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Comparison of series and parallel reactance to identify changes in intracellular water in response to physical training in athletes during a sports season

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ABSTRACT

Objective: Cross-sectional evidence has demonstrated that parallel reactance obtained by bioelectrical impedance analysis (BIA) may be an alternative to the regularly used series of measurements to predict intracellular water (ICW) in athletes. However, we are not aware of any studies that have determined the predictive role or compared the effectiveness of both series and parallel reactance for tracking ICW changes during an athletic season. The main aim of this study was to determine the predictive role and compare both series and parallel reactance (Xc) in tracking ICW during an athletic season.
Research methods and procedures: This longitudinal study analyzed 108 athletes in the preparatory and competitive periods. Using dilution techniques, total body water (TBW) and extracellular water (ECW) were determined and ICW was calculated. Resistance (R), Xc, and impedance (Z) standardized for height were obtained through BIA spectroscopy using a frequency of 50kHz in a series array and then mathematically transformed in a parallel array.
Results: Multiple regression analyses showed that only changes in parallel Xc and capacitance (CAP) ($P < 0.05$) were predictors of delta ICW during the sports season. In contracts, this was not the case for Xcs. Both changes in R and Z, series and parallel, predicted similarly the changes in ECW and TBW ($P < 0.05$) in athletes.
Conclusion: Our findings highlight the potential of parallel BIA values to detect changes in body water compartments over a competitive season. These data provide preliminary evidence that changes in parallel Xc/H, and ultimately CAP, represent valid markers of alterations in cell volume during a sports season.

Introduction

Bioelectrical impedance analysis (BIA) enables determination of the impedance (Z), representing the impediment of an applied alternating electrical current flux that is related to water and electrolytes in body fluids and tissues [1]. In particular, Z is comprised of two components: resistance (R) and reactance (Xc). The R represents the pure opposition of the conductor to the flow of the current [1] while Xc is a function of capacitance (CAP) and is frequency dependent. Capacitance causes the current to lag behind the voltage, creating a delay or phase shift that is geometrically represented as the phase angle (PhA). In other words, PhA is the angular index of the delay between current and voltage at the cell membrane or tissue junctions [1]. Through the use of these raw parameters,

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BIA is an easy, practical, and valid method for assessing and monitoring total body water (TBW), intracellular water (ICW), and extracellular water (ECW) [1], representing a proxy of hydration and membrane integrity [2,3]. Considering the observed link with athletic performance, raw BIA parameters emerge as a valuable tool to track athletes [4–6].

In practical terms, BIA assumes that body fluids (ICW and ECW) in the human body function as resistors, and the cell membranes as capacitors [1], assuming a network of resistors and capacitors. From a physiological standpoint, when the assessment is obtained with an alternating current at 50 kHz using a tetrapolar phase-sensitive device, data can be analyzed as in-series or parallel circuits and the interpretation relies on the biophysical model employed [7]. In both series and parallel circuits, R and Z refer to ECW and TBW, respectively, while Xc may assume different interpretations in the two circuits. Series Xc relates to cells and ICW collectively [8], being related to cell population and hence cells in water [1] and interpreted as cell membrane quality [2]. Parallel Xc provides additional associative fluid and cellular information describing cells and ICW separately and quantitatively [7]. Considering that cells are primarily composed of water [9], and that previous data [10] showed a direct relation between Xc and total body potassium (the reference method to assess body cell mass [11]), the current consensus is that Xc parallel represents a surrogate measure of ICW [8,12]. In respect of CAP, it has been pointed out as an indicator of cell concentration [13] and as it is derived from parallel Xc, we anticipate a relation between parallel Xc with ICW, separately [10] and additional information about cell content [13].

Although the parallel transformation of series measurements may be a physiological representation of the human body [14], the commercial BIA assumes a series-equivalent circuit. Moreover, all the predictive equations for estimating body water compartments have been developed using series data [15]. The first evidence of the potential value of parallel BIA parameters as indicators of changes in fluid distribution, namely ICW, was provided by Lukaski and Talluri [8]. More recently, the potential value of parallel Xc (and ultimately CAP) as a valid index of cell volume in athletes was recently demonstrated in an observational study, as an alternative to series measurements [12]. However, longitudinal data derived from series and parallel array configurations to detect ICW changes has not yet been explored. This becomes even more relevant when considering the physical and physiological demands that characterize the training periodization of most athletes involved in different types of sports.

Thus, the aim of this study was twofold: 1) to determine the predictive role of both changes in series and parallel Xc in tracking changes in ICW (determined by dilution techniques) and the role of changes in both series and parallel Z and R in tracking changes in TBW and ECW (determined by dilution techniques); 2) to compare (by testing correlation coefficients) series and parallel measurements to explore the best marker to track changes in ICW, ECW, and TBW during the course of the sports season. It was hypothesized that when compared to series BIA measurements, changes in parallel transformed values would better predict alterations in ICW throughout the sports season. In addition, it was hypothesized that both series and parallel Z and R values might be similarly effective for predicting TBW and ECW.

Materials and methods

Participants

This was a longitudinal observational investigation including 108 athletes (26 females) engaged in five different sports (basketball n = 26, judo n = 26, handball n = 12, volleyball n = 14, swimming n = 18, pentathlon n = 2, and triathlon n = 10) that were assessed in two periods throughout the course of one athletic season: 1) preparatory phase; and 2) competitive phase. The following inclusion criteria were considered: 1) 10 or more hours of training per week and 2) not taking any medications. All subjects (≥18 y) and their parents or guardians (if age < 18 y) 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 (n° 03/2010), University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association. Each subject visited to the laboratory during the morning period (8:00h–10:00h) after an overnight fast (12 h fast), refraining from vigorous exercise for at least 15 h, no caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before.

