UNIVERSITAT DE BARCELONA

Bioelectrical impedance vector analysis (BIVA) in exercise and sports practice

Jorge Castizo Olier

Jorge Castizo Olier

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA) IN EXERCISE AND SPORTS PRACTICE

INEFC
Barcelona
Generalitat de Catalunya
Universitat de Barcelona

UNIVERSIDAD DE BARCELONA

Facultad de Educación

INSTITUTO NACIONAL DE EDUCACIÓN FÍSICA DE CATALUÑA

Centro de Barcelona

PROGRAMA DE DOCTORADO

Activitat Física, Educació Física i Esport

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA) IN EXERCISE AND SPORTS PRACTICE

Tesis doctoral presentada por:

JORGE CASTIZO OLIER

Directores / Supervisors

DR. ALFREDO IRURTIA AMIGÓ

DR. MONÈM JEMNI

Tutor / Tutor

DR. FERRAN A. RODRÍGUEZ GUISADO

Tesis para la obtención del grado de Doctor por la Universitat de Barcelona

Dissertation on account for the degree of Doctor by the Universitat de Barcelona

BARCELONA, 2018

The PhD candidate is supported by a research grant from Generalitat de Catalunya, Agència de Gestió d'Ajuts Universitaris i de Recerca – AGAUR -, co-funded by the European Social Fund – ESF - (2015 FI_B 00217).

This research is funded by research grants from Generalitat de Catalunya, Institut Català de les Dones (U-34/10), Consejo Superior de Deportes (001/UPB10/11), and Grup de Recerca en Ciències de l'Esport de l'INEFC Barcelona (2017 SGR 1703). Furthermore, the work was conducted with the support of Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB).

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA) IN EXERCISE AND SPORTS PRACTICE

Jorge Castizo Olier

Institut Nacional d'Educació Física de Catalunya (INEFC) — Universitat de Barcelona (UB)
j.castizo9@gmail.com

Somos…

…el conjunto de personas que nos moldearon,

…la totalidad de experiencias que vivimos,

…la huella que dejamos el instante que existimos.

- Jorge Castizo Olier, 2018 -

ACKNOWLEDGEMENTS

Esta tesis doctoral no podría haber sido finalizada sin el soporte de muchas personas que, de forma directa o indirecta, dedicaron parte de su valioso tiempo a ayudarme. Por ello, me gustaría agradecer a las siguientes personas su apoyo:

A mi director de tesis, Alfredo Irurtia, por su paciencia y dedicación. Por su comprensión en momentos difíciles y por todas esas horas sin dormir para ayudarme. Pero, sobre todo, le doy las gracias por lo más valioso que me llevo de estos años a su lado: su amistad. Freddy, tengo en ti, en tu familia y en tu filosofía de vida el modelo al que aspiro llegar. Gracias por todo, amigo.

A mi co-director de tesis, Monèm Jemni, por abrirme la puerta a realizar una estancia de investigación internacional en la Universidad de Greenwich. Gracias a esta oportunidad crecí como investigador y, sobre todo, como persona.

A mi tutor de tesis, Ferran A. Rodríguez, por su amistad y apoyo a lo largo de todos estos años. Por compartir con nosotros todos esos enriquecedores momentos, repletos de experiencia y sabiduría. Por darle el punto de excelencia a todo aquello en lo que nos ha ayudado.

A Marta Carrasco, por introducirme en un proyecto que se inició con ella y que ha sido mi primera publicación internacional. Además, por confiar en mis posibilidades y abrirme la puerta a continuar desarrollándome como docente universitario. Pero sobre todo, por cuidar de Freddy y entenderlo aquellas veces en las que te pedía unos eternos "…cinco minutos más" para ayudarme.

A Xavier Iglesias, por ser la persona que me introdujo el gusanillo de la investigación, en aquellas prácticas de la licenciatura, allá por comienzos del 2011. También por enseñarme que se puede ser una gran persona, humilde, comprometida y con una sonrisa siempre en la boca, a la vez que se trabaja más horas que un reloj.

A Raúl Fernández, porque sin su ayuda no hubiese podido conseguir la beca de investigación que me permitiría dedicarme por completo a la presente tesis doctoral.

A Fernando Naclerio y al 'Department of Life & Sport Science', de la Universidad de Greenwich (Campus Medway), por abrirme las puertas de sus laboratorios y permitirme investigar en sus magníficas instalaciones.

A mis compañeras/os de doctorado, investigación y docencia, no solo por los conocimientos compartidos, sino también por las experiencias vividas. Ha sido un placer compartir estos años con vosotras/os.

A todo el equipo del Institut Nacional d'Educació Física de Catalunya (Barcelona), mi segunda casa, por toda la ayuda y el apoyo prestado durante mis años aquí.

A todos los participantes y colaboradores de los estudios presentes en la tesis, sin los que la misma no habría sido posible.

A mis amigas y amigos. A todas y todos vosotros, que me habéis acompañado y me acompañáis en el camino. Por estar ahí, incluso en la distancia. Por demostrarme que la familia no sólo tiene que ver con la sangre.

A Raúl Cabello. Porque gracias a ti descubrí aquellas prácticas de la licenciatura en INEFC-Barcelona, sin las que seguramente hoy no estaría aquí. Por contagiar allá por donde vayas ese inconformismo y ganas de progresar en la vida. Porque hemos vivido grandes momentos juntos y por los que aún quedan por llegar. Porque pase el tiempo que pase y nos separe la distancia que nos separe, estás ahí. Gracias, "cabesa".

A mis abuelas, abuelos, tías, tíos, primas y primos. Porque he crecido con vuestro cariño. Porque habéis estado con nosotros en los buenos momentos y, sobre todo, nos habéis apoyado en los tiempos más complicados. Porque os hace tanta ilusión

como a mí que finalice esta tesis doctoral. En definitiva, porque tengo la grandísima suerte de poder llamaros “Familia”.

A Pablo. Contigo las palabras sobran, ya que, seguramente, me conozcas mejor de lo que yo mismo me conozco. Simplemente, gracias por ser como eres. Por haber estado cerca de mamá y papá, cuidándolos cuando más lo necesitaban, mientras yo estoy lejos. Eres un ejemplo como persona y te admiro de corazón. Soy muy afortunado de haber crecido a tu lado. Mi equilibrio. Mi mejor amigo. Mi hermano.

A mi padre, por ser, junto con mi madre, mi modelo a seguir. La persona más leal, humilde, noble y paciente que he conocido nunca. Gracias, papá, por enseñarme tanto. Por dar todo de ti, sin pedir nada a cambio. Por inculcarme, junto con mamá, tu amor por el deporte. Por dar, desinteresadamente, tanto tiempo de vuestras vidas, llevándonos y trayéndonos de entrenamientos, partidos y campeonatos, sufriendo y disfrutando con nosotros. Por regalarnos tu amor incondicional. Gracias por ser mi padre.

A mi madre, una de las tres personas más importantes de mi vida, junto con mi padre y mi hermano. Por ser la leona de la casa, el pilar fundamental de nuestra familia. Por enseñarnos la máxima expresión del coraje y la valentía. Por hacernos fuertes y prepararnos para superar cualquier adversidad que pueda llegar. Por educarme, junto con papá, en los valores que hoy me guían. Por mostrarme que una persona puede abarcar al mismo tiempo el ser madre, trabajar, terminar una carrera y acabar un máster. Por inculcarme la sed del conocimiento. Esta tesis va por aquella que te hubiese encantado hacer. Gracias por ser mi madre.

A todas y todos vosotros, gracias de corazón. Sois ya, inevitablemente y para siempre, parte de mí.

ABSTRACT

Bioelectrical impedance analysis (BIA) is a non-invasive technique widely used in body composition assessment. Nevertheless, its accuracy is compromised because of its reliance on regression equations and assumptions that are not frequently met. The bioelectrical impedance vector analysis (BIVA or "classic BIVA") emerged as an alternative technique to overcome conventional BIA limitations, founding its main strength on the use of raw impedance variables. BIVA is nowadays a widely used technique in medicine for the assessment of hydration and nutritional status in different clinical conditions. Although there has been a rapid growth of interest regarding the application of BIVA in sport and exercise research and practice in the recent years, the current scientific literature is still scarce and very heterogeneous. For this reason, we first systematically reviewed the current knowledge on the bases, applications, usefulness and suitability of BIVA in sport and exercise (Study I). Furthermore, we outlined future perspectives in this field and suggested a research agenda. In Studies II and III, we aimed at providing the first description, in bioelectrical terms, of a group of young elite female synchronised swimmers and a group of experienced, well-trained, non-professional, ultra-endurance male triathletes, comparing them with a reference non-athletic population. Additionally, we assessed the variation in the whole-body bioimpedance vector and body mass after a training session in synchronised swimmers and after a competition in triathletes. We concluded that the bioimpedance vector analysis is a technique that has a great potential in sport and exercise, yet largely unexplored, especially for the identification of soft-tissue injury and its follow-up. However, "classic" BIVA is inconsistent in the assessment of two-compartment body composition and the vector position of athletes in relation to the reference population seems controversial in many cases. "Specific" BIVA, a method which proposes a

correction of bioelectrical values for body geometry, seems to overcome this limitation. In any case, specific bioelectrical distributions were found in synchronised swimmers and triathletes in comparison with their healthy, general reference population. In relation with this, Study II reports for the first time specific tolerance ellipses in a female sport group. Furthermore, BIVA showed bioelectrical differences between synchronised swimmers of different age and performance level. Accordingly, Study III also reported bioelectrical differences between triathletes of different performance level. Regarding the assessment of hydration status through "classic" BIVA, this is not a valid method to identify dehydration in individual athletes. Nevertheless, vector changes are consistent with fluid loss induced by high intensity synchronised swimming training and by an ultra-endurance triathlon competition, regardless of age and performance level. Furthermore, vector changes seem consistent with fluid recovery 48h after the triathlon event. However, more research is needed regarding the relationship between the bioelectrical signal and physiological adaptations induced by different types of exercise, especially in how the structure and function of the cell are altered and how these affect the behaviour of resistance, and in particular reactance.

RESUMEN

El análisis de impedancia bioeléctrica (BIA) es una técnica no invasiva ampliamente utilizada en la evaluación de la composición corporal. Sin embargo, su precisión se ve comprometida debido a la dependencia de ecuaciones de regresión y suposiciones que no se cumplen con frecuencia. El análisis del vector de impedancia bioeléctrica (BIVA o BIVA “clásico") surgió como una técnica alternativa para superar las limitaciones del BIA convencional, basando su principal fortaleza en el uso de parámetros primarios de impedancia. Hoy en día, BIVA es una técnica ampliamente utilizada en medicina como herramienta para la evaluación de la hidratación y el estado nutricional en diferentes condiciones clínicas. En cuanto a la aplicación de BIVA en la investigación y práctica de ejercicio y deporte, el interés ha crecido rápidamente en los últimos años, aunque la literatura científica actual es todavía escasa y muy heterogénea. Por esta razón, en la presente tesis realizamos primero una revisión sistemática sobre el conocimiento actual en relación a las bases, aplicaciones, utilidad e idoneidad de BIVA en el deporte y el ejercicio (Estudio I). Además, trazamos las perspectivas futuras en este campo y sugerimos una agenda de investigación. En los Estudios II y III, nuestro objetivo fue proporcionar la primera descripción, en términos bioeléctricos, de un grupo de jóvenes deportistas de élite de natación sincronizada y un grupo masculino no profesional de triatletas de ultra-resistencia, experimentados y bien entrenados, comparándolos con su población sana de referencia. Además, evaluamos la variación en el vector de bioimpedancia de cuerpo completo y la masa corporal después de un entrenamiento en las nadadoras y después de competición en los triatletas. Tras analizar los resultados obtenidos, concluimos que el análisis del vector de bioimpedancia es una técnica que tiene un gran potencial (aún apenas explorado) en el deporte y el ejercicio, especialmente para la identificación de lesiones de tejidos blandos y su seguimiento a lo

largo de la recuperación. Sin embargo, el BIVA "clásico" no es consistente en la evaluación bicompartimental de la composición corporal y la posición del vector de los atletas en relación a su población de referencia parece conflictiva en muchos casos. El BIVA "específico", un método que propone una corrección de los valores bioeléctricos en relación a la geometría del cuerpo, parece superar esta limitación. En cualquier caso, se encontraron distribuciones bioeléctricas específicas en nadadoras de natación sincronizada y en triatletas en comparación con su población sana de referencia. En relación a esto, el Estudio II genera por primera vez elipses de tolerancia específica en un grupo femenino de deportistas. Además, BIVA mostró diferencias bioeléctricas entre las nadadoras de diferentes edades y niveles de rendimiento. Asimismo, el Estudio III también informó sobre diferencias bioeléctricas entre los triatletas de diferentes nivel deportivo. Con respecto a la evaluación del estado de hidratación a través del BIVA “clásico”, este no es un método válido para identificar la deshidratación en atletas. Sin embargo, los cambios en el vector son consistentes con la pérdida de fluidos inducidos por un entrenamiento de natación sincronizada de alta intensidad y por una competición de triatlón de ultra-resistencia, independientemente de la edad y el nivel de rendimiento deportivo. Además, la migración del vector parece consistente con la recuperación de líquidos 48 horas después de la carrera de triatlón. Sin embargo, se necesita investigar más acerca de la relación entre la señal bioeléctrica y las adaptaciones fisiológicas inducidas por diferentes tipos de ejercicio, especialmente en cómo son alteradas la estructura y la función celular, y cómo éstas afectan al comportamiento de la resistencia y, en particular, al de la reactancia.

GLOSSARY

BCM	Body cell mass
BIA	Bioelectrical impedance analysis
BIS	Bioelectrical impedance spectroscopy
BIVA	Bioelectrical impedance vector analysis
BM	Body mass
BMI	Body mass index
C_{LC}	Circumference of the left calf
C_{LT}	Circumference of the left thigh
C_m	Cell membrane capacitance
C_o	Pre-junior synchronised swimmers
C_{RC}	Circumference of the right calf
C_{RT}	Circumference of the right thigh
DXA	Dual-energy X-ray absorptiometry
ECM	Extracellular mass
ECW	Extracellular water
ECW:TBW ratio	Extracellular / total body water ratio
Fc	Characteristic frequency
FFM	Fat-free mass
FM	Fat mass
H	Body height
Hotelling's T^2 test	Test comparing mean two group vectors
ICW	Intracellular water
J_r	Junior synchronised swimmers
Mahalanobis' D	Multidimensional distance between a point P and the mean of a group
MF-BIA	Multi-frequency bioelectrical impedance analysis
P_{osm}	Plasma osmolality
PA	Phase angle
R	Bioelectrical resistance (R/h when adjusted by height)
RPE	Rating of perceived exertion
RXc graph	R/h vs. Xc/h probabilistic plot
SD	Standard deviation

SF-BIA	Single-frequency bioelectrical impedance analysis
SS	Synchronised swimmers
TBW	Total body water
TRIMP	Training impulse
UET	Ultra-endurance triathlon event
Xc	Bioelectrical reactance (Xc/h when adjusted by height)
Z	Bioelectrical impedance
Z vector	Vector yield by the RXc graph

TABLE OF CONTENTS

ACKNOWLEDGEMENTS v

ABSTRACT ix

RESUMEN xi

GLOSSARY xiii

TABLE OF CONTENTS xvii

LIST OF PUBLICATIONS xxi

INTRODUCTION 1

Bioelectrical Impedance Analysis (BIA) 3

Bioelectrical Impedance Vector Analysis (BIVA) 5

Bioelectrical data acquisition 5

Data processing and analysis 10

RXc graph 11

RXc point graph 12

RXc score graph 13

RXc mean graph 14

RXc paired graph 15

Phase angle (PA) 16

Overview on the statistical analysis following BIVA assessment 17

Hotelling's T^2 test and RXc graph 17

Unpaired data analysis 17

Paired data analysis 17

Mahalanobis' generalised distance 18

JUSTIFICATION AND AIMS 19

METHODS 25
Systematic review (Study I) 27
Eligibility criteria 27
Information sources 27
Search strategy 28
Study records 28
Data items and prioritisation 29
Quasi-experimental studies (Studies II and III) 31
Participants 31
Procedures 31
Parameters and equipment 33
Anthropometric assessment 33
Temperature assessment 33
Whole-body bioimpedance assessment 34
Internal training/race load assessment 35
Performance 35
Energy deficit 35
Statistical analysis 36
Ethical considerations 37
RESULTS AND DISCUSSION 39
Systematic review (Study I) 41
Long-term vector changes 41
Injury identification and follow-up 45
Quasi-experimental studies (Studies II and III) 46
BIA vector characterisation of athletes 46

BIA vector changes evoked by training/competition 55
Short-term vector changes 55
Medium-term vector changes 64
CONCLUSIONS 69
CONCLUSIONES 73
LIMITATIONS 75
Systematic review (Study I) 77
Quasi-experimental studies (Studies II and III) 77
FUTURE PERSPECTIVES 81
REFERENCES 89
STUDIES 109
Study I 111
Study II 139
Study III 159

LIST OF PUBLICATIONS

This thesis is mainly based on the following publications, herein referred to by their Roman numerals:

I. Study I: **Castizo-Olier J**, Irurtia A, Jemni M, Carrasco-Marginet M, Fernández-García R, Rodríguez FA. Bioelectrical impedance vector analysis (BIVA) in sport and exercise: systematic review and future perspectives. PLoS One. 2018;13(6):e0197957. doi: 10.1371/journal.pone.0197957

II. Study II: Carrasco-Marginet M, **Castizo-Olier J**, Rodríguez-Zamora L, Iglesias X, Rodríguez FA, Chaverri D, Brotons D, Irurtia A. Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. PLoS One. 2017;12(6):e0178819. doi: 10.1371/journal.pone.0178819

III. Study III: **Castizo-Olier J**, Carrasco-Marginet M, Roy A, Chaverri D, Iglesias X, Pérez-Chirinos C, Rodríguez FA, Irurtia A. Bioelectrical impedance vector analysis (BIVA) and body mass changes in an ultra-endurance triathlon event. J Sports Sci Med. 2018;17:571-579.

The theoretical framework and contextualisation of the present doctoral thesis is based on the Study I, the systematic review about the bioelectrical impedance vector analysis in sport and exercise.

On the other hand, the quasi-experimental part of the doctoral thesis is represented by the Studies II and III, involving sports of different characteristics (such as synchronised swimming and ultra-endurance triathlon) and different ecological contexts (training and competition). Furthermore, Study II is included in the systematic review (Study I).

In addition, this thesis is supported by the following conferences presentations, herein referred to by their Roman numerals:

I. **Castizo-Olier J,** Sánchez A, Roy A, Vives M, Paloma V, Irurtia A. Whole-body bioelectrical impedance vector migration induced by a high intensity football training session: a field study report. 2nd International Symposium on Advances in Sport Science. Universidad Pablo de Olavide. 2013 May 3-4; Seville, Spain.

II. **Castizo-Olier J**, Roy A, Mediavilla A, Vives M, Paloma V, Cos F, Irurtia A. Bioimpedancia localizada en la práctica deportiva: análisis preliminar en futbolistas amateurs. 2nd Catalan Football Congress. Federació Catalana de Futbol, Institut Nacional d'Educació Física de Catalunya, el R.C.D. Espanyol and F.C. Barcelona. 2013 Jun 12-15; Barcelona, Spain.

III. **Castizo-Olier J**, Roy A, Vives M, Paloma V, Irurtia A. Changes in the whole-body bioelectrical impedance vector induced by training in amateur football players: preliminary results. 18th annual Congress of the European College of Sport Science; 2013 Jun 26-29; Barcelona, Spain. ISBN 978-84-695-7786-8.

IV. Molina S, Balcells J, Solà-Perez A, **Castizo-Olier J**, Vives M, Irurtia A. Whole-body and localized bioelectrical impedance vector analysis (BIVA) in professional soccer players. I Jornada Catalana de Recerca en Ciències de l'Activitat Física i l'Esport. Institut Nacional d'Educació Física de Catalunya. 2016 Jun 15; Barcelona, España.

V. **Castizo-Olier J**, Roca E, Brotons D, Subirats E, Brugada R, Porta J, Carrasco M, Mateu M, Solà-Perez A, Irurtia A. Whole-body bioelectrical impedance vector analysis (BIVA) in male trail runners: preliminary results. 21st annual Congress of the European College of Sport Science; 2016 Jul 6-9; Vienna, Austria. ISBN 978-3-00-053383-9.

VI. Sarola J, Bofill-Ródenas A, **Castizo-Olier J**, Cartes MA, Solà-Perez T, Carrasco-Marginet M, Porta M, Irurtia A. Anthropometric full profile and bioimpedance vector analysis (BIVA) in down syndrome: a preview. 15th International Society for the Advancement of Kinanthropometry Conference, held in conjunction with the World Conference in Kinanthropometry and Body Composition. ISAK-UADY 2016. 2016 Nov 31 Oct-2 Nov; Mérida, México. ISBN 978-607-9405-91-5.

VII. Solà-Perez T, **Castizo-Olier J**, Molina S, Balcells J, Sarola J, Carrasco-Marginet M, Porta M, Pérez-Chirinos C, Vives M, Irurtia A. Whole-body and localized bioelectrical impedance vector analysis (BIVA) in professional soccer players. 15th International Society for the Advancement of Kinanthropometry Conference, held in conjunction with the World Conference in Kinanthropometry and Body Composition. ISAK-UADY 2016. 2016 Nov 31 Oct-2 Nov; Mérida, México. ISBN 978-607-9405-91-5.

VIII. Roy A, **Castizo-Olier J**, Carrasco-Marginet M, Rodríguez FA, Porta J, Irurtia A. Análisis del vector de bioimpedancia en corredoras de montaña de diferentes niveles competitivos: resultados preliminares. Arch Med Dep. 2017;34(6):362-368.

IX. Irurtia A, Pérez-Chirinos C, Balius R, **Castizo-Olier J**, Sagasti N, Fernández-García R. Does ultrasound shear-wave elastography affect localized bioimpedance? A case study. 16th International Society for the Advancement of Kinanthropometry Conference. ISAK-UNAB 2018. 2018 29 Jun–1 Jul; Santiago de Chile, Chile.

X. Irurtia A, Roy A, **Castizo-Olier J**, Carrasco-Marginet M, Pérez-Chirinos C, Rodríguez FA, Porta J. (2018). Whole-body bioimpedance vector analysis in

endurance mountain male athletes with different competitive levels. 16th International Society for the Advancement of Kinanthropometry Conference. ISAK-UNAB 2018. 2018 29 Jun–1 Jul; Santiago de Chile, Chile.

INTRODUCTION

INTRODUCTION

Bioelectrical Impedance Analysis (BIA)

Bioelectrical impedance analysis (BIA) is a non-invasive technique widely used in body composition assessment (1-5), nutritional status (5-7), and hydration status (2, 8, 9), all considered areas of interest to monitor general health and well-being (10), but also training and performance levels. However, conventional BIA is limited by the use of models and algorithms that assume relations between body components are constant and correlated with each other during stable periods, which are used to estimate through simple or multiple regression equations an unknown body component from a related measured variable (bioimpedance) (11). Multiple validation studies demonstrated strong relationship between bodily impedance and fluid volume (e.g. compared to isotope dilution), but their prediction's validity and accuracy of prediction are population-specific (12). Furthermore, the standard errors of the best BIA regression equations were estimated to be ~3–8% for total body water (TBW) and ~3–6% for fat-free mass (FFM), both considered too large to be used in the clinical setting (12, 13). In the exercise and sport practice, this is especially relevant. For example, dehydration rates lower than these standard errors which may affect negatively the sport performance could be not adequately detected (14).

BIA measures body tissues' opposition to the flow of a low-level, alternating radiofrequency electric current. Bioelectrical impedance (Z)—i.e. the tissues opposition to the electric current flow—, the vector sum of the resistance (R)—i.e. the major resistance to the current through intra- and extracellular ionic fluids—and the reactance (Xc)—i.e. the additional opposition due to the capacitive elements such as cell membranes, tissue interfaces, and non-ionic substances. BIA has been performed using single- (SF-BIA) or multiple-frequency (MF-BIA) electrical current. Standard SF-BIA

uses a single frequency of 50 kHz to estimate TBW and FFM, but does not differentiate intracellular water (ICW), because at this frequency the current does not penetrate cells (15). In an attempt to overcome this, MF-BIA tries to estimate ICW and extracellular water (ECW) by measuring a spectrum of frequencies through different mathematical models (12). However, MF-BIA models have significant limitations, such as the required use of body mass (BM) as an independent variable. Most scientific evidence show that the use of both SF-BIA and MF-BIA lead to prediction errors in healthy people (5, 16-18) and even larger errors in people with clinical conditions (19, 20). In spite of the widespread use of BIA in the clinical and field settings, mainly in the estimation of body composition, such as fat mass (FM) and FFM, or TBW, ICW and ECW, its accuracy is compromised because of its reliance on regression equations, mostly derived from non-athletic or sport-specific populations (5), and assumptions such as constant tissue isotropy or constant tissue hydration, conditions that are not frequently met (5, 11). Alternative techniques such as the measure of the phase angle (PA) or the bioelectrical impedance vector analysis ("classic" BIVA or simply "BIVA") (21) emerged to overcome the above-mentioned BIA limitations, founding their main strength on the use of raw impedance variables. It has to be clarified that BIVA does not provide quantitative estimates of tissue mass (kg) or fluid volumes (L). Instead, it is qualitative and semi-quantitative evaluation of body cell mass (BCM) and hydration (22, 23).

The number of publications using BIVA in clinical practice increased exponentially during the last decade due to its strengths (11, 19, 24-30). Nowadays, BIVA is a widely used technique in medicine as a valid tool in the assessment of hydration and nutritional status (e.g. fluid imbalance and wasting of lean tissues, respectively) in different clinical conditions, such as renal disease (31), critically ill

patients (32), obesity (33) and morbid obesity (34), pulmonary disease (30), anorexia nervosa (26), cachexia (25), sarcopenia and sarcopenic obesity (27), Alzheimer's disease (29), heart failure (25), gastrointestinal disease (28), diabetes (24), wound healing (35), muscle injury assessment (36, 37), and pregnancy and postpartum (38). BIVA validation studies have shown a significant association of bioelectrical values with hydration (11, 39), and nutritional status (11) in clinical conditions. Several studies have compared BIVA variables with conventional BIA and other measures of body composition such as dual-energy X-ray absorptiometry (DXA), anthropometry (somatotype), and clinical evaluation in samples of healthy and sick populations with mixed results (for review see (4, 11, 40)).

