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Abstract

Purpose: To analyze the association between body-fluid changes evaluated by bioimpedance vector analysis (BIVA) and dilution techniques over a competitive season in athletes.

Methods: Fifty-eight athletes of both sexes (men, age 18.7 ± 4.0 y; women, age 19.2 ± 6.0 y) engaging in different sports were evaluated at the beginning (Pre) and 6 mo after (Post) the competitive season. Deuterium dilution and bromide dilution were used as the criterion methods to assess total body water (TBW) and extracellular water (ECW), respectively; intracellular water (ICW) was calculated as TBW minus ECW. Bioelectrical resistance and reactance were obtained with a phase-sensitive 50-kHz bioelectrical impedance analysis device; BIVA was applied. Dual-energy X-ray absorptiometry was used to assess fat mass and fat-free mass. The athletes were empirically classified considering TBW change (Pre – Post, increase or decrease) according to sex.

Results: Significant mean vector displacements in the Post groups were observed in both sexes. Specifically, reductions in vector length (Z/H) were associated with increases in TBW and ICW (r = –.718, $P < .01$; r = –.630, $P < .01$, respectively) and decreases in ECW:ICW ratio (r = .344, $P < .05$), even after adjusting for age, height, and sex. Phase-angle (PA) variations were positively associated with TBW and ICW (r = .458, $P < .01$; r = .564, $P < .01$, respectively) and negatively associated with ECW:ICW (r = –.436, $P < .01$). PA significantly increased in all the Post groups except in women in whom TBW decreased.

Conclusions: The results suggest that BIVA is a suitable method to obtain a qualitative indication of body-fluid changes during a competitive season in athletes.

Keywords: BIVA, intracellular water, phase angle, total body water, vector length

Introduction

In sports, as well as in daily life, hydration status plays an important role, as hypohydration and fluid accumulation may affect physical function, cognitive performance, and health status.[1–3] Although laboratory clinical tests are typically preferred over signs and symptoms for detecting changes in body fluids, the methods are expensive, involving specialized technicians to perform and analyze the required exams. Yet, practitioners, coaches and researchers face the common problem of a lack of valid and practical methods and techniques to monitor body fluids changes under various conditions. [3,4]

The bioelectrical impedance vector analysis (BIVA), described in detail by Piccoli et al. [5], Lukaski and Piccoli [6] and Buffa et al. [7], considers the impedance components [resistance (R) and reactance (Xc)] independently of regression predictions of fluid volumes or assumptions about the constant chemical composition of the fat-free body.[8] BIVA provides a classification (e.g., normal or not normal) and ranking (e.g., better or worse after treatment or intervention) tool; it does not provide estimates of volume or mass. The vectors, defined by their length [(R^2+Xc^2) ^ $^{0.5}$] and phase angle (PA) defining the angular transformation between Xc and R (arctan Xc/R 180/3.14) are plotted on the resistance-reactance (R-Xc) graph as a point and allows for the analysis of body composition characteristics relative to a reference group or among different samples. In classic BIVA [5], R and Xc are standardized for the subject's stature, to classify differences in total body water (TBW) (negatively related to vector length) and cell mass (positively related to PA). Even if the accuracy of classic BIVA in assessing the percentage of fat mass (%FM), and hydration status (i.e., detection of hyper or hypo-hydrated individuals) has been recently questioned in athletes [9], classic BIVA has been shown to correctly detect differences in absolute values for FM and fat-free mass (FFM) [10] compared to dual energy X-ray absorptiometry (DXA) and to detect TBW variations. [11] Furthermore, PA is negatively correlated with the extracellular to intracellular water ratio (ECW/ICW) [12–14] and may be used as a good tool for assessing the systemic efficiency exercise interventions and for looking at hydration status and cell functioning relevant for health and sports performance.[15]

Classic BIVA has been applied in different sports disciplines and practices. [3,16–20] In particular, it has shown to be able to identify changes of body fluids after an exercise session, compared to plasma osmolarity (a hydration biomarker), stable isotope dilution and body weight changes. [19,21]

However, to the best of our knowledge, no studies have explored the suitability of BIVA in evaluation long-term body fluid changes, through the comparison with dilution techniques, the gold standard method for determining total body water compartments.[22] Therefore, the aim of this investigation was to compare body fluid assessment obtained with dilution techniques and BIVA in athletes throughout a competitive season. Our hypothesis was that vector displacements could reflect changes in body fluid over the season.

