



Body composition, energy expenditure and physical activity

Lack of agreement of in vivo raw bioimpedance measurements obtained from two single and multi-frequency bioelectrical impedance devices

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Abstract

Background It is important for highly active individuals to accurately assess their hydration level. Bioelectrical impedance (BIA) can potentially meet these needs but its validity in active individuals is not well established.

Methods We compared whole-body bioimpedance measurements obtained from multi-frequency bioelectrical impedance spectroscopy (BIS, Xitron 4200) at a 50 kHz frequency with those determined by a phase-sensitive single-frequency device (SF-BIA, BIA-101, RJL/Akern Systems) in two populations: active adults and elite athletes.

Results One hundred twenty-six participants, including active males involved in recreational sports ($N = 25$, 20–39 yr) and elite athletes (females: $N = 26$, 18–35 yr; males: $N = 75$, 18–38 yr) participated in this study. Reactance (X_c), Resistance (R), Impedance (Z), and phase angle (PhA) were obtained by BIS and SF-BIA. Small but significant differences ($R: -9.91 \pm 15.09 \Omega$; $X_c: -0.97 \pm 2.56 \Omega$; $Z: -9.96 \pm 15.18 \Omega$; PhA: $0.12 \pm 0.2^\circ$) were observed between the bioimpedance equipment in all measured variables ($p < 0.05$) though differences were within the devices' technical error of measurements. Device-specific values were highly ($p < 0.0001$) correlated [R^2 ranged from 0.881 (X_c) to 0.833 (R)], but slopes and intercepts were different ($p < 0.0001$) from 1 and 0, respectively. Relatively large limits of agreement were observed for R (-40 to 21Ω), X_c (-6 to 4Ω), PhA (-0.4 to 0.5°), and impedance (-40 to 20Ω).

Conclusion: Bioimpedance measurements from the current single- and multi-frequency devices should not be used interchangeably. The lack of agreement between devices was observed in determining individual values of R , X_c , Z and PhA of highly active populations possibly due to methodological and biological factors.

Introduction

The assessment of balance fluid loss and intake is relevant in sports and should be monitored over the athletic season for assuring that athletes' performance is maximized while health is not compromised. Bioelectrical impedance

analysis (BIA) is a non-invasive and practical technique that can meet this need [1].

Several BIA methods and instruments have been widely used to assess the structure and function of biological entities [2]. Alternating current is introduced into the body by modern BI electronic devices at single or multiple frequencies. Passive bioelectrical measurements can be related to physiological or body composition parameters.

For whole-body and localized assessments, the BIA method uses a phase-sensitive impedance device that applies a low-level, constant alternating current with a tetrapolar surface electrode placement on the hands and feet [2, 3] or a defined region of the body [4, 5]. It measures impedance (Z), a complex quantity, that involves a purely resistive component, resistance (R) (from water and electrolytes in fluids and tissues) and the capacitance associated with cell membrane integrity and cell interfaces, reactance (X_c) [6].

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Measurement of the time delay between the application of voltage and current penetration at the cell membrane and tissue level is assessed by the complex electronic circuitry, and is identified as the phase angle (PhA). Using a simple mathematical approach, the impedance value for the body is distinguished into R and Xc components as Z (sin phase angle) and Z (cos phase angle), correspondingly, of a R–Xc series circuit. A 50-kHz frequency is usually utilized by the phase-sensitive BIA device (SF-BIA) to measure PhA and Z, and calculate R and Xc [6].

Tetrapolar multi-frequency BIA instruments, specifically bioelectrical impedance spectroscopy (BIS), determine frequency-specific Z ranging from 5 kHz to 1 MHz. At each frequency, the equipment measures R and Xc and calculates the Z and PhA [7].

Several factors such as sex, age, fluid distribution and body mass index (BMI) affect PhA values among healthy persons [8]. Therefore, PhA is considered an index of the cell membrane integrity and vitality with higher values indicating greater cellularity, cell function and integrity in individuals with normal hydration [6].

