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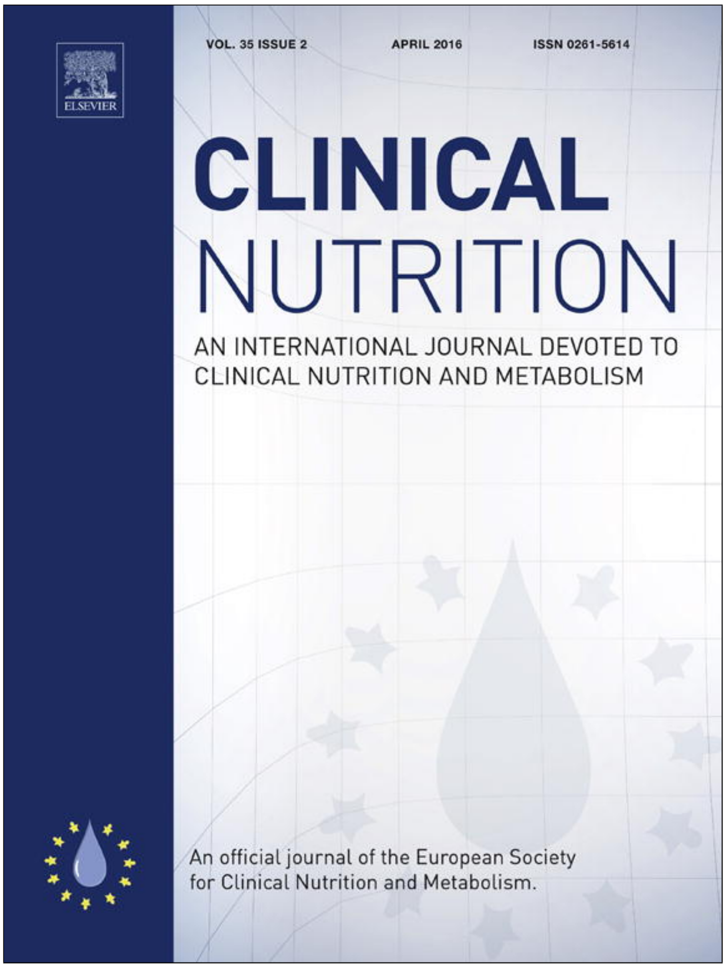
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Original article

Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models

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SUMMARY

Purpose: Bioelectrical impedance analysis (BIA) equations can predict total body water (TBW) and extracellular water (ECW) in non-athletic healthy populations. This study aimed: a) to develop BIA-based models for TBW and ECW prediction based on dilution methods in a sample of national level athletes; and b) to validate the new models with a cross-validation approach in a separate cohort using dilution methods as criterion.
Methods: Two hundred and eight highly trained athletes (21.3 ± 5.0 years) were evaluated during their respective competitive seasons. Athletes were randomly split into development (n = 139) and validation groups (n = 69). The criterion method for TBW was deuterium dilution and for ECW was bromide dilution, where ICW was the respective difference between both. Resistance (R) and reactance (Xc) were obtained with a phase-sensitive 50 kHz BIA device and used for the estimation of TBW and ECW.
Results: Athletic BIA-based models were developed for TBW and ECW [$TBW = 0.286 + 0.195*S^2/R + 0.385*Wt + 5.086*Sex$; $ECW = 1.579 + 0.055*S^2/R + 0.127*Wt + 0.006*S^2/Xc + 0.932*Sex$, where sex is 0 if female or 1 if male, Wt is weight (kg), S is stature (cm), and R and Xc are in ohm (Ω)]. Cross validation revealed R^2 of 0.91 for TBW and R^2 0.70 for ECW and no mean bias.
Conclusions: The new equations can be considered valid, with no observed bias, thus affording practical means to quantify TBW and ECW in national level athletes.

1. Introduction

The benefits and importance of an appropriate hydration for health and performance are well known with total body water (TBW) being comprised of both intracellular (ICW) and extracellular (ECW) with a flux existing between the two [1]. Criterion methods for measuring TBW (deuterium oxide) and ECW (sodium bromide) are expensive, impractical for most settings, and impossible for determining fluid shifts in short, discreet time intervals [2].