Dual–energy X–ray absorptiometry

Whole body fat-free mass (FFM) and fat mass (FM) were determined by dual–energy X–ray absorptiometry (Hologic Explorer W, QDR for Windows version 12.4, Waltham, MA, USA). In our laboratory, based on data derived from testing 10 healthy adults, the test–retest coefficient of variation for FFM and FM are 0.8% and 1.7%, respectively.

Body water compartments

TBW was measured by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). After a 12-h fast, the first urine specimen was collected in a urine container. Each participant was given an oral dose of 0.1g of 99.9% $^{2}H_2O$ per kg of body mass (Sigma–Aldrich; St. Louis, MO, USA). After a 4 and 5 h equilibration period, during which no food or beverage was consumed, a urine specimen was collected. Urine specimens were prepared for $^{1}H/^{2}H$ analysis using the equilibration technique of Prosser and Scrimgeour [16], using procedures described elsewhere [17]. After the second collection, in tubes with 0.5 mL of urine, urine samples were filled with hydrogen gas and remained in equilibrium overnight at room temperature. After this procedure, the hydrogen species are introduced into a constant flow of Helium and analyzed in the mass spectrometer to analyze the $^{1}H/^{2}H$ ratio. The coefficient of variation and the technical error of measurement based on 10 repeated measures for TBW with the stable isotope ratio mass spectrometry in our laboratory corresponds to 0.3% and 0.11 kg, respectively [18].

The ECW was determined through dilution of sodium bromide (NaBr). After the collection of a saliva sample, each participant was asked to drink 0.030 g of 99.0% NaBr (Sigma–Aldrich) per kg of body mass, diluted in 50 mL of distilled–deionized water, using procedures described elsewhere [17]. The saliva samples were collected into salivettes that were then centrifuged and frozen for posterior analyses. The coefficient of variation and the technical error of measurement in our laboratory based on 10 repeated measures for ECW using high–performance liquid chromatography are 0.4% and 0.11 kg, respectively [17].

For both analyses (TBW and ECW), single-use containers were used throughout the collection and analysis process, being stored in adequate conditions to avoid any contamination. Moreover, during the analysis process, duplicates, blanks, and multiple standards were used to help identify if any contamination had occurred.

Finally, ICW was determined as the difference between TBW and ECW using the dilution techniques (ICW = TBW − ECW) [19].

Bioelectrical impedance spectroscopy

Whole body R, Z, and Xc were obtained using a bioelectrical impedance spectroscopy (BIS) analyzer (model 4200, Xitron Technologies, San Diego, CA, USA) set at the frequency of 50 kHz. Measurements were performed after a 10–min period of rest with the participant in a supine position with a leg opening of 45° compared to the median line of the body and the upper limbs positioned 30° away from the trunk [20]. Two current injection electrodes were placed at the right hand and foot on the dorsal surfaces proximal to the metacarpal and metatarsal-phalangeal joints, respectively. Two voltage-detector electrodes were placed on the mid-line between the prominent ends of the right radius and ulna of the wrist, and mid-line between the medial and lateral malleoli of the right ankle. After electrode placement, it was ensured that current-injection and voltage detector electrodes were at least 5 cm apart [21,22]. Low-impedance electrodes (Impedimed, IU0GELTD, Pinkenba, QLD, Australia), specifically 27Ω, 0.9Ω, and 27Ω for R, Xc, and Z, respectively, were used. More details about the electrodes' placement can be seen elsewhere [20].

The coefficient of variation of these measurements in our laboratory (using six participants other than those included in this study) corresponds to 0.6 and 1.5% for R and Xc at 50kHz, respectively [22]. Because at the frequency of 50 kHz this impedance device measures series-equivalents R and Xc and then calculates the series Z, it was necessary to calculate parallel equivalents using the following formulas [23] where the letters s and p

indicate series and parallel, respectively:

$$Rp = Rs + \left[(Xcs)^2/(Rs)\right]$$

$$Xcp = Xcs + \left[(Rs)^2/(Xcs)\right]$$

$$Zp : 1/Zp^2 = 1/Rp^2 + 1/Xcp^2$$

$$CAP\ (pF) = \frac{1E12}{f_{50kHz} * X_{Cp} * 2\pi} = \frac{1E12}{50000 * X_{Cp} * 6.28}\ 1E12\ =\ 1 \times 10^{12}$$

Statistical analysis

The data were analyzed with the R-studio version 1.4.1717 (R Core Team, 2013, Vienna, Austria) using lmtest package. Descriptive analysis including means ± standard deviations was performed. Normality was checked using the Shapiro–Wilk test. Since the data showed a normal distribution, paired samples *t* tests were used to compare TBW, ICW, ECW, series, and parallel R, Xc, Z, and CAP in preparatory and competitive phase in males and females (separately) who lost and gained water (lost and gained more than the technical error of measurement, respectively). Then the delta values for independent (series and parallel R, Xc, Z, and CAP) and dependent variable (TBW, ICW, ECW, TBW/FFM) throughout the season were calculated, as demonstrated in the following example: Δ value = TBW in competitive phase − TBW in preparatory phase. Pearson's correlation coefficients were computed using the delta values obtained for the independent and dependent variables. The following criteria were used to classify the strength of the relationship between these specific variables: r = 0 to 0.19 as very weak, 0.20 to 0.39 as weak, 0.40 to 0.59 as moderate, 0.60 to 0.79 as strong, and 0.8 to 1.0 as very strong correlation [24].

