ORIGINAL ARTICLE

Total body water and water compartments assessment in athletes: Validity of multi-frequency bioelectrical impedance

Évaluation de l'eau corporelle totale et des compartiments d'eau chez les athlètes : validité de l'impédance bioélectrique multifréquence

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Summary Bioelectrical impedance provides an affordable assessment of the body's water compartments: total body water (TBW), extracellular (ECW) and intracellular water (ICW). However, little is known of its validity in athletes. We aim to validate TBW, ECW, and ICW obtained by multi-frequency bioelectrical impedance (MFBIA), using dilution techniques as reference (REF) in athletes. Thirty-six elite athletes (17 males and 19 females) with a mean age of 18.5 ± 4.1 years were evaluated. TBW, ECW, and ICW were assessed by MFBIA (Tanita MC-180MA) and dilution techniques. TBW_{REF} and ECW_{REF} were obtained by deuterium and bromide dilution techniques, respectively, while ICW_{REF} was calculated as their difference. MFBIA underestimated ECW ($\sim$1.3 kg) and overestimated TBW ($\sim$1 kg) and ICW ($\sim$3.5 kg), in relation to criterion. MFBIA explained 96%, 81%, and 83% of the total variability of results obtained from the reference for TBW, ECW, and ICW respectively. Standard error of the estimates ranged between

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1.6 kg (ECW) and 2.9 kg (ICW). Bland-Altman analyses revealed 95% confidence intervals: −3.1 to 5.1 kg for TBW, −5.0 to 2.3 kg for ECW, and −2.5 to 9.6 kg for ICW. Although methods are highly associated, these findings highlight that the MFBIA used is of limited accuracy at an individual level to estimate TBW, ECW and ICW, in an athletic population.

Résumé L'impédance bioélectrique fournit une évaluation abordable des compartiments de l'eau du corps : l'eau totale du corps (ETC), l'eau extracellulaire (EEC) et l'eau intracellulaire (EIC). Cependant, on sait peu de sa validité chez les athlètes. Nous visons à valider ETC, EEC, et EIC obtenus par impédance bioélectrique multifréquence (BIAMF), en utilisant des techniques de dilution comme référence (REF) chez les athlètes. Trente-six athlètes d'élite (17 garçons et 19 filles) âgés en moyenne de $18,5 \pm 4,1$ ans ont été évalués. ETC, EEC et EIC ont été évalués par BIAMF (Tanita MC-180MA) et des techniques de dilution. ETC_REF et EEC_REF ont été obtenus par des techniques de dilution de deutérium et de bromure, respectivement, tandis que EIC_REF a été calculé comme leur différence. Le BIAMF a sous-estimé EEC ($\sim 1,3$ kg) et surestimé ETC (~ 1 kg) et EIC ($\sim 3,5$ kg), par rapport au critère. BIAMF a expliqué 96 %, 81 % et 83 % de la variabilité totale des résultats obtenus à partir de la référence pour ETC, EEC et EIC respectivement. L'erreur-type des estimations variait entre 1,6 kg (EEC) et 2,9 kg (EIC). Les analyses de Bland-Altman ont révélé des intervalles de confiance de 95 % : −3,1 à 5,1 kg pour ETC, −5,0 à 2,3 kg pour EEC, et −2,5 à 9,6 kg pour EIC. Bien que les méthodes soient fortement associées, ces résultats soulignent que la BIAMF utilisée est d'une précision limitée au niveau individuel pour estimer ETC, EEC et EIC, dans une population sportive.

1. Introduction

Athletes require adequate water intake to offset their higher sweat losses and to maintain water balance [1]. The benefits and importance of body water are known and documented [2,3]. Total body water (TBW) is distributed into two main compartments: extracellular water (ECW) and intracellular water (ICW), comprising about 65% and 35% of TBW, respectively [4]. Water compartments assessment is extremely important, given the link between body water, exercise, and work performance [2,5—7]. It has been documented in elite judo athletes that a reduction in intracellular water resulted in decreased muscle power of the upper limbs and in maximal grip strength [5,6]. Additionally in a sample of basketball, handball and volleyball players' increases in ICW were associated with improvements in leg strength and jumping height (Silva et al., 2014).

