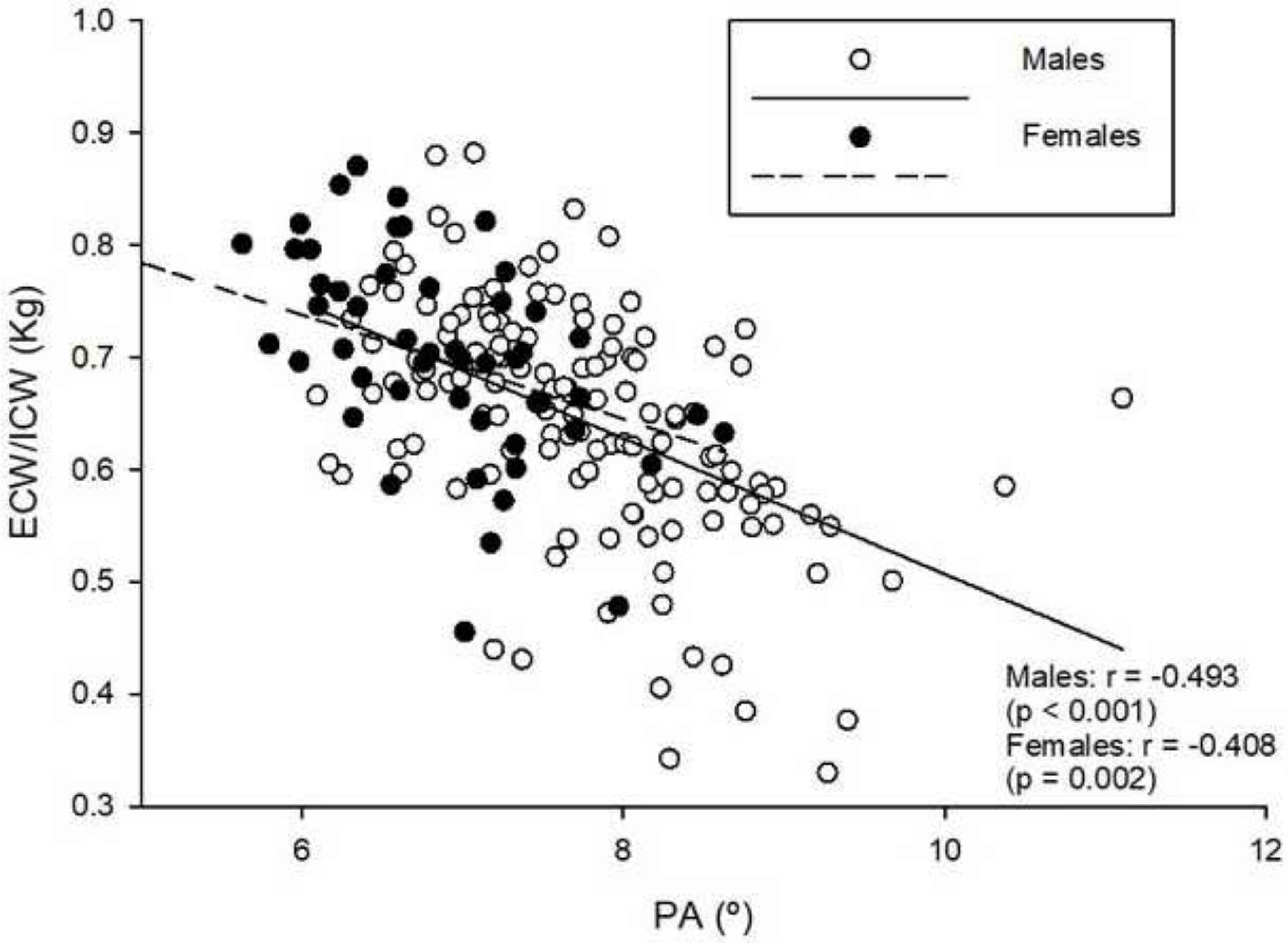

Highlights (for review)

- Classic and specific BIVA, and PA were tested against DXA and dilution techniques
- Classic BIVA correctly detected changes of TBW, but not those of %FM.
- Specific BIVA detected changes of %FM, but not those of TBW.
- PA (equal in classic and specific BIVA) was sensitive to ECW/ICW ratio and ICW.

Author Contribution Statement

Conceptualization (EM, RB, FC, AMS, LBS); Formal analysis (FC, CNM, RB, SS); Funding acquisition (LBS, AM); Investigation (LBS, CNM, AM); Methodology (FC, EM, AMS, LBS); Project administration (LBS, AM); Resources (SS, LBS, AM); Supervision (EM, AMS, LBS); Visualization (FC, CNM, SS, ST); Roles/Writing - original draft (FC, RB, SS, EM); Writing - review & editing (all authors).