For each independent variable separately, a multiple regression analysis was used to determine its association with the dependent variables using the unstandardized beta coefficient. The significance level was set at $P < 0.05$. To measure the quality of the statistical models, find the best-fit model, and compare between BIA parameters in series and parallel, the Akaike information criterion (AIC) was performed. A difference of ≥ 2 units was considered statistically significant, and the model with a lower AIC value is defined as the best-fit model [25].

Results

One hundred and eight athletes (26 females) were assessed during the preparatory and competitive phase. All the athletes were grouped according to ICW changes (those who lost and those who gained) during the sports season and those whose differences were below the technical error of measurement were excluded. Table 1 summarizes the series and parallel values of BIA measurements adjusted to athletes' height relative to changes in measured fluid volumes by reference techniques in athletes who lost (left panel) and gained ICW (right panel).

Table 2 displays Pearson's correlations between delta in raw BIA parameters and changes in body water compartments assessed by reference techniques. The results of the multiple regression analysis are displayed in Tables 3 and Table 4 for females and males, respectively.

In female athletes who lost ICW, parallel Xc and CAP ($P < 0.05$) were the only predictors of ICW changes. In male athletes who lost ICW, parallel Xc and CAP ($P < 0.05$) were also the only predictors of ICW changes, explaining between 10% and 13% of the variance in ICW (Fig. 1). Both parallel Xc and CAP showed similar AIC values for female and male athletes who lost ICW. In males who lost ICW over the sports season, changes in ECW were better predicted by both series and parallel R and Z, with similar AIC values ($P < 0.01$). In female athletes who lost ICW, changes in parallel Xc and CAP were shown to better predict TBW changes (lowest AIC score), while series R and both series and parallel Z showed similar AIC scores. Finally, in male athletes who lost ICW, no differences were found in predictive power (similar AIC scores) between series and parallel R, and series and parallel Z for predicting TBW changes.

In men who gained ICW throughout the season, only parallel Xc and CAP ($P < 0.05$) were the only predictors of ICW changes with CAP showing slightly a lower value (Fig. 1). In female athletes who gained ICW, changes in ECW were better predicted by series R, parallel Xc, series and parallel Z with similar AIC values ($P < 0.01$), while in men series and parallel R and parallel Z showed the lowest AIC scores and were considered the best predictors. Finally, in the female group who gained ICW, parallel Xc was shown to be the best predictor of TBW changes and both R and Z in series and parallel showed similar AIC values. Lastly, in the male group who gained ICW, series and parallel R, and parallel Z showed a better AIC and thus are the best predictors of TBW changes.

Discussion

In line with that hypothesized, the present results showed the following: 1) changes in parallel Xc adjusted for height and CAP have the strongest predictive value for tracking changes in ICW during a sports season in both females and males being congruent with previous research [12]; 2) changes in series and parallel R are equally effective for predicting changes in ECW and TBW in female and male athletes (regardless of losing or gaining water over the sports season), while Z in parallel was a better predictor (compared to Z in series) of ECW in the male group who gained ICW, and also of TBW in females who lost ICW.

As previously shown using a cross-sectional approach [12], parallel BIA parameters have the potential to identify fluid compartments in athletes, serving as an alternative to the regularly used series measurements. In specific, it was shown that Xc in parallel, and ultimately CAP, are valid indicators of cell volume. Adding to this information, the present findings indicate that Xc in parallel and CAP (derived from parallel Xc) also represent the best predictors of changes in cell volume throughout a sports season, and this is novel. Additionally, in line with that obtained in cross-sectional research [12], the present study also indicates that alterations in R (in both series and parallel) have predictive value for estimating changes in ECW and TBW during a sports season, with no major differences between them.

As shown in the Xc's equation [(Xc (Ohms) = 1/(2 x π x Frequency (Hz) x CAP (Farads)] [1], the value of Xc is frequency dependent and is described as capacitive resistance, which is inversely related to frequency and CAP [1]. Furthermore, it was previously demonstrated by Trebbels et al. [13] that there is a direct and positive relation between CAP and red blood cell content. Thus, while CAP indicates cell content, series Xc is indirectly related to the concentration of cells in water [3]. Also, as mentioned before, some studies [11,26] have suggested a direct correlation between total body potassium and both CAP and parallel Xc. Considering the relatively constant total body potassium/ICW [11,26], it can be concluded that parallel Xc is an indicator of ICW. As CAP is derived from parallel Xc ($CAP\ (pF) = \frac{1E12}{f_{50kHz} * X_{Cp} * 2\pi} = \frac{1E12}{50000 * X_{Cp} * 6.28}$), it can be said that parallel Xc is related to ICW and CAP is an indicator of cell mass. Hence, this information can be analyzed separately as cell content (as demonstrated by the relation of CAP and hematocrit) and ICW (as parallel Xc is related to ICW and ICW is correlated with total body potassium). It is also expected a direct relation between ICW and CAP as cell volume changes may directly affect the membrane thickness. Congruently, as demonstrated in the current and previous studies [12], CAP may be a useful, single, independent indicator of cell volume. Perhaps, this assumption explains our results as the changes in Xc in parallel and CAP were strong predictors of changes in ICW. Based on these findings, we can conclude that alterations in Xc in parallel and CAP over a sports season better predict changes in ICW. However, the same analysis of the group of females who gained ICW throughout the sports season was not possible due to a reduced sample size (n = 10) that