Technical concerns related with the use of different BIA instruments may compromise the use of reference or cut-off values proposed for PhA as an indicator of nutritional and physiological status. Genton et al. [9] found that PhA differed significantly in older adults measured with different BIA devices (Eugedia, RJL-101, and 4000 Xitron). In a multi-ethnic sample of children, Tanabe et al. [10] found significant differences in Xc values obtained with Xitron4200 and RJL BIA.

Recognizing the relevance of raw BIA parameters in health and disease along with the availability of different devices, it is still unclear whether SF-BIA and multi-frequency devices would provide similar R, Xc and PhA values, if obtained in a highly active population. Therefore, the present study aims to determine the accuracy of raw BIA values obtained from BIS (Xitron4200) compared to a phase-sensitive SF-BIA (BIA-101, RJL/Akern Systems).

Methods

Participants

A total of 126 participants (25 highly active, men involved in recreational sports and 101 national-level athletes (75 males and 26 females) from a multitude of sports (handball, volleyball, basketball, rugby, swimmers, athletics, triathlon, pentathlon, judo, tennis and soccer) participated in this study. Athletes were evaluated during the competitive period of the season.

The inclusion criteria were: (1) physical activity level > 2.0 or >10 h of sport-specific training per week [11]; (2)

negative test outcomes for performance-enhancing drugs; and (3) not taking any medications or supplements at the time of the measurements. Informed consent was obtained from each participant and/or guardian if under the age of legal consent prior to testing. All procedures were approved by the Ethics Committee of the University of Lisbon and the investigation was conducted according to the guidelines reported in the Declaration of Helsinki [12].

Body composition measurements

All body composition measurements were performed in the morning (8:00 to 10:00 a.m.) after an overnight fast lasting (≥ 12 h) with at least 15 h from the last exercise session.

Anthropometric measurements

All participants were weighed to the nearest 0.01 kg in minimal clothing using the scale interfaced with the plethysmograph (BOD POD[®] Cosmed, Rome, Italy), while stature was measured to the nearest 0.1 cm using a wall stadiometer (Seca, Hamburg, Germany) according to standardized procedures reported elsewhere [13]. Circumferences, skinfolds and breadths were obtained according to the International Society for the Advancement of Kinanthropometry protocols [14] by one certified anthropometrists. A total of four somatypes were identified based on Carter and Heath equations [13] namely: Central, Ectomorph and Mesomorph-Ectomorph, Endomorph and Endomorph-Mesomorph, and Mesomorph.

Body composition

Total and regional fat mass (FM), fat-free mass (FFM), lean soft tissue (LST) and bone mineral content (BMC) were determined by dual-energy X-ray absorptiometry (Hologic Explorer W, QDR for windows version 12.4, Waltham, MA, USA) as described in detail elsewhere [15].

Bioelectrical impedance

For both BI devices (SF-BIA and BIS), measurements were performed in a random order (time difference 30 s) after a 10-min period of rest with the participant in a supine position. Four electrodes were placed on the dorsal surfaces of right foot and ankle and right wrist and hand. A 240 μA_{RMS} alternating current at 50 kHz was introduced into the distal electrode of each pair (source electrode), and the voltage drop across the body was measured using the proximal electrode (detector electrode). Low-impedance electrodes (Impedimed, IU0GELTD, Pinkenba, QLD, Australia), specifically 27 Ω , 0.9 Ω and 27 Ω for R, Xc and