Body water is traditionally assessed by dilution techniques, methods that are considered the reference method in assessing TBW and its compartments [8]. Deuterium dilution technique is used to assess TBW while the sodium bromide dilution technique is used to assess the ECW compartment. Despite the known effects of water in exercise performance, understanding the effect of hydration and water distribution on athletic performance, using the techniques mentioned as the criterion, are scarce [1,9,10]. This gap in the literature is partly due to the length and complexity of the analytical procedures for sample processing, as well as the high cost of the dilution techniques [8]. It

is important to identify alternative methods for evaluating body water compartments that are less costly, faster and with a recognized accuracy. Bioelectrical impedance methods seem to be an easy to use and fairly inexpensive approach for body composition and hydration monitoring in athletes [11,12]. To date, few studies [9,13—15] have been conducted in this population using commercial impedance equipment's against dilution techniques as criterion. It has been recently said [16] that the bioelectrical impedance as a field method looks promising, regarding its ability to predict TBW and its compartments, yet there is limited research in athletes for the validity of bioelectrical impedance to estimate these hydration variables. Overall, the usefulness of the bioelectrical impedance approach for the prediction of the hydration status in the athletic population remains to be clarified [16]. Given the recognized simplicity in using this methodology, several devices appeared in the market to estimate TBW based on a single frequency, typically 50 kHz. However, the correct estimation of water compartments would require the use of lower and simultaneously higher frequencies [17]. A multi-frequency BIA (MFBIA) device developed by Tanita® (MC-180) was conceptualized to generate four frequencies 5, 50, 250 and 500 kHz (Tanita MC-180) to assess TBW, ECW and ICW separately.

Given the lack of validation studies conducted to test the accuracy of this MFBIA model, our aim was to analyze the validity of Tanita MC-180 to estimate TBW, ECW and ICW in athletes using dilution techniques as the criterion.

2. Methods

2.1. Participants

Using a cross-sectional design, a total of 36 athletes (17 males, 19 females) were evaluated during the in-season period. The sample consisted of athletes spanning 4 sports: basketball, handball, swimming, and volleyball. Female athletes were all in the folicular phase of the cycle when the evaluation occurred. The inclusion criteria were as follows:

- Tanner stage V or greater (determined by self-evaluation) [18];
- >10 hours of sport specific training per week;
- have negative anti-doping results, and;
- currently not taking any medication or dietary supplements.

All participants and parents or guardians were informed about the possible risks of the investigation. Minors provided assent to participate whereas adults (athletes, parents or guardians) provided written informed consent.

All procedures were approved by the Ethics Committee of the Faculty of Human Kinetics, University of Lisbon and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association [19].

2.2. Body composition measurements

All measurements were performed on the same day (8 a.m.) after an overnight fast (>12-hour) and at least 15 hours post-exercise. Further, alcohol and stimulant beverages consumption were not allowed for at least 15 hours prior testing.

2.2.1. Anthropometric measurements

Participants were weighed to the nearest 0.01 kg wearing a bathing suit, on an electronic scale connected to a plethysmograph computer (BOD POD© Cosmed, Rome, Italy). Stature was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany), using standardized procedures described elsewhere [20].

2.2.2. Hydration status

After a baseline urine sample was collected, the specific gravity (USG) was determined using a refractometer (Urisys 1100, Roche Diagnostics, Portugal) to ensure that all athletes were euhydrated (well hydrated USG < 1.010) [10]. The coefficient of variation (CV) of the urine specific gravity procedure in our laboratory based on 10 adults is 0.1% [21].