Table 1
Body water compartments, raw bioelectrical impedance parameters in series (s) and parallel (p) at 50 kHz frequency of athletes who lost (left panel) and who gained (right panel) intracellular water over the sports season

	Athletes who lost ICW					Athletes who gained ICW			
	Variable	Preparatory phase Mean ± SD	Competitive phase Mean ± SD	Δ Mean ± SD (%)		Variable	Preparatory phase Mean ± SD	Competitive phase Mean ± SD	Δ Mean ± SD (%)
Women (N = 16)	Body mass, kg	63.6 ± 8.1	63.6 ± 6.62	0.02 ± 2.3 (0.3 ± 3.3)	Women (N = 10)	Body mass, kg	63.0 ± 6.7	63.5 ± 5.6	0.5 ± 2.5 (0.5 ± 2.5)
	FFM, kg	46.9 ± 5.2	47.6 ± 5.0	0.8 ± 1.1* (1.7 ± 2.6)		FFM, kg	47.3 ± 5.6	48.3 ± 4.5	0.9 ± 1.5* (2.6 ± 3.4)
	ICW, L	21.8 ± 3.7	19.3 ± 2.37	−2.5 ± 2.3** (-10.7 ± 7.8)		ICW, L	19.8 ± 2.5	19.8 ± 2.6	1.5 ± 0.9* (8.6 ± 6.3)
	ECW, L	14.5 ± 1.3	14.9 ± 1.61	0.4 ± 1.3 (3.1 ± 8.5)		ECW, L	14.6 ± 1.3	14.6 ± 1.3	−0.7 ± 1.0 (-3.8 ± 5.5)
	TBW, L	36.3 ± 4.5	34.2 ± 3.61	−2.1 ± 1.7** (-5.5 ± 4.1)		TBW, L	34.4 ± 3.6	34.4 ± 3.7	0.8 ± 1.7 (2.8 ± 5.1)
	Δ Series R/H, Ω/m	328.9 ± 37.7	341.0 ± 34.8	12.1 ± 21.8* (4.0 ± 7.0)		Δ Series R/H, Ω/m	340.6 ± 45.6	338.4 ± 30.0	−2.1 ± 24.1 (0.3 ± 6.8)
	Δ Series Xc/H, Ω/m	39.9 ± 4.6	41.2 ± 4.5	1.3 ± 2.9 (3.7 ± 7.5)		Δ Series Xc/H, Ω/m	38.3 ± 5.2	40.7 ± 4.1	2.4 ± 4.3 (7.4 ± 12.2)
	Δ Series Z/H, Ω/m	331.3 ± 37.7	343.4 ± 34.9	12.0 ± 22.0* (4.0 ± 6.7)		Δ Series Z/H, Ω/m	342.7 ± 45.9	340.9 ± 30.1	−1.8 ± 24.5 (0.1 ± 6.8)
	Δ Parallel R/H, Ω/m	333.7 ± 37.9	346.0 ± 35.1	12.3 ± 21.8 (4.0 ± 6.9)		Δ Parallel R/H, Ω/m	344.8 ± 46.1	343.9 ± 30.1	−1.5 ± 24.8 (0.2 ± 7.0)
	Δ Parallel Xc/H, Ω/m	2772.8 ± 477.1	2876.3 ± 410.8	103.5 ± 310.1** (4.9 ± 13.1)		Δ Parallel Xc/H, Ω/m	3079.4 ± 519.0	2871.5 ± 430.2	−207.8 ± 134.4** (-6.5 ± 3.5)
	Δ CAP, pF	692.3 ± 115.1	660.9 ± 100.1	−31.2 ± 78.6 (-3.8 ± 10.4)		Δ CAP, pF	614.2 ± 79.3	654.6 ± 75.2	40.5 ± 22.3** (6.8 ± 4.0)
	Δ Parallel Z/H, Ω/m	331.3 ± 37.8	343.5 ± 34.9	12.2 ± 21.8* (4.0 ± 7.0)		Zp/H, ohm/m	342.7 ± 45.9	340.8 ± 30.1	−1.8 ± 24.5 (0.1 ± 6.8)
Men (N = 35)	Body mass, kg	76.8 ± 10.1	76.6 ± 10.0	−0.2 ± 2.8 (-0.3 ± 3.7)	Men (N = 47)	Body mass, kg	75.8 ± 11.0	76.2 ± 11.1	0.4 ± 1.8 (0.4 ± 1.8)
	FFM, kg	65.8 ± 7.8	66.1 ± 8.0	0.3 ± 2.0 (0.4 ± 3.1)		FFM, kg	64.8 ± 9.2	65.4 ± 9.7	0.7 ± 1.5* (0.9 ± 2.1)
	ICW, L	30.5 ± 4.9	28.5 ± 3.8	−2.1 ± 2.0** (-6.3 ± 5.1)		ICW, L	30.0 ± 4.8	30.0 ± 4.8	2.2 ± 1.5** (8.2 ± 5.3)
	ECW, L	19.9 ± 2.4	19.7 ± 2.5	−0.2 ± 1.6 (-1.0 ± 8.2)		ECW, L	19.1 ± 2.8	19.1 ± 2.8	−0.6 ± 1.1** (-3.0 ± 5.4)
	TBW, L	50.5 ± 6.9	48.2 ± 5.6	−2.3 ± 2.8** (-4.2 ± 4.6)		TBW, L	49.1 ± 7.2	49.1 ± 7.2	1.6 ± 1.7** (3.4 ± 3.6)
	Δ Series R/H, Ω/m	242.3 ± 27.5	247.6 ± 24.7	5.3 ± 13.2* (2.5 ± 6.0)		Δ Series R/H, Ω/m	258.5 ± 31.6	256.6 ± 32.5	−1.8 ± 10.5 (-0.7 ± 4.0)
	Δ Series Xc/H, Ω/m	33.1 ± 4.5	33.4 ± 4.1	0.4 ± 3.0 (1.7 ± 10.1)		Δ Series Xc/H, Ω/m	34.7 ± 4.8	35.3 ± 5.2	0.6 ± 2.2 (1.8 ± 6.5)
	Δ Series Z/H, Ω/m	244.6 ± 27.6	250.0 ± 24.8	5.4 ± 13.3* (2.5 ± 6.0)		Δ Series Z/H, Ω/m	262.0 ± 32.9	258.9 ± 32.8	2.1 ± 38.6 (-1.0 ± 5.2)
	Δ Parallel R/H, Ω/m	246.9 ± 27.8	252.1 ± 25.1	5.3 ± 13.6* (2.5 ± 6.0)		Δ Parallel R/H, Ω/m	263.2 ± 32.1	261.5 ± 33.1	−1.7 ± 10.8 (-0.6 ± 4.0)
	Δ Parallel Xc/H, Ω/m	1824.1 ± 290.7	1877.7 ± 247.3	53.6 ± 125.0** (3.6 ± 7.3)		Δ Parallel Xc/H, Ω/m	1972.2 ± 307.1	1912.6 ± 296.2	−59.6 ± 91.2** (-2.9 ± 4.5)
	Δ CAP, pF	987.9 ± 168.9	950.7 ± 134.7	−37.2 ± 70.8* (-3.1 ± 6.8)		Δ CAP, pF	903.1 ± 135.0	929.5 ± 137.1	26.5 ± 41.2** (3.1 ± 4.7)
	Δ Parallel Z/H, Ω/m	244.6 ± 27.6	249.9 ± 24.9	5.3 ± 13.4* (2.5 ± 6.0)		Δ Parallel Z/H, Ω/m	260.8 ± 32.0	259.0 ± 32.8	−1.8 ± 10.7 (-0.7 ± 4.0)