Table 1 Participants' characteristics and body composition

	Female	Male	Whole sample
	Mean \pm SD (<i>N</i> = 25)	Mean \pm SD (<i>N</i> = 101)	Mean \pm SD (<i>N</i> = 126)
Age (year)	208 \pm 4.4	22.7 \pm 5.2	22.3 \pm 5.1
Weight (kg)	62.5 \pm 8.1	76.5 \pm 11.5	73.6 \pm 12.2
Height (cm)	167.8 \pm 6.9	180.8 \pm 8.9	178.1 \pm 10.0
BMI (kg/m ²)	22.2 \pm 2.3	23.4 \pm 3.0	23.1 \pm 2.9
Waist circumference (cm)	71.9 \pm 2.1	77.6 \pm 4.5	76.2 \pm 4.7
Arm circumference (cm)	28.2 \pm 2.6	30.9 \pm 3.3	30.4 \pm 3.4
Calf circumference (cm)	37.9 \pm 10.7	38.2 \pm 6.0	38.2 \pm 7.2
BMC (kg)	2.5 \pm 0.5	3.1 \pm 0.6	3.0 \pm 0.6
Fat (kg)	14.4 \pm 4.2	11.0 \pm 4.7	11.7 \pm 4.8
Fat (%)	22.9 \pm 5.3	14.3 \pm 4.3	16.1 \pm 5.7
FFM (kg)	47.9 \pm 6.3	64.1 \pm 9.0	60.8 \pm 10.7
LST (kg)	45.5 \pm 5.9	61.2 \pm 8.5	58.0 \pm 10.2
R _{SF-BIA} (Ω)	497.7 \pm 74.3	482.1 \pm 54.9	485.3 \pm 59.4
R _{BIS50*} (Ω)	485.5 \pm 63.1	472.7 \pm 53.3	475.4 \pm 55.4
X _{CSF-BIA} (Ω)	65.3 \pm 8.1	65.4 \pm 7.4	65.4 \pm 7.6
X _{C_{BIS50*}} (Ω)	64.7 \pm 7.7	64.3 \pm 7.2	64.4 \pm 7.3
PhA _{SF-BIA} (Ω)	7.5 \pm 0.8	7.8 \pm 0.8	7.7 \pm 0.8
PhA _{BIS50*} (Ω)	7.6 \pm 0.8	7.8 \pm 0.8	7.8 \pm 0.8
Z _{SF-BIA} (Ω)	502.0 \pm 74.4	486.5 \pm 55.0	489.7 \pm 59.5
Z _{BIS50*} (Ω)	489.9 \pm 63.2	477.1 \pm 53.4	479.8 \pm 55.5

BMI body mass index, *BMC* bone mineral content, *FFM* fat free mass, *LST* lean soft tissue, *BIS₅₀* bioelectrical impedance spectroscopy at 50 kHz, *R* resistance, *Xc* reactance, *PhA* phase angle, *Z* impedance, *SF-BIA* single-frequency BIA

*Different from reference method, $p < 0.05$

Z, respectively, were used for measuring raw parameters obtained from single- and multi-frequency devices.

Single-frequency bioelectrical impedance analysis

Whole body *R* and *Xc* were obtained by BIA using a single-frequency, phase-sensitive 50 kHz (BIA-101, RJL/Akern Systems, Firenze, Italy) [16].

Prior to each test, the technical validity of SF-BIA instrument was determined with a precision circuit ($R = 383 \Omega$, and capacitance = 46Ω). Measured resistance and reactance values were within the tolerance of the precision circuit ($\leq 10 \Omega$ and $\leq 5 \Omega$, respectively). The biological reliability determined using low-impedance electrodes (Impedimed, 139 IUOGELTD, Pinkenba, QLD, Australia) in 10 participants in our laboratory was 0.3 and 0.9% for *R* and *Xc*, respectively [16].

Multispectral frequency bioelectrical impedance analysis

Whole-body *R*, *Xc*, *PhA* and *Z* at frequency 50 kHz were also determined by using a BIS model 4200 (Xitron Technologies, San Diego, CA, USA) [17]. Prior to each test, the technical validity of this device was determined using a manufacture-provided electronic Verification Module (option TS4201). The circuit consists of a 1% 681 Ω resistor in parallel with a series 1% 909 Ω resistor and 5% 3.3 nF capacitor. The manufacture's verification process did not yield raw BI values but modelled or calculated *R*, *Xc* and *Z* that were within the tolerance of the device [7]. The biological reliability determined in six participants in our laboratory for *R* and *Xc* at 50 Hz was 0.6 and 1.5%, respectively.