Methods

Participants

This was a longitudinal investigation of 58 athletes engaged in five sports [basketball (men = 20; women = 11), swimming (men = 5; women = 4), volleyball (men = 6; women = 4), handball (men = 6; women = 0) and triathlon (men = 2; women = 0)] (men: age 18.7±4.0 years; women: age 19.2±6.0 years). The following inclusion criteria were considered: 1) 10 or more hours of training per week, 2) negative test outcomes for performance-enhancing drugs and 3) not taking any medications. The results of a medical screening indicated that all subjects were in good health. All subjects (≥18 yrs) and their parents or guardians (if age < 18 yrs) were informed about the possible risks of the investigation before giving written informed consent to participate. All procedures were approved by the ethics committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association.

Procedures

Subjects were evaluated at the beginning (PRE) and after 6 months (POST), during the competitive season. The subjects came to the laboratory after an overnight fast (12 h fast), refraining from vigorous exercise at least 15 h, no caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before. All athletes were tested to ensure a well-hydrated state using the urine specific gravity test (refractometer Urisys 1100, Roche Diagnostics, Portugal), from a fasting baseline urine sample, according to Armstrong et al.[23]; a urine-specific gravity value <1.022 in the first urine was used to categorize euhydration. Body weight was measured with a scale without shoes and wearing minimal clothes, to the nearest 0.01 kg and stature was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). The intra-observer technical error of measurement (TEM) and the coefficient of variation (CV) were calculated in a subsample of ten subjects (height: TEM = 0.06 cm, CV =0.04; weight: TEM = 0.04 kg, CV = 0.07). Body mass index (BMI) was calculated as the ratio of body mass to height squared (kg/m^2).

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%. H_2O per kg of body weight (Sigma - Aldrich; St. Louis, MO) for the determination of total body water (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 (24). Urine samples were prepared for 1 H/^{2}H analyses using the equilibration technique by Prosser and Scrimgeour. [24] Our laboratory has reported a TEM and coefficient of variation (CV) in ten subjects for TBW of 0.11 and 0.3%, respectively. [25] The athletes were empirically divided considering TBW change (PRE-POST; increase or decrease), according to sex.

Extracellular water

Extracellular water (ECW) was assessed from 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. Baseline samples of saliva were collected before sodium bromide oral dose administration, and enriched samples were collected 3 h post-dose administration. Intracellular water (ICW) was calculated as the difference between TBW and ECW. The test-retest TEM and CV in 7 participants for the ECW using high performance liquid chromatography in our laboratory are 0.08 kg and 0.4%. [25]

Dual-energy X-ray absorptiometry

Athletes underwent a whole-body DXA scan according to the procedures recommended by the manufacturer on a Hologic Explorer-W fan-beam densitometer (Hologic, Waltham, MA, USA). The equipment measures the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. For athletes who were taller than the scan area, we used a validated procedure that consisted of the sum of a head and a trunk plus limbs scans. [26] The same technician positioned the participants, performed the scan, and executed the analysis (QDR for Windows software version 12.4; Hologic, Waltham, MA, USA) according to the operator's manual by using the standard analysis protocol. The DXA measurements included whole-body measurements of FM (kg) and FFM (kg). In our laboratory, in ten healthy adults, the test-retest TEM and CV for FM is 0.2 kg and 1.7% and for FFM is 0.3 kg and 0.8%, respectively.

Bioelectrical impedance analysis

The impedance measurements were performed with BIA (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made on an isolated cot from electrical conductors, the subjects were in the supine position with a leg opening of 45° compared to the median line of the body and the upper limbs, distant 30° from the trunk. After cleansing the skin with alcohol, two electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the right hand back and two electrodes on the corresponding foot. [6] Bioimpedance vector analysis was carried out using the classic BIVA method, normalizing R and Xc parameters for stature (H) in meters.[5] The measurements shown by the BIA 101 Anniversary device are R and Xc with Z calculated and then the values are adjusted for height R/H, Xc/H and the vector length (Z/H). The Z/H value was calculated as the hypotenuses of individual impedance normalized values. Bioelectrical PA was calculated as the arc-tangent of $Xc/R \times 180°/\pi$. 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.