CAP, capacitance; ECW, extracellular water; ICW, intracellular water; R/H, resistance standardized for height; TBW, total body water; Xc/H, reactance standardized for height; Z/H, impedance standardized for height
**$P < 0.001$; *$P < 0.05$.

Table 2
Pearson's correlation coefficients (r) between differences in raw bioelectrical impedance parameters in series (s) and parallel (p) adjusted to height (m) and differences in fluid volumes assessed by dilution techniques over the sport season in athletes who lost and gained ICW

	Athletes who lost ICW						Athletes who gained ICW					
	ΔICW		ΔECW		ΔTBW		ΔICW		ΔECW		ΔTBW	
Variable	Females *N* = 16	Males *N* = 35	Females *N* = 16	Males *N* = 35	Females *N* = 16	Males *N* = 35	Females *N* = 10	Males *N* = 47	Females *N* = 10	Males *N* = 47	Females *N* = 10	Males *N* = 47
Δ Series R/H, Ω/m	−0.46	−0.25	−0.15	**-0.71****	**-0.73***	**-0.61****	−0.44	−0.24	**-0.69***	**-0.54****	**-0.64***	**-0.55****
	P = 0.071	*P* = 0.142	*P* = 0.577	***P* < 0.001**	***P* = 0.001**	***P* < 0.001**	*P* = 0.207	*P* = 0.110	***P* = 0.027**	***P* < 0.001**	***P* = 0.047**	***P* < 0.001**
Δ Series Xc/H, Ω/m	0.31	−0.04	−0.41	**-0.45***	0.12	−0.29	−0.32	0.02	−0.58	**-0.50***	−0.51	**−0.33***
	P = 0.239	*P* = 0.843	*P* = 0.113	***P* = 0.007**	*P* = 0.669	*P* = 0.09	*P* = 0.366	*P* = 0.879	*P* = 0.079	***P* < 0.001**	*P* = 0.132	***P* = 0.022**
Δ Series Z/H, Ω/m	−0.45	−0.28	−0.16	**-0.70****	**-0.73***	**-0.62****	−0.43	−0.04	**-0.69***	**-0.38****	**-0.64***	−0.27
	P = 0.077	*P* = 0.104	*P* = 0.558	***P* < 0.001**	***P* = 0.001**	***P* < 0.001**	*P* = 0.210	*P* = 0.798	***P* = 0.028**	***P* = 0.008**	***P* = 0.048**	*P* = 0.063
Δ Parallel R/H, Ω/m	−0.45	−0.25	−0.16	**-0.71****	**-0.72***	**-0.60****	−0.43	−0.23	**-0.69***	**-0.55****	**-0.63***	**-0.54****
	P = 0.084	*P* = 0.156	*P* = 0.548	***P* < 0.001**	***P* = 0.002**	***P* < 0.001**	*P* = 0.212	*P* = 0.128	***P* = 0.028**	***P* < 0.001**	***P* = 0.049**	***P* < 0.001**
Δ Parallel Xc/H, Ω/m	**-0.69***	**-0.38***	0.05	**-0.54****	**-0.89****	**-0.60****	−0.55	**-0.33***	**-0.74***	−0.27	**-0.73***	**-0.46****
	P = 0.003	***P* = 0.021**	*P* = 0.843	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* = 0.096	***P* = 0.025**	***P* = 0.015**	*P* = 0.058	***P* = 0.016**	***P* = 0.001**
Δ CAP, pF	**-0.72***	**-0.37***	−0.11	**0.54****	**0.89****	**0.59****	−0.37	**-0.38***	**0.64***	0.21	0.57	**0.46****
	***P* = 0.002**	***P* = 0.028**	*P* = 0.680	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* = 0.294	***P* = 0.008**	***P* = 0.046**	*P* = 0.168	*P* = 0.084	***P* < 0.001**
Δ Parallel Z/H, Ω/m	−0.45	−0.25	−0.16	**-0.71****	**-0.73***	**-0.60****	−0.44	−0.23	**-0.69***	**-0.55****	**-0.64***	**-0.55****
	P = 0.077	*P* = 0.149	*P* = 0.562	***P* < 0.001**	***P* = 0.001**	***P* < 0.001**	*P* = 0.210	*P* = 0.119	***P* = 0.028**	***P* < 0.001**	***P* = 0.048**	***P* < 0.001**