Bioelectrical impedance vector analysis

Bioelectrical impedance vector analysis (BIVA) was applied using the SF-BIA device [17], adjusting individual vectors for height (*R/H*, Ω/m , and *Xc/H*, Ω/m) to eliminate the conductor length effect, and projecting the vectors in the cartesian plane defined by *R/H* and *Xc/H* (*R*–*Xc* graph). The characteristics of the individuals were compared with the concentric tolerance ellipses (50, 75 and 95% of cases) representing the variability of an Italian population used as the reference population to compare our Portuguese sample [18]. The major axis of the tolerance ellipses refers to hydration status (under-hydrated individuals tending towards the upper pole; over-hydrated individuals towards the lower one), and the minor axis indicates cell mass, where the left side corresponds to a high cell mass (i.e. more soft tissue). Individuals with values outside the 75% tolerance ellipse (three females and seven males) were removed from the sample in order to rule out possible bias in method comparison due to variations in hydration status.

Statistical analysis

Descriptive statistics were performed, and all variables were checked for normality. Paired sample *t*-tests were used to compare the mean values obtained from both devices. Comparison parameters included the analyses of the coefficient of determination and the standard error of estimations. It was also investigated if the slope and intercept differed from 1 and 0, respectively (line of identity). Additionally, agreement between methods was assessed using the Bland–Altman approach [19], including the analysis of the correlation between the mean and the difference of the methods.

IBM SPSS Statistics version 25.0, 2017 (IBM, Chicago, Illinois, USA) was used for data analysis. Statistical significance was set at $p < 0.05$.

Table 2 Regression and concordance correlation coefficient analysis for R, Xc, PhA and Z estimates using BIS at 50 kHz and the reference method (SF-BIA, Akern)

	<i>r</i>	SEE	Slope	Intercept	CCC	Precision (ρ)	Accuracy (C_b)
R	0.966	14.31	0.902 ^a	37.509 ^b	0.9498	0.9664	0.9829
Xc	0.939	2.53	0.906 ^a	5.159 ^b	0.9302	0.9307	0.9909
PhA	0.957	0.22	0.925 ^a	0.621 ^b	0.9548	0.9568	0.9979
Z	0.966	14.41	0.902 ^a	37.920 ^b	0.9495	0.9660	0.9829

BIS bioelectrical impedance spectroscopy, R resistance, Xc reactance, PhA phase angle, Z impedance, *r* coefficient of correlation, SEE standard error of estimation, CCC concordance correlation coefficient

^aSlope significantly different from 1, $p < 0.05$

^bIntercept significantly different from 0, $p < 0.05$

Results

Table 1 presents the demographic and body composition characteristics of the participants. Compared to the SF-BIA device, the BIS instrument provided significantly ($p < 0.05$) lower values of R, Xc and Z but higher values of PhA at 50 kHz.

Although the methods were highly correlated for all raw BIA outcomes, the slope and intercept significantly differed from 1 and 0, respectively (Table 2).

We observed a significant interaction term of somatotype and BIS in determining R ($p < 0.001$) and Xc obtained by SF-BIA ($p < 0.001$). For R, significant interactions ($p < 0.001$) were found for each somatotype category, with coefficients of 1.026 for central, 1.032 for ectomorph plus mesomorph ectomorph, 1.040 for endomorph plus endomorph mesomorph, and 1.022 for mesomorph somatotypes. For reactance, significant interactions ($p < 0.001$) were found for each somatotype category, with coefficients of 0.974 for central, 0.973 for ectomorph plus mesomorph ectomorph, 0.979 for endomorph plus endomorph mesomorph, and 0.974 for mesomorph somatotypes.

The Bland–Altman plots displayed in Fig. 1 illustrate agreement between methods according to somatotypes observed in the sample.