Statistical Analysis

Descriptive statistics including means ± SD were calculated for all outcome variables. Once the data were tested for normality (Shapiro-Wilks test), differences in body composition and bioelectrical variables between PRE and POST were analyzed by two-way analysis of covariance (ANCOVA) for repeated measures, considering athletes who increased and decreased body fluids as covariate. When F-ratio was significant, Bonferroni's post hoc test was used for the identification of specific differences in the variables. The paired, one-sample Hotelling's T2-test was performed to determine if the changes in the mean group vectors (measured at the first and second time points) were significantly different from zero (null vector). A 95% confidence ellipse excluding the null vector indicated a significant vector displacement. Single and multiple regression analyses were performed to understand the associations between changes in TBW, ICW and ECW/ICW ratio with vector length and PA. Model adjustments included age, stature and sex. Data were analyzed with IBM SPSS Statistics version 24.0 (IBM, Chicago, IL). For all tests, statistical significance was set at $p < 0.05$.

Results

General characteristics of the athletes are shown in Table 1. The majority of them (28 males and 11 females) significantly increased TBW from PRE to POST, while 11 men and 8 women showed a decrement.

INSERT TABLE 1 HERE

Table 2 shows the changes in the body composition and bioelectrical variables from the first (PRE) to the second (POST) measurement. In male and female athletes who significantly increased their fluids during the season, an increase in ICW, FFM and PA, and a reduction in R, R/H, and Z/H were measured. Otherwise, athletes who reduced TBW from PRE to POST, a reduction of ICW and an increase of all bioelectrical values (R, Xc, R/H, Xc/H, Z/H, and PA) were measured among men, and an increase of Xc and Xc/H among women. No significant interactions between gender and time were detected, whereas the gender and time effects were significant for all the variables.

INSERT TABLE 2 HERE

The vector displacements plotted on the R-Xc graph, from PRE to POST, and the results of the paired one-sample Hotelling's T2-test were significant and similar in men and women (figures 1 and 2).

INSERT FIGURE 1 AND 2 HERE

In Tables 3 and 4 results from single and multiple regression analysis are displayed. Vector length was negatively correlated with TBW and ICW and positively associated with the ECW/ICW ratio, even when adjusted for sex, age and stature. Phase angle was positively associated with TBW and ICW and negatively associated with the ECW/ICW ratio, independently of sex, age, and stature.

INSERT TABLE 3 AND 4 HERE

Discussion

The main finding of the present investigation is that changes in body fluids throughout a competitive season are associated with changes in bioelectrical vectors in athletes. In particular, decreases in TBW detected by deuterium dilution were accompanied by increases in Z/H and decreases in PA, and viceversa. Additionally, in all groups there was a significant increase in PA, except for the females whose TBW decreased, where a positive but not significant trend was observed. To be noted that groups showing higher PA values also showed higher values of FFM, significantly among those whose TBW increased. Using the smallest change observed for TBW (in L women group) the decrease of 1.5 kg is largely above the technical error of measurement in assessing TBW from the deuterium dilution (0.11kg). Additionally, using the smallest change observed for ECW (in H women group) the decrease of 0.2 kg is largely above the technical error of measurement in assessing ECW from the deuterium dilution (0.08 kg).

These results are consistent with the theoretical expectations considering the biophysical basis of bioimpedance, BIVA in particular, and the common use of BIVA for the classification of hydration.[27] Indeed, the resistive component of the classic impedance vector (R/H), highly correlated to Z/H, gives information on the physiological fluids and tissues containing water and electrolytes (which behave as resistors).[27] Hence, the vector length can be interpreted as inversely related to TBW. The other component of the impedance vector, the capacitive resistance, mainly responsible of PA values, can be considered proportional to cell membranes, which behave as capacitors in the human body.[27] Our results also support evidence provided in previous studies that highlighted that peripheral vectors lying on the left side of the minor axis of the tolerance ellipses, i.e. with higher PA, indicate more soft tissue. [5,7,8,10] Actually, higher PA values reflect higher cellularity, cell membrane integrity and better cell function[28], and are associated with improved power output in elite road cyclists.[29]

In our investigation, increases in PA were also associated with ECW/ICW ratio decrements and this is in line with the findings of Gonzalez et al.[13], who suggested that PA is inversely related to ECW/ICW ratio, and with our previous researches on athletes[14]. Carrasco-Marginet et al.[17] also showed that following a loss of fluids PA tends to increase. Also, in our research significant ICW reductions (men: -1.5 kg; women: -1.1 kg) occurred in athletes who decreased TBW (men: -2.3 kg; women: -1.5 kg). Although it was not our goal to investigate the causes of TBW changes in the athletes, our hypothesis is that the reductions of TBW and ICW can be due to the nutritional habits or the different demands of exercise and the respective recovery process.