Bioelectrical Impedance Vector Analysis (BIVA)

Bioelectrical data acquisition

BIVA has been performed with single-frequency, multi-frequency and bioelectrical impedance spectroscopy (BIS) devices using the frequency of 50 kHz because it provides the best information at a whole-body level, as it increases the signal-to-noise ratio and decreases the frequency dependent errors and the variability of electric flow paths (41). Furthermore, equivalence between information provided by the bioelectrical parameters at 50 kHz and that provided at other frequencies has been reported (42). Therefore, the appropriate way to perform BIVA is using a phase-sensitive bioimpedance device (in order to measure the PA and calculate R and Xc (11, 23)) at 50 kHz. The phase-sensitivity characteristic is important since non-phase-sensitive instruments do not measure Xc, and the proper way to apply BIVA needs both R and Xc. Another important requirement is the use of appropriate contact electrodes (i.e. electrodes with low intrinsic impedance) to obtain valid BIVA plots for evaluation,

since vectors have been shown to be significantly affected by the type of electrode used (43). Whole-body BIVA is performed through the standard tetra-polar electrode placement (Figure 1) (10). BIVA has also been used in segmental body parts, e.g. regional measurements of limbs and trunk (44), and localised muscle group measurements (36) although no standardised electrodes placement procedures exist for these techniques and there is no evidence that electrode placement different that hand-to-foot is a valid approach for application of BIVA. In the sport literature, the localised approach refers to the bioelectrical analysis of body segments of the lower limb which are composed by different muscle groups. The electrodes placement described is performed putting the four electrodes in line over the muscle group that is intended to be analysed (injectors externally and sensors internally), two at the beginning and two at the end of the segment. Nevertheless, other ways to place the electrodes have been described, such as locating them at certain distance from the point of maximum pain (36, 37). Therefore, a standardisation of the localised electrodes placement is needed. However, it should be taken into consideration that the penetration depth of the electric current increases with the separation of the current electrodes (45). Therefore, it should be also investigated the adequate distance between electrodes to assess the muscles which are intended to be analysed. To our knowledge, this is a critical point not considered in the current sport literature regarding the bioelectrical localised assessment.

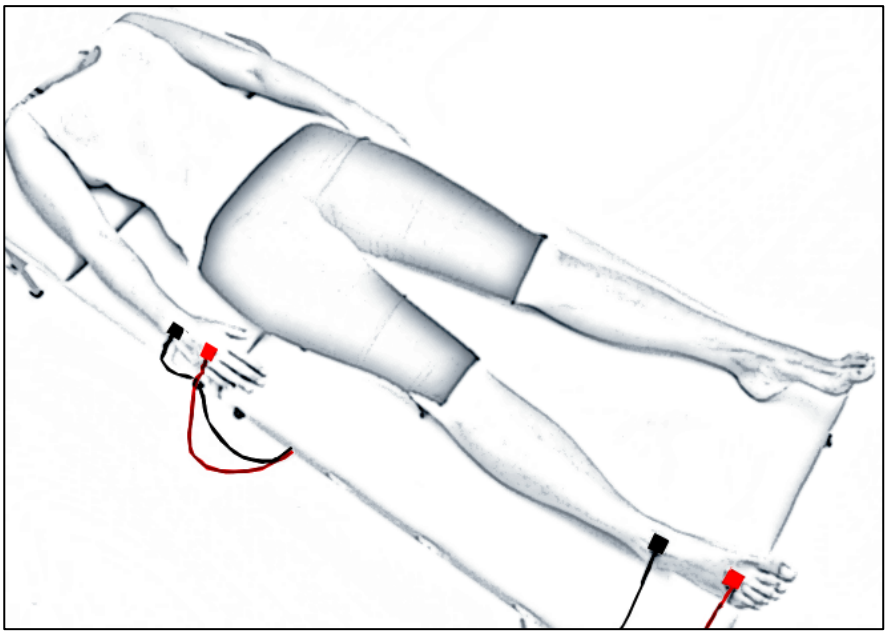

Figure 1. Standard tetra-polar electrode placement.

The limitations and biases of conventional BIA are well known and have been studied across multiple literature reports (10, 12, 13, 46, 47). Nevertheless, multiple factors need to be taken into consideration when it comes to using BIVA in sport and physical exercise applications to ensure the accuracy and reliability of bioelectrical signal acquisition; particularly within protocols measuring pre- and post-exercise (48-51). These considerations include: skin preparation (52); hydration status (53); variations in body fluid distribution (44); consumption of food or beverage (54-57); body position and posture during measurements (54, 58, 59); electrode impedance (43); electrode position and placement modification (54, 60-62); time of body fluid stabilisation (15, 63); variations in cutaneous blood flow and temperature (62, 64, 65); skin electrolyte accumulation produced by physical exercise (65); reproducibility of bioelectrical measurements influenced by biological intra-day (56, 57, 66) and inter-day variations (55, 66); environmental conditions (58, 59, 64); menstrual cycle (67-69) and injury conditions (37).

Therefore, the measurements must be performed in a room with neutral environment, where no strong electrical or magnetic fields can affect the assessment. Furthermore, metallic jewellery has to be removed and the subject must avoid the contact with metal frame of bed, in order to prevent electrical interferences (13). The minimal distance between electrodes must be 5 cm to avoid interaction between electric fields (60) and, in the case that is needed, the electrode which should be moved is the proximal one (13). Furthermore, before placing the electrodes, the skin must be prepared by shaving the electrode site to remove hair, rubbing with gel and cleaning with alcohol in order to reduce possible interferences in the assessment (70). For the evaluation, the subject must be euhydrated (unless the aim of the assessment is the evaluation of fluid variations after exercise), with no injuries or disease condition which can affect the measurement. The site of the electrodes should be changed in case that skin lesions are at the sight of the original electrodes location (13). The evaluation should be performed in fasting state (for at least 8 hours) and avoiding previous alcohol ingestion. Besides, the measurement should be performed once the bladder and rectum are voided (59) and after at least 10 minutes of stabilisation (63). In longitudinal protocols with different measurements, the position of the electrodes has to be marked, in order to preserve the same location, due to the influence of the electrode placement modification in the bioelectrical outputs (54). Variations in limb circumferences have to be controlled, since the whole-body impedance can be significantly reduced if a limb affected by swelling is in the same side as the electrodes (44). Furthermore, the temperature of the skin should be controlled and the environmental characteristics should be identical between assessments. As known, the increase in the skin temperature can lead to an important decrease in R (71). Temperature increases or decreases within the range of 1 ºC appear not to significantly affect the impedance (72)

and greater differences must be avoided. Before measuring after performing exercise, a shower (as cold as tolerable) should be performed in order to reduce cutaneous blood flow and temperature and remove accumulated electrolytes, which affect the bioelectrical signal (65). This measurement must be performed once the skin temperature, cutaneous blood flow and bioelectrical parameters have stabilised to baseline values. No food/drink should be consumed between measurements in the evaluation of acute variations after exercise (13). Nevertheless, in ecological protocols, where this condition is difficult to be followed, the quantity, moment and characteristics of the food/drink consumed should be registered. Regarding these type of protocols, it should be noted that the recent ingestion of a meal or beverage (< 1 h from the ingestion to BIA measurements) appears to be "electrically silent" and to have a minimal effect on the impedance value (73). On the other hand, with regard to the measurements in women, the menstrual cycle should be controlled and the comparison should be performed according to the cycle, in order to minimise the effect of body fluid fluctuations caused by the female hormonal kinetics (67). Moreover, the measurements should be performed at the same moment of the day, both for the comparison between subjects and for the intra-individual comparison between different assessments in order to minimise the effect of biological intra- and inter-day variations (55-57, 66). These are the principal technical requirements to perform valid measurements. More information regarding the specific recommendations for the bioimpedance analysis utilisation can be found in the European Society of Parenteral and Enteral Nutrition (ESPEN) Guidelines (13).

Finally, the type of sport and/or physical exercise, time of the season, and athlete’s characteristics (age, sex, competitive level, etc.), among other factors, may dramatically determine any approach aiming to provide rigorous, valid and reliable

information regarding the quality of the bioelectrical signal. In fact, although a pilot research has been published (48), we are not aware of any study in the sports field assessing the validity and reliability of BIVA as an indicator of changes in body composition and hydration status. However, two studies (22, 74) have evaluated the agreement of BIVA and DXA in adult and elderly, showing their inconsistency in the assessment of two-compartment body composition because of one of the limitations of "classic" BIVA methodology: the limited sensitivity in assessing the features of body composition (i.e. FM and FFM) due to the no consideration of the effect of cross-sectional areas of the body which interferes with bioelectrical values as well as lengths, according to the basic conductor theory (impedance is proportional to the conductor length and inversely related to its cross-sectional area) (58). This effect of cross-sectional areas is particularly relevant in sport sciences because athletes of different disciplines generally differ in their body shape. To overcome this limitation of "classic" BIVA, a relatively new procedure ("specific" BIVA) has been developed (27). This method proposes a correction of bioelectrical values for body geometry and it has proven to be effective in identifying the relative proportion of FM in adults and elderly (22, 74). Therefore, it should be further investigated in the sports field.

Data processing and analysis

As mentioned before, the fundamental advancement in recent BIA research is the use of raw impedance measurements (19). BIA relies on the conduction of a radio-frequency electrical current through the body's fluid (water) and electrolytes (58). Several approaches can be used to estimate body fluid volumes using BIA. Single- and multiple-frequency impedance devices calculate R, Xc, or Z, and use multiple-regression equations to predict TBW or ECW and, by calculation, ICW. BIS couples

MF-BIA with the Cole model (the mathematical model that is used most often to describe both theoretical and experimental data on skeletal muscle tissue) and mixture theory (used to model multiphase systems using the principles of continuum mechanics) to predict TBW and ECW (15). However, SF-BIA and MF-BIA methods seem inadequate to assess hydration status because of the large variability in individual predictions of fluid volumes (75) that yield unrealistic estimates of TBW and ECW in patients with altered hydration (12). Similarly, limitations in the application of the mixture theory in multicellular, physiological systems of the human body unfavourably limit the validity of BIS to estimate fluid volumes in adults with altered fluid status (61, 76). At present, BIVA, PA and regional BIS evaluate bioimpedance data relative to statistical-based reference norms for identification of physiological perturbation and evaluation of effects of intervention. Different analytical methods have been designed to graphically display and interpret bioelectrical data in order to interpret BIVA results.

RXc graph

This method consists in using raw R and Xc values, standardised for body height (h), to remove the effect of conductor length, and plotting them on a probabilistic graph —the so called RXc graph— that yields a Z vector that has length and direction. The vector length keeps an inverse relationship with the hydration status (38), where decreased R (shorter vector) means fluid overload and increased R (longer vector) means exsiccosis (bodily dehydration). Thus, it is consistent with body fluid changes but does not differentiate fluid shifts between compartments. It is important to highlight that any vector change is a function of ECW changes (77), since as already mentioned, a 50 kHz current does not penetrate cells. Therefore, vector migrations reflect ECW changes estimates only. On the other hand, a migration sideways of the vector due to low or high

Xc would indicate decreased or increased dielectric mass of soft tissues (membranes and tissue interfaces) (21). The sample size and the standard deviation (SD) of R/h and Xc/h shape the size of the ellipses (i.e. the bigger the sample size, the smaller the size; and the higher the SD, the bigger the size) and the correlation between R/h and Xc/h determines the ellipsoidal form of the bivariate probability distributions: confidence intervals for average vectors and tolerance for individual vectors (i.e. the higher the correlation, the narrower the ellipse) (21).

RXc point graph

The individual vector or the average vector of a group could be ranked in regard to tolerance ellipses representing 50%, 75% and 95% according to the values of a given reference population (Figure 2a) (21, 31). Besides, an individual's bioimpedance follow-up along successive measurements can be performed with the so-called "RXc path graph" (Figure 2b).

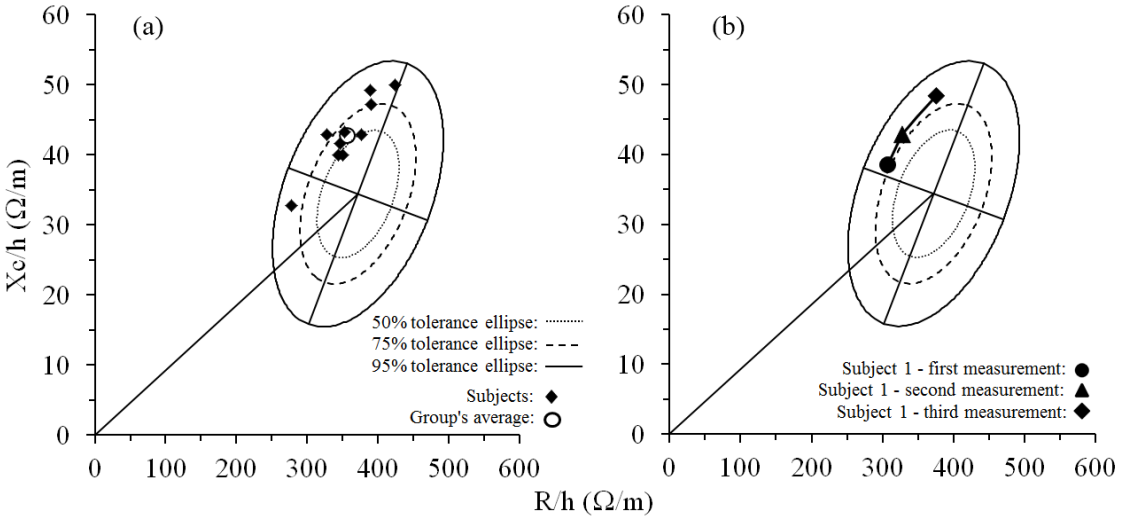

Figure 2. RXc point graph. Major axis refers to tissue hydration status, and minor axis refers to soft tissue status. The vector migration grid refers to changes in both hydration and soft tissue status (21). On the left side (a), example of standardised individual and mean impedance vectors plotted on the RXc point graph. On the right side (b), an example of an individual's bioimpedance follow-up along successive measurements plotted on the RXc path graph. R, resistance; Xc, reactance; h, height; Ω, ohms; m, metres.

Changes in hydration status without tissue structure variations are associated with the shortening (hyperhydration) or lengthening (dehydration) of the vector in the direction of the major axis of the tolerance ellipses (normal reference for sex). Changes in mass or soft tissues structure (thin and adipose) are associated to a vector displacement in the direction of the shorter axis of the ellipses, with increased PA (obese, athletes) or a decreased PA (malnutrition/cachexia, anorexia). Combined variations of hydration and nutrition status are associated to a vector migration towards the two combined main directions (78).

RXc score graph

After transforming vector components into bivariate Z-scores, measurements can be compared with any populations through its standard reference intervals using the so called RXc score graph (Figure 3) (79). This characteristic is especially relevant to assess how many standard deviations is an athlete or a group away from other

athletes/groups or to perform a follow-up along successive measurements. Additionally, the transformation into Z values allows the comparison of bioelectrical values measured with different devices.

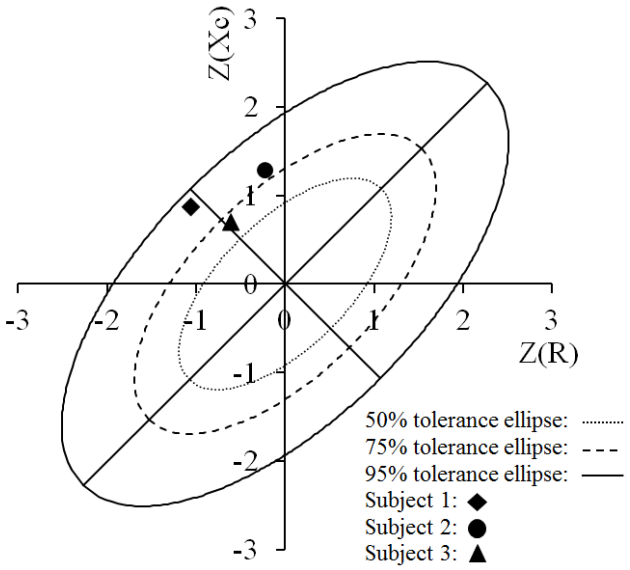

Figure 3. Standardised individual impedance vectors plotted on the RXc score graph. Z(R), standard resistance score; Z(Xc), standard reactance score.

RXc mean graph

The mean vector of different groups of subjects or the mean vector of different measurements can be plotted with the 95% confidence ellipse using the RXc mean graph (Figure 4) (80). This graph allows to clearly visualising the PA of different groups. It also shows the differences between groups according to the shape of their 95% confidence ellipses, conditioned by their sample size, standard deviation and R/h-Xc/h correlation.

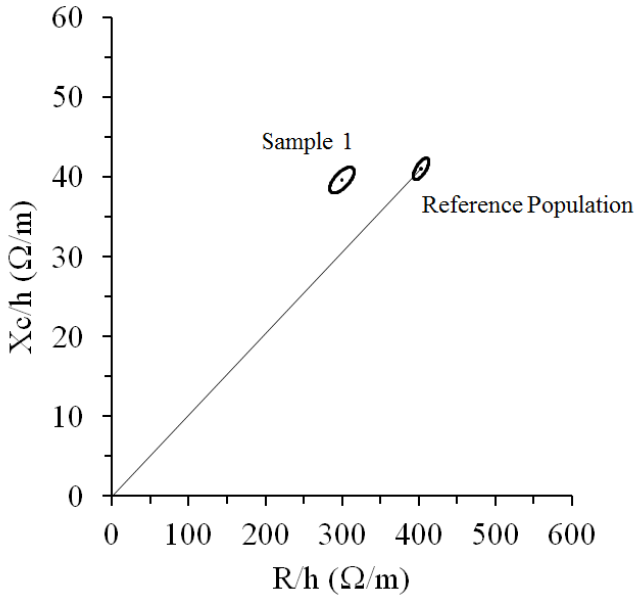

Figure 4. Comparative example of two mean impedance vectors plotted on the RXc mean graph: one sample (vector shifted to the left) vs. the corresponding reference population. R, resistance; Xc, reactance; h, height; Ω, ohms; m, metres.

RXc paired graph

The vector displacement of a group of subjects can be plotted with the 95% confidence ellipse using the RXc paired graph (Figure 5) (80). The main advantage of this graph is a clearly visualisation of the bioelectrical differences between two measurements (e.g. pre-post physical exercise protocols).

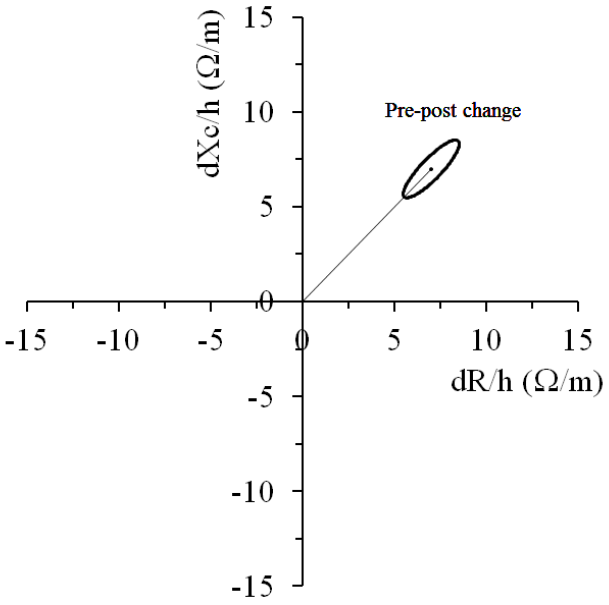

Figure 5. Example of pre-to-post intervention differences of a sample plotted on the RXc paired graph. dR, resistance difference; dXc, reactance difference; h, height; **Ω**, ohms; m, metres.

Phase angle (PA)

The PA is an impedance parameter also used to overcome BIA limitations. It is measured with a phase-sensitive device and is the geometric relationship between R and Xc (expressed as the arc tangent of Xc/R) (78). By definition, it is positively associated with Xc and negatively associated with R (81). PA expresses the quantity and quality of soft tissue (11) and it has been suggested to be an indicator of cellular health (82, 83), where higher values reflect higher cellularity, cell membrane integrity and better cell function.

Physical activity shows a positive relationship with the PA, where subjects who perform more physical activity have higher PA, probably due to greater muscle mass (11). This implies that higher hypertrophy levels of the skeletal muscle are related to greater PA (42) and the atrophy of the muscle mass entails lower PA (27). Regarding the behaviour of the PA relative to dehydration, higher angles have been observed after exercise (49, 51).

Nevertheless, the use of PA alone can provide biased information. For instance, obese and athletic subjects can theoretically produce identical PA values. BIVA allows the differentiation between these types of subjects with equal PA through the length of the vector and provides a more detailed understanding in terms of hydration status and cell mass (11).

Overview on the statistical analysis following BIVA assessment

Hotelling's T^2 test and RXc graph

Unpaired data analysis

The comparison between mean vectors from different groups of subjects is performed through the two-sample Hotelling's T^2 test. If the 95% confidence ellipses of two mean vectors do not overlap, their position is significantly different ($p < 0.05$). Generally, the reverse is true but not always, because there are some situations where confidence intervals overlap slightly, while Hotelling's test still finds a significant difference at the 5% confidence level (80).

Paired data analysis

The analysis of the mean difference between two impedance vectors measured in two conditions in the same group of people is performed through the paired one-sample Hotelling's T^2 test. A significant vector displacement ($p < 0.05$) is considered if the 95% confidence ellipse of the vector does not cover the origin of the RXc paired graph. In this case, the opposite is also true, due to the use of confidence intervals of the difference in paired analysis (80).

Mahalanobis' generalised distance

The Mahalanobis' distance (D) is a scale used to distinguish among groups by means of multivariate data set analysis (84). D is a multidimensional generalisation to measure how many standard deviations a point P is away from the mean of a given distribution. This distance is zero if P is at the mean of D, and grows as P moves away from the mean: along each principal component axis, it measures the number of standard deviations from P to the mean of the distribution, and uses within-groups variation (elliptical shape) as a yardstick for differences between means (e.g. if D = 4 between two vectors, then vectors differ by 4 within-group variation). Mahalanobis distance is unitless and scale-invariant, and takes into account the correlations of the data set.

JUSTIFICATION AND AIMS

JUSTIFICATION AND AIMS

There has been a rapid growth of interest in the application of BIVA in sport and exercise research in the recent years. On the one hand, “classic” BIVA is being used to characterise the body composition (i.e. hydration status and BCM) of athletes and active individuals (36, 42, 85, 86) and to monitor body composition longitudinal changes induced by exercise or sport practice (48, 49, 51, 87-97). On the other hand, the localised bioimpedance vector analysis is being applied for the identification and follow-up of muscle injuries (36, 37). The importance of assessing the body composition of athletes lies in the fact that the physical stress imposed during trainings and competitions may lead to body composition alterations, which can be detrimental to athletes (98). Furthermore, body composition has been suggested to discriminate athletes of different performance levels (99, 100) and has been shown to influence physical performance (101) and sport success (102). The importance of monitoring the hydration status in exercise and sport is because dehydration is recognised to impair sport performance (103, 104), as well as increasing the injury risk (105). Monitoring body fluid variations may help to adequately prescribe fluid intake and thus limit deleterious effects. Furthermore, the identification of injury and its follow-up during recovery until return-to-play depends on expensive methods which are not accessible to everyone. Therefore, the increase in the number of publications regarding BIVA in the exercise and sport field is justified in order to investigate the applicability of the method for assessments in real time and in a precise, accurate, reliable, non-invasive, portable, inexpensive, safe and simple way. Nevertheless, the current scientific literature in this field is still scarce and very heterogeneous, and a compilation of the current knowledge is needed in order to suggest a research agenda.

For these reasons, the present doctoral thesis is composed by three studies: one systematic review of the literature (Study I) and two quasi-experimental studies (Studies II and III).

The main objectives of Study I were to explain the bases and methodological principles of BIVA and to compile the current knowledge on the applications of the method in sport and exercise. Furthermore, the systematic review aimed to evaluate the usefulness and suitability of BIVA in assessing body composition, hydration status, and other physiological and clinical conditions in physically active and trained individuals. Ultimately, the investigation attempted to outline future perspectives in this field and to suggest a research agenda.

On the other hand, the quasi-experimental studies applied BIVA in two sport samples from an ecological perspective: young elite female synchronised swimmers and experienced, well-trained, non-professional ultra-endurance male triathletes. Study II analysed the training context and Study III applied BIVA in a competition.

Study II aimed at providing the first description, in bioelectrical terms, of two categories of synchronised swimming elite samples, comparing them with a reference non-athletic population. Furthermore, other objective of the investigation was to assess the variation in the whole-body bioimpedance vector and the BM of synchronised swimmers evoked during a training session. Finally, the study aimed to generate the synchronised swimming elite sample's 50%, 75% and 95% percentiles of the bioelectrical variables distribution, also known as tolerance ellipses.

Study III aimed at providing the first description, in bioelectrical terms, of a group of ultra-endurance triathletes, comparing them with a reference non-athletic population. Other objective of the investigation was to assess the variation in the whole-

body bioimpedance vector and the BM of the group of triathletes evoked during an ultra-endurance triathlon (UET) event.

METHODS

METHODS

Systematic review (Study I)

Study I followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to undertake the review (106). Besides, PRISMA checklist was also used to elaborate the systematic review protocol (107).

Eligibility criteria

The study reviewed and analysed methodological, clinical, and empirical studies using phase-sensitive devices to perform the analysis within the context of physical exercise and sport. Articles that have used BIVA in healthy sedentary people, physically active individuals and athletes of all levels were eligible for review. Studies were screened for eligibility on the following inclusion criteria: (a) empirical investigations with BIVA measures taken in human subjects performing acute or chronic exercise; (b) empirical investigations with BIVA measures taken in healthy sedentary people, physically active individuals and athletes; c) studies where data acquisition was performed with the appropriate methodology; (d) studies published in a peer-reviewed journal and/or in relevant congress proceedings; and (e) studies published in English language. No restrictions in terms of study design, setting, country or time frame were considered.

Information sources

A computer-based literature search was conducted for the period 1994-2017, ending by July 2017, of PubMed, SPORTDiscus and Scopus databases (Figure 6).

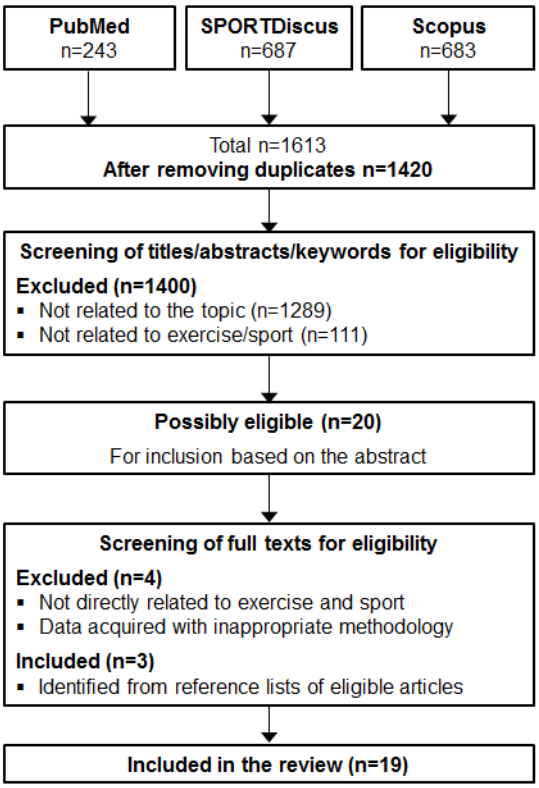

Figure 6. Flow chart of study identification and eligibility for the systematic review.

Search strategy

Title, abstract, and keyword fields were searched in each of the aforementioned databases using the following search terms and syntax: ("BIVA" OR "vector*") AND ("hydration" OR "body water").

Study records

Records were exported from the electronic databases to a reference management software (EndNote, v. X5, Thomson Reuters, 2011) and duplicate references were removed. Figure 6 displays the flow chart of study identification and eligibility for the systematic review.