The aforementioned limitations revealed the need for easier but valid field-based instruments, hence bioelectrical impedance serves this purpose (BIA) [3]. BIA is generally cheaper than more laboratory based methods and the maintenance is fairly straightforward. These advantages prompted the development of several predictive models based on 50 kHz single frequency phase-sensitive BIA using tracer dilution techniques as criterion. As reviewed by Kyle [3,4], for TBW estimation, 50 kHz single frequency BIA-based models were developed in children, in adults, in obese participants, and in chronic disease patients, while for the ECW estimation, 50 kHz single frequency BIA-based models were developed in healthy subjects, in paraplegia and in surgical

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patients. In addition ECW has been predicted using other frequencies, namely 5 kHz, 224 kHz, and the resistance of the extracellular fluid (R0 or Re) obtained by modelling procedures in bioelectrical impedance spectroscopy.

The ICW compartment is determined as the difference between the TBW and ECW compartments. It has been recently shown that reductions in the ICW compartment decrease strength and power in elite judo athletes and leg strength and jumping height over a season in basketball, handball and volleyball players [5–7]. These findings further support the important role of an effective monitoring of the water distribution volumes (TBW, ECW, and ICW) in physical performance.

Recently it has been suggested that assessment of water distribution volumes among athletes should use a phase-sensitive BIA instrument that provides measurements of R and Xc instead of volume estimations automatically calculated by the BIA device with generalized regression equations without justification or equations that were not validated for this specific population [8]. Athletes differ from the general population specifically with sport-specific differences in body geometry, that almost always are ignored by researchers despite awareness that geometry is a factor affecting bioimpedance measurements, and that BIA prediction equations are sample-specific [3]. Thus, to date there are no phase-sensitive BIA-based predictive models for TBW and ECW that have been specifically developed for athletes using reference dilution methods, and cross-validated in an athletic sample [8].

The purpose of this study was two-fold: develop a 50 kHz BIA-based model for estimating TBW and ECW in a convenient sample of national level athletes (male and female) using dilution techniques as the criterion method, and cross-validate the new prediction models, in a separate group of national level male and female athletes. A secondary objective was to determine the performance of previous non-specific published models in our sample of athletes.

2. Methods

2.1. Participants

Using a cross-sectional design, a total of 208 athletes (138 males, 70 females) were evaluated during their respective competitive seasons. The sample consisted of athletes spanning 15 sports: basketball (21 females, 26 males), handball (2 females, 16 males), judo and wrestling (2 females, 3 males), karate and taekwondo (4 females, 6 males), pentathlon (1 female, 2 males), rugby (9 males), soccer (1 male), swimming (14 females, 19 males), track and field athletics (sprinters, hurdlers, and jumpers) (6 females, 11 males), triathlon (5 females, 24 males), and volleyball (15 females, 17 males); tennis (2 males); sailing (2 males). The inclusion criteria were as follows: 1) Tanner stage V or greater (determined by self-evaluation) [9]; 2) > 10 h of sport specific training per week; 3) free from performance-enhancing drugs specifically and any medication in general and 4) free from the consumption of alcohol and caffeinated beverages for at least 15 h prior to testing. 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 [10].

2.2. Body composition measurements

Testing began promptly at 08:00 after an overnight fast lasting at least 12 h with at least 15 h from the last exercise session.

2.2.1. Anthropometric measurements

All participants were weighed (nearest 100th of a gram) in minimal clothing (i.e. swimsuit) using the scale interfaced with the plethysmograph (BOD POD© Cosmed, Rome, Italy), while stature was measured to the nearest 10th of a cm using a wall stadiometer (Seca, Hamburg, Germany) using standardized procedures as reported elsewhere [11].

2.2.2. Hydration status

The specific gravity (USG) was determined using a refractometer (Urisys 1100, Roche Diagnostics, Portugal) from a fasting baseline urine sample to ensure that all athletes were euhydrated (well hydrated USG <1.010) [12]. The coefficient of variation (CV) of the urine specific gravity procedure in our laboratory based on 10 active adults is 0.1% [13].

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

Body Composition, specifically % fat mass (%FM), total fat mass (FM), and fat free mass (FFM), was determined by dual-energy X-ray absorptiometry (Hologic Explorer W, QDR for windows version 12.4, Waltham, MA, USA) as described elsewhere [14]. In our laboratory, in ten healthy adults, the test–retest CV for both FM and FFM is 0.8% and 1.7%, respectively [14].

2.2.4. Reference total body water

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) 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 [2]. Urine samples were prepared for $^1H/^2H$ analyses using the equilibration technique by Prosser and Scrimgeour [15] as described by our group previously [16]. Our laboratory has reported a CV in ten subjects for TBW of 0.3% [16].

2.2.5. Reference extracellular water

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 (Sigma–Aldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water as described by our group previously [17].

