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Identifying Athlete Body Fluid Changes During a Competitive Season With Bioelectrical Impedance Vector Analysis

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# 1 Abstract

2

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

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

13 **Results:** Significant mean vector displacements in the Post groups were observed in both sexes.  
14 Specifically, reductions in vector length (Z/H) were associated with increases in TBW and ICW ( $r =$   
15  $-.718, P < .01$ ;  $r = -.630, P < .01$ , respectively) and decreases in ECW:ICW ratio ( $r = .344, P < .05$ ),  
16 even after adjusting for age, height, and sex. Phase-angle (PA) variations were positively associated  
17 with TBW and ICW ( $r = .458, P < .01$ ;  $r = .564, P < .01$ , respectively) and negatively associated with  
18 ECW:ICW ( $r = -.436, P < .01$ ). PA significantly increased in all the Post groups except in women in  
19 whom TBW decreased.

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

22

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

24

## 25 **Introduction**

26

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

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

50 used as a good tool for assessing the systemic efficiency exercise interventions and for looking at  
51 hydration status and cell functioning relevant for health and sports performance.<sup>15</sup>

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

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

## 62 **Methods**

### 63 **Participants**

64 This was a longitudinal investigation of 58 athletes engaged in five sports [basketball (men =  
65 20; women = 11), swimming (men = 5; women = 4), volleyball (men = 6; women = 4), handball (men  
66 = 6; women = 0) and triathlon (men = 2; women = 0)] (men: age 18.7±4.0 years; women: age 19.2±6.0  
67 years). The following inclusion criteria were considered: 1) 10 or more hours of training per week,  
68 2) negative test outcomes for performance-enhancing drugs and 3) not taking any medications. The  
69 results of a medical screening indicated that all subjects were in good health. All subjects (≥18 yrs)  
70 and their parents or guardians (if age < 18 yrs) were informed about the possible risks of the  
71 investigation before giving written informed consent to participate. All procedures were approved by  
72 the ethics committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were

73 conducted in accordance with the declaration of Helsinki for human studies of the World Medical  
74 Association.

## 75 **Procedures**

76 Subjects were evaluated at the beginning (PRE) and after 6 months (POST), during the  
77 competitive season. The subjects came to the laboratory after an overnight fast (12 h fast), refraining  
78 from vigorous exercise at least 15 h, no caffeine and alcohol during the preceding 24 h, and  
79 consuming a normal evening meal the night before. All athletes were tested to ensure a well-hydrated  
80 state using the urine specific gravity test (refractometer Urisys 1100, Roche Diagnostics, Portugal),  
81 from a fasting baseline urine sample, according to Armstrong et al.<sup>23</sup>; a urine-specific gravity value  
82 <1.022 in the first urine was used to categorize euhydration. Body weight was measured with a scale  
83 without shoes and wearing minimal clothes, to the nearest 0.01 kg and stature was measured to the  
84 nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). The intra-observer technical error of  
85 measurement (TEM) and the coefficient of variation (CV) were calculated in a subsample of ten  
86 subjects (height: TEM = 0.06 cm, CV = 0.04; weight: TEM = 0.04 kg, CV = 0.07). Body mass index  
87 (BMI) was calculated as the ratio of body mass to height squared ( $\text{kg/m}^2$ ).

## 88 **Total body water**

89 Following the collection of a baseline urine sample, each participant was given an oral dose  
90 of 0.1 g of 99.9%  $\text{H}_2\text{O}$  per kg of body weight (Sigma - Aldrich; St. Louis, MO) for the determination  
91 of total body water (TBW) by deuterium dilution using a Hydra stable isotope ratio mass spectrometer  
92 (PDZ, Europa Scientific, UK). Subjects were encouraged to void their bladder prior to the 4-h  
93 equilibration period and subsequent sample collection, due to inadequate mixing of pre-existing urine  
94 in the bladder (24). Urine samples were prepared for  $1\ \text{H}/^2\text{H}$  analyses using the equilibration  
95 technique by Prosser and Scrimgeour.<sup>24</sup> Our laboratory has reported a TEM and coefficient of

96 variation (CV) in ten subjects for TBW of 0.11 and 0.3%, respectively. <sup>25</sup> The athletes were  
97 empirically divided considering TBW change (PRE-POST; increase or decrease), according to sex.