The use of BIVA has become a very common practice in sports, to evaluate changes in body fluids in athletes during the competitive season or following an exercise program or a training session. Mascherini et al.,[30] showed that vector movements can occur during a competitive season, highlighting that increases in fluids occur at the end of the pre-season phase and at the end of the season, while fluid leaks can occur during the competitive period. The bioelectrical vector and PA changes have also been associated with increases in strength and decrease in FM after exercise training programs in adults.[15,31] In addition, several studies have proposed new BIVA references for sports such as soccer [32] and volleyball [18], highlighting that BIVA can identify significant differences based on the competitive level, due to different characteristics in athletes of several sports. Although the classic BIVA approach has shown to be weak in the distinction of the relative contribution of fat mass and fat free mass [7], the studies that validated BIVA with accurate laboratory tests for the evaluation of short-term fluid changes (as after a physical exercise) have concluded that BIVA was accurate to assess body fluid changes. [11,19] To our knowledge, this is the first investigation to examine vector changes over a competitive season in athletes, comparing the results obtained by BIVA with TBW and water compartments from dilution techniques.

Despite the encouraging results obtained in this investigation, some limitations should be addressed. First, our results are applicable to the actual BIA equipment using the 50 kHz frequency. In fact, 50 kHz single frequency 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. Secondly, it is important to underscore that since athletes were tested at the beginning and at the main stage of the competitive period, but it is unknown if these two measurements represent what happened during the entire season. In addition, water and beverage intake during the study period was uncontrolled. Lastly, as only five sports were included in this investigation, generalizability of these findings to other sports is limited.

Conclusion

This investigation has shown that vector changes convincingly mirror fluids loss or gain over a season. In particular, peripheral vectors lying on the left or right side of the minor axis of the tolerance ellipses, i.e. with higher or lower phase angles, indicate more or less soft tissue, respectively. In addition, PA is inversely related to fluid distribution assessed from the ECW/ICW ratio.

Practical Applications

Nutritionist and coaches might use BIVA shifts as a practical method to monitor body fluid changes and to adapt training and nutrition in athletes.

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Figure captions

Fig. 1. Paired graph and Hotelling's T^2 test that identify the mean vector displacements in athletes showing an increase (dashed line), or a decrease (solid line) of total body water over the competitive season. Panel a: men; panel b: women. The vector displacements after 6 months are significantly different from zero (p<0.05, 95% confidence ellipse not overlapping zero).

Fig. 2. R-Xc graph and mean impedance vectors plotted on the tolerance ellipses created from bioimpedance values measured at PRE in women (panel a) and men (panel b). Where circles and triangles represent the clusters that increase or decrease fluids from PRE (black clusters) to POST (white clusters), respectively.

Table 1. Participants' characteristics.

	Men (n=39)	Women (n=19)
Variable	Mean ± SD	Mean ± SD
Age (y)	18.7 ± 4.0	19.2 ± 6.0
Stature (cm)	79.58 ± 10.23	62.54 ± 8.52
Weight (kg)	188.52 ± 8.19	170.79 ± 4.87
BMI (kg/m^2)	22.36 ± 2.20	21.39 ± 2.26

Note: BMI, body mass index.

Table 2. Two-way analysis of covariance (ANCOVA) for the comparison at baseline (PRE) and during (POST) the competitive season after adjusting for athletes who increased (H) and decreased (L) body fluids as covariate.