The test–retest CV in 7 participants for the ECW using high performance liquid chromatography in our laboratory is 0.4% [17].

2.2.6. Reference intracellular water

ICW was calculated as the difference between TBW and ECW.

2.2.7. Single frequency bioelectrical impedance analysis

Whole body resistance (R) and reactance (Xc) were obtained by BIA using a single frequency, phase-sensitive 50 kHz (BIA-101, RJL/Akern Systems, Firenze, Italy), as described by others [18]. All subjects were instructed to lie in a supine position for 10 min (serving as an equilibration period) with electrodes placed on the right ankle and wrist. Using a 50 kHz frequency, a 0.8-mA alternating current was placed into the distal electrode (source electrode) of each pair, whereas using the proximal electrode (detector electrode) the voltage drop through the body is determined. Prior to each test the analyzer was calibrated 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 is 0.3% and 0.9%, respectively.

A few BIA models for TBW and ECW estimation in normal weight healthy adults were developed over the years against criterion methods, such as dilution techniques. Table 1 presents published TBW prediction equations.

2.3. Statistical analysis

Basic descriptive data were run to characterize the study participants. All variables were checked for normality, using Kolmogorov–Smirnov test. Participants were assigned to both a development group (n = 139) or a cross validation group (n = 69), based on sport and sex using a stratified random assignment.

The ability of the following variables (R, 1/R, Xc, 1/Xc, age, sex, stature, weight, impedance, stature^2/resistance, and stature^2/reactance) in predicting TBW and ECW in the development group was assessed using stepwise regression analysis. It was also included in the stepwise regression the athletes individual sport modality, as well as an athlete's categorization into 2 groups according to collective or individual sports, and an athlete's categorization into 5 groups, proposed according to the somatotype as: Endo-mesomorph, Balanced mesomorph, Ecto-mesomorph, Meso-morph-ectomorph, Meso-ectomotph [19].

During model development, normality of residuals and homogeneity of variance were tested. Significance at $p \leq 0.05$ was established as the criterion for inclusion of a predictor whereas removal criteria were set at $p \leq 0.1$. If more than one variable remained in the model, and to assess multi-collinearity, a variance inflation factor (VIF) was calculated for each independent variable [20]. No interactions were found between sex and other independent variables, therefore we used the whole sample in the models development.

To cross-validate the developed models, the resulting TBW and ECW models equations were then applied in the cross validation group according to the statistic methods described elsewhere [20]. Simultaneously, ICW estimation was determined as TBW minus ECW obtained in the developed models and the result of this calculation was validated against ICW assessed as TBW minus ECW from the reference method. A paired sample t-test was employed to compare the mean values obtained from the reference technique and from the new method. To assess the accuracy of BIA-based models, validation parameters included the analysis of the coefficient of determination and the pure error. The pure error was assessed using the following equation $\sum(\dot{Y}-Y)^2/n^{1/2}$, where $\dot{Y}$ is the predicted variable, Y is the observed variable and n represented the number of participants [20]. Using Lin's approach [21] the concordance correlation coefficient (CCC) was calculated with MedCalc Statistical Software v.11.1.1.0, 2009 (Mariakerke, Belgium). The CCC includes a measure of precision and accuracy ($\rho_c = \rho\ C_b$): where ρ represents the Pearson correlation coefficient, a measure of precision, which determines the deviation of each observation from the line of best-fit whereas C_b, a measure of accuracy, is a bias correction factor that determines the deviation from the best-fit line from the 45° line through the origin. Finally, agreement between BIA-based models and the criterion procedure was determined using the Bland-Altman method [22], including the analysis of the correlation between the mean and the difference of the methods and an estimate of the limits of agreement.

IBM SPSS Statistics version 21.0, 2012 (IBM, Chicago, Illinois, USA) was used to analyze the data. P < 0.05 was established as the statistical significance for all tests.

3. Results

Table 2 presents the physical characteristics and body composition variables for the developmental and cross validation groups,

Table 1
Predictive equations for water estimation in healthy adults.