## 98 **Extracellular water**

99 Extracellular water (ECW) was assessed from the sodium bromide (NaBr) dilution method  
100 after the subject consumed 0.030 g of 99.0% NaBr (Sigma - Aldrich; St. Louis, MO) per kg of body  
101 weight, diluted in 50 mL of distilled-deionized water. Baseline samples of saliva were collected before  
102 sodium bromide oral dose administration, and enriched samples were collected 3 h post-dose  
103 administration. Intracellular water (ICW) was calculated as the difference between TBW and ECW.  
104 The test-retest TEM and CV in 7 participants for the ECW using high performance liquid  
105 chromatography in our laboratory are 0.08 kg and 0.4%. <sup>25</sup>

## 106 **Dual-energy X-ray absorptiometry**

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

## 117 **Bioelectrical impedance analysis**

118 The impedance measurements were performed with BIA (BIA 101 Anniversary, Akern,  
119 Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made on an

120 isolated cot from electrical conductors, the subjects were in the supine position with a leg opening of  
121 45° compared to the median line of the body and the upper limbs, distant 30° from the trunk. After  
122 cleansing the skin with alcohol, two electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on  
123 the right hand back and two electrodes on the corresponding foot. <sup>6</sup> Bioimpedance vector analysis  
124 was carried out using the classic BIVA method, normalizing R and Xc parameters for stature (H) in  
125 meters.<sup>5</sup> The measurements shown by the BIA 101 Anniversary device are R and Xc with Z calculated  
126 and then the values are adjusted for height R/H, Xc/H and the vector length (Z/H). The Z/H value was  
127 calculated as the hypotenuses of individual impedance normalized values. Bioelectrical PA was  
128 calculated as the arc-tangent of  $Xc/R \times 180^\circ/\pi$ . Prior to each test the analyzer was calibrated with  
129 the calibration deemed successful if R value is 383  $\Omega$  and Xc equal to 46  $\Omega$ . The test-retest CV in 10  
130 participants in our laboratory for R and Xc is 0.3% and 0.9%, respectively.

## 131 **Statistical Analysis**

132 Descriptive statistics including means  $\pm$  SD were calculated for all outcome variables. Once  
133 the data were tested for normality (Shapiro-Wilks test), differences in body composition and  
134 bioelectrical variables between PRE and POST were analyzed by two-way analysis of covariance  
135 (ANCOVA) for repeated measures, considering athletes who increased and decreased body fluids as  
136 covariate. When F-ratio was significant, Bonferroni's post hoc test was used for the identification of  
137 specific differences in the variables. The paired, one-sample Hotelling's T2-test was performed to  
138 determine if the changes in the mean group vectors (measured at the first and second time points)  
139 were significantly different from zero (null vector). A 95% confidence ellipse excluding the null  
140 vector indicated a significant vector displacement. Single and multiple regression analyses were  
141 performed to understand the associations between changes in TBW, ICW and ECW/ICW ratio with  
142 vector length and PA. Model adjustments included age, stature and sex. Data were analyzed with IBM  
143 SPSS Statistics version 24.0 (IBM, Chicago, IL). For all tests, statistical significance was set at  $p <$   
144 0.05.

145 **Results**

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

149

150

\*\*\*INSERT TABLE 1 HERE\*\*\*

151

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

159

160

\*\*\*INSERT TABLE 2 HERE\*\*\*

161

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

165

166

\*\*\*INSERT FIGURE 1 AND 2 HERE\*\*\*

167

168           In Tables 3 and 4 results from single and multiple regression analysis are displayed. Vector length  
169 was negatively correlated with TBW and ICW and positively associated with the ECW/ICW ratio,



170 even when adjusted for sex, age and stature. Phase angle was positively associated with TBW and  
171 ICW and negatively associated with the ECW/ICW ratio, independently of sex, age, and stature.

172 \*\*\*INSERT TABLE 3 AND 4 HERE\*\*\*

173

## 174 **Discussion**

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

186 These results are consistent with the theoretical expectations considering the biophysical basis  
187 of bioimpedance, BIVA in particular, and the common use of BIVA for the classification of  
188 hydration.<sup>27</sup> Indeed, the resistive component of the classic impedance vector (R/H), highly correlated  
189 to Z/H, gives information on the physiological fluids and tissues containing water and electrolytes  
190 (which behave as resistors).<sup>27</sup> Hence, the vector length can be interpreted as inversely related to TBW.  
191 The other component of the impedance vector, the capacitive resistance, mainly responsible of PA  
192 values, can be considered proportional to cell membranes, which behave as capacitors in the human  
193 body.<sup>27</sup> Our results also support evidence provided in previous studies that highlighted that peripheral  
194 vectors lying on the left side of the minor axis of the tolerance ellipses, i.e. with higher PA, indicate