The eligible articles after removing duplicates were screened by two investigators, with disagreement settled by consensus. An initial screening of titles,

abstracts and keywords was performed in order to check for inclusion criteria and to exclude obviously irrelevant records using the eligibility criteria (Figure 6). Differences in study eligibility for review were compared and deviations were discussed with a third investigator until consensus could be reached. When a paper could not be rejected with certainty, it was included in the eligible papers for full text evaluation. Then, articles were assessed for eligibility through a full-text screening, and those meeting the established criteria were included in the review. The reference lists of articles retrieved for inclusion in the review up to this point were searched to identify other relevant investigations. The number of studies meeting the pre-specified inclusion criteria and those excluded and reasons for their exclusion were recorded (Figure 6).

Each selected article was reviewed for information on (1) bibliographic characteristics (type of publication, authors, year and journal); (2) aims of the investigation; (3) study design and methodology; (4) sample characteristics (number, population, gender, age, exercise activity, sport discipline, and sport competitive level of subjects); (5) BIA device employed; (6) electrode distribution; (7) BIVA approach (whole-body BIVA or localised bioimpedance vector analysis); (8) vector displacement and (9) comparative technique (e.g. other indicators to assess body composition and fluid status, injury assessment).

Data items and prioritisation

Full texts were reviewed in search for the following main variables: bioelectrical resistance (R, R/h), reactance (Xc, Xc/h), Z, PA, RXc graph, TBW, ICW, ECW, FM, FFM and BCM. Bioelectrical measures and directly derived parameters were considered the main outcome from the population studies or experimental interventions. From a methodological point of view, comparisons of BIVA outcomes with other measures of

body composition and fluid status assessment could underpin the validity of the technique and, therefore, the latter were considered additional outcomes.

Quasi-experimental studies (Studies II and III)

Participants

58 subjects were recruited for the different studies in this investigation. Participants were female synchronized swimmers -including the entire Spanish national junior team- (n=49) and experienced, well-trained, non-professional ultra-endurance male triathletes (n=9) from Spain. Selection criteria were as follows: Study II) to have competed at national and/or international level at least in the previous two years; to not present injuries or any clinical condition at the time of the study; to be in a postmenarcheal state with the ovarian cycle between days 5^{th} to 11^{th}; to not be under contraceptives or menstrual cycle pharmacological regulators treatment; Study III) to train at least 10 hours per week and the participation in a minimum of one UET event during the past 3 years. Their physical characteristics and training volume are presented in Table 1.

Table 1. Physical characteristics and training volume of the participants.

	Study II			**Study III**
	Pre-junior SS (n=34)	Junior SS (n=15)	All (n=49)	Ultra-endurance triathletes (n=9)
Age (years)	13.9 ± 0.9	16.3 ± 0.6	14.6 ± 1.4	36.6 ± 5.5
h (cm)	161.9 ± 8.2	166.3 ± 4.8	163.3 ± 7.6	175.0 ± 6.0
BM (kg)	47.2 ± 0.9	53.5 ± 5.2	49.1 ± 7.0	76.0 ± 6.9
TV (h/week)	15.0 ± 2.7	30.0 ± 3.8	19.4 ± 7.6	16.6 ± 2.9

Values are mean ± SD; h, body height; BM, body mass; TV, training volume; SS, synchronised swimmers.

Procedures

Studies II and III are both pre-post quasi-experimental studies, descriptive and correlational, which aimed to approach the topic from an ecological perspective. Furthermore, Study III collected data 48 hours post-race measurements and added a

multiple linear regression analysis to the statistical examination. Study II analysed short-term bioelectrical changes (i.e. <24 hours after the first measurement) after a high intensity synchronised swimming training and Study III investigated short-term and medium-term bioelectrical changes (i.e. <24 hours and <7 days after the first measurement, respectively) after an ultra-endurance triathlon race. Data collection took place within a 4-week precompetitive mesocycle in Study II and within a competitive mesocycle in Study III.

Figures 7 and 8 schematise data collection procedures.

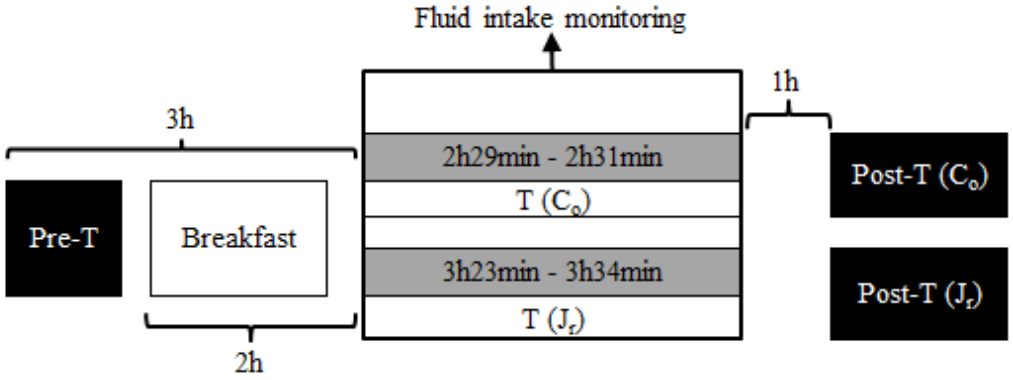

Figure 7. Study II protocol. Pre-T, pre-training measurements; Post-T, post-training measurements; C_o, pre-junior; J_r, junior.

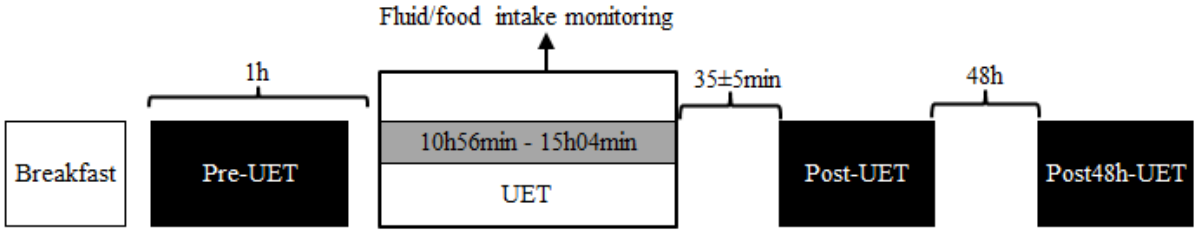

Figure 8. Study III protocol. Pre-UET, pre-competition measurements; Post-UET, post-competition measurements; Post 48h-UET, 48 hours post-competition measurements.

The characteristics of the training sessions and the race are presented in Table 2.

Table 2. Training and competition characteristics.

	Study II		**Study III**
	SS training (C_o)	SS training (J_r)	Ultra-endurance triathlon race
Duration (min)	149.6 ± 3.3	208.4 ± 10.3	752.2 ± 70.3
Internal load			
s-RPE (a.u.)	963.9 ± 78.5	1416.0 ± 129.0	-
TRIMP (a.u.)	-	-	1055.1 ± 172.3

Values are mean ± SD; SS, synchronised swimmers; C_o, pre-junior; J_r, junior; s-RPE, Rating of Perceived Exertion of the session; TRIMP, training impulse; a.u., arbitrary units.

Parameters and equipment

Anthropometric assessment

Anthropometric measurements were performed according to the standard criteria of The International Society for the Advancement of Kinanthropometry – ISAK - (108). Body height was assessed to the nearest 1 mm using a telescopic stadiometer (Seca 220®, Hamburg, Germany). BM was measured to the nearest 0.05 kg using a calibrated weighing scale (Seca 710®, Hamburg, Germany). Body mass index (BMI) was calculated as BM / h^2 (kg/m^2). The circumferences of the left and right thigh –CLT and CRT, respectively- (taken at mid-thigh) and the left and right calf –CLC and CRC, respectively- (taken at the greater perimeter of the calf) were measured in Study III to the nearest 1 mm using an anthropometric tape (Lufkin Executive®, Lufkin, USA), in order to evaluate possible variations between the different time points.

Temperature assessment

Core ($^oC_{core}$) and skin temperatures of the right hand ($^oC_{hand}$) and foot ($^oC_{foot}$) were measured using thermistors connected to a data logger (Squirrel 2010, Grant

Instruments Ltd, Cambridge, UK). Participants were instructed to take a cold shower (as cold as tolerable) for 10-15 minutes post-exercise, in order to reduce cutaneous blood flow and temperature and remove accumulated electrolytes (109). Skin temperature, as a surrogate for cutaneous blood flow (65), was measured just before BIA measurements; this verified the return to temperatures close to the baseline values ($p < 0.05$).

Whole-body bioimpedance assessment

R and Xc were measured using a previously calibrated phase-sensitive plethysmograph (Z-Metrix, BioparHom, Le Bourget-du-Lac, France) that emitted a 77 μA alternating sinusoidal current at seven operating frequencies (1, 5, 50, 150, 200, 250, and 325 kHz). The 50-kHz single frequency was selected for BIVA (110); meanwhile, multi-frequency capabilities were used to estimate TBW and ECW. In Study II, the ECW:TBW ratio was calculated by ECW÷TBW·100. The device provides impedance values with an accuracy average error of 0.95 ± 1.58% and average repeatability errors of 0.55 ± 0.38% for all the frequency range (1 to 1000 kHz) (111). Bioelectrical measurements were conducted under controlled conditions through the standard whole-body, tetrapolar, distal BIA technique (10). All the participants arrived to the measurements after voiding their bladder and rectum. Triathletes were tested pre-race and 48 h post-race in euhydration state according to a standardised 8-point urine colour chart (112). Before placing the electrodes (Red Dot™, 3M Corporate Headquarters, St. Paul, MN, USA), the skin was prepared by shaving the electrode site to remove hair, rubbing with gel and cleaning with alcohol. The anatomical sites for electrodes were marked with a waterproof pen (48). Bioimpedance assessments in pre-training/race and 48h after the race were performed after 10 minutes of stabilisation (63). Measurements were repeated

until they were stable to within 1 Ω (usually up to three times within an interval of 20 ± 30 seconds). The average value was used in calculations (59). Post-training/race measurements were performed once the core and skin temperatures were close to the baseline values (± 1 ºC).

Internal training/race load assessment

The individual training session-RPE (s-RPE) was chosen for rating the perceived exertion during training in Study II (113). The CR-10 RPE scale (114) was shown to the participants immediately after the training was completed. Scores were computed by multiplying the duration of the training by the relative RPE values. To estimate the total work load of exercise performed by each participant in the race, the training impulse (TRIMP) method was used in Study III (115).

Performance

Performance was evaluated in Study III by recording the racing time of each participant, showing greater performance participants with lower racing time.

Energy deficit

In Study III, the individually derived linear relationship between heart rate (HR) and oxygen uptake (VO_2) was used to estimate the oxygen cost during the work efforts for each segment. Three different individualised equations were established. These were three linear regression equations derived from data of three preliminary incremental exercise tests (as described in Barrero et al. (116)). To estimate energy expenditure during the race, an energy equivalent of oxygen based on the mean intensity during racing time was used, as described in a previous study (117). All the wraps and bottles

of each participant were collected in order to calculate the energy intake during the race. Finally, energy deficit was calculated as energy intake minus energy expenditure.

Statistical analysis

Descriptive statistics (mean ± SD) for each independent variable were calculated. Once the data were tested for normality of the distributions (Shapiro–Wilks test) and homogeneity of variance (Levene tests), differences in anthropometric (BM and circumferences of the thighs and calves), temperature and bioelectrical variables (R, Xc, Z, R/h, Xc/h, Z/h and PA) between the different points of measurement were analysed by the Student's paired t-test (Study II) and the repeated-measures one-way ANOVA (Study III). In Study III, post-hoc analyses were performed using Bonferroni correction and *p*-value was adjusted at $p_{adj} = 0.017$. The magnitude of ratio changes was computed as delta values (Δ%). Effect sizes (ES) were calculated using Cohen's d (118) and defined as small, $d \leq 0.2$; medium, $d \leq 0.5$; and large, $d \leq 0.8$. Pearson's correlation coefficient was used to determine possible statistical associations between: a) bioelectrical baseline values vs. bioelectrical delta values; b) bioelectrical data (baseline and delta values) vs. racing time, internal workload, energy deficit and BM delta values; c) BM delta values vs. racing time, internal workload, energy deficit; d) PA vs. ECW:TBW ratio; e) PA vs. chronological age. Study III added a multiple linear regression analysis in order to explain the changes at the different points of measurements of each bioelectrical (dependent) variable in relation to performance (independent) variables: racing time, internal workload, energy deficit, and BM changes. To add information to the multivariate analysis, the process was further applied in reverse, alternating the role between dependent and independent variables (i.e. the analysis was performed to explain the performance variables results in relation

to the changes of each bioelectrical variable). Whole-body bioimpedance vectors were analysed by the RXc graph method (21) using the BIVA software (119). Study II applied the RXc point graph to plot each athlete in the tolerance ellipses (50%, 75% and 95%) of the reference population. Whole-body individual vectors were analysed in Study III by the RXc score graph. Studies II and III used the RXc mean graph to compare: a) whole-body vectors of the sport groups analysed; b) whole-body vectors of the sport groups analysed vs. reference population. Study II executed the RXc paired graph to analyse longitudinal changes in the vectors of the athletes. The paired one-sample Hotelling's T^2 test (120) was used in Study II to analyse longitudinal vector changes through the 95% confidence ellipses. Both studies used the two-sample Hotelling's T^2 test (120) to determine BIA vector differences between: a) whole-body vectors of the sport groups analysed; b) whole-body vectors of the sport groups analysed vs. reference population. In both studies, the level of significance was set at $p < 0.05$. Statistical analyses were conducted using SPSS for Windows (v. 18, SPSS Inc., PASW Statistics for Windows, Chicago, USA).

Ethical considerations

The quasi-experimental protocols had received the approval from the Ethics Committee for Clinical Sport Research of Catalonia and follow the legal requirements and the 2013 Declaration of Helsinki. All participants volunteered and did not receive economic compensation. They were fully informed of the procedures, measurements and potential risk, after which they gave their written informed consent to participate in the study.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Systematic review (Study I)

In Study I, after removal of duplicates, 1420 records were identified, which were reduced to 20 after screening titles, abstracts and keywords for eligibility (Figure 6). After full-text evaluation, 19 studies matched the selection criteria and were included in the qualitative synthesis analysis. Publication date ranged from 1996 to 2017, yet only two studies were published before 2011, corroborating the novelty of the technique in the field of sport science.

Three of these studies were aimed at analysing short-term changes (<24 hours) in the hydration status induced by exercise and training, eleven assessed body composition changes induced by exercise at the long term (≥ 7 days), three compared athletic groups or populations, and two of the articles related bioelectrical patterns to athletic injury or muscle damage.

From the current applications of BIVA in sport and exercise, the results and discussion of the studies characterising sporting group samples and the articles investigating short-term vector changes will be integrated and further developed in the section of the quasi-experimental studies of the present thesis (Studies II and III), in order not to overlap the information of the investigations.

Long-term vector changes (≥7 days after the first measurement)

These type of studies, which investigate long-term (≥7 days) vector adaptations, have some protocol-specific advantages in comparison with investigations focused on acute vector changes, mainly because the quality of the bioelectrical signal can be assessed independently from the acute adaptations related to exercise.

BCM and extracellular mass (ECM) have been proposed as representatives of ICW and ECW, respectively (89). Nevertheless, it is important to note that the estimation of fluid volumes and cell mass with BIA prediction models is inappropriate when discussing changes in vector positions after interventions or treatments. Gatterer et al. (89), in their study assessing body composition using "classic" BIVA in the 2008 European Football Championship, found a significant lengthening of the vector within a period between 1 and 2 weeks. They attributed it to changes in BCM and ECW in both starters and non-starters after the first match with respect to baseline values, indicating body fluid loss. After the second match, only the athletes who played more (starters) showed a significant lengthening of the vector possibly due to a decrease in ECW. Therefore, they concluded that changes in body composition were mainly due to changes in ECW. However, their results should be taken with caution, since only analysis with appropriate reference methods (e.g. isotope dilution) can support them.

Similarly to the results of Gatterer et al. (89), rapid loss of BM protocols within a few days before competition in boxers (92) was found to be achieved mainly by isotonic dehydration (they attributed it principally due to changes in ECW), as identified by the significant vector lengthening on the RXc point graph and the decreases in plasma and blood volume. Nevertheless, as mentioned before, their results should be further investigated with appropriate reference methods for the estimation of fluid volumes, since BIA prediction models are inappropriate to discuss changes in vector positions. According to the results of Reljic et al. (92), Piccoli et al. (91) also found a significant lengthening of the vector with isotonic dehydration at high altitude (5500 m). Nevertheless, although a subsequent hypertonic dehydration was identified by a decreased BM (-3.0 kg) and several hydration biochemical markers, the vector lengthening was not significant. The causes that explain why the vector remained

unchanged after such a BM loss were not elucidated, and the authors recognised the difficulty of explaining the metabolic reasons that led to such BM reduction. In any case, emphasis should be placed on the importance of not considering body fluids quantitatively only (i.e., volume), but also regarding their qualitative composition, due to the biological adaptations generated by different types of exercise. For instance, after descent to sea level, the impedance vector underwent a significant shortening and returned close to baseline values. Lastly, significant relationships were found between changes in bioelectrical variables (R/h and Xc/h) and changes in the following hydration biomarkers along measurements performed at altitude and at sea level: BM, urine volume, plasma osmolality (P_{osm}), serum Na^+, K^+, Cl^- and glucose, and urine osmolar excretion (91).

On the other hand, two studies (96, 97) found significant shortening of the vector along three weeks of multistage road bicycle race, indicating fluid gain during the tour and attributing these results to muscle oedema, haemodilution, released water from muscle glycogen oxidation, and excess fluid intake. Although the vector shortening was not related to power output or rating of perceived exertion (97), it was negatively associated with performance during the last stages (96), suggesting the authors that increases in plasma volume and improved thermoregulatory capacity could explain these outputs. Nevertheless, their results should be taken with caution, since measurements were performed approximately two hours after exercise and this could have altered the data.

Regarding studies analysing longer-term vector adaptations, Mascherini et al. (90) analysed a football team across a sport season and reported a significant shortening of the vector in the pre-season associated with an improvement in endurance performance possibly due to plasma volume expansion and enhanced glycogen storage.

These results are in agreement with other studies (88, 95) which also found significant bioelectrical differences in the pre-season, hypothesising that they were due to fluid expansion. Bonuccelli et al. (88) and Macherini et al. (90) found a significant lengthening of the vector in the mid-season compared to pre-season results. This could indicate a reduced body fluid volume (i.e., decreased plasma or interstitial volume) despite an increased intracellular fluid associated with an increase in BCM, and consequently in PA (85). However, while Mascherini et al. (90) reported a significant shortening of the vector at the end of the season compared to the mid-season, Bonuccelli et al. (88) observed a significant water content decrease. Sport calendars could have led to adopt training strategies inducing different performance status and evoked opposite vector displacements.

On the other hand, regarding the age-related decreases in Xc and PA (121), improvements have been reported after six months of resistance training in elderly women (93), suggesting increased amount and quality of soft tissues. These improvements were accompanied by increases in leg strength and thigh circumference. Along with these changes, BIVA showed a significant vector migration with greater PA after the training program.

With regard to children, one study (94) evaluated the body composition in participants of swimming and gymnastics along one year. The baseline measurement (T_0) was performed at a period preceding races and sporting events, just as the third measurement (T_2) one year later. The second measurement (T_1) was executed six months after T_0 in a period characterised by a softer daily training. They found a significant increase in Xc from T_0 to T_1, along with increased PA and ICW (derived from ECW:TBW ratio). The authors hypothesised that this was due to an improvement in the muscular trophism with higher levels of intracellular proteins and glycogen and to

a lower stress from training program. After one-year follow-up, no significant differences were found in R, Xc and PA. However, again, their hypotheses should be taken with caution, since fluid estimations were calculated from BIA prediction models. Variables as the type of sport and training strategy should be taken into account when monitoring along a season, since they might influence the bioelectrical measures. Moreover, also intra-group comparisons between seasons should be analysed with caution, since inter-seasonal bioelectrical variations could be effected by factors such as biological maturation.

Injury identification and follow-up

These studies (36, 37) consisted in single cross-sectional protocols aiming to identify bioelectrical patterns of change depending on the injury type and grade, and longitudinal protocols aiming at assessing bioimpedance vector sensitivity to monitor injuries and their recovery. R and Xc were found to be decreased in the injured muscles due to the oedema and to the disruption of the muscle structure, respectively. Additionally, the more severe the injury was, the more R and Xc were decreased. On the other hand, a bioelectrical symmetry between muscular groups in lower-limbs was found. The follow-up of the injury identified bioelectrical patterns of changes similar to those in wound healing and an increase of R and Xc values were observed to values close to pre-injury.

Overall, localised bioimpedance vector analysis appears as an alternative method that could help to assess soft tissue injury and to monitor the injury recovery process (36, 37).

Quasi-experimental studies (Studies II and III)

BIA vector characterisation of athletes

Studies II and III performed a cross-sectional analysis aiming to describe sporting group samples in terms of bioelectrical data.

Both studies reported specific BIA vector distribution of the athletes in comparison with their reference populations (Figures 9 and 10).

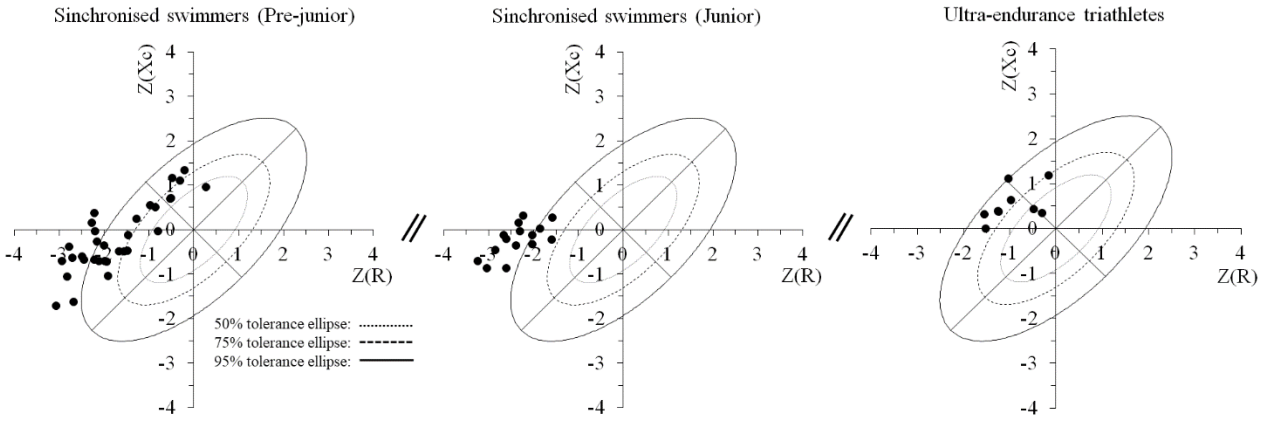

Figure 9. BIVA score graph. Individual vector score values of synchronised swimmers and ultra-endurance triathletes are plotted on the 50%, 75%, and 95% tolerance ellipses of the corresponding reference populations. Z(R), resistance Z score; Z(Xc), reactance Z score.

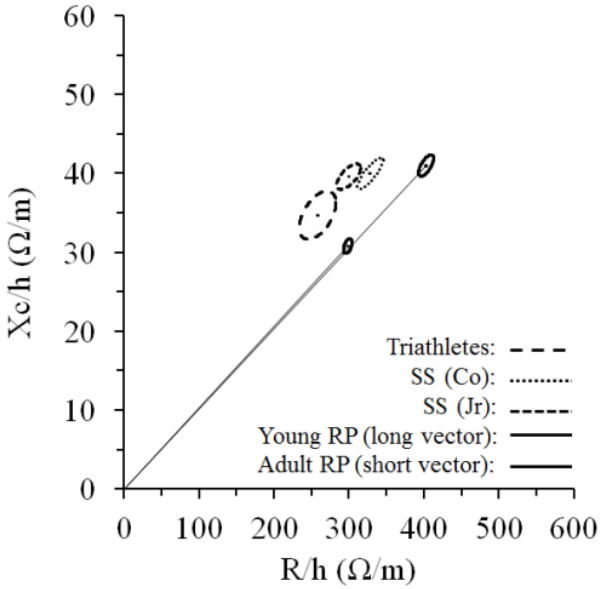

Figure 10. RXc mean graph. The 95% confidence ellipses for the mean impedance vectors of synchronised swimmers, ultra-endurance triathletes, the healthy young female reference population (solid line ellipse with long vector) (122) and the healthy adult male reference population (solid line ellipse with short vector) (123) are shown. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance; Ω, ohms; m, metres; C_o, pre-junior; J_r, junior; SS, synchronised swimmers; RP, reference population.

Vectors shifted to the left with greater PA were found in both young and adult athletes compared to the corresponding reference populations (Figures 9 and 10), which is consistent with the results reported by other studies (85, 86, 124, 125). The shift to the left indicates increased BCM and fluid content, and might reflect a better cell functioning (11, 85). It suggests that these differences are due to sport-specific adaptations (85). Athletes generally possess increased soft tissue mass and differing fluid content compared to the sedentary population (126). Since total body fluid is affected by factors such as training (127), trained athletes have a greater amount of body fluid and different fluid distribution between the intracellular and extracellular compartments. This could be because of their larger muscle mass, increased plasma volume and muscle glycogen reserves (65, 128), which could increase water transport into the muscle (129) and fluid-regulating hormone adaptations (i.e., aldosterone and sensitivity) (130). In relation with this, Study II found a negative correlation between

the ECW:TBW ratio and PA in both synchronised swimmers, indicating a differing fluid distribution (i.e. increased ICW content) (131), likely due to the hypertrophy of muscle fibres (85). However, since the ECW:TBW ratio was obtained through BIA, the results should be taken with caution and further research including other hydration markers is needed.

On the other hand, when sport samples where compared, the mean vector of older athletes also showed a shift to the left with regard to the younger athletes (Figure 10). Additionally, Study II found that with increasing performance level of the athletes, a displacement to the left was also observed (Figure 10). Vectors shifted to the left have been already reported with increasing age (86, 132) and performance level (85, 125) in sport samples. The differences could be the result of vector displacement due to the increase in metabolic tissues because of the biological maturation (122), to the specific training process (85) or a combination of both. In relation with this, Koury et al. (132) demonstrated a shift to the left in early adolescent football athletes compared to the late adolescent ones, according to bone age and erythrocyte zinc. This finding opens the discussion of performing bioelectrical comparisons in young populations according to the biological age or the chronological age. On the other hand, Study III found a significantly positive relationship between basal R/h (and therefore, Z/h) and racing time in the triathletes ($r = 0.68$; $p > 0.05$). This implies that the vector of triathletes who had better performance in the race (and that, presumably, had higher performance levels pre-race) would be displaced to the left, due to lower R/h values. Accordingly, it was observed that triathletes who registered lower basal R/h (and Z/h), racing time and internal workload showed lower changes in these parameters after completing the race ($r = 0.8$, $p \leq 0.02$), experiencing lower levels of dehydration. This particularly relevant finding highlights the need of further research regarding this matter, since the

application of a non-invasive technique could help to discriminate between performance levels of athletes according to the position of their vectors.