Author	Equation for water estimation	Sample	Age range (Years)	R^2	SEE	Cross-validation	Bias	95% LoA	Trend
TBW									
Kushner and Schoeller, 1986 [18]	$0.5561*(S^2/R) + 0.0955*Wt + 1.726$	Obese and non obese adults (N = 58)	25–67	0.97	1.748 L	Yes	−1.0	−4.763; 2.763	N.A.
Van Loan and Mayclin, 1987 [23]	$9.9868 + 0.000724*S^2 + 0.2822*Wt - 0.0153*R - 2.3313*Sex^a - 0.1319*Age$	Healthy adults (N = 118)	18–64	0.87	2.918 L	No	–	–	–
Lukaski and Bolonchuk, 1988 [24]	$0.377*(S^2/R) + 0.14*Wt - 0.08*Age + 2.9*Sex + 4.65$	Healthy adults (N = 110)	20–73	0.98	1.50 L	Yes	0.2	−9.333; 9.733	N.A.
Kushner, Schoeller et al., 1992 [29]	$0.59*S^2/R + 0.065*Wt + 0.04$	From Neonates to adults (N = 175)	0.02–67	0.99	1.41 kg	Yes	−1.6	−11.865; 15.065	N.A.
Schoeller and Luke, 2000 [26]	$0.499*S^2/R + 0.080*Wt + 2.9$	Caucasian (N = 125)	14–53	0.93	2.5 kg	No	–	–	–
Sun, Chumlea et al., 2003 [30]	$1.20 + 0.45*S^2/R + 0.18*Wt$	Males (N = 712)	12–94	0.84	3.8 L	Yes	3.8	5.598; −13.498	N.A.
	$3.75 + 0.45*S^2/R + 0.11*Wt$	Females (N = 1089)		0.79	2.6 L		2.9	−5.505; 11.305	N.A.
ECW									
Lukaski and Bolonchuk, 1988 [24]	$0.189*(S^2/R) + 0.052*Wt - 0.0002*(S^2/Xc) + 1.03$	Healthy adults (N = 110)	20–73	0.88	1.01 L	Yes	0.2	−5.586; 5.986	N.A.
Sergi, Bussolotto et al., 1994 [25]	$-5.22 + 0.2*S^2/R + 0.005/Xc + 0.08*Wt + 1.9 + 1.86*Sex^a$	Healthy and fluid retention adults (N = 40)	21–81	0.89	1.7 L	No	–	–	–

Abbreviations: S, Stature (cm); Wt, Weight (kg); R, resistance; Xc, reactance; R^2, coefficient of determination; SEE, standard error of estimation.
[a] 0 if female; 1 if male.

Table 2
Descriptive characteristics and body composition of development and cross-validation groups (mean ± sd).

	Development group			Cross-validation group		
	Male (n = 92)	Female (n = 47)	Whole sample (n = 139)	Male (n = 46)	Female (n = 23)	Whole sample (n = 69)
Age (yrs)	21.2 ± 4.7	20.6 ± 5.1	21.0 ± 4.8	22.5 ± 5.3	20.8 ± 5.4	21.9 ± 5.4
Weight (kg)	77.1 ± 11.1	64.1 ± 8.9	72.7 ± 12.1	79.6 ± 12.8	64.4 ± 8.1	74.5 ± 13.5
Stature (cm)	183.6 ± 12.0	171.1 ± 8.7	179.4 ± 12.5	183.0 ± 10.1	170.8 ± 7.1	178.9 ± 10.8
BMI (kg/m^2)	22.7 ± 2.4	21.8 ± 1.9	22.4 ± 2.3	23.9 ± 3.0	22.1 ± 2.1	23.3 ± 2.8
FM (kg)	10.2 ± 3.7	15.1 ± 4.5	11.9 ± 4.6	12.2 ± 5.5	15.6 ± 3.7	13.3 ± 5.2
FM (%)	13.2 ± 3.3	23.5 ± 4.6	16.7 ± 6.2	15.1 ± 5.0	24.3 ± 4.5	18.2 ± 6.5
FFM (kg)	66.2 ± 8.8	48.5 ± 6.0	60.2 ± 11.6	66.6 ± 9.5	48.3 ± 6.0	60.5 ± 12.1
TBW_{Ref} (kg)	49.5 ± 7.1	35.1 ± 4.6	44.6 ± 9.3	50.0 ± 7.4	35.8 ± 4.6	45.2 ± 9.4
ECW_{Ref} (kg)	19.5 ± 3.0	15.0 ± 1.9	18.0 ± 3.4	19.5 ± 3.2	15.2 ± 1.9	18.0 ± 3.5
ICW_{Ref} (kg)	30.1 ± 5.1	20.1 ± 3.2	26.7 ± 6.6	30.4 ± 5.7	20.6 ± 3.8	27.2 ± 6.9

Abbreviations: Abbreviations: BMI: body mass index; FM, fat-mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Ref, reference; sd, standard deviation.

as well as for the whole sample with no differences observed between the two groups (i.e. developmental and cross-validation) ($p > 0.05$).