195 more soft tissue.<sup>5,7,8,10</sup> Actually, higher PA values reflect higher cellularity, cell membrane integrity  
196 and better cell function<sup>28</sup>, and are associated with improved power output in elite road cyclists.<sup>29</sup>

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

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

220 Despite the encouraging results obtained in this investigation, some limitations should be  
221 addressed. First, our results are applicable to the actual BIA equipment using the 50 kHz frequency.  
222 In fact, 50 kHz single frequency devices are among the most used equipment, yet similar studies  
223 should be conducted to test other frequencies resulting from multifrequency equipment as predictors  
224 of TBW and its compartments. Secondly, it is important to underscore that since athletes were tested  
225 at the beginning and at the main stage of the competitive period, but it is unknown if these two  
226 measurements represent what happened during the entire season. In addition, water and beverage  
227 intake during the study period was uncontrolled. Lastly, as only five sports were included in this  
228 investigation, generalizability of these findings to other sports is limited.

229

## 230 **Conclusion**

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

235

## 236 **Practical Applications**

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

239

## 240 **References**

241

- 242 1. Maughan RJ, Shirreffs SM. Dehydration and rehydration in competitive sport. *Scand J Med*  
243 *Sci Sports*. 2010;20 Suppl 3:40-47. doi:10.1111/j.1600-0838.2010.01207.x

- 244 2. Cutrufello PT, Dixon CB, Zavorsky GS. Hydration assessment among marathoners using urine  
245 specific gravity and bioelectrical impedance analysis. *Res Sports Med.* 2016;24(3):234-242.  
246 doi:10.1080/15438627.2016.1202831
- 247 3. Campa F, Gatterer H, Lukaski H, Toselli S. Stabilizing Bioimpedance-Vector-Analysis  
248 Measures With a 10-Minute Cold Shower After Running Exercise to Enable Assessment of  
249 Body Hydration. *Int J Sports Physiol Perform.* January 2019:1-13. doi:10.1123/ijsp.2018-  
250 0676
- 251 4. Armstrong LE. Assessing hydration status: the elusive gold standard. *J Am Coll Nutr.*  
252 2007;26(5 Suppl):575S-584S.
- 253 5. Piccoli A, Rossi B, Pillon L, Bucciante G. A new method for monitoring body fluid variation  
254 by bioimpedance analysis: the RXc graph. *Kidney Int.* 1994;46(2):534-539.
- 255 6. Lukaski HC, Piccoli A. Bioelectrical impedance vector analysis for assessment of hydration in  
256 physiological states and clinical conditions. In: Preedy V, ed. *Handbook of Anthropometry.*  
257 London: Springer; 2012:287-305.
- 258 7. Buffa R, Mereu E, Comandini O, Ibanez ME, Marini E. Bioelectrical impedance vector  
259 analysis (BIVA) for the assessment of two-compartment body composition. *Eur J Clin Nutr.*  
260 2014;68(11):1234-1240. doi:10.1038/ejcn.2014.170
- 261 8. Lukaski HC. Evolution of bioimpedance: a circuitous journey from estimation of physiological  
262 function to assessment of body composition and a return to clinical research. *Eur J Clin Nutr.*  
263 2013;67 Suppl 1:S2-9. doi:10.1038/ejcn.2012.149
- 264 9. Castizo-Olier J, Irurtia A, Jemni M, Carrasco-Marginet M, Fernandez-Garcia R, Rodriguez  
265 FA. Bioelectrical impedance vector analysis (BIVA) in sport and exercise: Systematic review  
266 and future perspectives. *PLoS One.* 2018;13(6):e0197957. doi:10.1371/journal.pone.0197957
- 267 10. Marini E, Sergi G, Succa V, et al. ( Biva ) for Assessing Body Composition in the Elderly. *J*  
268 *Nutr Health Aging.* 2013;17(6):515-521.
- 269 11. Heavens KR, Charkoudian N, O'Brien C, Kenefick RW, Cheuvront SN. Noninvasive