CAP, capacitance; ECW, extracellular water; ICW, intracellular water; R/H, resistance standardized for height; TBW, total body water; Xc/H, reactance standardized for height; Z/H, impedance standardized for height
**$P < 0.001$; *$P < 0.05$

Table 3
Linear regression between raw bioelectrical impedance parameters in series (s) and parallel (p) adjusted to height (m) (independent variables) and fluid volumes assessed by dilution techniques (dependent variables) in females

Females															
Who lost ICW (*N* = 16)															
	ΔICW					ΔECW					k				
Independent variable	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC
Δ Series R/H, Ω/m	–	–	–	–	–	–	–	–	–	–	−0.057	0.014	0.50	**0.001**	55.05
Δ Series Xc/H, Ω/m	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Δ Series Z/H, Ω/m	–	–	–	–	–	–	–	–	–	–	−0.056	0.014	0.49	**0.001**	66.84
Δ Parallel R/H, Ω/m	–	–	–	–	–	–	–	–	–	–	−0.056	0.015	0.48	**0.002**	55.78
Δ Parallel Xc/H, Ω/m	−0.005	0.001	0.44	**0.003**	66.31	–	–	–	–	–	−0.005	0.001	0.78	**<0.001**	42.32
Δ CAP, pF	0.021	0.005	0.49	**0.002**	64.97	–	–	–	–	–	0.019	0.003	0.77	**<0.001**	42.71
Δ Parallel Z/H, Ω/m	–	–	–	–	–	–	–	–	–	–	−0.057	0.014	0.49	**0.001**	55.41
Who gained ICW (*N* = 10)															
	ΔICW					ΔECW					ΔTBW				
Independent variable	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC
Δ Series R/H, Ω/m	–	–	–	–	–	−0.027	0.010	0.47	**0.027**	25.89	−0.044	0.019	0.33	**0.047**	38.38
Δ Series Xc/H, Ω/m	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Δ Series Z/H, Ω/m	–	–	–	–	–	−0.027	0.010	0.41	**0.028**	25.95	−0.043	0.019	0.33	**0.048**	38.44
Δ Parallel R/H, Ω/m	–	–	–	–	–	−0.026	0.010	0.40	**0.028**	25.98	−0.043	0.018	0.33	**0.049**	38.48
Δ Parallel Xc/H, Ω/m	–	–	–	–	–	−0.005	0.002	0.49	**0.015**	24.47	−0.009	0.003	0.48	**0.001**	35.92
Δ CAP, pF	–	–	–	–	–	0.027	0.012	0.04	**0.046**	27.08	–	–	–	–	–
Δ Parallel Z/H, Ω/m	–	–	–	–	–	−0.27	0.010	0.41	**0.028**	25.94	−0.044	0.019	0.28	**0.048**	38.43

CAP, capacitance; ECW, extracellular water; ICW, intracellular water; R/H, resistance standardized for height; TBW, total body water; Xc/H, reactance standardized for height; Z/H, impedance standardized for height.

Table 4
Linear regression between raw bioelectrical impedance parameters in series (s) and parallel (p) adjusted to height (m) (independent variables) and fluid volumes assessed by dilution techniques (dependent variables) in males