The present investigation found that the distance between the confidence ellipses of adolescent and adult athletes was lower than between the ellipses among their respective reference populations (pre-junior synchronised swimmers vs. triathletes: $d = 2.01$; junior synchronised swimmers vs. triathletes: $d = 2.19$; young vs. adult reference population: $d = 2.42$; $p < 0.05$). This is in agreement with the results of Koury et al. (86). The authors speculated that the intense training reduced the differences between young and adult individuals, although this is still to be elucidated.

Regarding the vector position on the RXc graph, the trend is to be outside the 50% tolerance ellipse of the respective reference population in both young and adult athletes (Figure 9). Furthermore, many vectors are plotted outside the 95% tolerance ellipse. Piccoli et al. (42) also found the mean impedance vector of bodybuilders almost completely outside the 95% tolerance ellipse of the reference population, reflecting a specific body composition, characterised by greater soft tissue mass and different fluid content. This suggests that specific tolerance ellipses are needed for sport populations (36, 85). Study II is the first investigation generating specific reference tolerance ellipses for a female sporting group (i.e. synchronised swimmers) (Figure 11). To our knowledge, only two other studies (85, 125) have generated new specific tolerance ellipses for sport populations (football and road cycling, respectively).

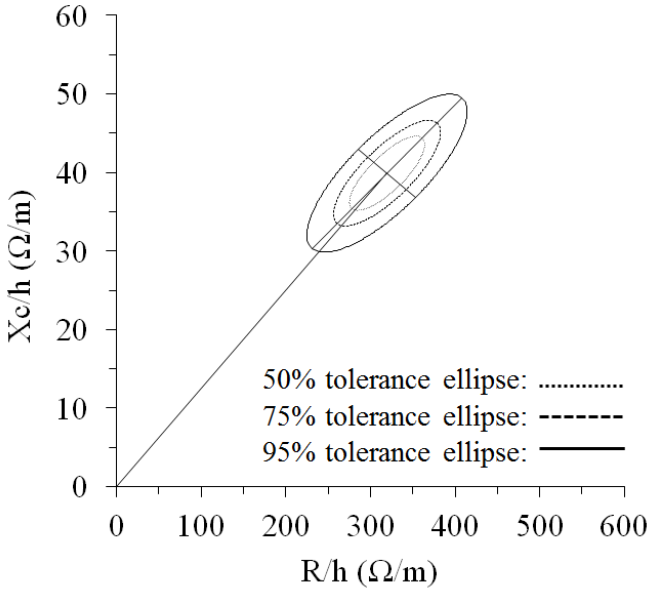

Figure 11. Tolerance ellipses. 50%, 75%, and 95% tolerance ellipses generated of the entire group of synchronised swimmers. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance; Ω, ohms; m, metres.

The relationship between the new specific tolerance ellipses (for each sport, gender, age and race) and the hydration status, body composition and sport performance level should be analysed, in order to represent significant hydration changes (that compromise health or performance) or target zones of impedance vectors for athletes. Nevertheless, it is possible that a new approach is required for the exercise and sports field, beyond the current BIVA point graph, based on 50-95% tolerance ellipses and quadrants related to clinical outputs. With regard to the hydration assessment, it should be noted that fluid overload (overhydration) is not common in healthy athletes. Therefore, the analysis of the hydration status should be related to euhydration and physiological dehydration processes. In this way, as mentioned in Heavens et al. (50) regarding the identification of dehydration with single measurements according to the tolerance ellipses of the reference population, the limits for "normal hydration" (individuals positioned within the 50% tolerance ellipses, according to the literature (19, 78)) should be reviewed, since subjects experiencing high levels of fluid loss can still be

identified as euhydrated (50). Other studies related to sport and exercise (48, 91) and Studies II and III (Figure 12, post-training/race measurement) identified some individuals as euhydrated after significant BM decreases. Accordingly, as shown in Study I, the majority of the studies applying "classic" BIVA in sport and exercise identify the athletes outside the 50% tolerance ellipse. This is probably due to a range of "normal hydration" comprised by the ellipses wider than a hydration status considered as "dehydration" through other methodologies (50). Therefore, the current BIVA point graph is not a valid method to detect euhydration and dehydration status in individual athletes with single measurements.

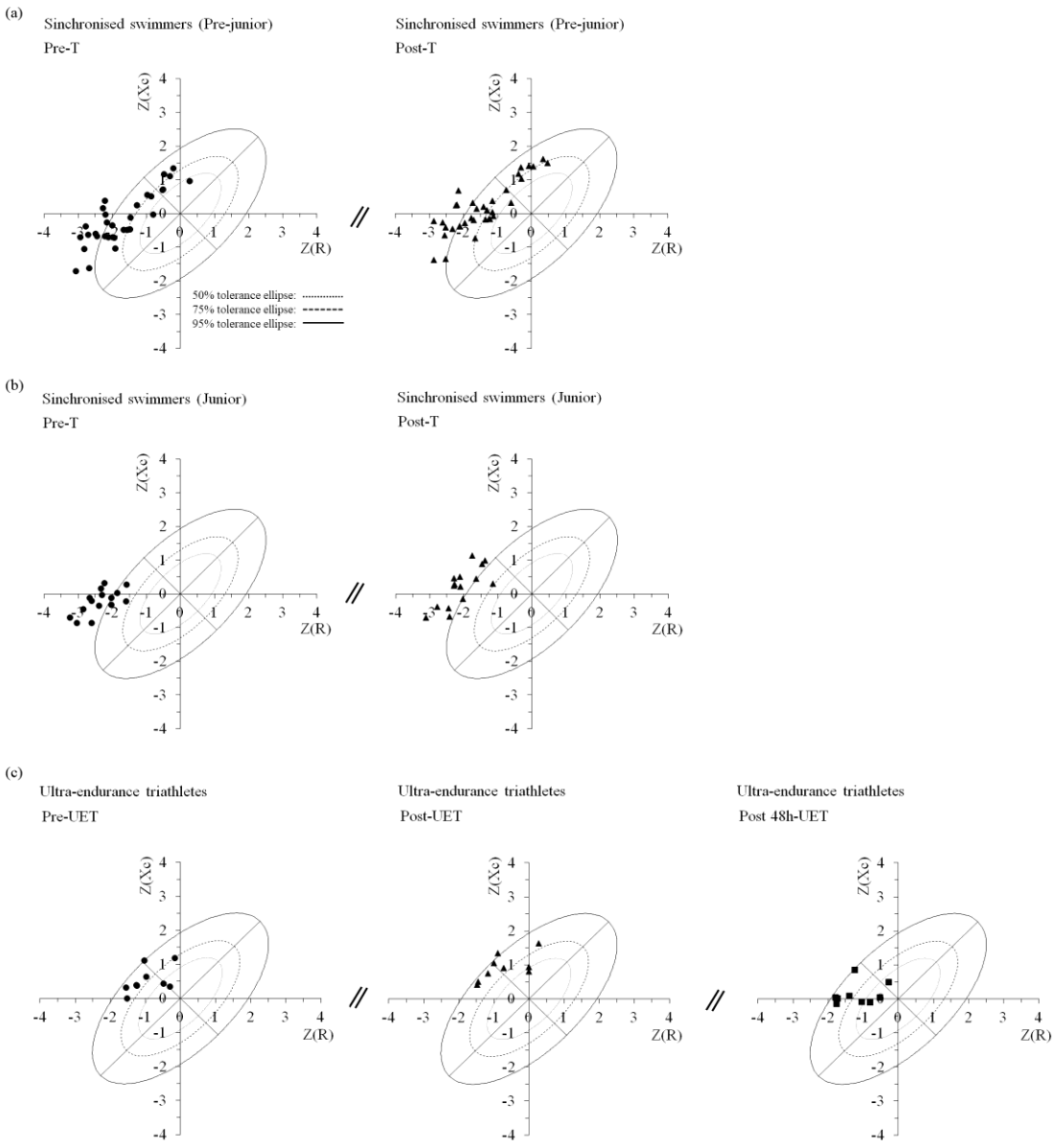

Figure 12. BIVA patterns before and after training/competition. Individual impedance score vectors of the (a) pre-junior and (b) junior synchronised swimmers, and (c) ultra-endurance triathletes, plotted on the 50%, 75%, and 95% tolerance ellipses of the corresponding healthy reference population, are displayed for pre-, post-training/competition and post 48 hours-competition. Z(R), resistance Z score; Z(Xc), reactance Z score; Pre-T, pre-training; Post-T, post-training; Pre-UET, pre-triathlon race measurements; Post-UET, post-triathlon race measurements; Post 48h-UET, 48 hours post-triathlon race measurements.

On the other hand, it should be investigated the relationship between the new specific tolerance ellipses and different sport performance levels. Maybe different sectors of the tolerance ellipses identify target zones for the athletes.

With regard to the body composition assessment and in accordance with "classic BIVA", athletes have been identified in the upper left quadrant of the reference population and obese individuals in the lower left quadrant. This would generally imply greater R/h and Xc/h values of the athletes. Nevertheless, as mentioned in the literature (22, 74), according to the electro-physical assumptions, FFM is characterised by a greater conductivity in comparison with the poorly hydrated adipose tissue, not justifying the relative shortness of vectors of obese individuals with respect to the athletes, unless contemplating their generally greater FM, fluid overload and body size. Furthermore, the vector position of athletes regarding the tolerance ellipses of the general reference population is controversial (4). As mentioned by Buffa et al. (4), athletic individuals are not always plotted in the "athlete" quadrant of the reference population and their vectors often overlap the "obesity" area. This controversy can be observed in Study I: from the eighteen investigations analysed, six studies did not report vectors distribution with regard to the reference population and only four found the majority or all the vectors of athletes positioned in the "athlete" area (48, 92, 95, 96). Comparable vector position of athletes and obese individuals would imply similar values of R/h and Xc/h. The already mentioned factors FM and fluid overload could compensate the bioelectrical values between both individuals, not being "classic" BIVA able to detect the differences (e.g. discriminating fluids distribution between compartments, with greater ICW content in athletes). Moreover, as shown in the literature (22, 74), "classic BIVA" would be characterised by a limited sensitivity in assessing the features of body composition due to the no consideration of the effect of cross-sectional areas of the body. "Specific" BIVA, a method which performs a correction of bioelectrical values for body geometry, emerges as the key to overcome this limitation. Although the inclusion of anthropometric measurements can make these

plots more sample-specific and perhaps less generalizable than "classic" BIVA, this adaptation may be an advance when comparing athletes with different body composition (in terms of FM and FFM). Therefore, it should be further investigated in the sports field.

Finally, regarding the bioelectrical parameters that determine the vector position, the interpretation is also controversial and more research is needed in order to clarify the matter. When athletes present a vector shifted to the left with greater PA in comparison to the reference population, due to a decrease in R/h with no differences in Xc/h (the case of synchronised swimmers - Study II), it has been suggested that it reflects different ICW content (85). On the other hand, when the vector shifted to the left with greater PA of athletes compared to the reference population is due to a decreased R/h and an increased Xc/h (the case of triathletes – Study III), the following explanation have been suggested: the decreased R/h is probably due, among other factors, to a greater muscle mass, muscle glycogen reserves and plasma volume (65, 126) and the increased Xc/h may be due to an increase in the size and number of muscle cells (hypertrophy and hyperplasia, respectively), although the last one is still a controversial topic (133). However, since a decreased R/h is also related to greater FM (33), further research is needed in order to clarify the reason for this behaviour. Moreover, Xc/h is not only conditioned by the cell size, but also by the thickness and composition of the cell membrane and also by the distance between them, due to its relationship with cell membrane capacitance (C_m) (134). In this way, lower Xc/h values have been documented in bodybuilders (the best model of extreme muscle hypertrophy) compared to healthy active people and with no differences with the healthy reference population (42). On the other hand, vectors shifted to the left with lower PA have been reported in competitive children in comparison with healthy control groups due to significantly

lower Xc/h values in absence of differences in R/h (94). Meleleo et al. (94) suggested that it could be due to an increase in the size of the section of the limbs or to a greater 'sufferance' in cell membranes maybe due to bad response to the workloads (over-training). Therefore, the interpretation of these parameters (R/h, and especially Xc/h) in these cases remains unresolved.

BIA vector changes evoked by training/competition

To date, Studies II and III are the only ones in the sport literature that applied BIVA with a longitudinal, quasi-experimental, ecological design, to assess vector and BM variations evoked by a training session or a competition. Both studies reported BM loss after an intense training session and an ultra-endurance triathlon event and the vector displacements observed between measurements through "classic" BIVA were consistent with fluid loss.

Short-term vector changes (<24 h after the first measurement)

These types of studies are those which currently face more difficulties, since their validity can be easily compromised, mostly because of the already mentioned factors that may affect the accuracy of the measurements despite any attempts to control them.

Regarding the changes induced by a high intensity synchronised swimming training and an ultra-endurance triathlon event, Studies II and III show a mild dehydration in synchronised swimmers (average loss <1% BM) and mild-moderate dehydration in triathletes (average loss ~5% BM) (135). Furthermore, individual vectors' migration along the major axis was observed due to an increase in R and Xc (Tables 3 and 4; Figure 12), which is consistent with fluid loss according to the theory

(21, 79). Accordingly, RXc paired graphs and Hotelling's test showed significant vector changes after exercise in all groups (Figure 13).

Table 3. Anthropometric and bioelectrical variables of synchronised swimmers before (Pre-T) and after (Post-T) training.

	Pre-T	Post-T	Δ-value (%)	Cohen's d (*d*)
Pre-junior (n = 34)				
Anthropometric				
BM (kg)	47.2 ± 7.0	46.9 ± 7.0	-0.8 ± 0.6*	0.20[a]
Bioelectrical				
R (Ω)	529.5 ± 46.1	548.8 ± 48.5	3.7 ± 3.0*	0.39[b]
Xc (Ω)	64.4 ± 5.1	67.7 ± 5.0	5.2 ± 3.3*	0.67[c]
R/h (Ω/m)	328.4 ± 38.8	340.5 ± 41.0	3.7 ± 3.0*	0.30[b]
Xc/h (Ω/m)	40.0 ± 4.5	42.0 ± 4.6	5.2 ± 3.3*	0.43[b]
PA (Ω)	7.0 ± 0.5	7.1 ± 0.5	1.5 ± 2.5*	0.20[a]
Z (Ω/m)	330.9 ± 38.9	343.1 ± 41.2	3.7 ± 3.0*	0.20[a]
r (R/h, Xc/h)	0.84	0.84	--	
Junior (n = 15)				
Anthropometric				
BM (kg)	53.5 ± 5.2	53.2 ± 5.1	-0.6 ± 0.4*	0.17[a]
Bioelectrical				
R (Ω)	498.5 ± 35.1	518.5 ± 38.9	4.0 ± 3.3*	0.53[c]
Xc (Ω)	65.8 ± 2.9	70.2 ± 4.8	6.6 ± 3.9*	0.82[c]
R/h (Ω/m)	299.9 ± 21.6	311.9 ± 23.4	4.0 ± 3.3*	0.53[c]
Xc/h (Ω/m)	39.6 ± 2.2	42.2 ± 3.4	6.6 ± 3.9*	0.62[c]
PA (Ω)	7.5 ± 0.4	7.7 ± 0.4	2.4 ± 3.3*	0.45[b]
Z (Ω/m)	302.5 ± 21.7	314.8 ± 23.5	4.1 ± 3.3*	0.51[c]
r (R/h, Xc/h)	0.66	0.76	--	

Values are mean ± SD (95% CI); BM, body mass; R, resistance; Xc, reactance; h, height; PA, phase angle; Z, impedance vector module; Pre-T, pre-training; Post-T, post-training; r, Pearson correlation coefficient between R/h and Xc/h; %Δ, percent differences Pre to Post; CI, 95% confidence interval; *, significant differences between Pre-T and Post-T, p-value < 0.05 (paired t-test Pre-T vs. Post-T); a, small effect size (≤ 0.2); b, medium effect size (d ≤ 0.5); c, large effect size (d ≤ 0.8).

Table 4. Anthropometric and bioelectrical variables before (Pre-UET), after (Post-UET), and 48 hours after the race (Post 48h-UET).

				Δ-value (%)			Cohen's d (*d*)		
	Pre-UET	**Post-UET**	**Post 48h-UET**	Pre-UET vs. Post-UET	Post-UET vs. Post 48h-UET	Pre-UET vs. Post 48h-UET	Pre-UET vs. Post-UET	Post-UET vs. Post 48h-UET	Pre-UET vs. Post 48h-UET
Anthropometric									
BM (kg)	76.0 ± 6.9	72.1 ± 6.1	75.0 ± 6.3	-5.0 ± 0.9*	4.0 ± 0.9*	-1.3 ± 1.1*	3.9[b]	-4.3[b]	1.1[b]
C_{RT} (mm)	54.0 ± 1.7	53.3 ± 1.9	53.9 ± 1.7	-1.3 ± 1.7	1.1 ± 1.8	-0.2 ± 0.3	0.8[b]	-0.6[a]	0.8[b]
C_{LT} (mm)	54.2 ± 1.8	53.5 ± 2.0	54.1 ± 1.7	-1.2 ± 1.7	1.1 ± 1.7	-0.2 ± 0.3	0.7[a]	-0.6[a]	0.5[a]
C_{RC} (mm)	38.1 ± 1.3	37.7 ± 1.5	38.0 ± 1.3	-1.1 ± 1.6	0.9 ± 1.6	-0.3 ± 0.4	0.7[a]	-0.6[a]	0.8[b]
C_{LC} (mm)	38.3 ± 1.3	37.8 ± 1.5	38.2 ± 1.2	-1.2 ± 1.6	1.0 ± 1.8	-0.2 ± 0.4	0.7[a]	-0.5[a]	0.6[a]
Bioelectrical									
R (Ω)	452.6 ± 45.8	470.0 ± 56.6	435.2 ± 46.4	3.7 ± 2.3*	-7.2 ± 2.9*	-3.9 ± 2.1*	-1.4[b]	2.2[b]	1.9[b]
Xc (Ω)	60.6 ± 4.3	65.6 ± 4.6	55.5 ± 3.7	8.3 ± 3.7*	-15.2 ± 4.5*	-8.2 ± 4.8*	-2.3[b]	3.0[b]	1.7[b]
R/h (Ω/m)	258.4 ± 22.4	268.3 ± 28.3	248.6 ± 24.0	3.7 ± 2.3*	-7.2 ± 2.9*	-3.9 ± 2.1*	-1.5[b]	2.3[b]	1.9[b]
Xc/h (Ω/m)	34.7 ± 2.8	37.5 ± 2.8	31.8 ± 2.4	8.3 ± 3.7*	-15.2 ± 4.5*	-8.2 ± 4.8*	-2.3[b]	3.0[b]	1.7[b]
PA (Ω)	7.7 ± 0.5	8.0 ± 0.7	7.3.0 ± 0.7	3.7 ± 4.9	-8.7 ± 4.9*	-5.4 ± 6.4	-0.8[b]	1.7[b]	0.9[b]
Z (Ω)	456.6 ± 45.8	474.6 ± 56.5	438.8 ± 46.2	3.8 ± 2.3*	-7.4 ± 2.9*	-3.9 ± 2.0*	-1.5[b]	2.2[b]	2.0[b]
r (R/h, Xc/h)	0.52	0.65	0.27	–	–	–	–	–	–

Values are mean ± SD; BM, body mass; C_{RT}, circumference of the right thigh; C_{LT}, circumference of the left thigh; CRC, circumference of the right calf; C_{LC}, circumference of the left calf; R, resistance; Xc, reactance; Z, impedance vector module; h, body height; PA, phase angle; r, Pearson's correlation coefficient between R/h and Xc/h; time-point differences: *RM-ANOVA, repeated measures analysis of variance with Bonferroni post-hoc test, significance at $p_{adj} < 0.017$; a, medium effect size ($d \leq 0.5$); b, large effect size ($d \leq 0.8$).

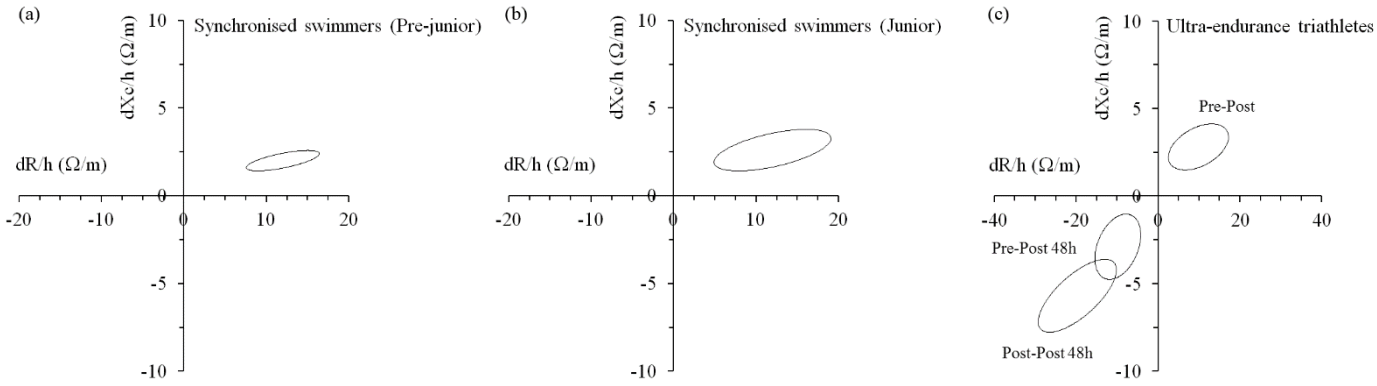

Figure 13. BIVA patterns before and after training/competition. Mean vector displacements of (a) pre-junior synchronised swimmers, (b) junior synchronised swimmers and (c) ultra-endurance triathletes, from Pre- to Post-training/race, Pre- to Post 48h-race and Post to Post 48h-race are shown. dR, resistance difference; dXc, reactance difference; h, height; Ω, ohms; m, metres.

Nevertheless, no correlation was observed between changes in BM and BIA vector migration. This could be due to the fluid/food intake of the athletes during training and race, which was maintained due to the ecological study design. Only two studies (48, 50) have investigated the short-term vector changes induced by exercise, apart from Study II and III. Although the ingestion of food or fluid was not allowed, the studies found results similar to ours with exercise-induced dehydration. The authors observed increased resistance and reactance, as well as a vector migration after performing physical exercise. Furthermore, Gatterer et al. (48) found no correlation between changes in BM and bioelectrical parameters. As they noted, this could be influenced by inadequate criteria for stable bioelectrical impedance measurements or by exercise-related factors, such as sweat rate, respiratory water loss and oxidative water production, that may lead to BM loss without an effective net negative fluid balance (136). However, these results differ from other studies (91, 137) that found a significant relationship between changes in bioelectrical values and BM induced passively and/or chronically. It is possible that greater changes could have been observed in Studies II and III if the athletes had not ingested fluids/food during the exercise. The ingestion of a

meal or beverage has an influence on Z, which may decrease over a 2- to 4-h period after a meal, generally representing a change of < 3% in Z values (47). Thus, an underestimation of ~9-10 Ω and ~14 Ω in Z values (Study II and Study III, respectively) could have occurred in the post-exercise BIVA measurements.

On the other hand, the multiple regression analysis allows the study of the bioelectrical vector's behaviour from a multifactorial perspective. Thus, while BM did not correlate with bioelectrical variables as an isolated variable, it was selected as an explanatory factor in a multivariate model in Study III (Table 5). When the bioelectrical variables were analysed as dependent variables, the changes observed in R/h and Z/h after the race were significantly explained by the behaviour of BM, the racing time and the estimated internal workload, which makes sense. When the process was further applied in reverse in order to add information to the multivariate analysis, alternating the role between dependent and independent variables, the racing time was significantly explained by the behaviour of Z/h from pre- to post-race. In the event that, in the future, this methodology could be validated, it could be a possible indicator of the training/competition load. In addition, using the multivariate analysis, some variables (e.g. race time) could be estimated analysing the behaviour of other parameters, with a certain degree of error. Therefore, this type of analysis should be taken into consideration both in the interpretation of certain variables related to the vector behaviour, and in future studies about this topic. However, the scarce sample analysed in the present study forced us to be cautious in drawing robust conclusions.

Table 5. Multiple linear regression analysis of bioelectrical, anthropometric and performance parameters before (Pre) and after (Post) the race.

Dependent variables	**Explanatory equations**	**r_m^2**		**ANOVA**				**SE_E**
		Exact	Adjusted	F	df_1	df_2	*p*	
$R/h_{Pre\text{-}Post}$ (Δ%)	$-28.40 - (1.14 \cdot BM_{Pre\text{-}Post}) + (0.021 \cdot time) + (0.010 \cdot TRIMP)$	0.92	0.88	19.95	3	5	0.003	0.80
$Z/h_{Pre\text{-}Post}$ (Δ%)	$-28.62 - (1.17 \cdot BM_{Pre\text{-}Post}) + (0.022 \cdot time) + (0.010 \cdot TRIMP)$	0.93	0.88	29.42	3	5	0.003	0.79
Racing time (min)	$660.3 + (24.4 \cdot Z/h_{Pre\text{-}Post})$	0.63	0.58	11.914	1	7	0.01	45.7

F_{in} ($p \leq 0.05$), F_{out} ($p \geq 0.10$). R: resistance; Z: impedance module; h: height; BM: body mass; TRIMP: training impulse (a.u., arbitrary units); time: racing time; r_m^2: multiple regression coefficient squared; SE_E, standard error of estimation.

In opposition to Study II, Study III and Gatterer et al. (48), one study (51) only found a tendency to reduction of fluids (the authors related it to an extracellular water decrease given by a significant increase in Xc) along with an increased BM in a group of men and no differences in women after approximately 10 hours of subterranean exploration (caving). Nevertheless, factors affecting protocols measuring Pre- and Post-exercise (such as dietary intake during cave activity or the skin temperature in the post measurement) could have influenced their observations.

Regarding the bioelectrical changes observed in Studies II and III after exercise, resistance is pure opposition of the conductor to the flow of current (58). Therefore, the significantly increased resistance experienced by the athletes would indicate a decrease in body fluids (65), which is supported by the decrease in BM, and is also probably followed—which we cannot prove—by changes in electrolyte concentration (91). With regard to the reactance, Gatterer et al. (48) suggested that the increased reactance after exercise could indicate fluid shifts between intra- and extracellular compartments. As mentioned before, Xc maintains a relationship with C_m, which is affected by the size, thickness, composition and distance between cell membranes (134). Exercise generates processes which modify the characteristics of muscle cells (such as changes in fluid distribution). As suggested, when cell membrane becomes thinner, the cell swells and C_m increases, and the opposite happens as the cell shrinks (138), thus affecting Xc. Moreover, in accordance with De Lorenzo et al. (15), variations in fluid distribution would modify the characteristic frequency (Fc)—i.e., the frequency at which Xc is maximal—. Because Xc is highly dependent on the relationship between the frequency of measurement and Fc, changes in Fc would evoke great variations in Xc at 50 kHz, simply because this frequency is a fixed point on the changing impedance locus (139, 140). Nonetheless, De Lorenzo and collaborators' hypothesis should be considered with

caution because it refers to the Hanai's model, which relays on assumptions such as spherical cell shape. Therefore, multiple factors may affect Xc values and further research should focus on this parameter in exercise. Despite the fact that the vector changes after fluid removal and overload (the wet–dry cycle of dialysis) as a non-physiological process is clinically well-established (78), every dehydration process induced by physical exercise is consequence of scarcely explored physiological adaptations as regard of the vector behaviour, especially at cellular level (and therefore, affecting R and Xc). In literature, Xc is an indicator of dielectric mass (membranes and tissue interfaces) in soft tissues (58). Given the results observed in sport, it is possible that the behaviour of Xc could be due to other factors and, thus, its meaning remains to be clarified.