2.2.3. Fat mass (FM) and fat free mass (FFM)

Percent fat mass (%FM), total fat mass (FM), and fat free mass (FFM) were determined by dual-energy X-ray absorptiometry (DXA) fan-beam densitometer (Hologic Explorer W, QDR for windows version 12.4, Waltham, MA, USA). The test-retest CV in 10 participants in our laboratory for FM and FFM is 0.8% and 1.7%, respectively [22].

2.2.4. Reference total body water

Total body water (TBW) was assessed by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). Following the collection of a baseline urine sample, each participant was given an oral dose of 0.1 g of 99.9% ^2H_2O per kg of body weight (Sigma-Aldrich; St. Louis, MO). Because of the bladder memory effect, voids after tracer administration were performed before the enriched sample was collected. After a 4-hour equilibration period, a second urine sample was collected [8]. Urine samples were prepared for $^1H/^2H$ analyses using the equilibration technique by Prosser and Scrimgeour [23] as described by our group previously [13]. The test-retest CV in 10 participants in our laboratory for TBW is 0.3% [13].

2.2.5. Reference extracellular water

Extracellular water (ECW) was assessed by sodium bromide (NaBr) dilution. A baseline saliva sample was obtained prior to dosing where each participant was asked to drink 0.030 g of 99.0% NaBr (Sigma-Aldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water, using procedures described elsewhere [24].

The test-retest CV in 7 participants for the ECW using high performance liquid chromatography in our laboratory is 0.4% [24].

2.2.6. Reference intracellular water

Intracellular water (ICW) was calculated as the difference between the measured TBW and ECW for each participant.

2.2.7. Multi-Frequency Bioelectrical Impedance Analysis (MFBIA)

An eight-electrode system was applied for measuring TBW and water compartments, at four distinct frequencies: 5, 50, 250 and 500 kHz (Tanita MC-180 MA, Tokyo, Japan). TBW and water compartments was estimated in total body and by segmental sections (right arm, left arm, trunk, right leg, and left leg) by the software through a generated algorithm and according to previously displayed individual data. In this study the ''athlete'' mode was considered in all participants, which is defined by the manufacturer as the equivalent of a person involved in intense physical activity for more than 12 hours per week [25]. The sampling procedures were performed according to manufactures instructions, using minimal clothing. Clean bare feet positioned on the heel and toe electrodes on each side in roughly equal proportions; arms straight down during measurement. Participants were instructed to avoid contact between the arms and torso, and between the inner thighs.

Before each testing session, the analyser was checked with a calibration circuit simulating a human body of known proportions. The test-retest CV in 10 participants in our laboratory for TBW, ECW, and ICW is 0.3%, 0.7%, and 0.3%, respectively.

2.3. Statistical analysis

Descriptive statistics were performed and all variables were checked for normality. Independent-sample t-tests were used for parametric variables and whenever a non-parametric distribution was observed, Mann-Whitney U test

was applied to compare sex. A paired sample *t*-test was used to compare the mean values between the reference technique and the alternative method, if variables were normally distributed. Wilcoxon test was performed if the distribution is not normal. Multiple regression analysis was performed to test the influence of sex alone and in interaction with TBW, ECW, and ICW. If an interaction term between sex and the alternative method was non-significant, linear regression analysis was performed using the whole sample.

To assess the accuracy of the alternative methods, validation parameters included the analyses of the coefficient of determination and the standard error of the estimation. The concordance correlation coefficient (CCC) was performed using the Lin approach [26] with MedCalc® vs. 11.1.1.0 (2009) software. The CCC (ρc) contains a measurement of precision ρ and accuracy (ρc = ρ Cb) where ρ is the Pearson correlation coefficient, which measures how far each observation deviates from the best-fit line, and is a measure of precision, and Cb is a bias correction factor that measures how far the best-fit line deviates from the 45° line through the origin, and is a measure of accuracy. Agreement between methods was assessed using the Bland-Altman method [27], including the analysis of the correlation between the mean and the difference of the methods. In addition, the correlation between the differences of the methods and potential variables were performed. Data were analysed with PASW for Windows version 21.0 (SPSS Inc., an IBM Company, Chicago). For all tests, statistical significance was set at $P < 0.05$.