Males														
	Who lost ICW (*N* = 35)													
	ΔICW					ΔECW					ΔTBW			
Independent variable	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC
Δ Series R/H, Ω/m	–	–	–	–	–	−0.088	0.015	0.49	**<0.001**	114.16	−0.127	0.029	0.35	**<0.001**	159.64
Δ Series Xc/H, Ω/m	–	–	–	–	–	−0.247	0.085	0.18	**0.007**	130.92	–	–	–	–	–
Δ Series Z/H, Ω/m	–	–	–	–	–	−0.086	0.015	0.48	**<0.001**	114.88	−0.129	0.028	0.37	**<0.001**	158.62
Δ Parallel R/H, Ω/m	–	–	–	–	–	−0.085	0.015	0.48	**<0.001**	114.71	−0.122	0.029	0.34	**<0.001**	160.27
Δ Parallel Xc/H, Ω/m	−0.006	0.003	0.13	**0.021**	148.31	−0.007	0.002	0.27	**<0.001**	127.03	−0.013	0.003	0.34	**<0.001**	159.97
Δ CAP, pF	0.011	0.005	0.11	**0.028**	148.83	0.012	0.003	0.26	**<0.001**	126.99	0.023	0.006	0.33	**<0.001**	160.75
Δ Parallel Z/H, Ω/m	–	–	–	–	–	−0.087	0.015	0.49	**<0.001**	114.43	−0.125	0.029	0.34	**<0.001**	159.95
	Who gained ICW (*N* = 47)													
	ΔICW					ΔECW					ΔTBW			
Independent variable	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC	β	SE	$r^2_{adjusted}$	*P*	AIC
Δ Series R/H, Ω/m	–	–	–	–	–	−0.057	0.013	0.28	**<0.001**	130.22	−0.091	0.021	0.29	**<0.001**	173.17
Δ Series Xc/H, Ω/m	–	–	–	–	–	−0.243	0.063	0.23	**<0.001**	133.27	−0.259	0.109	0.09	**0.022**	184.51
Δ Series Z/H, Ω/m	–	–	–	–	–	−0.011	0.004	0.13	**0.008**	139.40	–	–	–	–	–
Δ Parallel R/H, Ω/m	–	–	–	–	–	−0.055	0.013	0.30	**<0.001**	130.00	−0.087	0.020	0.29	**<0.001**	173.31
Δ Parallel Xc/H, Ω/m	−0.122	0.047	0.11	**0.013**	172.37	–	–	–	–	–	−0.009	0.003	0.20	**<0.001**	178.85
Δ CAP, pF	0.113	0.045	0.10	**0.016**	170.29	–	–	–	–	–	0.019	0.006	0.20	**0.001**	178.77
Δ Parallel Z/H, Ω/m	–	–	–	–	–	−0.056	0.013	0.28	**<0.001**	130.11	−0.089	0.020	0.28	**<0.001**	173.43

CAP, capacitance; TBW, total body water; ECW, extracellular water; ICW, intracellular water; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Z/H, impedance standardized for height.

may limit the actual power in detecting associations between the BIA parameters and ICW.

Finally, our data shows that there are no noticeable differences between changes in both series and parallel R to predict ECW and TBW during the course of the sports season. As explained before, the physiological interpretation of BIA measurements obtained with an alternating current at 50 kHz depends on the biophysical model employed [1]. Series Z is defined as $Z^2 = R^2 + Xc^2$, so changes in series R and Xc directly influence the value of Z. In contrast, changes in parallel R and Xc values indirectly affect parallel Z values as it is defined as $1/Z^2 = 1/R^2 + 1/Xc^2$ [1,8]. Still, in both series and parallel circuits, R and Z are related respectively with ECW and TBW [1,8,12]. Contrary to what was hypothesized because the interpretation of parallel and series Z are supposed to be similar [1,3], Z in parallel was a better predictor (compared to Z in series) of TBW in females who lost ICW and of ECW in males who gained ICW. These results were found probably because in these groups, parallel Xc values had higher changes compared to series values thus affecting the Z parallel. Although the series and parallel Z values showed similar mean values, this highlights that parallel Z measurements are better predictors than the commonly used values. Furthermore, our findings point to Xc in parallel and CAP being the best predictors (lowest AIC value) of changes in TBW in female athletes who lost ICW. As our first aim was to test the predictive role of alterations in parallel Xc of changes in ICW, the sample was divided by ICW changes and thus this division may confound the results. Considering that ICW is the main compartment of TBW, and the predictive power of Xc in parallel in tracking ICW loss and gain, the strong relationship between Xc in parallel and TBW may be related to ICW changes.

Despite the novelty of the current study, it is important to acknowledge some limitations. First, the results are limited to the use of the same equipment (BIS model 4200 from Xitron Technologies). Although this device is no longer in production, many research groups, laboratories, and professionals still use it and can benefit from our findings. Furthermore, despite the equipment has been discontinued, this study continues to be valuable from a scientific point of view due to its novelty and new insights. This is the first evidence showing that alterations in Xc in parallel and CAP are predictors of changes in ICW over a sports season and potentially useful, simple, and independent markers of changes in cell volume. This new evidence (despite using a discontinued equipment) should be valued and will allow for new advances in the future. This information could be useful for researchers and the technology industry who may aim to replicate these results with newer and more sophisticated equipment. We encourage further studies to test our hypotheses with newer equipment currently in use.

Second, according to the user manual of the Xitron [27], this device is not a phase-sensitive device and thus measures the R and Xc and then calculates the reciprocal Z and PhA at the selected frequency (in this study 50 kHz). In opposition, devices such as Akern use different technology and are phase-sensitive instruments. It means that they use a single frequency (50 kHz) to measure PhA and Z and calculate R and Xc based on a trigonometric equation [28]. It is known that raw BIA data should not be used interchangeably given the individual errors between different technologies as previously demonstrated [22]. Thus, the extrapolation, interpretation, and comparison with other studies are limited. For example, Silva et al. [22] observed that somatotype among athletes was a factor that may affect the differences in measurements of bioimpedance variables between both devices, probably because body shape is generally accepted to be constant in BIA theory [29]. This