We found no significant interaction with sex for any of the main independent predictors and, thus, combined males and females for development of the prediction models.

3.1. Phase I – models development

Table 3 shows the BIA-based models for TBW and ECW generated in the national level athletes.

Two different models were developed for TBW and ECW estimates. In both cases, a preliminary model was developed including anthropometric and bioelectrical impedance variables, namely age, weight, stature, BMI, R, Xc, impedance, resistance index, and impedance index, as well as individual sport modality and athlete's categorization into 2 and 5 groups. Only variables contributing to the estimates using backward stepwise approach were used in the model. Accuracy of the developed model can be observed by the high coefficients of determination ($R^2 \geq 0.84$) and low standard errors of estimation (SEE ≤ 2.42 kg).

The resulting prediction models included:

$$TBW = 0.286 + 0.195{*}S^2/R + 0.385{*}Wt + 5.086{*}Sex\ (SEE = 2.42\ kg)$$

$$ECW = 1.579 + 0.055{*}S^2/R + 0.127{*}Wt + 0.006{*}S^2/Xc + 0.932{*}Sex\ (SEE = 1.33\ kg)$$

where sex is 0 if female or 1 if male, Wt is weight in kg, S is stature in cm, R is resistance, and Xc is reactance in ohm (Ω).

Table 3
Developed models for total body water (TBW) and extracellular water (ECW) prediction.

(N = 139, 92 M and 47 F)	Coefficient	R^2	SEE (kg)	CV (%)
TBW Model		0.93	2.42	5.4
Intercept	0.286			
S^2/R	0.195			
Wt	0.385			
Sex[§]	5.086			
ECW Model		0.84	1.33	7.4
Intercept	1.579			
S^2/R	0.055			
Wt	0.127			
S^2/Xc	0.006			
Sex[§]	0.932			

Abbreviations: M, male; F, female; TBW, total body water; ECW, extracellular water; Wt, weight (kg); S, stature (cm); R, resistance (Ω); Xc, reactance (Ω); R^2, coefficient of determination; SEE, standard error of the estimate; CV, coefficient of variation.
[§]0 if female; 1 if male.

3.2. Phase II – cross validation of derived prediction models

A cross validation was performed and the results of the regression parameters, CCC, and agreement analyses between the TBW and ECW estimated from the new BIA-based models and the reference method are presented in Table 4 (cross-validation panel). Table 4 presents the regression results, CCC, and agreement analyses between the ICW calculated as TBW minus ECW estimated from the new BIA-based models and the ICW calculated as the difference of the TBW and ECW estimated from the reference procedures.

No differences between methods were observed for TBW, ECW, or ICW estimation; with the methods highly correlated ($p < 0.001$). The BIA-based models developed in phase I for TBW and ECW explained 91% and 70% of the variability observed in the values of the reference methods, respectively. The ICW, calculated as their difference, explained 80% of the variability observed in the criterion method. Pure errors ranged from 1.90 kg in ECW to 3.12 kg for ICW. The precision and accuracy of the methods was higher than 0.83 and 0.99, respectively, with a CCC between the new method and the reference procedure superior to 0.83 (Table 4 – cross-validation panel). From the agreement analysis, we observed no bias between the methods for TBW, ECW and ICW.

3.3. Phase III – comparison with other TBW and ECW published equations

Table 4 (validation panel) shows the results from regression parameters, CCC, and agreement analysis between our athletes TBW and ECW estimated from other predictive equations (Table 1) and the comparison to reference methods.

Differences between predicted and reference values were observed for all TBW and ECW predictive models, with the exception of Kushner and Schoeller equation [18].

TBW estimation by the literature equations was highly correlated (R^2 ranged from 0.90 to 0.91) with the reference values, with the exception of the estimates ($R^2 = 0.75$) obtained by the Van Loan and Mayclin equation [23]. The ECW predictive equations explained 70% of the variability observed in the reference methods. Pure errors for TBW ranged from 2.77 kg [24] to 4.64 kg [23], while the PE for ECW was 1.91 kg [25] and 1.93 kg [24].

A CCC value exceeding 0.75 was observed between the existent BIA-based models and the reference method for TBW. For ECW the observed CCC was higher than 0.78.

Concerning agreement analysis, a trend was verified between the mean and the difference of methods for the Van Loan and Mayclin equation, Schoeller and Luke equation, and Sergi equation [23,25,26] with wide limits of agreement being observed.

Table 4
Cross-validation of the total body water (TBW) and extracellular water (ECW) predictive models and intracellular water (ICW) estimation, and validation of other published equations.