3. Results

Participants' characteristics and body composition variables are presented in Table 1. All variables were different between males and females, with the exception of age and USG.

Differences between reference and alternative methods were observed in all compartments, with the exception of TBW in the male subsample. In males, MFBIA underestimated ECW and overestimated ICW, while in females MFBIA overestimated TBW and ICW and underestimated ECW. Considering the whole sample, MFBIA underestimated ECW and overestimated TBW and ICW (Table 1).

We have determined if a sex by water compartments interaction contributed to the relationships between MFBIA water estimation and the reference measurements. A non-significant contribution for a sex interaction term was observed *(P > 0.05)* for TBW and water compartments. Therefore, we have tested the accuracy of MFBIA to TBW, ECW, and ICW compared to the reference method using the whole sample.

Results of the regressions and CCC analyses between the methods for TBW, ECW, and ICW estimates are illustrated in Fig. 1. TBW, ECW, and ICW estimated from MFBIA explained 96%, 81%, and 83%, respectively, of the total variability observed from the reference method. Standard error of the estimates ranged from 1.61 (ECW) to 2.89 kg (ICW). The regression line did not differ from the line of identity for TBW and ICW, with slope and intercept values, respectively, of 1.042 and −2.855 for TBW and 0.864 and 0.377 for ICW. The regression line differed from the line of identity for ECW, with a slope of 1.359 and an intercept value of −4.341.

The CCC values were 0.973 for TBW, 0.753 for ECW, and 0.817 for ICW.

The agreement between methods was verified using the Bland-Altman method (Fig. 2).

For TBW and water compartments, relatively large limits of agreement (95% confidence intervals) were observed. An underestimation of 3.1, 5.0, and 2.5 kg or an overestimation of 5.1, 2.3, and 9.6 kg were observed, respectively for TBW, ECW, and ICW.

We further explored if the differences between methods were related with USG, FM, and FFM. The only significant independent variable was FFM, where a negative association ($r = -0.727$, $P < 0.001$) was observed between FFM and the difference of the methods in estimating the ECW compartment. Considering the other potential confounders, no associations were found with the differences of the methods in estimating TBW, ECW, and ICW.

4. Discussion

This investigation analysed the validity of a specific MFBIA model, Tanita MC-180, in the estimation of TBW, ECW, and ICW in a sample of elite athletes, using the dilution techniques as the reference method. Differences between the alternative and the reference methods were observed.

To date, only two studies [14,15], validated BIA devices in the athletic population using deuterium dilution as the reference method. No literature was found regarding the specific MFBIA model used in the present study (Tanita MC-180) in the assessment of TBW and its compartments, in comparison to dilution techniques. Quiterio and colleagues found that the Tanita TBF-model 310 explained 87 and 88% of the total variability in TBW, respectively in athletic boys and girls [15]. At an individual analyses, Quiterio and colleagues [15] found larger limits of agreement ranging from −4.8 to 3.7 kg and −6.6 kg for the 5.1 kg in girls and boys, respectively.

Matias and colleagues [14] developed a BIA-based model, using a single frequency BIA method, specific for athletes. The cross validation of that equation was performed against dilution techniques.

No differences between methods were observed for TBW, ECW, or ICW estimation, and the methods were highly correlated ($r^2 \geq 0.83$). The BIA-based models developed for TBW and ECW explained 85% and 83% of the variability observed in the values of the reference methods, respectively. The precision and accuracy of the methods according with the CCC analysis of agreement [26] was higher than 0.84 and 0.99, respectively, with a CCC between the new method and the reference procedure superior to 0.84 (moderate strength of agreement). From the Bland-Altman individual agreement analysis, no trend between the mean and the differences of the methods for TBW, ECW and ICW was observed by authors.