We observed a significant difference of -9.91Ω for R, -0.97Ω for Xc, 0.42° for PhA and -0.95Ω for Z. The limits of agreement ranged from -40.1 to 20.6Ω for R, -6.1 to 4.2Ω for Xc, from -0.4 to 0.5° for PhA and -40.3 to 20.4Ω for Z. A significant trend between the mean and the difference of the methods was found for R ($r = -0.26$; $p = 0.003$) and Z ($r = -0.26$, $p = 0.004$).

The greatest magnitude of differences was observed for the category endomorph and endomorph mesomorph (R: -14.95Ω , Xc: -1.32Ω , PhA: 0.08° and Z: -14.95Ω), whereas the lowest differences (R: -7.58Ω , PhA: 0.02° and Z: -7.63Ω) was observed for mesomorph participants.

We additionally investigated the effect of age, weight, height, total and regional BMC, FM, FFM and LST on differences between the methods. Age was associated with the difference of the methods for R ($r = 0.264$, $p = 0.007$) and Z ($r = 0.264$, $p = 0.007$), which means that in younger

participants BIS tend to display lower values of R and Xc compared to SF-BIA, whereas in older participants the opposite is observed. Legs and appendicular FM, separately, were negatively associated with the difference of the methods for R (arms = -0.189 , $p = 0.033$; legs = -0.231 , $p = 0.009$; appendicular = -0.227 , $p = 0.010$) and Z (arms = -0.186 , $p = 0.03$; legs = -0.228 , $p = 0.010$; appendicular = -0.225 , $p = 0.011$). These observations mean that in athletes with a higher adiposity in the limbs, BIS tend to display lower values of R and Z, exhibiting higher values of R and Z, compared to SF-BIA, in those with a lower appendicular FM.

Discussion

The main finding of this study was the lack of agreement between Akern and Xitron 4200 at 50 kHz in the individual determination of raw measured parameters, despite the high association observed at the group level in a highly active populations.

The main difficulty in understanding the differences between methods in determining R, Xc, Z, PhA using Akern and Xitron instruments is related to different technology used to provide the validity and reliability of these values. Akern is a phase-sensitive instrument that measures PhA and Z, and calculates R and Xc [6] based on the trigonometric equation. According to the Xitron manual [7] R and Xc are determined and Z and PhA are calculated. Akern uses a single frequency (50 kHz) whereas Xitron employs a best-fit evaluation over many frequencies.

Several validation studies were performed using BIA methods for water estimation in healthy adults, SF-BIA-RJL/AKERN [20–22] or Xitron BIS [22–25], but only four studies compared raw parameters measures using single- and multi-frequency BIA devices in haemodialyses patients [26], body builders [27], older adults [9] and children [10]; though only Genton et al. [9] and Tanabe et al. [10] provided comparison parameters between devices for the raw data. The authors compared BIS with RJL-101 in a sample of multi-ethnic infants and pre-school children observing an R^2 of 95% for Xc [10]. A nonsignificant mean bias was