- 270 assessment of extracellular and intracellular dehydration in healthy humans using the  
271 resistance-reactance-score graph method. *Am J Clin Nutr.* 2016;103(3):724-729.  
272 doi:10.3945/ajcn.115.115352
- 273 12. Chertow GM, Lowrie EG, Wilmore DW, et al. Nutritional assessment with bioelectrical  
274 impedance analysis in maintenance hemodialysis patients. *J Am Soc Nephrol.* 1995;6(1):75-  
275 81.
- 276 13. Gonzalez MC, Barbosa-Silva TG, Bielemann RM, Gallagher D, Heymsfield SB. Phase angle  
277 and its determinants in healthy subjects: influence of body composition. *Am J Clin Nutr.*  
278 2016;103(3):712-716. doi:10.3945/ajcn.115.116772
- 279 14. Marini E, Campa F, Buffa R, et al. Phase angle and bioelectrical impedance vector analysis in  
280 the evaluation of body composition in athletes. *Clin Nutr.* February 2019.  
281 doi:10.1016/j.clnu.2019.02.016
- 282 15. Sardinha LB. Physiology of exercise and phase angle: another look at BIA. *Eur J Clin Nutr.*  
283 2018;72(9):1323-1327. doi:10.1038/s41430-018-0215-x
- 284 16. Koury JC, Trugo N MF, Torres AG. Phase angle and bioelectrical impedance vectors in  
285 adolescent and adult male athletes. *Int J Sports Physiol Perform.* 2014;9(5):798-804.  
286 doi:10.1123/ijsp.2013-0397
- 287 17. Carrasco-Marginet M, Castizo-Olier J, Rodríguez-Zamora L, et al. Bioelectrical impedance  
288 vector analysis (BIVA) for measuring the hydration status in young elite synchronized  
289 swimmers. Barbosa TM, ed. *PLoS One.* 2017;12(6). doi:10.1371/journal.pone.0178819
- 290 18. Campa F, Toselli S. Bioimpedance Vector Analysis of Elite, Sub-Elite and Low-Level Male  
291 Volleyball Players. *Int J Sports Physiol Perform.* March 2018:1-13. doi:10.1123/ijsp.2018-  
292 0039
- 293 19. Gatterer H, Schenk K, Laninschegg L, Schlemmer P, Lukaski H, Burtscher M. Bioimpedance  
294 identifies body fluid loss after exercise in the heat: a pilot study with body cooling. *PLoS One.*  
295 2014;9(10):e109729. doi:10.1371/journal.pone.0109729

- 296 20. Koury JC, Ribeiro MA, Massarani FA, Vieira F, Marini E. Fat-free mass in adolescent athletes:  
297 Accuracy of bioimpedance equations and identification of new predictive equations. *Nutrition*.  
298 2019;60:59-65. doi:<https://doi.org/10.1016/j.nut.2018.09.029>
- 299 21. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspán J, Klein PD. Total body water  
300 measurement in humans with <sup>18</sup>O and <sup>2</sup>H labeled water. *Am J Clin Nutr*. 1980;33(12):2686-  
301 2693. doi:10.1093/ajcn/33.12.2686
- 302 22. Shoeller D. Hydrometry. In: *Human Body Composition*. Champaign: Human Kinetics Books;  
303 2005:35-49.
- 304 23. Armstrong LE, Pumerantz AC, Fiala KA, et al. Human hydration indices: acute and  
305 longitudinal reference values. *Int J Sport Nutr Exerc Metab*. 2010;20(2):145-153
- 306 24. Prosser SJ, Scrimgeour CM. High-Precision Determination of <sup>2</sup>H/<sup>1</sup>H in H<sub>2</sub> and H<sub>2</sub>O by  
307 Continuous-Flow Isotope Ratio Mass Spectrometry. *Anal Chem*. 1995;67(13):1992-1997.  
308 doi:10.1021/ac00109a014
- 309 25. Matias CN, Silva AM, Santos DA, Gobbo LA, Schoeller DA, Sardinha LB. Validity of  
310 extracellular water assessment with saliva samples using plasma as the reference biological  
311 fluid. *Biomed Chromatogr*. 2012;26(11):1348-1352. doi:10.1002/bmc.2702
- 312 26. Matias CN, Santos DA, Gonçalves EM, Fields DA, Sardinha LB, Silva AM. Is bioelectrical  
313 impedance spectroscopy accurate in estimating total body water and its compartments in elite  
314 athletes? *Ann Hum Biol*. 2013;40(2):152-156. doi:10.3109/03014460.2012.750684
- 315 27. Lukaski HC, Vega Diaz N, Talluri A, Nescolarde L. Classification of Hydration in Clinical  
316 Conditions: Indirect and Direct Approaches Using Bioimpedance. *Nutrients*. 2019;11(4): pii:  
317 E809. doi: 10.3390/nu11040809
- 318 28. Norman K, Stobaus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance  
319 vector analysis--clinical relevance and applicability of impedance parameters. *Clin Nutr*.  
320 2012;31(6):854-861. doi:10.1016/j.clnu.2012.05.008
- 321 29. Pollastri L, Lanfranconi F, Tredici G, Burtscher M, Gatterer H. Body Water Status and Short-