In conclusion, directional changes in vector values from serial measurements seem to be consistent with fluid loss. Nevertheless, regarding the validity of BIVA in order to identify dehydration, BIVA is not currently able to identify type and magnitude of fluid loss. It is possible that a new approach is required beyond the current BIVA point and score graphs, since, as mentioned above, subjects experiencing high levels of fluid loss can still be identified as euhydrated, according to the tolerance ellipses of the reference population (48, 50, 91). Thus, research investigating different levels of dehydration and their relationship with the new specific tolerance ellipses is needed in order to identify the limit of "normal hydration". Furthermore, different types of dehydration can be experienced in sport: a) hypertonic dehydration (i.e. primarily a loss of water) is a common type of dehydration developed after exercise in which heavy sweating occurs; b) hypotonic dehydration (i.e. primarily a loss of electrolyte) and c) isotonic dehydration (i.e. equal losses of electrolytes and water), both may be developed by athletes competing in aesthetic-type sports and in weight classification sports in

which fasting, vomiting and diuretic use are common behaviours (105). Therefore, research is needed related to the sensitivity of BIVA to each type of dehydration, as well as the behaviour of each one with regard to the tolerance ellipses. As mentioned in Heavens et al. (50), the analysis of the vector length could be the key for serial measurements of hydration status.

Medium-term vector changes (<7 days after the first measurement)

As already mentioned, Study III collected data 48 hours post-race. This type of analysis, in which two basal measurements are compared and no exercise is performed immediately before the second measurement, has fewer limitations than the short-term vector changes analysis.

Regarding the bioelectrical changes observed 48 hours after performing an ultra-endurance triathlon race, Study III showed individual vectors' migration along the major axis due to significant decreases in R and Xc (Table 4; Figure 12), indicating fluid accumulation (78), while BM values were still significantly lower than at baseline. Accordingly, RXc paired graphs and Hotelling's test showed significant vector changes in the group of triathletes (Figure 13). Triathletes followed individual uncontrolled Post-UET to Post 48h-UET recovery strategies (nutrition, hydration, physical activity, environmental conditions, etc.). However, the other mentioned factors that could affect the accuracy and reliability of BM and BIA measurements were controlled, being Pre-UET and Post 48h-UET on equal terms. Thus, while BM alone does not detect a return to basal conditions, significant bioelectrical decreases below basal values were observed, indicating fluid retention in comparison with Pre-UET. This highlights the potential advantage of BIVA in providing additional information about hydration changes in comparison with BM alone. However, it is important to clarify that neither

BM nor BIVA can really identify what happened to TBW. As already mentioned, BIVA changes reflect ECW loss estimates only. Thus, ICW may still be reduced (captured by BM at this time point) with a migration of fluids to the ECW space.

Fluid retention has been already reported in ultra-endurance events (141-143). As explained by Knechtle et al. (143), although the reasons and mechanisms for the fluid increase are still unknown, it could be due to several factors which will be discussed below.

One explanation for this fluid retention could be an increase in plasma volume due to a higher activity of aldosterone and antidiuretic hormone. Transient expansion of plasma volume after endurance events has been commonly reported in the literature (130, 144, 145). Prolonged exercise generates an increased loss of fluids by sweating and respiration (146) and causes the activation of the renin-angiotensin-aldosterone system. Furthermore, physical exercise leads to an elevated plasma antidiuretic hormone concentration, probably due to an increased P_{osm} and a decreased plasma volume (147). Antidiuretic hormone and aldosterone are increased after intense exercise (148, 149) and the concentration of both hormones is higher with increasing exercise intensity (150). Therefore, the activation of both hormone systems leads to an enhanced retention of sodium and fluids, resulting in an increase of plasma volume (151).

Moreover, protein catabolism with consequent fluid shifts (hypoproteinemic oedema) might occur in an ultra-endurance performance. Lehmann et al. (152) suggested a fluid shift from intra- to extracellular compartment after an ultra-endurance event and proposed the decrease in cellular hydration level as a protein-catabolic signal.

Another possible factor for the observed fluid expansion could be the increased plasma protein concentration –especially albumin– inducing an increase in plasma oncotic pressure. An increase of total protein and albumin after prolonged exercise has

been reported (145, 153). Nevertheless, more research is needed regarding this topic, since the contrary was observed in other studies (151, 154, 155).

On the other hand, the increase of body fluids could also be a result of the impairment of the kidney due to the rhabdomyolysis occurring in ultra-endurance events (156). Rhabdomyolysis during ultra-endurance events has been demonstrated (157, 158) and an association between skeletal muscle damage and impaired renal function has been suggested. Strenuous exercise including running leads to damage of muscle cells (159). If severe muscle damage occurs, creatine kinase and myoglobin from muscle cells will be released into the blood and myoglobinuria can result. Myoglobin can reach the kidneys and trigger an acute renal failure (158). However, the pathophysiology of acute renal failure is multifactorial and is the combined effect of different factors, such as rhabdomyolysis, dehydration, hypotension, nonsteroidal anti-inflammatory drugs, and hyperuricemia (158). In general, acute renal failure in an ultra-endurance event is very rare (160). The duration of the exercise and the performance level of the athlete might be of importance (143).

Finally, regarding the putative factors explaining the fluid increase 48 hours after the race, the already mentioned individual uncontrolled Post-UET to Post 48h-UET recovery strategies may have affected these findings.

Therefore, the identification of fluid retention after ultra-endurance events through BIVA should be further studied together with tests investigating the aforementioned mechanisms, in order to analyse possible relationships.

Regarding the behaviour of the reactance, the significant decrease in Xc/h values from Pre- to Post 48h-UET (as already mentioned, two basal measurements with no exercise performed immediately before the second measurement which may affect the bioelectrical signal) could indicate a significant decrease in soft tissues, since Xc is

related to cell membranes and tissue interfaces. As already mentioned, there is evidence of muscle damage after prolonged exercise. Significant modifications in markers of muscle damage and inflammation two days after the end of the event have been reported (161-163). Further studies should investigate the relationship between the behaviour of Xc and muscle damage biomarkers after completing this type of events. It would be interesting to analyse the validity of this parameter (obtained from a non-invasive method) in order to control the muscle disruption recovery after exercise. Nevertheless, taking into account the aforementioned limitation of not controlling individual recovery strategies, it is difficult at present to elucidate the reasons why this behaviour occurs due to the already mentioned problems in the interpretation of Xc when the fluid distribution changes.

CONCLUSIONS

CONCLUSIONS

From the present doctoral thesis, the following conclusions were derived:

1. The bioimpedance vector analysis is a technique that has a great potential in sport and exercise, yet largely unexplored, especially for the identification of soft-tissue injury and its follow-up.
2. With regard to the body composition assessment, “classic” BIVA is inconsistent in the assessment of two-compartment body composition and the vector position of athletes with regard to the reference population seems controversial in many cases. “Specific” BIVA seems to overcome this limitation, since this method considers the effect of cross-sectional areas of the body.
3. However, regarding the body composition assessment using BIVA, specific bioelectrical distributions were found in synchronised swimmers and triathletes in comparison with their healthy, general reference population. In relation with this, Study II reports for the first time specific tolerance ellipses in a female sport group.
4. Furthermore, BIVA showed bioelectrical differences between synchronised swimmers of different age and performance level. A vector shifted to the left (due to significantly lower R/h) was observed in junior athletes compared to the pre-junior ones. Accordingly, bioelectrical differences between triathletes of different performance level were found. The best athletes were characterised by lower basal R/h and Z/h values and registered lower changes in these parameters after the competition.
5. Regarding the validity of “classic” BIVA for the assessment of hydration status, the method is not currently able to identify type and magnitude of fluid loss in individual athletes and a new approach is needed.

6. Nevertheless, vector migration after a high intensity synchronised swimming training and a UET event is consistent with fluid loss, regardless of age and performance level. Furthermore, vector changes 48h after the UET seem consistent with fluid recovery.
7. Although with the utmost caution, there seems to be a relationship between the behaviour of the bioelectrical vector and certain performance parameters, specifically in relation to a UET event. The multivariate analysis may help to better understand the bioelectrical vector's behaviour pre- to post-exercise.
8. Currently, the relationship between the bioelectrical signal and physiological adaptations induced by different types of exercise remain largely unresolved, especially in how the structure and function of the cell are altered and how these affect the behaviour of R, and in particular Xc.

CONCLUSIONES

A raíz de los resultados de la presente tesis doctoral, se obtuvieron las siguientes conclusiones:

1. El análisis del vector de bioimpedancia es una técnica con gran potencial, aunque aún inexplorado, en el deporte y el ejercicio, especialmente para la identificación y seguimiento de lesiones de tejidos blandos.
2. Con respecto a la evaluación de la composición corporal, el BIVA "clásico" no es consistente en la evaluación bicompartimental de la composición corporal y la posición del vector de los atletas en relación a su población de referencia parece conflictiva en muchos casos. El BIVA "específico" parece superar esta limitación, ya que este método considera el efecto de las áreas transversales del cuerpo.
3. En cualquier caso, se encontraron distribuciones bioeléctricas específicas en nadadoras de natación sincronizada y triatletas en comparación con su población sana de referencia. En relación a esto, el Estudio II generó por primera vez elipses de tolerancia específica en un grupo femenino de deportistas.
4. Además, BIVA mostró diferencias bioeléctricas entre nadadoras de diferentes edades y niveles de rendimiento. Se observó un vector desplazado hacia la izquierda (debido a una R/h significativamente menor) en las nadadoras junior, en comparación con las pre-junior. Asimismo, se encontraron diferencias bioeléctricas entre triatletas de diferente nivel deportivo. Los mejores deportistas se caracterizaron por tener menores valores basales de R/h y Z/h y registraron cambios menores en estos parámetros después de la competición.

5. Con respecto a validez del BIVA “clásico” en relación a la evaluación del estado de hidratación, el método no es actualmente capaz de identificar el tipo y la magnitud de la pérdida de fluidos en atletas y se necesita un nuevo enfoque.
6. Sin embargo, la migración del vector después de un entrenamiento de natación sincronizada de alta intensidad y de una carrera de triatlón de ultra-resistencia es consistente con una pérdida de fluidos, independientemente de la edad y el nivel de rendimiento. Además, los cambios en el vector 48 horas después de la carrera de triatlón parecen consistentes con una recuperación de fluidos.
7. Aunque con la máxima precaución, parece haber una relación entre el comportamiento del vector bioeléctrico y ciertos parámetros de rendimiento, específicamente en relación a una carrera de triatlón de ultra-resistencia. El análisis multivariante puede ayudar a comprender mejor el comportamiento del vector bioeléctrico antes y después del ejercicio.
8. Actualmente, la relación entre la señal bioeléctrica y las adaptaciones fisiológicas inducidas por diferentes tipos de ejercicio sigue sin estar resuelta, especialmente en la forma en que se modifican la estructura y función de la célula y cómo éstas afectan el comportamiento de R y, en particular, Xc.

LIMITATIONS

LIMITATIONS

Systematic review (Study I)

The main limitations derived from the literature analysis about the use of BIVA in the sport context are: 1) the difficulty of controlling multiple sources of error that may influence the bioelectrical signal; 2) the lack of tests correlating the bioelectrical signal (vector) with other variables studied in the literature; 3) the limited scientific evidence explaining the bioelectrical behaviour of human tissues induced by exercise; 4) the formulation of possible explanations for the bioelectrical behaviour of human tissues induced by exercise with inappropriate methodologies (e.g. the use of estimated fluid volumes with BIA prediction models to discuss vector variations); 5) the limited sensitivity of "classic" BIVA for the assessment of a) individual dehydration in exercise and b) two-compartment body composition; and 6) the scarcity of scientific information related to the use of BIVA in sport and exercise. Furthermore, the systematic review did not consider investigations in languages other than English, so an information bias might have existed.

Quasi-experimental studies (Studies II and III)

In protocols measuring acute vector changes (before and after exercise), some factors should be controlled in order to avoid measurement errors and provide accurate and reliable results. As for Studies II and III, both attempted to control these factors. Nevertheless, some of them could not be avoided. The main limitation of both studies is the ecological constraints of the protocols. In order to respect the ecological design, the consumption of food and beverage was allowed. As mentioned in the discussion, the ingestion of a meal or beverage has an influence on Z, which may decrease over a 2- to 4-h period after a meal, generally representing a change of < 3% in Z values. With

regard to this, both studies have different characteristics. The ultra-endurance triathlon race started at 6:00 a.m. and lasted for many hours. Therefore, each participant had their own nutritional strategies before competition (usually they have breakfast 2-3 hours before the competition) and we could not control them. This could have influence the bioelectrical values obtained pre-race. Furthermore, due to the free consumption of food and beverage during the race, an underestimation of ~14 Ω in Z values could have occurred in the post-exercise BIVA measurements. Nevertheless, the amount of water intake at the end of the race should not have affected the BIVA measurements because the recent ingestion of a meal or beverage (< 1h from the ingestion to BIA measurements) appears to be "electrically silent" and to have a minimal effect on whole-body Z. On the other hand, synchronised swimming trainings started at 10:00 a.m. and swimmers had the breakfast set two hours before starting the training. Therefore, pre-training measurements had to be taken before the breakfast. Furthermore, fluid ingestion was allowed during the trainings. Thus, post-exercise BIVA measurements could have been influenced by breakfast and water intake during the training session, possibly underestimating Z values by ~9 ± 10 Ω. The ecological design of the studies implied also other limitations. In the case of Study III, the post-race measurement was notably the most difficult assessment because of the multiple factors that must be controlled due to their influence on the bioelectrical signal at a moment in which athletes are extremely tired and less motivated (after more than 11 hours of competition). Other limitations of Study III that should be taken into consideration in further studies are: 1) the time at which the fluid/food intakes were performed during the race was not registered; 2) there were no records of each individual's recovery strategies, which could have influence the bioelectrical

measurements; 3) the sample size was low, which limits the possibility of reaching stronger inferences, especially in the multiple regression analysis.

Finally, with regard to the vector analysis, Study II plotted the synchronised swimmers sample on the tolerance ellipses of the healthy reference population closest in age, since no tolerance ellipses of the healthy reference population have been published for their specific age range. Moreover, no sport-specific tolerance ellipses for synchronised swimmers and ultra-endurance triathletes exist yet to allow a more enriching analysis.

FUTURE PERSPECTIVES

FUTURE PERSPECTIVES

BIVA in sports and exercise science is an emerging area of research with great potential.

From the methodological standpoint, closely related to the quality, reliability and validity of the bioelectrical signal, some issues should be deeper investigated. For example, adequate hydration protocols are required in order to assess participants in a euhydrated state. Related to this, rigorous fluid intake control before bioelectrical measurements should be performed and reported. In studies assessing BIVA after exercise, adequate protocols of cold water application before testing with different duration and temperatures in order to reduce the sources of error in bioelectrical measurements should also be investigated, adapting the protocol to the type, intensity and duration of the exercise. Core and skin temperature should be monitored pre- and post-exercise. In sport practice, baseline values for BIVA should be established before the start of any follow-up protocol (e.g. to monitor changes along a sport competition) in the attempt to guarantee an optimal hydration status and to avoid excessive fluid loss.

Further research is also required on how much some factors affect the bioelectrical signal, especially in exercise-induced acute vector change assessment (e.g. exhaustive control of quantity and composition of fluids and food intake, and time between fluids/food intake and the bioelectrical measurements). With regard to differences in the bioelectrical signal among type of electrodes, distribution of the electrodes (e.g. whole-body standard placement or eight-polar tactile distribution), and BIA devices, further research is required. Standardisation of contact electrodes is necessary for valid BIA measurements.

As for the bioelectrical parameters, especially Xc, it will be difficult to obtain conclusions as valid and accurate as possible concerning to their patterns until the

behaviour of cells in the human body is not well explained using simulated circuit models (in series, in parallel or mixed), for both homeostatic and non-homeostatic conditions. Regarding Xc changes after exercise, further research is needed in order to clarify the causes of these behaviour. As for PA, its relationship with cell functioning in sport should also be addressed.

Another critical point needing further investigation is the assessment of the validity and reliability of "classic" BIVA as a method for monitoring BCM and hydration status in sports and exercise. New specific tolerance ellipses for each sport, sex, age and race, should be generated and it should be investigated whether they can be used for the classification of an individual vector (in terms of hydration status, body composition and sport performance level) and if they represent significant hydration changes (that compromise health or performance) or target zones of impedance vectors for athletes. With regard to the hydration assessment, the analysis of the hydration status should be related to euhydration and physiological dehydration processes. In this way, as for the identification of dehydration according to the tolerance ellipses of the reference population, the limits for "normal hydration" should be reviewed. Research investigating different levels of dehydration and their relationship with the vector length and new specific tolerance ellipses is needed in order to identify the limit of "normal hydration". Furthermore, research is needed related to the sensitivity of "classic" BIVA to each type of dehydration, as well as the behaviour of each one with regard to the tolerance ellipses. On the other hand, research investigating the relationship between the new specific tolerance ellipses and different sport performance levels is required. With regard to the body composition assessment, it should be further investigated the effect on the bioelectrical signal of the FM, fluid overload and cross-sectional areas of the body. Furthermore, future investigations should seek to clarify if BCM changes shown

by “classic” BIVA mean actually BCM variations, different fluid distribution between compartments, or a combination of both. More research is needed with regard to the application of “specific” BIVA in the sports field. Comparisons of BIVA outcomes with validated body composition and fluid status assessment are to be undertaken to better define the basis for interpretation and application of this technique. These types of analyses should be undertaken in both laboratory and field conditions adjusted to the reality of sport. On the other hand, it is surprising to realise how few reliability studies in BIVA there are, this being a critical factor in establishing its practical application as a diagnostic tool.

With regard to the localised bioimpedance vector analysis, it seems necessary to standardise the distribution of the electrodes and generate muscle-specific ellipses in order to improve the reproducibility of bioelectrical measurements. This standardisation should consider the muscle length instead of the body height to normalise the bioelectrical values, since differences in the proportionality between subjects may lead to greater errors. Besides, the symmetry between limbs should be determined for each sport and discipline, particularly in relation with differences between dominant and non-dominant limbs and asymmetrical sports (e.g. jumps, throws, team sports, tennis). When speaking of localised assessment in injured muscles, further research is needed in order to establish ranges of alterations in bioelectrical vector outcomes, as well as the time course of injury recovery and return-to-play.

Regarding sports practice, PA and “classic” BIVA showed that the intense training changed functional and hydration parameters of the athletes (86). It should be analysed if BCM and fluid content reflect the sport-specific adaptations of BM and body composition. Furthermore, the utility of integrated evaluation of PA and BIVA to identify possible risks derived by different training loads in athletes should be

investigated. Further research is also required to assess the relationship between BIVA and other body composition techniques.

Related tests in acute and long-term designs (e.g. muscle function, glycogen storage, haematological and biochemical markers, etc.) should be performed to correlate them with vector displacements, in order to understand better the cause of vector migration. In addition, vector changes at the medium term (< 7 days) should be further investigated. Finally, it would be interesting to investigate whether the vector position is an indicator of different individual biological responses to the training load or if it is the result of optimised training activity and/or recovery strategy.

REFERENCES

1. Lukaski HC, Bolonchuk WW, Siders WA, Hall CB. Body composition assessment of athletes using bioelectrical impedance measurements. J Sports Med Phys Fitness. 1990;30(4):434-40.

2. Segal KR. Use of bioelectrical impedance analysis measurements as an evaluation for participating in sports. Am J Clin Nutr. 1996;64(3):469S-471S.

3. Kerr A, Slater G, Byrne N, Chaseling J. Validation of Bioelectrical Impedance Spectroscopy to Measure Total Body Water in Resistance-Trained Males. Int J Sport Nutr Exerc Metab. 2015;25(5):494-503.

4. Buffa R, Mereu E, Comandini O, Ibanez ME, Marini E. Bioelectrical impedance vector analysis (BIVA) for the assessment of two-compartment body composition. Eur J Clin Nutr. 2014;68(11):1234-40.

5. Moon JR. Body composition in athletes and sports nutrition: an examination of the bioimpedance analysis technique. Eur J Clin Nutr. 2013;67(1):S54-9.

6. Loenneke JP, Wilson JM, Wray ME, Barnes JT, Kearney ML, Pujol TJ. The estimation of the fat free mass index in athletes. Asian J Sports Med. 2012;3(3):200-3.

7. Papadopoulou SK, Gouvianaki A, Grammatikopoulou MG, Maraki Z, Pagkalos IG, Malliaropoulos N et al. Body Composition and Dietary Intake of Elite Cross-country Skiers Members of the Greek National Team. Asian J Sports Med. 2012;3(4):257-66.

8. Fernandez-Elias VE, Martinez-Abellan A, Lopez-Gullon JM, Moran-Navarro R, Pallares JG, De la Cruz-Sanchez E et al. Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. PLoS One. 2014;9(4):e95336.

9. Matias CN, Judice PB, Santos DA, Magalhaes JP, Minderico CS, Fields DA et al. Suitability of Bioelectrical Based Methods to Assess Water Compartments in Recreational and Elite Athletes. J Am Coll Nutr. 2016;35(5):413-421.

10. Yanovski SZ, Hubbard VS, Heymsfield SB, Lukaski HC. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement. Am J Clin Nutr. 1996;64(3):524S-532S.

11. Norman K, Stobaus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis--clinical relevance and applicability of impedance parameters. Clin Nutr. 2012;31(6):854-61.

12. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM et al. Bioelectrical impedance analysis-part I: review of principles and methods. Clin Nutr. 2004;23(5):1226-43.

13. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gomez J et al. Bioelectrical impedance analysis-part II: utilization in clinical practice. Clin Nutr. 2004;23(6):1430-53.

14. Maughan RJ, Shirreffs SM. Hydrometry, Hydration Status, and Performance. In: Lukaski HC, editor. Body Composition: Health and Performance in Exercise and Sport. Florida: CRC Press, Taylor & Francis Group; 2017. pp. 49-68.

15. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J Appl Physiol. 1997;82(5):1542-58.

16. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 1986;60(4):1327-32.

17. Lukaski HC. Applications of bioelectrical impedance analysis: a critical review. Basic Life Sci. 1990;55:365-74.

18. Lukaski HC, Siders WA. Validity and accuracy of regional bioelectrical impedance devices to determine whole-body fatness. Nutrition. 2003;19(10):851-7.

19. Lukaski HC. Evolution of bioimpedance: a circuitous journey from estimation of physiological function to assessment of body composition and a return to clinical research. Eur J Clin Nutr. 2013;67(1):S2-9.

20. Oppliger RA, Nielsen DH, Shetler AC, Crowley ET, Albright JP. Body composition of collegiate football players: bioelectrical impedance and skinfolds compared to hydrostatic weighing. J Orthop Sports Phys Ther. 1992;15(4):187-92.

21. Piccoli A, Rossi B, Pillon L, Bucciante G. A new method for monitoring body fluid variation by bioimpedance analysis: the RXc graph. Kidney Int. 1994;46(2):534-9.

22. Buffa R, Saragat B, Cabras S, Rinaldi AC, Marini E. Accuracy of specific BIVA for the assessment of body composition in the United States population. PLoS One. 2013;8(3):e58533.

23. Lukaski HC, Kyle UG, Kondrup J. Assessment of adult malnutrition and prognosis with bioelectrical impedance analysis: phase angle and impedance ratio. Curr Opin Clin Nutr Metab Care. 2017;20(5):330-9.

24. Buffa R, Saragat B, Succa V, Ruggiu R, Carboni L, Putzu PF et al. Elderly subjects with type 2 diabetes show altered tissue electrical properties. Nutrition. 2013;29(1):132-7.

25. Castillo-Martínez LC-R, E.; Orea-Tejeda, A.; González Islas, D. G.; Rodríguez García, W. D.; Santillán Díaz, C.; Gutiérrez Rodríguez, A. E.; Vázquez Durán, M.; Keirns Davies, C. Cachexia assessed by bioimpedance vector analysis as a prognostic indicator in chronic stable heart failure patients. Nutrition. 2012;28(9):886-91.

26. Haas V, Riedl A, Hofmann T, Nischan A, Burghardt R, Boschmann M et al. Bioimpedance and bioimpedance vector analysis in patients with anorexia nervosa. Eur Eat Disord Rev. 2012;20(5):400-5.

27. Marini E, Buffa R, Saragat B, Coin A, Toffanello ED, Berton L et al. The potential of classic and specific bioelectrical impedance vector analysis for the assessment of sarcopenia and sarcopenic obesity. Clin Interv Aging. 2012;7:585-91.

28. Norman K, Smoliner C, Kilbert A, Valentini L, Lochs H, Pirlich M. Disease-related malnutrition but not underweight by BMI is reflected by disturbed electric tissue properties in the bioelectrical impedance vector analysis. Br J Nutr. 2008;100(3):590-5.

29. Saragat B, Buffa R, Mereu E, Succa V, Cabras S, Mereu R et al. Nutritional and psycho-functional status in elderly patients with Alzheimer's disease. J Nutr Health Aging. 2012;16(3):231-6.

30. Walter-Kroker A, Kroker A, Mattiucci-Guehlke M, Glaab T. A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. Nutr J. 2011;10(1):1.

31. Piccoli A. Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status. Kidney Int. 2004;65(3):1050-63.

32. Baldwin CE, Paratz JD, Bersten AD. Body composition analysis in critically ill survivors: a comparison of bioelectrical impedance spectroscopy devices. J Parenter Enteral Nutr. 2012;36(3):306-15.

33. Piccoli A, Brunani A, Savia G, Pillon L, Favaro E, Berselli M et al. Discriminating between body fat and fluid changes in the obese adult using bioimpedance vector analysis. Int J Obes. 1998;22(2):97-104.

34. Savastano S, Belfiore A, Di Somma C, Mauriello C, Rossi A, Pizza G et al. Validity of bioelectrical impedance analysis to estimate body composition changes after bariatric surgery in premenopausal morbidly women. Obes Surg. 2010;20(3):332-9.

35. Lukaski HC, Moore M. Bioelectrical impedance assessment of wound healing. J Diabetes Sci Technol. 2012;6(1):209-12.

36. Nescolarde LY, J.; Medina, D.; Rodas, G.; Rosell-Ferrer, J. Assessment and follow-up of muscle injuries in athletes by bioimpedance: preliminary results. Conf Proc IEEE Eng Med Biol Soc. 2011;1137-40.

37. Nescolarde L, Yanguas J, Lukaski H, Alomar X, Rosell-Ferrer J, Rodas G. Localized bioimpedance to assess muscle injury. Physiol Meas. 2013;34(2):237-45.

38. Lukaski HC, Hall CB, Siders WA. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 2007;23(7-8):543-50.

39. Bronhara B, Piccoli A, Pereira JC. Fuzzy linguistic model for bioelectrical impedance vector analysis. Clin Nutr. 2012;31(5):710-6.

40. Barbosa-Silva MC, Barros AJ. Bioelectrical impedance analysis in clinical practice: a new perspective on its use beyond body composition equations. Curr Opin Clin Nutr Metab Care. 2005;8(3):311-7.