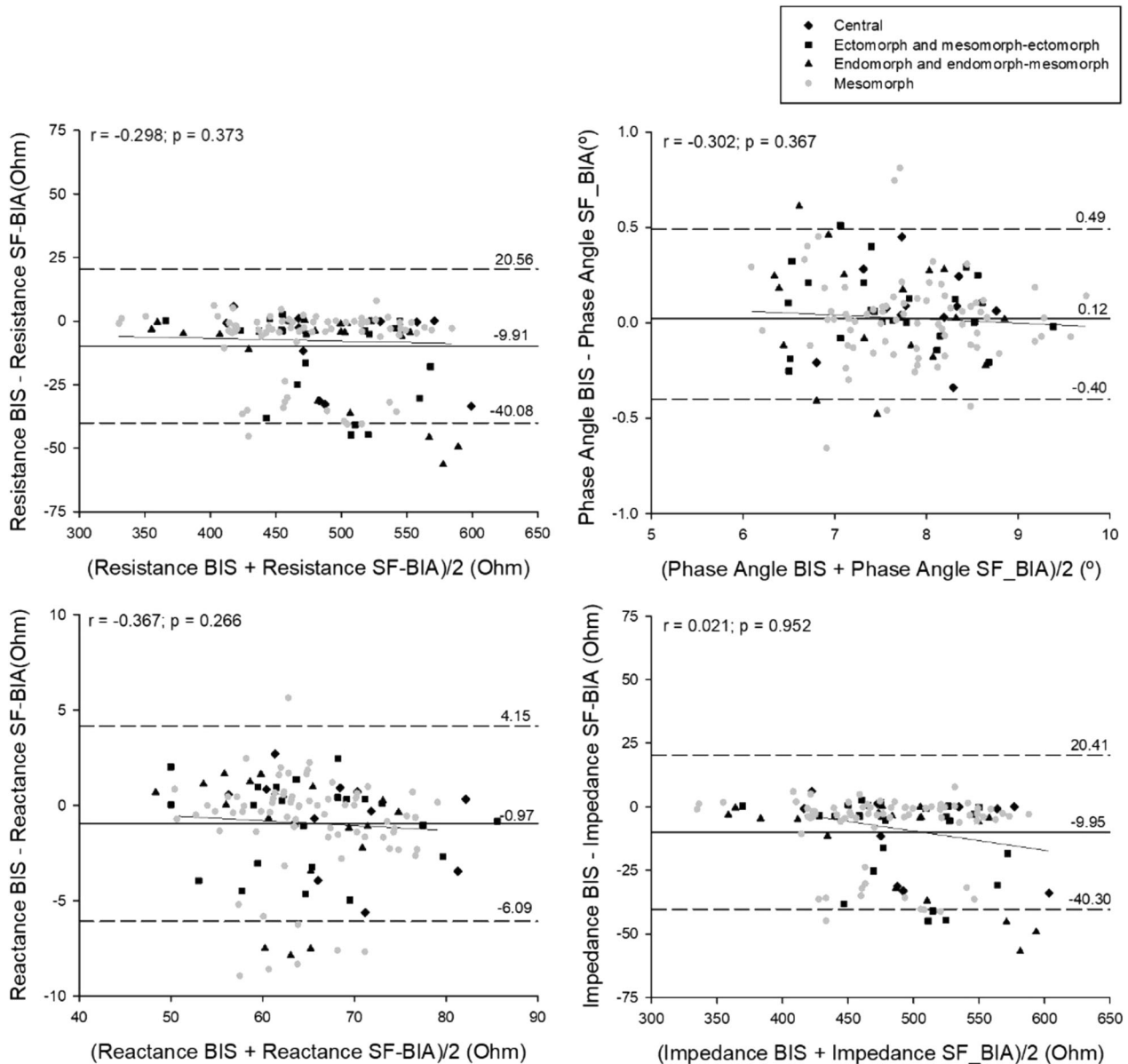


Fig. 1 Bland–Altman analysis of the agreement between methods for resistance, reactance, phase angle and impedance. The middle solid line represents the mean differences between bioelectrical impedance spectroscopy (BIS) at 50 kHz and the reference method (SF-BIA, Akern). The upper and lower dashed line represents 95% limits of

agreement (± 1.96 SD). The trend line represents the degree of association between the differences of the methods and the mean of both methods, as illustrated by the coefficient of correlation (r), and according to somatotype

observed for R with limits of agreement of $\pm 16 \Omega$, whereas BIS significantly underestimated X_c by 3.84Ω with limits of agreement of $\pm 3.6 \Omega$. Our results partially extend Tanabe et al. [10] findings for X_c as an R^2 of 81% was observed. Genton et al. [9] found that PhA was significantly underestimated in older adults measured by Xitron4000 and RJL-101 instruments, with a difference of $-1.50^\circ \pm 0.24$, which is appreciable.

The differences observed may be due to the effects of modelling but it could be argued that modelling provides a better measure at any single frequency since the effects of

individual error at any discrete frequency of measurement is minimized by the averaging effect of the modelling [28]. However, errors associated with modelling are recognized, and technical inadequacy issues, namely the effects of stray capacitance and lead position reported [29, 30]. Nevertheless, these modelling associated errors occur at higher frequencies and unlikely at frequency 50 kHz which was virtually identified in our equipment.