Matias et al., 2016 used a single frequency BIA, while this paper relies on the use of a multi-frequency BIA. Based on a literature review, evidence has suggested that 50 kHz may not be the ideal frequency at which to estimate body composition, as, at 50 kHz, the electrical pathway is not solely extracellular or intracellular but rather primarily extracellular with some unknown intracellular penetration [28,29], therefore single frequency BIA analysers are limited in their ability to distinguish the distribution of body water into

Table 1 Participant's characteristics and body composition.

	Male (n = 17)	Female (n = 19)	Whole (n = 36)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (years)	21.3 ± 4.4	22.8 ± 5.3	22.1 ± 4.9
Weight (cm)	82.7 ± 12.2[a]	66.3 ± 9.7	74.1 ± 13.6
Height (cm)	186.6 ± 10.8[a]	172.3 ± 9.2	179.0 ± 12.2
BMI (kg/m^2)	23.7 ± 2.1[a]	22.3 ± 1.9	22.9 ± 2.1
USG	1.021 ± 0.003	1.019 ± 0.006	1.020 ± 0.005
FFM (kg)	68.8 ± 11.1[a]	48.1 ± 6.2	57.6 ± 13.6
Fat mass (%)	13.6 ± 4.5[a]	17.4 ± 4.5	15.7 ± 4.8
Fat mass (kg)	16.5 ± 4.7[a]	26.4 ± 4.5	21.9 ± 6.7
TBW$_{REF}$ (kg)	50.9 ± 7.9[a]	34.8 ± 4.5	42.4 ± 10.3
TBW$_{MFBIA}$ (kg)	51.2 ± 7.7[a]	36.5 ± 4.6[b]	43.5 ± 9.7[b]
ECW$_{REF}$ (kg)	19.8 ± 3.3[a]	14.9 ± 1.9	17.2 ± 3.6
ECW$_{MFBIA}$ (kg)	17.8 ± 1.7[a,b]	14.1 ± 1.3[b]	15.9 ± 2.4[b]
ICW$_{REF}$ (kg)	31.1 ± 5.0[a]	19.9 ± 2.9	25.2 ± 6.9
ICW$_{MFBIA}$ (kg)	34.3 ± 6.5[a,b]	23.7 ± 3.2[b]	28.7 ± 7.3[b]

SD: standard deviation; BMI: body mass index; USG: urine specific gravity; FFM: fat free mass; TBW: total body water; ECW: extracellular water; ICW: intracellular water; REF: reference method (dilution techniques); MFBIA: multi-frequency bioelectrical impedance.
[a] Significantly different between sex, $P < 0.001$.
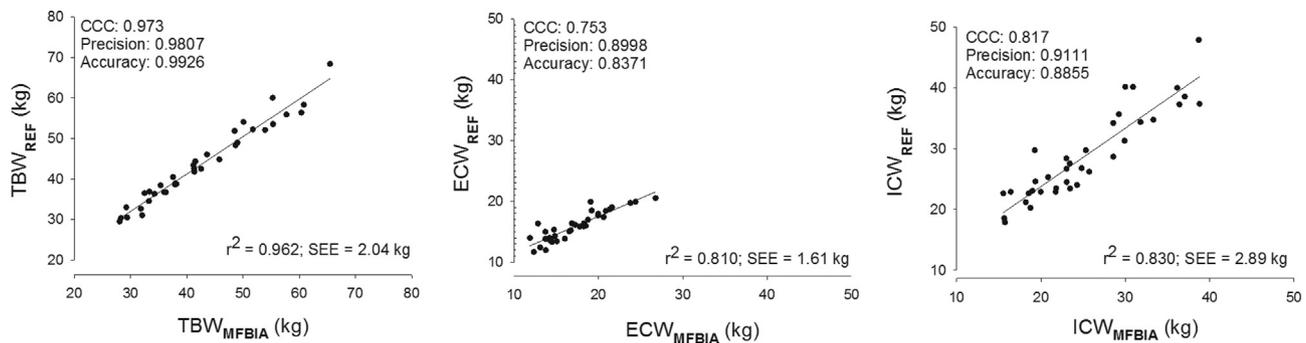[b] Significantly different from the reference method, $P < 0.05$.