41. Martinsen OG, Grimnes S. Bioimpedance and bioelectricity basics. London: Academic press; 2011.

42. Piccoli A, Pastori G, Codognotto M, Paoli A. Equivalence of information from single frequency v. bioimpedance spectroscopy in bodybuilders. Br J Nutr. 2007;97(1):182-92.

43. Nescolarde L, Lukaski H, De Lorenzo A, de-Mateo-Silleras B, Redondo-del-Río M, Camina-Martín M. Different displacement of bioimpedance vector due to Ag/AgCl electrode effect. Eur J Clin Nutr. 2016;70(12):1401–7.

44. Codognotto M, Piazza M, Frigatti P, Piccoli A. Influence of localized edema on whole-body and segmental bioelectrical impedance. Nutrition. 2008;24(6):569-74.

45. Walker D, Brown B, Smallwood R, Hose D, Jones D. Modelled current distribution in cervical squamous tissue. Physiol Meas. 2002;23(1):159.

46. Dehghan M, Merchant AT. Is bioelectrical impedance accurate for use in large epidemiological studies? Nutr J. 2008;7(1):1.

47. Kushner RF, Gudivaka R, Schoeller DA. Clinical characteristics influencing bioelectrical impedance analysis measurements. Am J Clin Nutr. 1996;64(3):423S-427S.

48. Gatterer H, Schenk K, Laninschegg L, Schlemmer P, Lukaski H, Burtscher M. Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. PLoS One. 2014;9(10):e109729.

49. Carrasco-Marginet M, Castizo-Olier J, Rodriguez-Zamora L, Iglesias X, Rodriguez FA, Chaverri D et al. Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. PLoS One. 2017;12(6):e0178819.

50. Heavens KR, Charkoudian N, O'Brien C, Kenefick RW, Cheuvront SN. Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the resistance-reactance-score graph method. Am J Clin Nutr. 2016;103(3):724-9.

51. Antoni G, Marini E, Curreli N, Tuveri V, Comandini O, Cabras S et al. Energy expenditure in caving. PLoS One. 2017;12(2):e0170853.

52. Laferriere P, Lemaire ED, Chan AD. Surface electromyographic signals using dry electrodes. IEEE Trans Instrum Meas. 2011;60(10):3259-68.

53. Berneis K, Keller U. Bioelectrical impedance analysis during acute changes of extracellular osmolality in man. Clin Nutr. 2000;19(5):361-6.

54. Gualdi-Russo E, Toselli S. Influence of various factors on the measurement of multifrequency bioimpedance. Homo. 2002;53(1):1-16.

55. Deurenberg P, Weststrate JA, Paymans I, van der Kooy K. Factors affecting bioelectrical impedance measurements in humans. Eur J Clin Nutr. 1988;42(12):1017-22.

56. Rodríguez G, Moreno LA, Sarría A, Fleta J, Bueno M. Assessment of nutritional status and body composition in children using physical anthropometry and bioelectrical impedance: influence of diurnal variations. J Pediatr Gastroenterol Nutr. 2000;30(3):305-9.

57. Slinde F, Rossander-Hulthén L. Bioelectrical impedance: effect of 3 identical meals on diurnal impedance variation and calculation of body composition. Am J Clin Nutr. 2001;74(4):474-8.

58. Lukaski HC. Biological indexes considered in the derivation of the bioelectrical impedance analysis. Am J Clin Nutr. 1996;64(3):397S-404S.

59. Rush EC, Crowley J, Freitas IF, Luke A. Validity of hand-to-foot measurement of bioimpedance: standing compared with lying position. Obesity. 2006;14(2):252-7.

60. Gartner A, Maire B, Delpeuch F, Sarda P, Dupuy RP, Rieu D. Importance of electrode position in bioelectrical impedance analysis. Am J Clin Nutr. 1992;56(6):1067-8.

61. Thomas B, Ward L, Cornish B. Bioimpedance spectrometry in the determination of body water compartments: accuracy and clinical significance. Appl Radiat Isot. 1998;49(5):447-55.

62. Foster KR, Lukaski HC. Whole-body impedance–what does it measure? Am J Clin Nutr. 1996;64(3):388S-396S.

63. Slinde F, Bark A, Jansson J, Rossander-Hulthén L. Bioelectrical impedance variation in healthy subjects during 12 h in the supine position. Clin Nutr. 2003;22(2):153-7.

64. O'Brien C, Young AJ, Sawka MN. Bioelectrical impedance to estimate changes in hydration status. Int J Sports Med. 2002;23(5):361-6.

65. Buono MJ, Burke S, Endemann S, Graham H, Gressard C, Griswold L et al. The effect of ambient air temperature on whole-body bioelectrical impedance. Physiol Meas. 2004;25(1):119.

66. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr. 1986;44(3):417-24.

67. Gleichauf C, Roe D. The menstrual cycle's effect on the reliability of bioimpedance measurements for assessing body composition. Am J Clin Nutr. 1989;50(5):903-7.

68. Lusseveld E, Peters ETJ, Deurenberg P. Multifrequency bioelectrical impedance as a measure of differences in body water distribution. Ann Nutr Metab. 1993;37(1):44-51.

69. Mitchell CO, Rose J, Familoni B, Winters S, Ling F. The use of multifrequency bioelectrical impedance analysis to estimate fluid volume changes as a function of the menstrual cycle. In: Ellis K, Eastman J, editors. Human Body Composition. New York: Springer; 1993. pp. 189-91.

70. Hermens H, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, et al. European recommendations for surface electromyography: Results of the SENIAM Project. Enschede: Roessingh Research and Development; 1999, pp. 1-122.

71. Caton JR, Mole PA, Adams WC, Heustis DS. Body composition analysis by bioelectrical impedance: effect of skin temperature. Med Sci Sports Exerc. 1988;20(5):489-91.

72. Liang M, Norris S. Effects of skin blood flow and temperature on bioelectric impedance after exercise. Med Sci Sports Exerc. 1993;25(11):1231-9.

73. Evans W, McClagish H, Trudgett C. Factors affecting the in vivo precision of bioelectrical impedance analysis. Appl Radiat Isot. 1998;49(5-6):485-7.

74. Marini E, Sergi G, Succa V, Saragat B, Sarti S, Coin A, Manzato E, Buffa R. Efficacy of specific bioelectrical impedance vector analysis (BIVA) for assessing body composition in the elderly. J Nutr Health Aging. 2013;17(6):515-21.

75. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. Am J Clin Nutr. 2003;77(2):331-40.

76. Buchholz AC, Bartok C, Schoeller DA. The validity of bioelectrical impedance models in clinical populations. Nutr Clin Pract. 2004;19(5):433-46.

77. Segal KR, Burastero S, Chun A, Coronel P, Pierson Jr RN, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. Am J Clin Nutr. 1991;54(1):26-9.

78. Lukaski HC, Piccoli A. Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions. In: Preedy RV, editor.

Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease. New York, NY: Springer New York; 2012. pp. 287-305.

79. Piccoli A, Pillon L, Dumler F. Impedance vector distribution by sex, race, body mass index, and age in the United States: standard reference intervals as bivariate Z scores. Nutrition. 2002;18(2):153-67.

80. Pillon L & Piccoli A. (2003). U.S. Patent Application No. 10/740,911.

81. Baumgartner RN, Chumlea WC, Roche AF. Bioelectric impedance phase angle and body composition. Am J Clin Nutr. 1988;48(1):16-23.

82. Mattar J. Application of total body bioimpedance to the critically ill patient. Brazilian Group for Bioimpedance Study. New Horiz. 1996;4(4):493-503.

83. Zdolsek HJ, Lindahl OA, Sjöberg F. Non-invasive assessment of fluid volume status in the interstitium after haemodialysis. Physiol Meas. 2000;21(2):211.

84. Mahalanobis PC. On the generalized distance in statistics. Proc Natl Inst Sci India. 1936;2:49-55.

85. Micheli ML, Pagani L, Marella M, Gulisano M, Piccoli A, Angelini F et al. Bioimpedance and impedance vector patterns as predictors of league level in male soccer players. Int J Sports Physiol Perform. 2014;9(3):532-9.

86. Koury JC, Trugo NMF, Torres AG. Phase Angle and Bioelectrical Impedance Vectors in Adolescent and Adult Male Athletes. Int J Sports Physiol Perform. 2014;9(5):798-804.

87. Bonuccelli A, Marzatico F, Stesina G, Stefanini L, Buonocore D, Rucci S et al. Bioelectrical impedance vector analysis (BIVA) to evaluate seasonal variations in body composition of elite soccer players. J Int Soc Sports Nutr. 2011;8(1):P37.

88. Bonuccelli A, Causarano A, Marzatico F, Catanese S, D'Urbano G, Beschi S et al. Innovative assessment of seasonal variations in body composition of elite soccer players with the integrated analysis DXA-BIVA. J Int Soc Sports Nutr. 2012;9(1):P1.

89. Gatterer H, Schenk K, Ferrari P, Faulhaber M, Schopp E, Burtscher M. Changes in hydration status of soccer players competing in the 2008 European Championship. J Sports Med Phys Fitness. 2011;51(1):89-94.

90. Mascherini G, Gatterer H, Lukaski H, Burtscher M, Galanti G. Changes in hydration, body-cell mass and endurance performance of professional soccer players through a competitive season. J Sports Med Phys Fitness. 2014;55(7-8):749-55.

91. Piccoli A, Piazza P, Noventa D, Pillon L, Zaccaria M. A new method for monitoring hydration at high altitude by bioimpedance analysis. Med Sci Sports Exerc. 1996;28(12):1517-22.

92. Reljic D, Hassler E, Jost J, Friedmann-Bette B. Rapid weight loss and the body fluid balance and hemoglobin mass of elite amateur boxers. J Athl Train. 2013;48(1):109-17.

93. Fukuda DH, Stout JR, Moon JR, Smith-Ryan AE, Kendall KL, Hoffman JR. Effects of resistance training on classic and specific bioelectrical impedance vector analysis in elderly women. Exp Gerontol. 2016;74:9-12.

94. Meleleo D, Bartolomeo N, Cassano L, Nitti A, Susca G, Mastrototaro G et al. Evaluation of body composition with bioimpedence. A comparison between athletic and non-athletic children. Eur J Sport Sci. 2017;17(6):710-9.

95. Mascherini G, Petri C, Galanti G. Integrated total body composition and localized fat-free mass assessment. Sport Sci Health. 2015;11(2):217-25.

96. Pollastri L, Lanfranconi F, Tredici G, Schenk K, Burtscher M, Gatterer H. Body fluid status and physical demand during the Giro d'Italia. Res Sports Med. 2016;24(1):30-8.

97. Pollastri L, Lanfranconi F, Tredici G, Burtscher M, Gatterer H. Body Water Status and Short-term Maximal Power Output during a Multistage Road Bicycle Race (Giro d'Italia 2014). Int J Sports Med. 2016;37(4):329-33.

98. Silvestre R, Kraemer WJ, West C, Judelson DA. Body composition and physical performance during a National Collegiate Athletic Association Division I men's soccer season. J Strength Cond Res. 2006;20(4):962.

99. Kalapotharakos V, Strimpakos N, Vithoulka I, Karvounidis C. Physiological characteristics of elite professional soccer teams of different ranking. J Sports Med Phys Fitness. 2006;46(4):515.

100. Melchiorri G, Monteleone G, Andreoli A, Calla C, Sgroi M, De Lorenzo A. Body cell mass measured by bioelectrical impedance spectroscopy in professional football (soccer) players. J Sports Med Phys Fitness. 2007;47(4):408.

101. Silvestre R, West C, Maresh CM, Kraemer WJ. Body composition and physical performance in men's soccer: a study of a National Collegiate Athletic Association Division I team. J Strength Cond Res. 2006;20(1):177.

102. Arnason A, Sigurdsson SB, Gudmundsson A, Holme I, Engebretsen L, Bahr R. Physical fitness, injuries, and team performance in soccer. Med Sci Sports Exerc. 2004;36(2):278-85.

103. Cian C, Barraud P, Melin B, Raphel C. Effects of fluid ingestion on cognitive function after heat stress or exercise-induced dehydration. Int J Psychophysiol. 2001;42(3):243-51.

104. Maughan R. Impact of mild dehydration on wellness and on exercise performance. Eur J Clin Nutr. 2003;57(S2):S19.

105. Oppliger RA, Bartok C. Hydration Testing of Athletes. Sports Med. 2002;32(15):959-71.

106. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

107. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

108. Stewart A, Marfell-Jones M, Olds T, de Ridder H. International standards for anthropometric assessment. Murcia, Spain: International Society for the Advancement of Kinanthropometry. 2011.

109. Peiffer JJ, Abbiss CR, Nosaka K, Peake JM, Laursen PB. Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. J Sci Med Sport. 2009;12(1):91-6.

110. Piccoli A. Bioelectric impedance measurement for fluid status assessment. Contrib Nephrol. 2010;164:143-52. doi: 10.1159/000313727.

111. Moreno MV, Ribbe-Cornet E, Rebeyrol J, Vannicatte A, Krief L. Evaluation of a new impedancemeter to independently measure extracellular, intracellular and total body water volumes: application to the measurement of hydration. Med Biol Eng Comput. 2015;53(10):989-99. doi: 10.1007/s11517-015-1305-8.

112. Armstrong LE, Maresh CM, Castellani JW, Bergeron MF, Kenefick RW, LaGasse KE, et al. Urinary indices of hydration status. Int J Sport Nutr. 1994;4(3):265-79.

113. Foster C, Daines E, Hector L, Snyder AC, Welsh R. Athletic performance in relation to training load. Wis Med J. 1996;95(6):370-4.

114. Borg G, Hassmén P, Lagerström M. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. Eur J Appl Physiol Occup Physiol. 1987;56(6):679-85.

115. Bescós R, Rodríguez F-A, Iglesias X, Knechtle B, Benítez A, Marina M, et al. Physiological demands of cyclists during an ultra-endurance relay race: a field study report. Chin J Physiol. 2011;54(5):339-46.

116. Barrero A, Erola P, Bescós R. Energy balance of triathletes during an ultra-endurance event. Nutrients. 2015;7(1):209-22. doi: 10.3390/nu7010209.

117. Zuntz N. Ueber die Bedeutung der verschiedenen Nährstoffe als Erzeuger der Muskelkraft. Pflugers Arch Gesamte Physiol Menschen Tiere. 1901;83(10-12):557-71.

118. Cohen J. A power primer. Psychol Bull. 1992;112(1):155-9.

119. Piccoli A, Pastori G. BIVA software. Padova: Department of Medical and Surgical Sciences, University of Padova, Italy. 2002.

120. Hotelling H. The generalization of Student's ratio. Breakthroughs in Statistics: Springer; 1992. p. 54-65.

121. Saragat B, Buffa R, Mereu E, De Rui M, Coin A, Sergi G, et al.. Specific bioelectrical impedance vector reference values for assessing body composition in the Italian elderly. Exp Gerontol. 2014;50:52-6. doi: 10.1016/j.exger.2013.11.016.

122. De Palo T, Messina G, Edefonti A, Perfumo F, Pisanello L, Peruzzi L, et al. Normal values of the bioelectrical impedance vector in childhood and puberty. Nutrition. 2000;16(6):417-24.

123. Piccoli A, Nigrelli S, Caberlotto A, Bottazzo S, Rossi B, Pillon L, et al. Bivariate normal values of the bioelectrical impedance vector in adult and elderly populations. Am J Clin Nutr. 1995;61(2):269-70.

124. Campa F, Toselli S. Bioimpedance Vector Analysis of Élite, Sub-Élite and Low-Level Male Volleyball Players. Int J Sports Physiol Perform. 2018:1-13.

125. Giorgi A, Vicini M, Pollastri L, Lombardi E, Magni E, Andreazzoli A, et al. Bioimpedance patterns and bioelectrical impedance vector analysis (BIVA) of road cyclists. J Sports Sci. 2018:1-6.

126. Andreoli A, Monteleone M, Van Loan M, Promenzio L, Tarantino U, De Lorenzo A. Effects of different sports on bone density and muscle mass in highly trained athletes. Med Sci Sports Exerc. 2001;33(4):507-11.

127. Convertino VA. Blood volume: its adaptation to endurance training. Med Sci Sports Exerc. 1991;23(12):1338-48.

128. Meyer F, Volterman KA, Timmons BW, Wilk B. Fluid balance and dehydration in the young athlete assessment considerations and effects on health and performance. Am J Lifestyle Med. 2012;6(6):489-501.

129. Sawka MN. Physiological consequences of hypohydration: exercise performance and thermoregulation. Med Sci Sports Exerc. 1992;24(6):657-70.

130. Fellmann N. Hormonal and plasma volume alterations following endurance exercise. Sports Med. 1992;13(1):37-49.

131. Chertow GM, Lowrie EG, Wilmore DW, Gonzalez J, Lew NL, Ling J, et al. Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. J Am Soc Nephrol. 1995;6(1):75-81.

132. Koury JC, de Oliveira-Junior AV, Portugal MRC, de Oliveira KDJF, Donangelo CM. Bioimpedance parameters in adolescent athletes in relation to bone maturity and biochemical zinc indices. J Trace Elem Med Biol. 2018;46:26-31.

133. Brumitt J, Cuddeford T. Current Concepts of Muscle and Tendon Adaptation to Strength and Conditioning. Int J Sports Phys Ther. 2015;10(6):748.

134. Sperelakis N. Cell physiology source book: essentials of membrane biophysics. Elsevier; 2012.

135. McDermott BP, Anderson SA, Armstrong LE, Casa DJ, Cheuvront SN, Cooper L, et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. J Athl Train. 2017;52(9):877-95.

136. Maughan RJ, Shirreffs SM, Leiper JB. Errors in the estimation of hydration status from changes in body mass. J Sports Sci. 2007;25(7):797-804.

137. Gatterer H, Wille M, Faulhaber M, Lukaski H, Melmer A, Ebenbichler C, et al. Association between body water status and acute mountain sickness. PLoS One. 2013;8(8):e73185. doi: 10.1371/journal.pone.0073185.

138. Gerth WA, Montgomery LD, Wu Y-C. A computer-based bioelectrical impedance spectroscopic system for noninvasive assessment of compartmental fluid redistribution, Conf Proc Third Annual IEEE Symposium on Computer-Based Medical Systems. 1990;446-456.

139. Matthie J, Withers P. The ambiguities of predicting total body water and body cell mass with a single frequency (50KHz) measurement of bioimpedance. J Am Soc Nephrol. 1995;6(6):1682-5.

140. Lofgren B. The electrical impedance of a complex tissue and its relation to changes in volume and fluid distribution; a study on rat kidneys. Acta Physiol Scand Suppl. 1951;81:1-51.

141. Knechtle B, Duff B, Schulze I, Kohler G. The effects of running 1,200 km within 17 days on body composition in a female ultrarunner-Deutschlandlauf 2007. Res Sports Med. 2008;16(3):167-88. doi: 10.1080/15438620802104120.

142. Knechtle B, Fraire OS, Andonie J, Kohler G. Effect of a multistage ultra-endurance triathlon on body composition: World Challenge Deca Iron Triathlon 2006. Br J Sports Med. 2008;42(2):121-5.

143. Knechtle B, Wirth A, Knechtle P, Rosemann T. Increase of total body water with decrease of body mass while running 100 km nonstop—formation of edema? Res Q Exerc Sport. 2009;80(3):593-603.

144. Fellmann N, Ritz P, Ribeyre J, Beaufrère B, Delaître M, Coudert J. Intracellular hyperhydration induced by a 7-day endurance race. Eur J Appl Physiol Occup Physiol 1999;80(4):353-9. doi: 10.1007/s004210050603.

145. Maughan R, Whiting P, Davidson R. Estimation of plasma volume changes during marathon running. Br J Sports Med. 1985;19(3):138-41.

146. Deuster PA, Singh A, Hofmann A, Moses FM, Chrousos GC. Hormonal responses to ingesting water or a carbohydrate beverage during a 2 h run. Med Sci Sports Exerc. 1992;24(1):72-9.

147. Schrier RW, Hano J, Keller HI, Finkel RM, Gilliland PF, Cirksena WJ, et al. Renal, metabolic, and circulatory responses to heat and exercise: Studies in military recruits during summer training, with implications for acute renal failure. Ann Intern Med. 1970;73(2):213-23.

148. Freund BJ, Claybaugh JR, Dice MS, Hashiro GM. Hormonal and vascular fluid responses to maximal exercise in trained and untrained males. J Appl Physiol. 1987;63(2):669-75.

149. Melin B, Eclache J, Geelen G, Annat G, Allevard A, Jarsaillon E, et al. Plasma AVP, neurophysin, renin activity, and aldosterone during submaximal exercise performed until exhaustion in trained and untrained men. J Appl Physiol Occup Physiol. 1980;44(2):141-51.

150. Freund BJ, Shizuru EM, Hashiro GM, Claybaugh JR. Hormonal, electrolyte, and renal responses to exercise are intensity dependent. J Appl Physiol. 1991;70(2):900-6.

151. Neumayr G, Pfister R, Hoertnagl H, Mitterbauer G, Prokop W, Joannidis M. Renal function and plasma volume following ultramarathon cycling. Int J Sports Med. 2005;26(1/02):2-8.

152. Lehmann M, Huonker M, Dimeo F, Heinz N, Gastmann U, Treis N, et al. Serum amino acid concentrations in nine athletes before and after the 1993 Colmar ultra triathlon. Int J Sports Med. 1995;16(3):155-9.

153. Mischler I, Boirie Y, Gachon P, Pialoux V, Mounieri R, Rousset P, et al. Human albumin synthesis is increased by an ultra-endurance trial. Med Sci Sports Exerc. 2003;35(1):75-81. doi: 10.1097/00005768-200301000-00013.

154. Fellmann N, Bedu M, Giry J, Pharmakis-Amadieu M, Bezou M-J, Barlet J-P, et al. Hormonal, fluid, and electrolyte changes during a 72-h recovery from a 24-h endurance run. Int J Sports Med. 1989;10(6):406-12.

155. Wu H-J, Chen K-T, Shee B-W, Chang H-C, Huang Y-J, Yang R-S. Effects of 24 h ultra-marathon on biochemical and hematological parameters. World J Gastroenterol. 2004;10(18):2711.

156. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. Eur J Appl Physiol. 2007;99(4):443-7.

157. Skenderi KP, Kavouras SA, Anastasiou CA, Yiannakouris N, Matalas A. Exertional rhabdomyolysis during a 246-km continuous running race. Med Sci Sports Exerc. 2006;38(6):1054-7.

158. Uberoi H, Dugal J, Kasthuri A, Kolhe V, Kumar A, Cruz S. Acute renal failure in severe exertional rhabdomyolysis. J Assoc Physicians India. 1991;39(9):677-9.

159. Koller A, Mair J, Schobersberger W, Wohlfarter T, Haid C, Mayr M, et al. Effects of prolonged strenuous endurance exercise on plasma myosin heavy chain fragments

and other muscular proteins. Cycling vs running. J Sports Med Phys Fitness. 1998;38(1):10-7.

160. MacSearraigh ET, Kallmeyer JC, Schiff HB. Acute renal failure in marathon runners. Nephron. 1979;24(5):236-40.

161. Carmona G, Roca E, Guerrero M, Cussó R, Irurtia A, Nescolarde L, et al. Sarcomere disruptions of slow fiber resulting from mountain ultramarathon. Int J Sports Physiol Perform. 2015;10(8):1041-7.

162. Millet GY, Tomazin K, Verges S, Vincent C, Bonnefoy R, Boisson R-C, et al. Neuromuscular consequences of an extreme mountain ultra-marathon. PLoS One. 2011;6(2):e17059.

163. Overgaard K, Lindstrøm T, Ingemann-Hansen T, Clausen T. Membrane leakage and increased content of Na+-K+ pumps and Ca2+ in human muscle after a 100-km run. J Appl Physiol. 2002;92(5):1891-8.

STUDIES

STUDY I

PLOS ONE

RESEARCH ARTICLE

Bioelectrical impedance vector analysis (BIVA) in sport and exercise: Systematic review and future perspectives

Jorge Castizo-Olier[1,2], Alfredo Irurtia[1,2]*, Monèm Jemni[3], Marta Carrasco-Marginet[1,2], Raúl Fernández-García[4], Ferran A. Rodríguez[1]

1 INEFC-Barcelona Sport Sciences Research Group, National Institute of Physical Education of Catalonia (INEFC), University of Barcelona (UB), Barcelona, Spain, **2** Catalan School of Kinanthropometry, National Institute of Physical Education of Catalonia (INEFC), University of Barcelona (UB), Barcelona, Spain, **3** Department of Sport Science, Qatar University, Doha, Qatar, **4** Department of Electronic Engineering, Polytechnic University of Catalonia, Barcelona, Spain

* airurtia@gencat.cat

Citation: Castizo-Olier J, Irurtia A, Jemni M, Carrasco-Marginet M, Fernández-García R, Rodríguez FA (2018) Bioelectrical impedance vector analysis (BIVA) in sport and exercise: Systematic review and future perspectives. PLoS ONE 13(6): e0197957. https://doi.org/10.1371/journal.pone.0197957

Editor: Antoine Nordez, Universite de Nantes, FRANCE

Received: November 6, 2017

Accepted: May 13, 2018

Published: June 7, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: Jorge Castizo-Olier was a pre-doctoral researcher supported by a grant from Generalitat de Catalunya, Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR; http://agaur.gencat.cat/ca/inici/) (2015FI_B00217). Furthermore, this study was conducted with the support of the National Institute of Physical

Abstract

Background

Bioelectrical impedance vector analysis (BIVA) is a general concept that includes all methodologies used in the analysis of the bioelectrical vector, whereas the "classic" BIVA is a patented methodology included among these methods of analysis. Once this was clarified, the systematic review of the literature provides a deeper insight into the scope and range of application of BIVA in sport and exercise.

Objective

The main goal of this work was to systematically review the sources on the applications of BIVA in sport and exercise and to examine its usefulness and suitability as a technique for the evaluation of body composition, hydration status, and other physiological and clinical relevant characteristics, ultimately to trace future perspectives in this growing area, including a proposal for a research agenda.

Methods

Systematic literature searches in PubMed, SPORTDiscus and Scopus databases up to July, 2017 were conducted on any empirical investigations using phase-sensitive bioimpedance instruments to perform BIVA within exercise and sport contexts. The search included healthy sedentary individuals, physically active subjects and athletes.

Result

Nineteen eligible papers were included and classified as sixteen original articles and three scientific conference communications. Three studies analysed short-term variations in the hydration status evoked by exercise/training through whole-body measurements, eleven assessed whole-body body composition changes induced by long-term exercise, four

Education of Catalonia (INEFC), University of Barcelona (UB). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: BCM, Body cell mass; BIA, Bioelectrical impedance analysis; BIS, Bioelectrical impedance spectroscopy; BIVA, Bioelectrical impedance vector analysis; BM, Body mass; BMI, Body mass index; Cm, Cell membrane capacitance; DXA, Dual-energy X-ray absorptiometry; ECM, Extracellular mass; ECW, Extracellular water; ECW/TBW ratio, Extracellular / total body water ratio; FFM, Fat-free mass; FM, Fat mass; H, Body height; Hotelling's T^2test, Test comparing mean two group vectors; ICW, Intracellular water; Mahalanobis' D, Multidimensional distance between a point P and the mean of a group; MF-BIA, Multi-frequency bioelectrical impedance analysis; P_{osm}, Plasma osmolality; PA, Phase angle; R, Bioelectrical resistance (R/h when adjusted by height); RXc graph, R/h vs. Xc/h probabilistic plot; SD, Standard deviation; SF-BIA, Single-frequency bioelectrical impedance analysis; TBW, Total body water; Xc, Bioelectrical reactance (Xc/h when adjusted by height); Z, Bioelectrical impedance; Z vector, Vector yield by the RXc graph.

compared athletic groups or populations using the whole-body assessment, and two analysed bioelectrical patterns of athletic injuries or muscle damage through localised bioimpedance measurements.