	Regression analysis			CCC analysis			Agreement analysis		
	R^2	PE (kg)	CV (%)	CCC	ρ	C_b	Bias	95% LoA	Trend
Cross-validation									
TBW (kg)	0.91	2.77	6.1	0.95	0.9548	0.9999	−0.017	−5.631; 5.597	r = −0.042 (p = 0.733)
ECW (kg)	0.70	1.90	9.5	0.83	0.8343	0.9925	0.222	−3.627; 4.072	r = −0.192 (p = 0.134)
ICW (kg)	0.80	3.12	11.5	0.88	0.8904	0.9938	−0.193	−6.487; 6.102	r = −0.231 (p = 0.056)
Validation									
TBW Kushner and Schoeller, 1986 [18]	0.90	3.02	6.7	0.94	0.9458	0.9972	0.269	−5829; 6368	r = −0.208 (p = 0.086)
TBW Van Loan and Mayclin, 1987 [23]	0.75	4.64	10.3	0.75	0.8671	0.8600	−3.059[a]	−13,124; 7006	r = −0.639 (p < 0.001)
TBW Lukaski and Bolonchuk, 1988 [24]	0.91	2.77	6.1	0.90	0.9664	0.9319	−3.572[a]	−8454; 1311	r = −0.094 (p = 0.444)
TBW Kushner, Schoeller et al., 1992 [29]	0.90	3.08	6.8	0.93	0.9439	0.9856	−1.454[a]	−7652; 4744	r = −0.175 (p = 0.151)
TBW Schoeller and Luke, 2000 [26]	0.90	3.03	6.7	0.86	0.9455	0.9080	−3.478[a]	−9961; 3006	r = −0.499 (p < 0.001)
TBW Sun, Chumlea et al., 2003 [30]	0.91	2.78	6.2	0.94	0.9581	0.9832	−1.611[a]	−6989; 3767	r = −0.180 (p = 0.139)
ECW Lukaski and Bolonchuk, 1988 [24]	0.70	1.93	10.7	0.80	0.8310	0.9710	−0.766[a]	−5773; 3829	r = −0.140 (p = 0.251)
ECW Sergi, Bussolotto et al., 1994 [25]	0.70	1.91	10.6	0.78	0.8332	0.9460	−0.972[a]	−4675; 3143	r = 0.378 (p < 0.001)

Abbreviations: TBW, total body water; ECW, extracellular water; ICW, intracellular water; R^2, coefficient of determination; PE, pure error; CV, coefficient of variation; CCC, concordance correlation coefficient; ρ, precision; C_b, accuracy; LoA, limits of agreement.
[a] Significant differences between predicted model and reference method ($p < 0.05$).

4. Discussion

The overall intent of this study was to investigate a complete lack of BIA-based models to predict TBW, ECW, and consequently ICW as their difference, using dilution techniques as the criterion method in highly trained athletes.

Prediction models for TBW and ECW were based on weight, stature, sex, R, and Xc. As body shape differs among athletes due to the respective sport, athletes' categorization according to the individual sport was expected to be a predictor in the development of the mathematical model for the water estimates because body shape is generally accepted to be constant in BIA theory [27].

A cross validation was performed and a very strong correlation was observed between the developed methods and the reference method ($R^2 \geq 0.80$) for the TBW and ICW estimates, while the ECW estimation by the new model presented a strong correlation with the reference method. Additionally, precision and accuracy between the new methods and the dilution techniques were analyzed with concordance correlation coefficient analysis. A substantial strength of agreement [28] between the methods was observed in estimating TBW (CCC = 0.95), while for the ICW and ECW, a less strong agreement was found between the methods (CCC = 0.88 and CCC = 0.83, respectively). The measurement errors (±2.5 kg error for TBW and ±2 kg error for ECW) are cumulative, which means that the uncertainty in estimating ICW is typically ±3 kg. ICW is the difference between TBW and ECW and thus the error is anticipated to exceed the individual errors (technical and biological) of the TBW and ECW dilutional methods. Bland-Altman analyses was used to determine potential bias between the predictive and reference models [22]. Although no significant trend was observed, the 95% confidence intervals were relatively large. In other words, TBW could be over- or underestimated by ~5.6 kg, ECW could be underestimated by ~3.6 kg or overestimated by ~4 kg and ICW could be over- or underestimated by ~6 kg. For instance, considering an athlete who has an actual value of TBW of 45 kg and 20 kg of ECW, the individual error based on the 95% confidence intervals observed indicate that TBW could range between 40 and 50 kg while ECW can vary between 16 and 24 kg. Despite the relatively large limits of agreement observed in our cross validation, the 95% confidence intervals are smaller than those reported by other authors (Table 1), namely Lukaski and Bolonchuk [24], Kushner and colleagues [29], and Sun and colleagues [30]. Only Kushner and Schoeller [18] presented similar 95% confidence intervals relative to those obtained in our investigation for TBW determination. Regarding ECW estimates, the limits of agreement obtained in our investigation are smaller than those reported by Lukaski and Bolonchuk [24].