Figure 1 The figure illustrates the results of the regression analysis for total body water (TBW), extracellular water (ECW), and intracellular water (ICW), obtained by multi-frequency bioelectrical impedance (MFBIA) and dilution techniques as the reference method (REF). r^2 and SEE represent coefficient of determination and standard error of the estimates, respectively. CCC represents the concordance correlation coefficient.
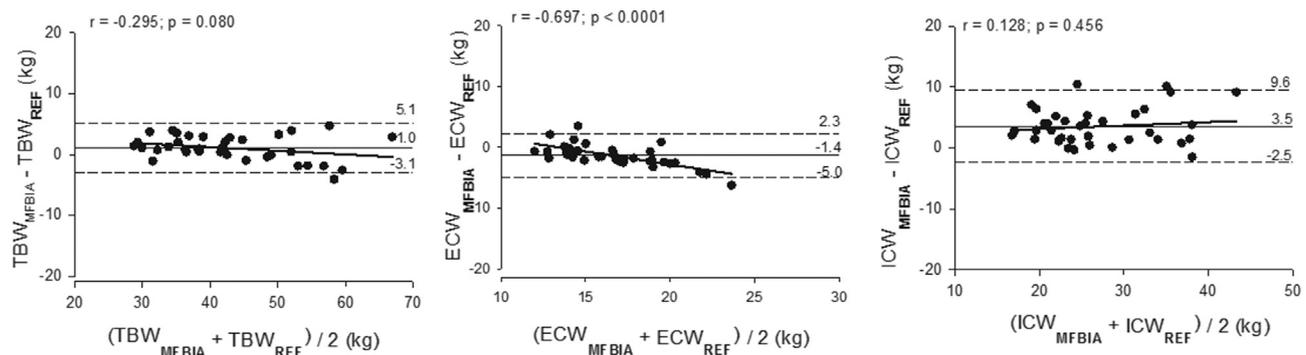

Figure 2 The figure illustrates the Bland-Altman analysis of the agreement between methods for total body water (TBW), extracellular water (ECW), and intracellular water (ICW) assessment. The middle solid line represents the mean differences between multi-frequency bioelectrical impedance (MFBIA) and the reference method (REF). 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).

its intra- and extracellular compartments (Chumlea & Sun, 2005).

Multi-frequency BIA uses several discrete frequencies or a combination of frequencies to evaluate TBW, ECW and ICW (Baracos et al., 2012; Kyle et al., 2004). Low frequencies, such as 1 or 5 kHz, are typically used to predict ECW because it is believed that there is a negligible penetration of the ICW at this frequency. Higher frequencies, such as 100 or 500 kHz, are used to predict TBW, assuming there is nearly complete penetration of cells by the current (Buchholz, Bartok, & Schoeller, 2004). The use of more than one frequency led the researchers to examine which frequencies may be more reliable on to estimate water pools and, as reviewed elsewhere (Buchholz et al., 2004), the results are so variable that no conclusions can be made regarding the validity of one frequency over another in the prediction of body fluid compartments. Nevertheless, the observed reliability at all multiple frequency impedance was showed to be good at all tested frequencies and similar to those reported for 50 kHz impedance instruments (Buchholz et al., 2004).