Variable	H				L				Gender Effect *P*-value	Time Effect *P*-value	Gender x Time interaction *P*-value
	Men (n = 28)		Women (n = 11)		Men (n = 11)		Women (n = 8)				
	PRE	POST	PRE	POST	PRE	POST	PRE	POST			
TBW (kg)	49.2 ± 6.1	51.6 ± 6.0*	31.8 ± 3.1	33.3 ± 3.2*	52.1 ± 6.6	49.8 ± 6.3*	36.7 ± 4.6	35.2 ± 4.5*	<0.001	<0.001	0.403
ECW (kg)	20.3 ± 2.5	21.0 ± 2.5*	14.1 ± 1.8	14.3 ± 1.5	20.4 ± 2.5	19.7 ± 2.0	15.1 ± 1.7	14.7 ± 1.5	<0.001	0.005	0.521
ICW (kg)	28.9 ± 4.1	30.6 ± 4.3*	17.7 ± 1.7	19.0 ± 2.0*	31.7 ± 4.6	30.2 ± 4.9*	21.6 ± 3.2	20.5 ± 3.2*	<0.001	0.001	0.240
ECW/ICW	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.003	0.824	0.279
FM (kg)	11.8 ± 4.7	11.5 ± 4.3	16.3 ± 3.8	16.3 ± 4.2	12.2 ± 3.2	11.8 ± 2.6	15.7 ± 4.8	15.0 ± 4.6	0.034	0.728	0.876
FFM (kg)	67.6 ± 7.4	70.0 ± 7.9*	44.1 ± 4.8	45.8 ± 4.4*	65.5 ± 8.5	67.2 ± 8.2	48.8 ± 6.0	48.8 ± 5.4	<0.001	<0.001	0.712
R (ohm)	491.0 ± 49.9	463.1 ± 45.6*	617.0 ± 51.4	591.4 ± 54.0*	447.9 ± 34.0	461.1 ± 37.1*	557.5 ± 77.2	576.4 ± 84.0	<0.001	<0.001	0.687
Xc (ohm)	60.3 ± 6.3	60.1 ± 6.0	71.0 ± 8.2	71.0 ± 8.3	59.5 ± 4.9	62.9 ± 4.9*	68.1 ± 11.4	72.0 ± 12.5*	<0.001	0.004	0.869
R/H (ohm/m)	258.4 ± 27.0	243.1 ± 24.0*	363.4 ± 36.1	347.7 ± 37.3*	243.6 ± 22.0	250.4 ± 23.0*	324.7 ± 44.4	334.7 ± 46.6	<0.001	<0.001	0.878
Xc/H (ohm/m)	31.8 ± 3.9	31.6 ± 3.8	41.8 ± 5.3	41.7 ± 5.2	32.3 ± 2.9	34.1 ± 2.8*	39.7 ± 6.7	41.8 ± 7.0*	<0.001	0.002	0.879
PA (°)	7.1 ± 0.7	7.5 ± 0.8*	6.6 ± 0.3	6.9 ± 0.4*	7.6 ± 0.7	7.9 ± 0.7*	7.0 ± 0.6	7.2 ± 0.6	0.005	0.034	0.345
Z/H (ohm/m)	260.4 ± 27.1	245.1 ± 24.1*	365.8 ± 36.5	350.2 ± 37.6*	245.8 ± 22.0	252.7 ± 23.0*	327.1 ± 44.8	337.4 ± 47.0	<0.001	<0.001	0.884

Note: Data are expressed as mean and standard deviation; * p< 0.05 vs. PRE; TBW, total body water; ECW, extracellular water; ICW, intracellular water; FM, far mass; FFM, fat free mass; R, resistance; Xc, reactance; R/H, resistance adjusted for stature; Xc/H, reactance adjusted for stature; PA, phase angle; Z/H, vector length adjusted for stature.

Table 3. Regression analyses for body fluids with vector length

	Model	Model [a]
	β (CI 95%)	β (CI 95%)
Δ TBW		
Δ ZL	-0.718 (-0.142; -0.080)**	-0.672 (-0.137; -0.071)**
Δ ICW		
Δ ZL	-0.630 (-0.134; -0.064)**	-0.531 (-0.119; -0.047)**
Δ ECW/ICW		
Δ ZL	0.344 (0.000; 0.004)*	0.217 (0.000; 0.003)

β, standardized beta coefficient; CI, confident interval; R, correlation coefficient; Δ, changes; TBW, total body water; ICW, intracellular water; ECW, extracellular water; ZL, vector length;

* Significant at $p < 0.05$

** Significant at $p < 0.01$

[a] Adjusted for sex, age and stature.