Conclusions

BIVA is a relatively new technique that has potential in sport and exercise, especially for the assessment of soft-tissue injury. On the other hand, the current tolerance ellipses of "classic" BIVA are not a valid method to identify dehydration in individual athletes and a new approach is needed. "Specific" BIVA, a method which proposes a correction of bioelectrical values for body geometry, emerges as the key to overcome "classic" BIVA limitations regarding the body composition assessment. Further research establishing standardised testing procedures and investigating the relationship between physiology and the bioelectrical signal in sport and exercise is needed.

Introduction

Bioelectrical impedance analysis (BIA) is a non-invasive technique widely used in body composition assessment [1–5], nutritional status [5–7], and hydration status [2, 8, 9], all considered areas of interest to monitor general health and well-being [10], but also training and performance levels. However, conventional BIA is limited by the use of models and algorithms that assume relations between body components are constant and correlated with each other during stable periods, which are used to estimate through simple or multiple regression equations an unknown body component from a related measured variable (bioimpedance) [11]. Multiple validation studies demonstrated solid relationship between bodily impedance and fluid volume (e.g. compared to isotope dilution), but their validity and accuracy of prediction are population-specific [12]. Furthermore, the standard errors of the best BIA regression equations were estimated to be, for instance, ~3–8% for total body water (TBW) and ~3–6% for fat-free mass (FFM), both considered too large to be used in clinical setting [12, 13]. In the exercise and sport practice, this is especially relevant. For example, dehydration processes lower than these standard errors which may affect negatively the sport performance could be not adequately detected [14].

BIA measures body tissues opposition to the flow of a low-level, alternating radiofrequency electric current. Bioelectrical impedance (Z)—i.e. the tissues opposition to the electric current flow—, the vector sum of the resistance (R)—i.e. the major resistance to the current through intra- and extracellular ionic fluids—and the reactance (Xc)—i.e. the additional opposition due to the capacitive elements such as cell membranes, tissue interfaces, and non-ionic substances. BIA has been performed using single- (SF-BIA) or multiple-frequency (MF-BIA) electrical current. Standard SF-BIA uses a single frequency of 50 kHz to estimate TBW and FFM, but does not differentiate intracellular water (ICW) and extracellular water (ECW), respectively. In an attempt to overcome this, MF-BIA tries to estimate ICW and ECW by measuring a spectrum of frequencies through different mathematical models [12]. However, MF-BIA models have significant limitations, such as the required use of body mass (BM) as an independent variable. Most scientific evidence shows that the use of both SF-BIA and MF-BIA lead to prediction errors in healthy people [5, 15–17] and even larger errors in people with clinical conditions [18, 19]. In spite of the widespread use of BIA in the clinical and field settings,

mainly in the estimation of body composition, such as fat mass (FM) and FFM, or TBW, ICW and ECW, its accuracy is compromised because of its reliance on regression equations, mostly derived from non-athletic or sport-specific populations [5], and assumptions such as constant tissue isotropy or constant tissue hydration, conditions that are not frequently met [5, 11].

Alternative techniques such as the measure of the phase angle (PA) or the "classic" bioelectrical impedance vector analysis ("classic" BIVA) [20] emerged to overcome the above-mentioned BIA limitations, basing their main strength on the use of raw impedance parameters. It is important to mention that the present review distinguished between the term "classic" BIVA (commonly termed BIVA in the literature), the methodology patented by Pillon and Piccoli [21], and a more general concept that include all methodologies using vector analysis, i.e. bioelectrical impedance vector analysis (BIVA in the present review). This general concept include the whole-body assessment methods "classic" BIVA and "specific" BIVA (which is a methodology that tries to overcome some limitations of "classic" BIVA), and the localised bioelectrical impedance vector analysis (which is a method proposed for the identification and follow-up of muscle injuries). Once this was noted, it has to been clarified that "classic" BIVA does not provide quantitative estimates of tissue mass (kg) or fluid volumes (L). Instead, it is qualitative and semi-quantitative evaluation of body cell mass (BCM) and hydration [22, 23]. The number of publications using "classic" BIVA in clinical practice increased exponentially during the last decade due to its strengths [11, 18, 24–30]. Nowadays, "classic" BIVA is a widely used technique in medicine as a tool for the assessment of hydration and nutritional status (e.g. fluid imbalance and wasting of lean tissues, respectively) in different clinical conditions, such as renal disease [31], critically ill patients [32], obesity [33] and morbid obesity [34], pulmonary disease [30], anorexia nervosa [26], cachexia [25], sarcopenia and sarcopenic obesity [27], Alzheimer's disease [29], heart failure [25], gastrointestinal disease [28], diabetes [24], wound healing [35], muscle injury assessment [36, 37], and pregnancy and postpartum [38]. Validation studies of "classic" BIVA have shown a significant association of bioelectrical values with hydration [11, 39], and nutritional status [11] in clinical conditions. Several studies have compared "classic" BIVA parameters with conventional BIA and other measures of body composition such as dual-energy X-ray absorptiometry (DXA), anthropometry, and clinical evaluation in samples of healthy and sick populations with mixed results (for review see [4, 11, 40]).

There has been a rapid growth of interest in the application of BIVA in sport and exercise research and practice in the recent years. On the one hand, "classic" BIVA is being used to characterise the body composition (i.e. hydration status and BCM) of athletes and active individuals [36, 41–43] and to monitor body composition longitudinal changes induced by exercise or sport practice [44–57]. On the other hand, the localised bioimpedance vector analysis is being applied for the identification and follow-up of muscle injuries [36, 37]. The importance of assessing the body composition of athletes lies in the fact that the physical stress imposed during trainings and competitions may lead to body composition alterations, which can be detrimental to athletes [58]. Furthermore, body composition has been suggested to discriminate athletes of different performance levels [59, 60] and has been shown to influence physical performance [61] and sport success [62]. On the other hand, the importance of monitoring the hydration status in exercise and sport is because dehydration is recognised to impair sport performance [63, 64], as well as increasing the injury risk [65]. Monitoring body fluid variations may help to adequately prescribe fluid intake and thus limit deleterious effects. Furthermore, the identification of injury and its follow-up during recovery until return-to-play depends on expensive methods, which are not accessible to everyone. Therefore, the increase in the number of publications regarding BIVA in the exercise and sport field seems justified in order to investigate the applicability of the method for assessments in real time and in a precise, accurate, reliable, non-invasive, portable, inexpensive, safe and simple way. In addition, since the

current scientific literature in this field is still scarce and very heterogeneous, a compilation of the current knowledge is needed in order to suggest a research agenda.

Objectives

This systematic review aims to summarise the current knowledge on the applications of BIVA in sport and exercise, and to evaluate the usefulness and suitability of the method in assessing body composition, hydration status, and other physiological and clinical conditions in healthy sedentary people, physically active and trained individuals. Ultimately, this review attempts to outline future perspectives in this field and to suggest a research agenda.

Methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied to undertake the present review [66]. PRISMA checklist was also used to elaborate the systematic review protocol [67].

Eligibility criteria

This study reviewed and analysed methodological, clinical, and empirical studies using phase-sensitive devices to perform the analysis within the context of exercise and sport. The phase-sensitivity characteristic is important since non phase-sensitive instruments do not measure Xc, and the proper way to apply BIVA needs both R and Xc. Articles that have used BIVA in healthy sedentary people, physically active individuals and athletes of all levels were eligible for review. Studies were screened for eligibility on the following inclusion criteria: (a) empirical investigations with BIVA measures taken in human subjects performing acute or chronic exercise; (b) empirical investigations with BIVA measures taken in healthy sedentary people, physically active individuals and athletes; c) studies where data acquisition was performed with the appropriate methodology; (d) studies published in a peer-reviewed journal and/or in relevant congress proceedings; and (e) studies published in English language. No restrictions in terms of study design, setting, country or time frame were considered.

Information sources

A computer-based literature search was conducted for the period 1994–2017, ending by July 2017, of PubMed, SPORTDiscus and Scopus databases (Fig 1).

Search strategy

Title, abstract, and keyword fields were searched in each of the aforementioned databases using the following search terms and syntax: ("BIVA" OR "vector*") AND ("hydration" OR "body water").

Study records

Records were exported from the electronic databases to a reference management software (EndNote, v. X5, Thomson Reuters, 2011) and duplicate references were removed. Fig 1 displays the flow chart of study identification and eligibility for the systematic review.

The eligible articles after removing duplicates were screened by two investigators (JCO, AI), with disagreement settled by consensus. An initial screening of titles, abstracts and keywords was performed in order to check for inclusion criteria and to exclude obviously irrelevant records using the eligibility criteria (Fig 1). Differences in study eligibility for review were compared and deviations were discussed with a third investigator (FAR) until consensus could be

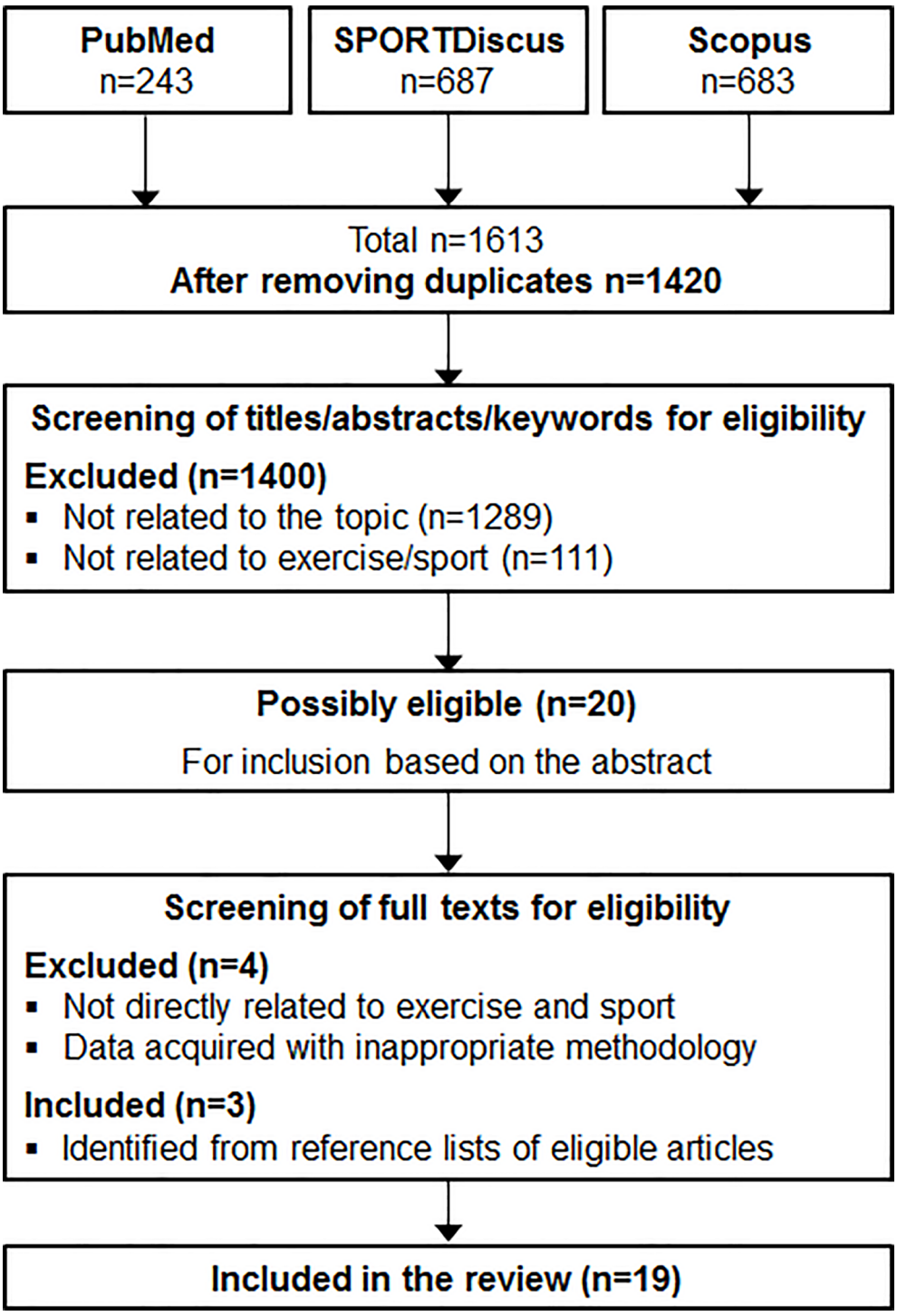

Fig 1. Flow chart of study identification and eligibility for the systematic review.

https://doi.org/10.1371/journal.pone.0197957.g001

reached. When a paper could not be rejected with certainty it was included in the eligible papers for full text evaluation. Then, articles were assessed for eligibility through a full-text screening, and those meeting the established criteria were included in the review. The reference lists of articles retrieved for inclusion in the review up to this point were searched to

identify other relevant investigations. The number of studies meeting the pre-specified inclusion criteria and those excluded and reasons for their exclusion were recorded (Fig 1).

Each selected article was reviewed for information on (1) bibliographic characteristics (type of publication, authors, year and journal); (2) aims of the investigation; (3) study design and methodology; (4) sample characteristics (number, population, gender, age, exercise activity, sport discipline, and sport competitive level of subjects); (5) BIA device employed; (6) electrode distribution; (7) BIVA approach (whole-body BIVA or localised bioimpedance vector analysis); (8) vector displacement and (9) comparative technique (e.g. other indicators to assess body composition and fluid status, injury assessment).

Data items and prioritisation

Full texts were reviewed in search for the following main variables: bioelectrical resistance (R, R/h), reactance (Xc, Xc/h), Z, PA, RXc graph, TBW, ICW, ECW, FM, FFM and BCM. Bioelectrical measures and directly derived parameters were considered the main outcome from the population studies or experimental interventions. From a methodological point of view, comparisons of BIVA outcomes with other measures of body composition and fluid status assessment could underpin the validity of the technique and, therefore, the latter were considered additional outcomes.

Results

Search outcome

After removal of duplicates, 1420 records were identified, which were reduced to 20 after screening titles, abstracts and keywords for eligibility (Fig 1). After full-text evaluation, 19 studies matched the selection criteria and were included in the qualitative synthesis analysis and summarised in Tables 1–4. Table 5 compiles the information about the baseline bioelectrical parameters and vector position of the participants analysed in the studies included in the present review.

The reviewed studies were sixteen original articles and three scientific conference communications. Publication date ranged from 1996 to 2017, yet only two studies were published before 2011, corroborating the novelty of the technique in the field of sport science.

Participants

A total number of 1667 subjects participated in the different studies, yet most took part in a soccer population study (n = 893) [41] an athletic vs. non-athletic comparative investigation (n = 219) [53] and a multisport comparative research (n = 195) [43]. Most studies were performed in males and only four included females [51–53, 57]. Only three studies analysed non-adult populations [43, 53, 57]. Fourteen studies were carried out with elite or professional athletes.

Finding outcomes

Three studies were aimed at analysing short-term changes (<24 hours) in the hydration status induced by exercise and training [47, 51, 57] (Table 1), eleven assessed body composition changes induced by exercise at the long term (≥ 7 days) [44–46, 48–50, 52–56] (Table 2), three compared athletic groups or populations [41–43] (Table 3), and two of the articles related bioelectrical patterns to athletic injury or muscle damage [36, 37] (Table 4).

Table 1. BIVA studies analysing short-term changes (<24 hours) in the hydration status induced by exercise and training.

Study	Publication	Aim	Design	Methodology	n	Sex	Age	Sport/Exercise	Level	BIA device	Electrode distribution	Vector/BIA differences (Yes / No)*	Comparative technique
Gatterer et al. 2014 [47]	Original article	To analyse bioelectrical changes induced by exercise under heat stress (environmental chamber) with hydration biomarkers	Short-term vector changes (1 h of exercise)	Analysis of intra-individual and intra-group differences. Comparison with the healthy reference population	14	M	24.1±1.7	Self-rated intensity (Borg Scale) cycle ergometer test	Well trained subjects	BIA 101 ASE, Akern/RJL (P-S)	Whole-body	Yes	Directional changes in vector values towards the upper pole of the ellipses occurred along with BM and plasma osmolality changes after exercise
Antoni et al. 2017 [51]	Original article	To analyse bioelectrical changes induced by a subterranean exploration	Short-term vector changes (~10 h of physical activity)	Analysis of intra-group and inter-group differences. Comparison with the healthy reference population	40	F, M	44.0±19	Caving	Beginners, amateurs and experts	BIA 101 ASE, Akern/RJL (P-S)	Whole-body	Yes (Xc and PA, only in men)	Directional changes in vector values towards the upper pole of the ellipses occurred along with a significant increase in BM in the group of men
Carrasco-Marginet et al. 2017 [57]	Original article	To analyse bioelectrical changes induced by a synchronised swimming training	Short-term vector changes (~2.5–3.5 h of exercise)	Analysis of intra-group and inter-group differences. Comparison with the healthy reference population	49	F	Pre-junior (n = 34): 13.9±0.9 Junior (n = 15): 16.3±0.6	Synchronised swimming	Elite	Z-Metrix, Bioparhom (P-S)	Whole-body	Yes	Directional changes in vector values towards the upper pole of the ellipses and significant mean vector differences occurred along with BM changes after exercise

M: males; F: females; P-S: phase-sensitive device; BIA: bioelectrical impedance analysis; BIVA: bioelectrical impedance vector analysis; Xc: reactance; h: height; BM: body mass

* Significance level: $p<0.05$

https://doi.org/10.1371/journal.pone.0197957.t001

Table 2. BIVA studies analysing long-term (≥7 days) changes in body composition induced by exercise and training.

Study	Publication	Aim	Design	Methodology	N	Sex	Age	Sport/ Exercise	Level	BIA device	Electrode distribution	Vector/BIA differences (Yes / No)*	Comparative technique
Piccoli et al. 1996 [49]	Original article	To analyse bioelectrical changes induced by a high altitude climbing expedition	Long-term vector changes (~12 weeks)	Analysis of intra-individual and intra-group differences. Comparison with the healthy reference population	7	M	25 (22–28)	Climbing	Healthy subjects	BIA-101, Akern/ RJL Systems (P-S)	Whole-body	Yes	Bioelectrical changes correlated with changes in BM and hydration biomarkers
Gatterer et al. 2011 [46]	Original article	To analyse bioelectrical changes induced by two soccer matches	Long-term vector changes (~1–2 weeks)	Analysis of intra-group differences. Comparison with the healthy reference population	14	M	Starters (n = 7): 24.3±3.0 Non-starters (n = 7): 26.0±5.0	Soccer	Elite	BIA 2000-M, Data Input GmbH (P-S)	Whole-body	Yes	Significant vector displacement along with BM changes were observed in the starters group between the first and the second match
Bonuccelli et al. 2011 [44]	Scientific congress communication	To analyse bioelectrical changes induced by a soccer season	Long-term vector changes (whole season)	Analysis of intra-group differences	18	M	27.6±4.9	Soccer	Elite	BIA-101, Akern/ RJL Systems (P-S)	Whole-body	Yes	No comparative technique was reported
Bonuccelli et al. 2012 [45]	Scientific congress communication	To analyse bioelectrical and DXA changes induced by a soccer season	Long-term vector changes (whole season)	Analysis of intra-group differences	10	M	26.7±3.0	Soccer	Elite	BIA-101, Akern/ RJL Systems (P-S)	Whole-body	Yes	BIVA was sensitive to body composition changes (identified by DXA) through a soccer season
Reljic et al. 2013 [50]	Original article	To analyse bioelectrical changes with hydration biomarkers	Long-term vector changes (unspecified duration)	Analysis of intra-group differences. Comparison with the healthy reference population	17	M	Weight-loss group (n = 10): 19.7±3.2 Control (n = 7): 18.4±2.2	Boxing	Elite	BIA-101, Akern/ RJL Systems (P-S)	Whole-body	Yes	Directional changes in vector values towards the upper pole of the ellipses occurred along with significant changes in BM and blood parameters within few days before competition
Mascherini et al. 2014 [48]	Original article	To analyse bioelectrical changes induced by a soccer season	Long-term vector changes (whole season)	Analysis of intra-group differences. Comparison with the soccer specific reference population	18	M	21.8±3.0	Soccer	Professional	BIA-101 ASE, Akern/ RJL Systems (P-S)	Whole-body	Yes	Changes in the vector length correlated with changes in the endurance performance

(*Continued*)

Table 2. (Continued)

Study	Publication	Aim	Design	Methodology	N	Sex	Age	Sport/ Exercise	Level	BIA device	Electrode distribution	Vector/BIA differences (Yes / No)*	Comparative technique
Mascherini et al. 2015 [54]	Original article	To analyse bioelectrical changes induced by a soccer training program	Long-term bioelectrical changes (50 days)	Analysis of intra-group differences. Comparison with the healthy reference population	59	M	22.5±5.6	Soccer	Elite	BIA-101 ASE, Akern/ RJL Systems (P-S)	Whole-body and localised	Yes	Bioelectrical differences in the whole-body and localised assessments were found along with some anthropometric measures changes after 50 days of training
Fukuda et al. 2016 [52]	Original article	To analyse bioelectrical changes induced by a resistance training program	Long-term vector changes (6 months)	Analysis of intra-group differences	20	F	71.9±6.9	Full-body resistance training program	Healthy, ambulatory subjects	Quantum II, RJL Systems (P-S)	Whole-body	Yes	Significant training effects were found for PA after the training program. No relationship was observed between changes in strength and BIA after 6 months.
Pollastri et al. 2016 [55]	Original article	To analyse bioelectrical changes induced by a multistage road bicycle race (Giro d'Italia 2014)	Long-term vector changes (3 weeks)	Analysis of intra-group differences. Comparison with the healthy reference population	9	M	28.2±4.7	Cycling	Professional	BIA-101 ASE, Akern/ RJL Systems (P-S)	Whole-body	Yes	BIA vector changes were not related to power output or RPE
Pollastri et al. 2016 [56]	Original article	To analyse bioelectrical changes induced by a multistage road bicycle race (Giro d'Italia 2014)	Long-term vector changes (3 weeks)	Analysis of intra-group differences	8	M	28.8±4.7	Cycling	Elite	BIA-101 ASE, Akern/ RJL Systems (P-S)	Whole-body	Yes	BIA vector changes correlated with maximal mean power of different time durations depending on the stage
Meleleo et al. 2017 [53]	Original article	To analyse bioelectrical changes induced by daily competitive sport	Long-term vector changes (1 year)	Analysis of intra-group and inter-group differences	219	F, M	Non-athletic group: 9.3 (8.2–10.5) Athletic group: 9.5 (8.0–10.5)	Swimming Gymnastics	Healthy subjects	BIA-101 ASE, Akern/ RJL Systems (P-S)	Whole-body	Yes	Bioelectrical differences were found along with a lack of difference in BMI between groups

M: males; F: females; P-S: phase-sensitive device; BIA: bioelectrical impedance analysis; BIVA: bioelectrical impedance vector analysis; DXA: dual-energy X-ray absorptiometry; R: resistance; Xc: reactance; PA: phase angle; h: height; BM: body mass; RPE: rating of perceived exertion; BMI: body mass index

* Significance level: $p<0.05$

https://doi.org/10.1371/journal.pone.0197957.t002

Table 3. BIVA studies analysing bioelectrical differences between populations.

Study	Publication	Aim	Design	Methodology	N	Sex	Age	Sport/ Exercise	Level	BIA device	Electrode distribution	Vector/BIA differences (Yes / No)*	Comparative technique
Piccoli et al. 2007 [42]	Original article	To assess the equivalence of information between BIA (50 kHz) and BIS in two different groups	Single measure	Inter-group analysis. Comparison with the healthy reference population	60	M	Bodybuilders (n = 30): 32.1 ±5.7 Controls (n = 30): 25.2 ±5.3	Bodybuilding	Professional	SEAC SFB3, UniQuest-SEAC (P-S); BIA-101, RJL Systems (P-S)	Whole-body	Yes	R and Xc (50 kHz) correlated with other frequencies. Estimated TBW with BIS correlated with Sun's formula (50 kHz)
Micheli et al. 2014 [41]	Original article	To assess BIVA in soccer players and establish new specific tolerance ellipses	Single measure	Inter-group analysis. Comparison with the healthy reference population	893	M	24.1±5.1	Soccer	Elite and professional	BIA-101, Akern/RJL Systems (P-S)	Whole-body	Yes	Elite and high-level soccer players registered significant bioelectrical and BM differences compared with lower performance levels
Koury et al. 2014 [43]	Original article	To assess BIVA in adolescent and adult athletes	Single measure	Inter-group analysis. Comparison with the healthy reference population	195	M	Adolescents (n = 105): 15.1 ±2.1 Adults (n = 90): 28.9±7.3	Athletics (n = 25) Soccer (n = 50) Swimming (n = 22) Water polo (n = 15) Triathlon (n = 20) Basketball (n = 20) Adventure running (n = 6) Cycling (n = 15) Marathon (n = 15) Judo (n = 7)	Elite	Quantum BIA-101Q, RJL-101 (P-S)	Whole-body	Yes	PA correlated with BM and age

M: males; F: females; P-S: phase-sensitive device; BIA: bioelectrical impedance analysis; BIVA: bioelectrical impedance vector analysis; BIS: bioelectrical impedance spectroscopy; R: resistance; Xc: reactance; PA: phase angle; BM: body mass; TBW: total body water

* Significance level: $p<0.05$

https://doi.org/10.1371/journal.pone.0197957.t003

Table 4. BIVA studies analysing bioelectrical changes induced by injury.

Study	Publication	Aim	Design	Methodology	N	Sex	Age	Sport/ Exercise	Level	BIA device	Electrode distribution	Vector/BIA differences (Yes / No) *	Comparative technique
Nescolarde et al. 2011 [36]	Scientific congress communication	To analyse whole-body and localised bioelectrical differences between two sports, and to assess muscle injuries	Single measure	Inter-group analysis. Comparison with the healthy reference population	14	M	>18.0	Soccer (n = 10) Basketball (n = 4)	Professional	BIA-101, Akern-RJL Systems (P-S)	Whole-body and localised	Yes	Localised BIA was sensitive to different types of injury diagnosed by magnetic resonance imaging
Nescolarde et al. 2013 [37]	Original article	To analyse bioelectrical changes induced by injury and its recovery	Long-term bioelectrical changes (9 to 75 days)	Analysis of intra-individual differences (injury identification and follow-up)	3	M	22.0 ±3.6	Soccer	Professional	BIA-101, Akern/ RJL Systems (P-S)	Localised	Yes	Localised BIA was consistent with reference magnetic resonance imaging diagnoses with differing levels of injury severity

M: males; P-S: phase-sensitive device; BIA: bioelectrical impedance analysis; BIVA: bioelectrical impedance vector analysis

* Significance level: $p<0.05$

https://doi.org/10.1371/journal.pone.0197957.t004

Table 5. Baseline bioelectrical parameters and vector position of the participants analysed in the studies included in the present review.