- 322 term Maximal Power Output during a Multistage Road Bicycle Race (Giro d'Italia 2014). *Int*  
323 *J Sports Med.* 2016;37(4):329-333. doi:10.1055/s-0035-1565105
- 324 30. Mascherini G, Gatterer H, Lukaski H, Burtscher M, Galanti G. Changes in hydration, body-  
325 cell mass and endurance performance of professional soccer players through a competitive  
326 season. *J Sports Med Phys Fitness.* 2015;55(7-8):749-755.
- 327 31. Campa F, Silva AM, Toselli S. Changes in Phase Angle and Handgrip Strength Induced by  
328 Suspension training in Older Women. *Int J Sports Med.* April 2018. doi:10.1055/a-0574-3166
- 329 32. Micheli ML, Pagani L, Marella M, et al. Bioimpedance and impedance vector patterns as  
330 predictors of league level in male soccer players. *Int J Sports Physiol Perform.* 2014;9(3):532-  
331 539. doi:10.1123/ijsp.2013-0119

332

### 333 **Figure captions**

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

338

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

**Table 1** Participants' Characteristics

<b>Variable</b>	<b>Men (n = 39)</b>	<b>Women (n = 19)</b>
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 <sup>2</sup>	22.36 (2.20)	21.39 (2.26)

Abbreviation: BMI, body mass index. Note: Values are presented as mean (SD).



**Table 2 Two-Way ANCOVA for the Comparison at Baseline (PRE) and During the Competitive Season (POST) 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 × time interaction P value
	Men (n = 28)		Women (n = 11)		Men (n = 11)		Women (n = 8)		PRE	POST	PRE	POST			
	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)*	<.001	<.001	.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)	<.001	<.001	.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)*	<.001	<.001	.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)	.003	.003	.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)	.034	.034	.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)	<.001	<.001	.712				
R, Ω	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)	<.001	<.001	.687				
Xc, Ω	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)*	<.001	<.001	.869				
R/H, Ω/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)	<.001	<.001	.878				
Xc/H, Ω/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)*	<.001	<.001	.879				
PA, deg	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)	.005	.005	.345				
Z/H, Ω/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)	<.001	<.001	.884				

Abbreviations: ANCOVA, analysis of covariance; ECW, extracellular water; FFM, fat-free mass; FM, fat mass; ICW, intracellular water; PA, phase angle; R, resistance; R/H, resistance adjusted for stature; TBW, total body water; Xc, reactance; Xc/H, reactance adjusted for stature; Z/H, vector length adjusted for stature. Note: Values are presented as mean (SD).

\*P < .05 versus PRE.

**Table 3 Regression Analyses for Body Fluids With Vector Length**

	Model	Model <sup>a</sup>
	$\beta$ (95% CI)	$\beta$ (95% CI)
$\Delta$ TBW		
$\Delta$ ZL	-0.718 (-0.142 to -0.080)**	-0.672 (-0.137 to -0.071)**
$\Delta$ ICW		
$\Delta$ ZL	-0.630 (-0.134 to -0.064)**	-0.531 (-0.119 to -0.047)**
$\Delta$ ECW/ICW		
$\Delta$ ZL	0.344 (0.000 to 0.004)*	0.217 (0.000 to 0.003)

Abbreviations:  $\Delta$ , changes;  $\beta$ , standardized beta coefficient; CI, confidence interval; ECW, extracellular water; ICW, intracellular water; TBW, total body water; ZL, vector length.

<sup>a</sup>Adjusted for sex, age, and stature. \*Significant at  $P < .05$ . \*\*Significant at  $P < .01$ .