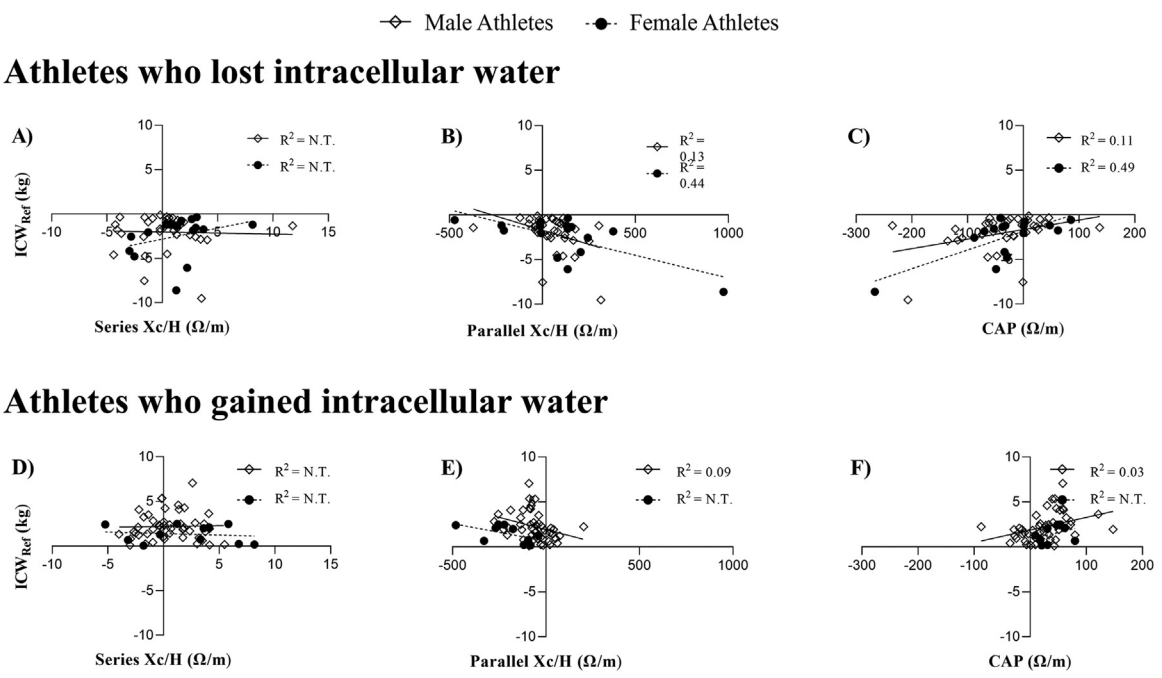

Fig. 1. The association of alterations in series Xc/H, parallel Xc/H, and CAP with tracer dilution-determined ICW. (A) Alterations in series Xc/H versus changes in ICW in athletes who lost ICW; (B) Changes in parallel Xc/H versus changes in ICW in athletes who lost ICW; (C) Changes in CAP versus changes in ICW in athletes who lost ICW; (D) Alterations in series Xc/H versus changes in ICW in athletes who gained ICW; (E) Changes in parallel Xc/H versus changes in ICW in athletes who gained ICW; (F) Changes in CAP versus changes in ICW in athletes who gained ICW. ICW, intracellular water; Xc/H, reactance standardized for height; Z/H, impedance standardized for height; CAP, capacitance.

observation showed to be 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 model 4200 compared to Akern single frequency. Nevertheless, differences between both technologies may be due to the effects of modeling and technical inadequacy issues by Xitron, namely the effects of stray capacitance and lead position reported [30,31]. Still, these errors associated with modeling are more likely at higher frequencies and unlikely to occur at a frequency of 50 kHz to assess the raw BIA parameters. Lastly, the number of female athletes was considerably lower than the number of male athletes. The lack of statistical significance in part of the analyses conducted in women may be due to a smaller sample size, limiting the generalizability of these findings for female athletes given the insufficient power to detect differences. We strongly recommend conducting additional research using observational prospective or experimental designs with a larger sample size or focusing on female athletes. It is important to consider the study's limitations and to interpret the results cautiously, acknowledging the need for further investigation to ensure generalizability across female athletes.

Conclusion

In conclusion, alterations in Xc in parallel and CAP are the best predictors of changes in ICW over a sports season and thus they might be useful, simple, and independent markers of changes in cell volume. However, both R and Z indirectly predict changes in ECW and TBW in athletes using series or parallel arrays. This study has implications for researchers, clinicians, and practitioners as they can use either series or parallel changes in R and Z to predict ECW and TBW over a sports season; however, regarding Z values, in some instances parallel values seem to represent a more valid option. Moreover, a mathematical transformation of series Xc to parallel Xc or CAP should be used to predict cell volume and cell content changes over the sports season. In other words, the practical use of transforming parallel bioimpedance values lies in enhancing the accuracy, sensitivity, and capability of bioimpedance measurements for various applications, including the possibility of separately analyzing the Xc and CAP as indicators of cell volume and cell content. This provides useful and practical information to sport-related professionals as the gold standard methods to assess cell volume and cell content are expensive and require specialized expertise, limiting its widespread availability to only a few laboratories. This study also includes important implications for athletes as they will benefit from a better diagnosis of their cellular hydration changes, allowing them to improve their performance and health.

Declaration of competing interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Rúben Francisco: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Filipe Jesus:** Writing – review & editing, Investigation, Formal analysis. **Catarina L. Nunes:** Investigation, Formal analysis, Conceptualization. **Marta Alvim:** Supervision, Conceptualization. **Francesco Campa:** Supervision, Conceptualization. **Luís B. Sardinha:** Supervision, Methodology, Investigation, Conceptualization. **Henry Lukaski:** Supervision, Methodology, Investigation, Conceptualization. **Analiza M. Silva:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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