As body shape differs among athletes participating in different sports, individuals were categorized according to the somatotype. We observed that somatotype was a factor

affecting the differences in measurements of bioimpedance variables between methods, probably because body shape is generally accepted to be constant in BIA theory [19]. This observation was particularly relevant in endomorph and endomorph-mesomorph participants, as a trend to display lower values of R, Xc and Z, and higher values of PhA when using BIS compared to SF-BIA was found. The significant trend in displaying lower values of R and Z in participants with higher adiposity in the limbs, should be noted. Taking together these observations are difficult to be explained. Indeed, differences in raw data between devices, regardless of using the single- and multi-frequency approach have been previously observed [31].

Differences in biological variability in determining R and Xc from BIS-Xitron and SF-BIA Akern should be underscored, with a larger error observed by Xitron (0.6% for R and 1.5% for Xc) compared to Akern (0.3% for R and 0.9% for Xc). The differences of 9.91 Ω observed for R (representing a 2.0% mean difference) and of $\sim 1 \Omega$ for Xc (representing a 1.5% mean difference) are relatively close to the biological variability of the devices. Therefore, we should recognize that the technical error of measurements observed in both devices might actually question the meaning of the significant differences observed. Still, small differences in PhA ($< 0.5^\circ$) may compromise an accurate classification of athletes by performance level [32], as well survival prediction in advanced cancer patients [33]. Additionally, these discrepancies reinforce the need for using device-specific reference values of BIA raw data such as PhA and BIVA.

Despite the high association observed between devices, raw BIA data should not be used interchangeably given the individual errors and the significant trend between the differences and the magnitude of R and Z values, making it difficult to develop calibration models. Many laboratories and clinical centres still use the BIS-Xitron equipment. The point of this comparison is to demonstrate that not all BI devices yield comparable measurements *in vivo*. Thus, understanding the degree of agreement between devices is important, in particular, if we expect to understand the magnitude of the error involved in data collected, interchangeably, by these devices. A comprehensive review of the factors affecting impedance measurements and the call for standardization has been recently highlighted by Brantlov et al. [34]. As reported by Lukaski et al. [6], a mandatory future goal for impedance companies is to establish international manufacturing standards, synchronization of technology and cross-calibration of the electrical accuracy of different instruments.

It is important to underscore strengths of this study. Specifically, the unique sample of active adults and elite athletes with varying body physiques and the exploration of possible confounders in the between-methods differences,

and the use of classic BIVA to eliminate dehydrated participants, rolling out potential bias due to variations in hydration status. However, a few limitations should also be addressed. The results of the between-methods agreement are limited to a highly active population. When BIVA was used to eliminate dehydrated participants, we assumed that our Portuguese participants presented similar characteristics of the reference population (Italian individuals). Another concern is the physical characteristics of the electrodes used, as manufacturers of BIAs recommend the use of specific electrodes. Although the current electrodes (Impedimed) provide low impedance values (27.14 Ω) they were not specifically designed to be used in an Akern or Xitron instruments and rather by an IU0GELTD device. Lastly, given the cross-sectional design, future longitudinal studies are required to determine between-devices agreement, tracking raw-BIA the season.

In conclusion, BIS and SF-BIA-Akern raw parameters were highly related in very active males and elite athletes. However, due to the relatively large limits of agreement the methods should not be used interchangeably. Methodological and biological underlying assumptions, specifically with respect to the electronic accuracy of the instruments used and the different somatotypes observed, may be responsible for the lack of agreement between BIS and SF-BIA for measuring raw BIA parameters in highly active individuals.

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Author contributions AMS conceptualize and designed the study. DAS, CNM and CLN acquired the data, performed the data analysis and interpretation. EM, HL and LBS revised the manuscript critically for important intellectual content. All authors contributed to the final approval of the version to be submitted.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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