Also in TBW, using a sample of adolescent athletes, Quitério and colleagues [31] found limits of agreements that ranged from −4.7–2.9 kg in girls and from −6.3–5.9 kg in boys. No bias was found in these adolescent athletes by the aforementioned authors. The absolute values reported by Quitério and colleagues were similar to ours, and despite their athletes were lighter, the relative limits of agreement were similar (−13%; 8% in girls and −14%; 3% in boys) to ours (−12%, 12%).

Considering the absolute and relative differences between methods, the results should be interpreted with caution for an individual assessment of body water compartments, whereas the developed prediction equations are appropriate for groups of athletes (e.g., before and after training). Additionally, the large coefficient for weight tends to cause large changes in predicted TBW or ECW with any change in weight. Therefore, the accuracy of these models for athletes needing to achieve a specified weight, or weight range may be compromised and requires more study.

Nevertheless, this new approach will fulfil a lack in the literature as it was recently stated that a specific and appropriate equation for water estimation in athletes should be developed, using state of the art methods as criterion [4,8]. Moon [8] suggested that athletes use a BIA system that provides raw data (R and Xc) instead of the predefined values obtained in the device software. Additionally the aforementioned author also stated that the equations used are generally population specific; this is to say if the predictive equations were obtained in middle aged sedentary individuals the predictive capacity is limited if using athletic populations [4,8], highlighting the need for a specific equation.

A recent Body Composition and Performance research working group under the auspices of the International Olympic Committee, recently recognized the need to monitor body composition and TBW (including both ICW and ECW) in determining the effectiveness of any exercise and dietary intervention [32]. In the sport science field there are no simple, inexpensive, and valid methods for estimating TBW or their respective water pools and this is an important omission as a simple field based method allows coaches to better gauge both their training programs effectiveness as well as to better follow the specific trends of their athletes (i.e. overtraining by be addressed by noticeable decreases in strength and performance). It has been shown recently that reductions in the ICW decreased both power and strength in athletes [5–7], reinforcing the need to accurately assess water compartments with simple, easy and inexpensive methods.

The 50 kHz phase-sensitive BIA is the most common model for in vivo body composition and water analysis due to its simplicity in operating and the reduced costs. Almost all single frequency impedance analyzers operate at a current frequency of 50 kHz. It is important to note that using the frequency of 50 kHz an acceptable estimation of TBW is obtained. However, the capability of distinguish the water pools distribution at this frequency is limited [33]. In fact, at 50 kHz frequency, the resulted impedance is a mixture of both R and Xc, which means that the electrical pathway is mainly ECW with unknown ICW penetration [4,33]. Despite the used frequency, the only way to convert impedance values of resistance and reactance into estimates of water and therefore body composition is applying those in a BIA regression model validated against a criterion method, in a specific population [8,33].

Even though no published studies regarding the development and cross validation of prediction models for TBW and its compartments based on BIA measurements using dilution techniques as reference in athletes are available, several similar studies have been conducted in different populations. To further debate, we limited the discussion to the studies that accomplish samples of healthy adults with normal weight and that were performed against deuterium and bromide dilution techniques as the criterion methods (Table 1).

Kushner and Schoeller [18] obtained a R^2 of 0.97 and an SEE of 1.75 L for TBW estimates in obese and non-obese adults. Cross-validation was performed in a smaller sample and good coefficient of determination was observed by the authors ($R^2 \geq 0.93$; PE $\leq$ 2.25 L). Van Loan and Mayclin [23] developed a model for TBW based on a sample of 188 healthy adults ($R^2 = 0.87$; SEE = 2.92 L). Using a development group of 116 participants and a cross validation group comprising 59 participants, a new predictive equation was developed by Kushner and colleagues [29], ($R^2 \geq 0.99$ and SEE of 1.41 kg) and good results remained in the cross-validation group. Schoeller and Luke [26], using a pooled sample from their laboratory, including 125 Caucasians and 89 African-Americans, developed a BIA-model for TBW, with a 93% of explained variance and SEE = 2.5 kg. Sun and colleagues [30] developed a sex-specific BIA-based equation ($R^2 \geq 0.79$ and SEE < 3.8 L) and cross validation was performed obtaining a $R^2 \geq 0.77$ and PE $\leq$ 3.8 L. Using a sample of 110 healthy adults (aged 20–73 years) Lukaski and Bolonchuk [24] developed a mathematical model for TBW assessment and for ECW estimation. A cross validation of the model was performed with a $R^2 = 0.98$ and SEE = 1.50 L for TBW, and $R^2 = 0.88$ and SEE = 1.01 L in the ECW estimates. A predictive equation for ECW estimation was developed by Sergi and colleagues [25] in 40 adults with $R^2 = 0.89$ and SEE = 1.7 L.