**Table 4 Regression Analyses for Body Fluids With Phase Angle**

	Model	Model <sup>a</sup>
	$\beta$ (95% CI)	$\beta$ (95% CI)
$\Delta$ TBW		
$\Delta$ PA	0.458 (1.228 to 4.324)**	0.396 (0.780 to 4.024)*
$\Delta$ ICW		
$\Delta$ PA	0.564 (2.013 to 4.929)**	0.455 (1.307 to 4.293)**
$\Delta$ ECW/ICW		
$\Delta$ PA	-0.436 (-0.166 to -0.042)**	-0.433 (-0.171 to -0.007)*

Abbreviations:  $\Delta$ , changes;  $\beta$ , standardized beta coefficient; CI, confidence interval; ECW, extracellular water; ICW, intracellular water; PA, phase angle; TBW, total body water.

<sup>a</sup>Adjusted for sex, age, and stature. \*Significant at  $P < .05$ . \*\*Significant at  $P < .01$ .

Figure 1

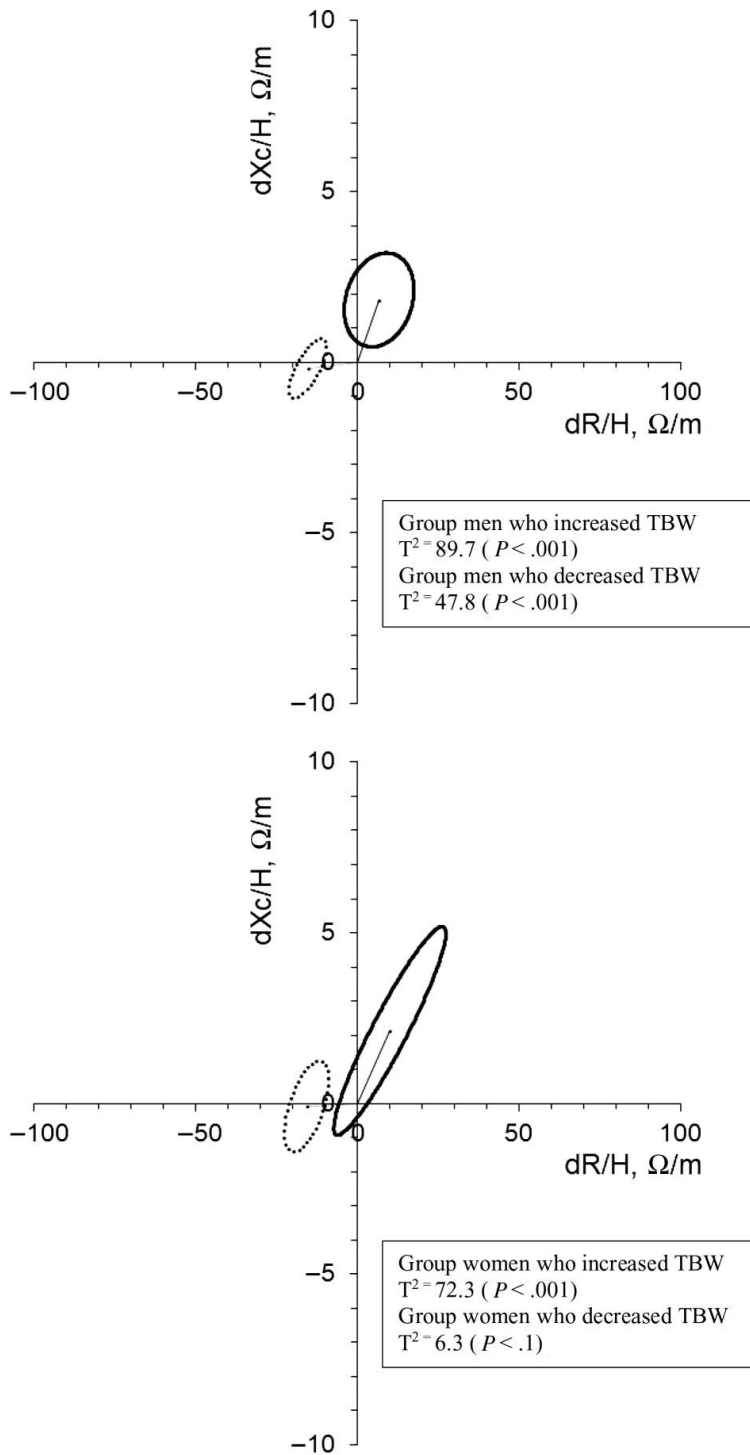


Figure 2