To our knowledge eleven validation studies were performed using multi-frequency BIA to predict TBW or ECW (Bedogni, Merlini, Ballestrazzi, Severi, & Battistini, 1996; Bedogni et al., 1996; Borghi et al., 1996; Desport et al., 2000; Deurenberg & Schouten, 1992; Deurenberg, Wolde-Gebriel, & Schouten, 1995; Janssen, Deurenberg, & Roelfsema, 1997; Olde Rikkert, Deurenberg, Jansen, van't Hof, & Hoefnagels, 1997; Ritz, 2001; Vache et al., 1998; Visser, Deurenberg, & van Staveren, 1995) and, as reviewed elsewhere (Buchholz et al., 2004; Chumlea & Sun, 2005), a common conclusion was that multi-frequency impedance has improved body composition estimates over the single frequency impedance, as it has been able to provide accurate and precise estimates of TBW and compartments, which were limited with single frequency devices.

So, one can state that our results are similar to those reported in the aforementioned investigations [14,15], probably due to the fact that the sample is similar, as both groups were athletes. Nevertheless, considering the age of the athletes of the Quiterio study [15], conclusions and applicability in adults should be taken with careful. Despite all this, this type of equipment lack validity in athletes, which is the main point of our paper.

It has been suggested that TBW estimated by BIA method may be influenced by internal factors that can change conductivity properties of the body, such as variations in plasma and extracellular tonicity [30,31], therefore influencing the electric potential and consequently the resistance and reactance of the fluids and thus resulting in altered TBW estimates. This fact can help justify why the individual values were different from those observed by the reference methods. Nevertheless, it is important to highlight the fact that this particular device did not use a single frequency 50 kHz, as in previously reported studies, but incorporated four discrete frequencies of 5, 50, 250, and 500 kHz. According to Buchholz and colleagues [32] measures obtained at lower frequencies (5 kHz) are associated with ECW while higher frequencies are able to penetrate through the cell membranes, allowing the quantification of both the ECW and ICW. Unlike bioelectrical impedance spectroscopy that reports the use of the biophysical model proposed by Cole and Cole [33], in commercial devices such

as the present equipment, the algorithm for TBW, ECW, and ICW is unknown.

For body water compartments, we have observed an underestimation of ECW and an overestimation of ICW when the MFBIA (Tanita MC-180) was used, in comparison to dilution techniques. However, the methods were highly associated and, in fact, ECW and ICW obtained by MFBIA (Tanita MC-180) explained 81 and 83%, respectively, of the values observed by the reference method. Despite the relatively large limits of agreement observed, which limits its accuracy at the individual level, an additional analysis was conducted using the concordance coefficient correlation (CCC) and values of precision and accuracy between MFBIA and dilution techniques were verified. The CCC values indicate a substantial strength of agreement between methods for TBW, while both extra- and intracellular compartments presented a poor strength of agreement (<0.90) [34].

Our findings were similar to those observed by Matias et al. [13] using bioelectrical impedance spectroscopy in the estimation of TBW and its compartments in a group of athletes. The authors reported an acceptable accuracy at a group level but the impedance method is a less valid approach at the individual level.

The main strength of this investigation lies in the use of dilution techniques as the criterion for body water compartment assessment in a unique sample of highly trained athletes. However some limitations need to be addressed. The results are only applicable to the MFBIA Tanita MC-180 model and to this particular sample and may not be generalized to other BIA devices or populations. Moreover, the validity of this MFBIA model was tested using a cross-sectional design and it would be relevant to explore the accuracy of this method in longitudinally monitored population. Additionally, one should take into account that in commercial devices such as the present equipment, the algorithm for TBW, ECW, and ICW is unknown.

5. Conclusion

Although TBW, ECW, and ICW obtained by MFBIA (Tanita MC-180) were highly associated with the reference dilution techniques, the large limits of agreement restrict the accuracy of the estimates at the individual level. Therefore MFBIA (Tanita MC-180) should be limited to larger epidemiological studies for estimating TBW and its compartments in an athletic population.

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Contribution

A. M. Silva: conception and design of the study, interpretation of data, revised the article, and gave final approval of the version to be published.

Disclosure of interest

The authors declare that they have no competing interest.

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