Comparing our regression parameters to the discussed investigations, the predictive model developed for this specific population behave better than the equations developed by Van Loan and Mayclin [23] and by Sun and colleagues [30]; similarly to Kushner and Schoeller [18] and to Schoeller and Luke [26]; and worse than Kushner and colleagues [29] and then Lukaski and Bolonchuk [24], when considering TBW prediction. Comparing the results obtained in the ECW estimation, our BIA based equation presented a lower coefficient of determination than Lukaski and Bolonchuk [24] and Sergi [25] equations.

After applying the literature equations to our cross-validation group of athletes, we observed that all TBW equations showed to be highly correlated to the reference methods, with the exception of the Van Loan and Mayclin equation [23]. However, all the equations with the exception of Kushner and Schoeller equation [18], presented significant differences to the criterion method (deuterium dilution). The CCC analysis indicated a poor strength of agreement [28] in two of the equations [23,26] and a moderate strength of agreement in the others [18,24,29,30]. Concerning Bland-Altman analysis a trend was observed between the mean and the difference of the methods in the application of the equations proposed by Van Loan and Mayclin and by Schoeller and Luke [23,26]. Wide limits of agreement were observed in the application of the literature equation to our cross validation group.

When applied to our cross-validation group, the ECW literature equations [24,25] presented a $R^2 = 0.70$ with significant differences between the predictive equation and the reference method and a poor strength of agreement was verified by the CCC analysis [28]. Also large limits of agreement were observed for the Sergi equation [25]. Despite some positive results obtained when the literature equations were applied to our cross-validation athletic group, the mathematical model developed in this investigation presented no mean bias and no trend between methods.

Considering the behaviour of the literature models once applied to our athletes sample, we can consider that the sample specific equations developed in this study are valid thus affording a practical mean to quantify TBW and its compartments in national level athletes.

Like all body composition methods, bioelectrical impedance depends on static assumptions and dynamic relationships regarding electrical properties of the body, its composition, hydration and density, age, race, sex and physical condition of the participant [3,4,8]. The major assumption that underlies the measurement is that the human body has uniform cross-sectional area. The body is seen as the sum of 5 uniform conductive cylinders whose dimensions are proportional to the participant's stature [4,33] with homogeneous conductivity. The tissue composition of the limbs and trunk result in regional differences in specific resistivity [4,33] but in commercial instruments the specific resistivity is assumed to be a constant for whole body [4,8,33]. However, considering all advantages and disadvantages of BIA and the logistically complex reference methods such as dilution techniques for hydration assessment, BIA-based models developed specifically to a population seem to be a good alternative in estimating TBW and its compartments. Future longitudinal studies to determine the validity of these new BIA based models in tracking water and its compartments in highly trained athletes over a season are required to be conducted.

Despite the encouraging results obtained in this study, some limitations are present and should be considered. First, our results are applicable to BIA equipment using the 50 kHz frequency. In fact, 50 kHz unifrequency devices are among the most used equipment, yet similar studies should be conducted to test other frequencies resulting from multifrequency equipment as predictors of TBW and its compartments. Second, the two groups were unbalanced with respect to gender and sport played. Though no interaction was found between sex and each predictor to explain the criterion method, additional studies should be performed to test the accuracy of these models in female athletes. Finally, the training process is different between sports and future research should be conducted to refine the developed models with athletes from further sports than those included in the present study.

5. Conclusions

The high cost of dilution techniques precludes their use in most clinical/athletic settings. Using a portable, safe, quick, and easy to perform BIA device, our findings provide new valid and non-biased models for TBW, ECW, and ICW estimates in national level athletes of various sports. The new models will have practical hydration monitoring with applications and benefits to researchers in the athletic and sports medicine field, as TBW and its compartments are of high significance in the framework of health and sports performance.

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Conflict of interest

None.

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