Table 4. Regression analyses for body fluids with phase angle

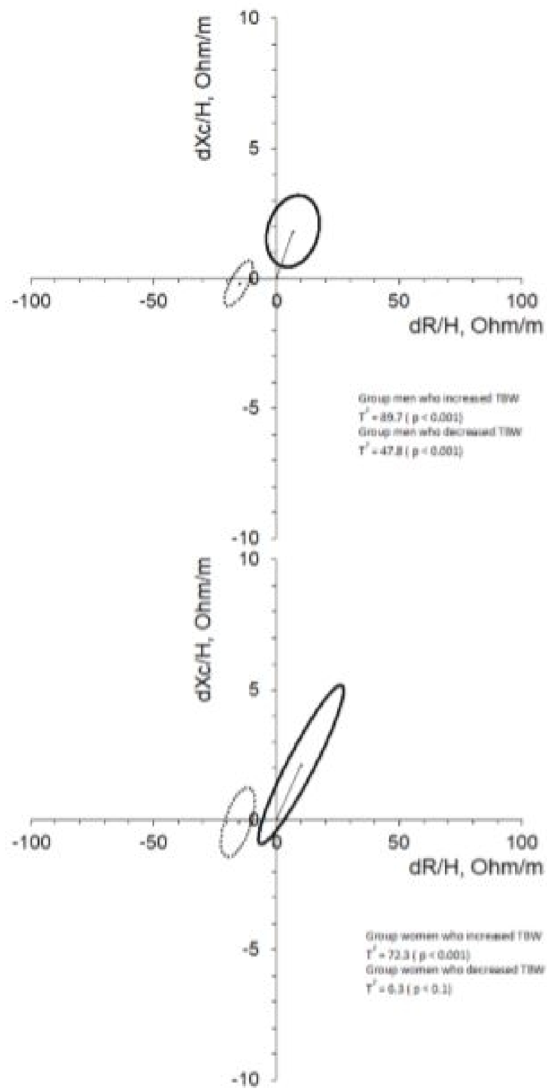
	Model	Model [a]
	β (CI 95%)	β (CI 95%)
Δ TBW		
Δ PA	0.458 (1.228; 4.324)**	0.396 (0.780; 4.024)*
Δ ICW		
Δ PA	0.564 (2.013; 4.929)**	0.455 (1.307; 4.293)**
Δ ECW/ICW		
Δ PA	-0.436 (-0.166; -0.042)**	-0.433 (-0.171; -0.007)*

β, standardized beta coefficient; CI, confident interval; R, correlation coefficient; Δ, changes; TBW, total body water; ICW, intracellular water; ECW, extracellular water; PA, phase angle.

* Significant at $p < 0.05$

** Significant at $p < 0.01$

[a] Adjusted for sex, age and stature.



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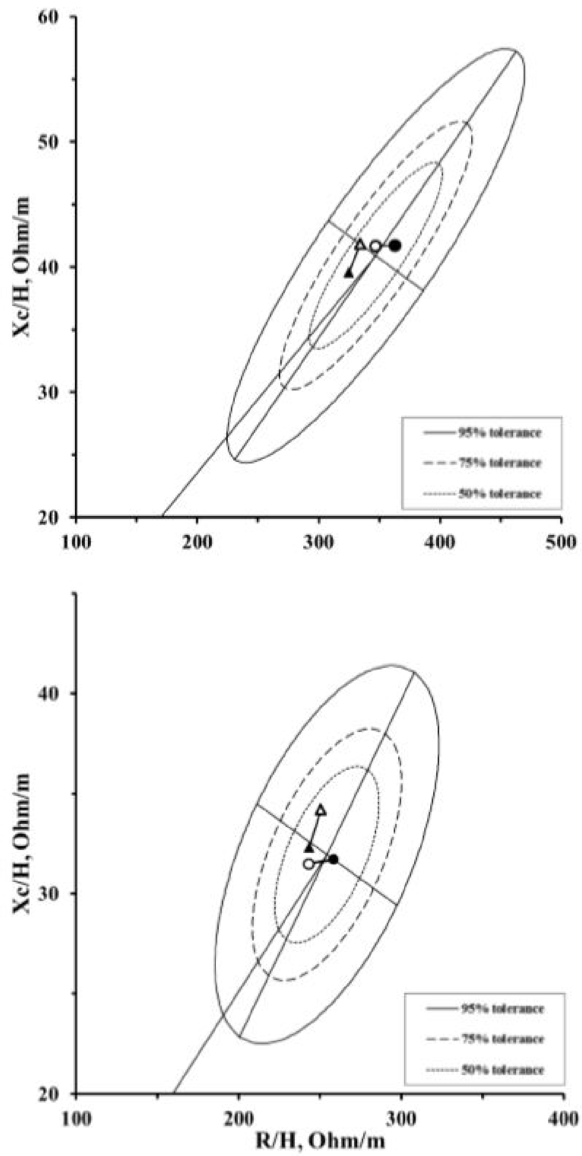

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