Study	BMI (kg/m^2)	R/h (Ω/m)	Xc/h (Ω/m)	PA (°)	Vector position on the BIVA point graph	Other comments
Nescolarde et al. 2011 [36]	Soccer: 23.2 ±1.5 Basketball: 24.3±1.1	Soccer: 268.9 ±22.4 Basketball: 221.8±22.9	Soccer: 37.4 ±3.8 Basketball: 28.8±4.9	Soccer: 7.9 ±0.7 Basketball: 7.4±0.6	Soccer: The mean vector was plotted inside the "athlete" quadrant of the reference population, outside the range of normal hydration Basketball: The mean vector was plotted inside the "obese" quadrant of the reference population, outside the range of normal hydration	
Nescolarde et al. 2013 [37]	NR	NR	NR	NR	NR	
Micheli et al. 2014 [41]	All: 23.3±1.6	All: 263.9 ±26.2	All: 33.8±3.9	All: 7.3±0.6	The individual vectors were scattered in both "athlete" and "obese" quadrants of the reference population, outside and inside the range of normal hydration	Some individual vectors were plotted inside the "lean" quadrant of the reference population, outside and inside the range of normal hydration
Piccoli et al. 2007 [42]	BB: 28.9±3.6	BB: NR	BB: NR	BB: 8.6±1.1	The mean vector was plotted in the limit of the 95% ellipse of the "obese" quadrant of the reference population, outside the range of normal hydration	
Koury et al. 2014 [43]	Adolescent: 20.2±3.0 Adult: 22.7 ±2.7	Adolescent: 302.0±71.0 Adult: 252.4 ±33.8	Adolescent: 36.2±6.7 Adult: 35.4 ±4.9	Adolescent: 6.9±0.9 Adult: 8.0 ±0.7	Adolescent: The majority of the individual vectors were scattered inside the "obese" quadrant of the reference population, either when all the participants were plotted and when the comparison was performed according to paired sport modalities. Most of them were plotted outside the range of normal hydration Adult: The majority of the individual vectors were scattered in both "athlete" and "obese" quadrants of the reference population, either when all the participants were plotted and when the comparison was performed according to paired sport modalities. Most of them were plotted outside the range of normal hydration	
Bonuccelli et al. 2011 [44]	NR	NR	NR	NR	NR	
Bonuccelli et al. 2012 [45]	NR	NR	NR	NR	NR	
Gatterer et al. 2011 [46]	S: 23.5±0.9 NS: 24.3±1.1 All: 23.9±1.1	NR	NR	NR	The mean vectors of both groups were plotted inside the "obese" quadrant of the reference population, close to the "athlete" one, outside the range of normal hydration	
Gatterer et al. 2014 [47]	NR	284.1±23.0	37.5±3.3	NR	Mean and individual vectors were plotted inside the "athlete" quadrant of the reference population, the majority of them outside the range of normal hydration	Only one individual vector was plotted inside the "obese" quadrant of the reference population, close to the "athlete" area, outside the range of normal hydration
Mascherini et al. 2014 [48]	NR	272.7±24.9	36.0±4.0	7.5±0.5	The mean vector was plotted inside the "lean" quadrant of the reference population, within the range of normal hydration	

(*Continued*)

Table 5. (Continued)

Study	BMI (kg/m^2)	R/h (Ω/m)	Xc/h (Ω/m)	PA (°)	Vector position on the BIVA point graph	Other comments
Piccoli et al. 1996 [49]	22.9 (21.8–25.6)	256.5	31.2	NR	The mean vector was plotted inside the "obese" quadrant of the reference population, in the limit of the range of normal hydration	The article shows two examples of individual vectors, one plotted inside the "athlete" quadrant of the reference population (outside the range of normal hydration) and the other inside the "obese" one (within the range of normal hydration)
Reljic et al. 2013 [50]	NR	NR	NR	NR	The mean vectors of both groups were plotted inside the "athlete" quadrant of the reference population, within the range of normal hydration	
Antoni et al. 2017 [51]	F: 21.8±2.1 M: 24.7±3.0	F: 388.6±34.1 M: 296.6±38.5	F: 33.7± 3.2 M: 28.1± 5.9	F: 8.7± 0.8 M: 9.4± 1.3	F: The mean vector of women was plotted between the "cachexic" and the "lean" quadrants of the reference population, close to the left ones, within the range of normal hydration M: The mean vector of men was plotted inside the "cachexic" quadrant of the reference population, close to the "obese" one, within the range of normal hydration	
Carrasco-Marginet et al. 2017 [57]	Co: 18.0±1.9 Jr: 19.3±1.3 All: 18.4±1.8	Co: 328.4 ±38.8 Jr: 299.9±21.6 All: 319.7 ±36.7	Co: 40.0±4.5 Jr: 39.6±2.2 All: 39.9±3.9	Co: 7.0±0.5 Jr: 7.5±0.4 All: 7.1±0.5	Co: The majority of the individual vectors were plotted outside and inside the 95% tolerance ellipse of the "obese" quadrant of the reference population, outside the range of normal hydration Jr: The majority of the individual vectors were plotted outside the 95% tolerance ellipse of the "obese" quadrant of the reference population, outside the range of normal hydration. None of them were located inside the "athlete" quadrant	Some of the Co individual vectors were plotted inside the "athlete" quadrant of the reference population, most of them outside the range of normal hydration
Fukuda et al. 2016 [52]	24.5±3.0	376.9±45.4	31.6±5.5	4.8±0.6	NR	
Meleleo et al. 2017 [53]	F: 17.68 M: 19.68	F: 465.6±13.7 M: 418.7±14.9	F: 46.8±1.6 M: 40.6± 1.7	F: 5.8± 0.1 M: 5.6± 0.2	NR	
Mascherini et al. 2015 [54]	23.3±1.5	259.8±27.0	35.5±3.5	7.8±0.6	The mean vector was plotted inside the "athlete" quadrant of the reference population, outside the range of normal hydration	
Pollastri et al. 2016 [55]	NR	NR	NR	NR	NR	
Pollastri et al. 2016 [56]	NR	NR	NR	NR	Mean and individual vectors were plotted inside the "athlete" quadrant of the reference population, outside the range of normal hydration	

BMI: body mass index; R: resistance; Xc: reactance; h: height; PA: phase angle; BIVA: bioelectrical impedance vector analysis; NR: not reported; BB: bodybuilders; S: starters; NS: non-starters; Co: pre-junior; Jr: junior; F: females; M: males

https://doi.org/10.1371/journal.pone.0197957.t005

Bioelectrical measures

Most studies used whole-body electrode distribution, one used localised electrode distribution to analyse injury-induced bioelectrical changes [37], and two combined the standard whole-body and the localised techniques [36, 54]. The majority of the investigations used single-

frequency impedance devices (50 kHz), two used multiple frequency bioimpedance analysers [46, 57] and one used both types of devices [42].

Discussion

BIVA applications in sport and exercise

Sporting population studies. These types of studies (Table 3) consist of single measure, cross-sectional protocols aiming to characterise sporting group samples in terms of bioelectrical data. As observed by Koury et al. [43], athletes exhibit similar trends of PA variation with age to those of the general population of the same sex and age, with a positive correlation ($r = 0.63$, $p = 0.0004$) in adolescents and a negative correlation ($r = -0.27$, $p = 0.009$) in adults. Vectors shifted to the left and with greater PA were found in both adolescent and adult athletes compared to the corresponding reference populations, which is consistent with the results reported by other studies for soccer players [41] and synchronised swimmers [57], suggesting that these differences are due to sport-specific adaptations [41]. In comparison with adolescent athletes, the mean vector of adult athletes also showed a shift to the left. Both shifts to the left indicate increased BCM and fluid content, and might reflect a better cell functioning [41].

Regarding the vector position on the RXc graph, the trend is to be outside the 50% tolerance ellipse of the respective reference population in both adolescent and adult athletes. According to this, Piccoli et al. [42] also found the mean impedance vector of bodybuilders almost completely outside the 95% tolerance ellipse of the reference population. This reflects a specific body composition and suggests that specific tolerance ellipses are needed for sport populations [36, 41, 57]. To date, only two studies [41, 57] have characterised sport-specific populations. The relationship between the new specific tolerance ellipses (for each sport, gender, age and race) and the hydration status, body composition and sport performance level should be analysed, in order to represent significant hydration changes (that compromise health or performance) or target zones of impedance vectors for athletes. Nevertheless, it is possible that a new approach is required for the exercise and sports field, beyond the current BIVA point graph, based on 50–95% tolerance ellipses and quadrants related to clinical outputs. With regard to the hydration assessment, it should be noted that fluid overload (overhydration) is not common in healthy athletes. Therefore, the analysis of the hydration status should be related to euhydration and physiological dehydration processes. In this way, as mentioned in Heavens et al. [68] regarding the identification of dehydration with single and serial measurements according to the tolerance ellipses of the reference population, the limits for "normal hydration" (individuals positioned within the 50% tolerance ellipses, according to the literature [18, 69]) should be reviewed, since subjects experiencing high levels of fluid loss can still be identified as euhydrated [68]. Other studies related to sport and exercise [47, 49] identified some individuals as euhydrated after significant BM decreases. Moreover, as shown in Table 5, the majority of the studies analysed identify the athletes outside the 50% tolerance ellipse. This is probably due to a range of "normal hydration" comprised by the ellipses wider than a hydration status/change considered as "dehydration" through other methodologies [68]. Nevertheless, the conclusions of Heavens et al. [68] should be confirmed with the appropriate methodology, since the study was not performed with a phase-sensitive device, and therefore, they could not obtain the real value of Xc. Therefore, although directional changes in vector values from serial measurements seem to be consistent with fluid loss, the current BIVA point graph is not a valid method to detect dehydration in individual athletes. Research investigating different levels of dehydration and their relationship with the new specific tolerance ellipses is needed in order to identify the limit of "normal hydration". Furthermore, different types of dehydration can be experienced in sport: a) hypertonic dehydration (i.e. primarily a loss of

water) is a common type of dehydration developed after exercise in which heavy sweating occurs; b) hypotonic dehydration (i.e. primarily a loss of electrolyte) and c) isotonic dehydration (i.e. equal losses of electrolytes and water), both may be developed by athletes competing in aesthetic-type sports and in weight classification sports in which fasting, vomiting and diuretic use are common behaviours [65]. Thus, research is needed related to the sensitivity of "classic" BIVA to each type of dehydration, as well as the behaviour of each one with regard to the tolerance ellipses. On the other hand, it should be investigated the relationship between the new specific tolerance ellipses and different sport performance levels. Maybe different sectors of the tolerance ellipses identify target zones for the athletes. With regard to the body composition assessment and in accordance with "classic BIVA", athletes have been identified in the upper left quadrant of the reference population and obese individuals in the lower left quadrant. This would generally imply greater R/h and Xc/h values of the athletes. Nevertheless, as mentioned in the literature [22, 70], according to the electro-physical assumptions, FFM is characterised by a greater conductivity in comparison with the poorly hydrated adipose tissue, not justifying the relative shortness of vectors of obese individuals with respect to the athletes, unless contemplating their generally greater FM, fluid overload and body size. Furthermore, the vector position of athletes regarding the tolerance ellipses of the general reference population is controversial [4]. As mentioned by Buffa et al. [4], athletic individuals are not always plotted in the "athlete" quadrant of the reference population and their vectors often overlap the "obesity" area. This controversy can be observed in Table 5. From the nineteen investigations analysed, six studies did not report vectors distribution with regard to the reference population and only four found the majority or all the vectors of athletes positioned in the "athlete" area [47, 50, 54, 56]. Comparable vector position of athletes and obese individuals would imply similar values of R/h and Xc/h. The already mentioned factors FM and fluid overload could compensate the bioelectrical values between both individuals, not being "classic" BIVA (50 kHz) able to detect the differences (e.g. discriminating fluids distribution between compartments, with greater ICW content in athletes). Moreover, as mentioned in the literature [22, 70], "classic BIVA" would be characterised by a limited sensitivity in assessing the features of body composition (i.e. FM and FFM) due to the no consideration of the effect of cross-sectional areas of the body which interferes with bioelectrical values as well as lengths, according to the basic conductor theory (impedance is proportional to the conductor length and inversely related to its cross-sectional area) [71]. This effect of cross-sectional areas is particularly relevant in sport sciences because athletes of different disciplines generally differ in their body shape. To overcome this limitation of "classic" BIVA, a relatively new procedure ("specific" BIVA) has been developed [22, 70]. This method proposes a correction of bioelectrical values for body geometry and it has proven to be effective in identifying the relative proportion of FM in adults and elderly [22, 70]. Although the inclusion of anthropometric measurements can make these plots more sample-specific and perhaps less generalizable than "classic" BIVA, this adaptation may be an advance when comparing athletes with different body composition (in terms of FM and FFM). Therefore, it should be further investigated in the sports field.

Koury et al. [43] observed that the distance between the confidence ellipses of adolescent and adult athletes was lower than between the ellipses among their respective reference populations, either considering all sport modalities or only paired modalities. The authors speculated that the intense training reduced the differences between adolescent and adult individuals, although this is still to be elucidated. In their study, vector and PA differences were due to differences in R/h, significantly lower in adult athletes than in adolescent athletes, with no differences in Xc/h. Similar to these findings, Micheli et al. [41] reported that in soccer players of higher competitive level, vectors shifted to the left due to a decrease in R/h, with no

difference in Xc/h compared to those in lower soccer divisions. This shift to the left was also found between elite and high-level players. As suggested by Micheli et al. [41], these results reflect different ICW content (adult > adolescent; higher > lower sport levels), since the ECW/TBW ratio is inversely related to PA [72], and it could be due to the hypertrophy of muscle fibres. Furthermore, despite similar training loads among players of the highest level, differences may be due to different individual responses to the training load, or they could also be an indicator of better training and/or recovery strategies in elite teams [41]. Carrasco-Marginet et al. [57] also reported a shift to the left, with no difference in Xc/h, in young synchronised swimmers of higher competitive level. Nevertheless, since higher-level swimmers were older than the lower-level ones, it should be investigated whether the differences were due to biological maturation, to specific training or a combination of both.

As noted, a greater PA accompanying a vector shifted to the left has been observed in adult athletes compared to the healthy reference population [36, 41–43, 46, 49, 50]. This is due to i) a decreased R/h as a result of a different body composition, probably due, among other factors, to a greater muscle mass, muscle glycogen reserves and plasma volume [73, 74], and ii) an increased Xc/h, probably due to an increase in the size and number of muscle cells (hypertrophy and hyperplasia, respectively), although the last one is still a controversial topic [75]. However, since a decreased R/h is also related to greater FM [33], further research is needed in order to clarify the reason for this behaviour. Furthermore, Xc/h is not only conditioned by the cell size, but also by the thickness and composition of the cell membranes and also by the distance between them, due to their relationship with membrane capacitance (Cm) [76]. In this way, lower Xc/h values have been documented in bodybuilders (the best model of extreme muscle hypertrophy) compared to healthy active people and with no differences with the healthy reference population [42]. However, vectors shifted to the left with lower PA have been reported in competitive children in comparison with healthy control groups due to significantly lower Xc/h values in absence of differences in R/h [53]. The authors suggested that it could be due to an increase in the size of the section of the limbs or to a greater 'sufferance' in cell membranes maybe due to bad response to the workloads (over-training). Therefore, the interpretation of Xc/h in these cases remains unresolved.

Nescolarde et al. [36] reported differences in both whole-body and localised mean Z vectors of soccer and basketball players, attributed to the different body structure between both disciplines. Soccer players presented a whole-body vector shifted to the right on the BIVA graph compared to basketball players, due to greater R/h and Xc/h. Regarding the localised vectors, soccer players showed a shift to the left of quadriceps and hamstrings vectors, due to a decrease in R/h and an increase in Xc/h. On the other hand, gastrocnemius vectors of soccer players showed a shift to the right, due to an increase in R/h and Xc/h. The muscle groups in lower-limbs were found to be symmetrical in athletes and this could be used to detect changes in hydration and/or muscular structure.

Short-term vector changes (<24 h after exercise). These types of studies (Table 1) are those which currently face more difficulties, since their validity can be easily compromised, mostly because of several factors that may affect the accuracy of the measurements despite any attempts to control them. To date, two studies have investigated the vector adaptations using this type of design.

Gatterer et al. [47] analysed the short-term bioelectrical adaptations in well-trained subjects after 1 hour of self-rated intensity cycle ergometer test in the heat (environmental chamber). They reported an increase in both R/h and Xc/h after exercise, as well as significant vector migration indicating fluid loss. Besides, they pointed out a negative relationship between changes in Xc/h and in plasma osmolality (P_{osm}) (r = -0.58). The authors concluded that "classic" BIVA changes mirrors water loss during exercise in the heat, and that changes in Xc/h

values reflect fluid shifts between intracellular and extracellular compartments. As mentioned before, Xc is related to Cm, which is affected by the size, thickness, composition and distance between cell membranes [76]. Exercise generates processes which modify the characteristics of muscle cells (such as changes in fluid distribution). As suggested, the cell membrane becomes thinner as the cell swells and Cm increases, and the opposite happens as the cell shrinks [77], thus affecting Xc. Besides, as the cell swells, the distance to the adjacent cell membranes decreases and Cm increases (the opposite happens as the cell shrinks), also affecting Xc. Moreover, in accordance with De Lorenzo et al. [78], variations in fluid distribution would change the impedance locus and, consequently, the characteristic frequency (Fc), defined as the frequency at which Xc presents a greater value and that it is close to 50 kHz. Thus, these variations would evoke considerable changes in Xc at 50 kHz, the frequency used in BIVA [79, 80]. Nonetheless, De Lorenzo and collaborators' hypothesis should be considered with caution because it refers to the Hanai's model, which relays on assumptions such as spherical cell shape. Therefore, multiple factors may affect Xc values and further research should focus on this parameter in exercise.

According to Gatterer et al. [47], Carrasco-Marginet et al. [57] reported significant vector displacements along to the major axis after exercise due to significant increases in R and Xc. Furthermore, the mentioned study showed that BIVA paired graph seems to identify significant vector differences after exercise inducing mild dehydration (average loss of $<1\%$ BM) in different groups of athletes.

In opposition to both studies [47, 57], Antoni et al. [51] only found a tendency to reduction of fluids (the authors related it to an extracellular water decrease given by a significant increase in Xc) along with an increased BM in a group of men and no differences in women after approximately 10 hours of subterranean exploration (caving). Factors affecting protocols measuring pre- and post-exercise (such as dietary intake during cave activity or the skin temperature in the post measurement) could have influenced their observations. Nevertheless, despite the fact that the vector changes after fluid removal and overload (the wet–dry cycle of dialysis) as a non-physiological process is clinically well-established [69], every dehydration process induced by physical exercise is consequence of scarcely explored physiological adaptations as regard of the vector behaviour, especially at cellular level (and therefore, affecting R and Xc). In literature, Xc is an indicator of dielectric mass (membranes and tissue interfaces) in soft tissues [71]. Given the results observed in sport, it is possible that the behaviour of Xc could be due to other factors and, thus, its meaning remains to be clarified.

Long-term vector changes. Studies investigating long-term ($\geq$7 days) vector adaptations (Table 2), have some protocol-specific advantages in comparison with investigations focused on acute vector changes, mainly because the quality of the bioelectrical signal can be assessed independently from the acute adaptations related to exercise.

BCM and extracellular mass (ECM) have been proposed as representatives of ICW and ECW, respectively [46]. Nevertheless, it is important to note that the estimation of fluid volumes and cell mass with BIA prediction models is inappropriate when discussing changes in vector positions after interventions or treatments. Gatterer et al. [46], in their study assessing body composition using "classic" BIVA in the 2008 European Soccer Championship, found a significant lengthening of the vector within a period between 1 and 2 weeks. They attributed it to changes in BCM and ECW in both starters and non-starters after the first match with respect to baseline values, indicating body fluid loss. After the second match, only the athletes who played more (starters) showed a significant lengthening of the vector possibly due to a decrease in ECW. Therefore, they concluded that changes in body composition were mainly due to changes in ECW. However, their results should be taken with caution, since only analysis with appropriate reference methods (e.g. isotope dilution) can support them.

Similarly to the results of Gatterer et al. [46], rapid loss of BM protocols within a few days before competition in boxers [50] was found to be achieved mainly by isotonic dehydration (they attributed it principally due to changes in ECW), as identified by the significant vector lengthening on the RXc point graph and the decreases in plasma and blood volume. Nevertheless, as mentioned before, their results should be further investigated with appropriate reference methods for the estimation of fluid volumes, since BIA prediction models are inappropriate to discuss changes in vector positions. According to the results of Reljic et al. [50], Piccoli et al. [49], also found a significant lengthening of the vector with isotonic dehydration at high altitude (5500 m). Nevertheless, although a subsequent hypertonic dehydration was identified by a decreased BM (-3.0 kg) and several hydration biochemical markers, the vector lengthening was not significant. The causes that explain why the vector remained unchanged after such a BM loss were not elucidated, and the authors recognised the difficulty of explaining the metabolic reasons that led to such BM reduction. In any case, emphasis should be placed on the importance of not considering body fluids quantitatively only (i.e., volume), but also regarding their qualitative composition, due to the biological adaptations generated by different types of exercise. For instance, after descent to sea level, the impedance vector underwent a significant shortening and returned close to baseline values. Lastly, significant relationships were found between changes in bioelectrical variables (R/h and Xc/h) and changes in the following hydration biomarkers along measurements performed at altitude and at sea level: BM, urine volume, P_{osm}, serum Na^+, K^+, Cl^- and glucose, and urine osmolar excretion [49].

On the other hand, two studies [55, 56] found significant shortening of the vector along three weeks of multistage road bicycle race, indicating fluid gain during the tour and attributing these results to muscle oedema, haemodilution, released water from muscle glycogen oxidation, and excess fluid intake. Although the vector shortening was not related to power output or rating of perceived exertion [55], it was negatively associated with performance during the last stages [56], suggesting the authors that increases in plasma volume and improved thermoregulatory capacity could explain these outputs. Nevertheless, their results should be taken with caution, since measurements were performed approximately two hours after exercise and this could have altered the data.

Regarding studies analysing longer-term vector adaptations, Mascherini et al. [48] analysed a soccer team across a sport season and reported a significant shortening of the vector in the pre-season associated with an improvement in endurance performance possibly due to plasma volume expansion and enhanced glycogen storage. These results are in agreement with other studies [45, 54] which also found significant bioelectrical differences in the pre-season, hypothesising that they were due to fluid expansion. Bonuccelli et al. [45] and Macherini et al. [48] found a significant lengthening of the vector in the mid-season compared to pre-season results. This could indicate a reduced body fluid volume (i.e., decreased plasma or interstitial volume) despite an increased intracellular fluid associated with an increase in BCM, and consequently in PA [41]. However, while Mascherini et al. [48] reported a significant shortening of the vector at the end of the season compared to the mid-season, Bonuccelli et al. [45] observed a significant water content decrease. Sport calendars could have led to adopt training strategies inducing different performance status and evoked opposite vector displacements.

On the other hand, regarding the age-related decreases in Xc and PA [81], improvements have been reported after six months of resistance training in elderly women [52], suggesting increased amount and quality of soft tissues. These improvements were accompanied by increases in leg strength and thigh circumference. Along with these changes, BIVA showed a significant vector migration after the training program.

With regard to children, one study [53] evaluated the body composition in participants of swimming and gymnastics along one year. The baseline measurement (T0) was performed at a period preceding races and sporting events, just as the third measurement (T2) one year later. The second measurement (T1) was executed six months after T0 in a period characterised by a softer daily training. They found a significant increase in Xc from T0 to T1, along with increased PA and ICW (derived from ECW/TBW ratio). The authors hypothesised that this was due to an improvement in the muscular trophism with higher levels of intracellular proteins and glycogen and to a lower stress from training program. After one-year follow-up, no significant differences were found in R, Xc and PA. However, again, their hypotheses should be taken with caution, since fluid estimations were calculated from BIA prediction models. Variables as the type of sport and training strategy should be taken into account when monitoring along a season, since they might influence the bioelectrical measures. Moreover, also intra-group comparisons between seasons should be analysed with caution, since inter-seasonal bioelectrical variations could be effected by factors such as biological maturation.

Injury identification and follow-up. These studies [36, 37] consisted in single cross-sectional protocols aiming to identify bioelectrical patterns of change depending on the injury type and grade, and longitudinal protocols aiming at assessing bioimpedance vector sensitivity to monitor injuries and their recovery. R and Xc were found to be decreased in the injured muscles due to the oedema and to the disruption of the muscle structure, respectively. Furthermore, the more severe the injury was, the more R and Xc were decreased. On the other hand, a bioelectrical symmetry between muscular groups in lower-limbs was found. The follow-up of the injury identified bioelectrical patterns of changes similar to those in wound healing and an increase of R and Xc values were observed to values close to pre-injury.

Overall, localised bioimpedance vector analysis appears as an alternative method that could help to assess soft tissue injury and to monitor the injury recovery process [36, 37].

Prospective research applications in sport and research agenda

BIVA in sports and exercise science is an emerging area of research with potential. The present document aims, not only to systematically overview the available scientific information, but also to outline areas of priority, future perspectives and a research agenda on this topic.

From the methodological standpoint, closely related to the quality, reliability and validity of the bioelectrical signal, some issues should be deeper investigated. For example, adequate hydration protocols are required in order to assess participants in a euhydrated state. Related to this, rigorous fluid intake control before bioelectrical measurements should be performed and reported. In studies assessing BIVA after exercise, adequate protocols of cold water application before testing with different duration and temperatures in order to reduce the sources of error in bioelectrical measurements should also be investigated, adapting the protocol to the type, intensity and duration of the exercise. Core and skin temperature should be monitored pre- and post-exercise. In sport practice, baseline values for BIVA should be established before the start of any follow-up protocol (e.g. to monitor changes along a sport competition) in the attempt to guarantee an optimal hydration status and to avoid excessive fluid loss.

Further research is also required on how much some factors affect the bioelectrical signal, especially in exercise-induced acute vector change assessment (e.g. exhaustive control of quantity and composition of fluids and food intake, and time between fluids/food intake and the bioelectrical measurements). With regard to differences in the bioelectrical signal among type of electrodes, distribution of the electrodes (e.g. whole-body standard placement or eight-polar tactile distribution), and BIA devices, further research is required. Standardisation of contact electrodes is necessary for valid BIA measurements.

As for the bioelectrical parameters, especially Xc, it will be difficult to obtain conclusions as valid and accurate as possible concerning to their patterns until the behaviour of cells in the human body is not well explained using simulated circuit models (in series, in parallel or mixed), for both homeostatic and non-homeostatic conditions. Regarding Xc changes after exercise, further research is needed in order to clarify the causes of these behaviour. As for PA, its relationship with cell functioning in sport should also be addressed.

Another critical point needing further investigation is the assessment of the validity and reliability of "classic" BIVA as a method for monitoring BCM and hydration status in sports and exercise. New specific tolerance ellipses for each sport, sex, age and race, should be generated and it should be investigated whether they can be used for the classification of an individual vector (in terms of hydration status, body composition and sport performance level) and if they represent significant hydration changes (that compromise health or performance) or target zones of impedance vectors for athletes. With regard to the hydration assessment, the analysis of the hydration status should be related to euhydration and physiological dehydration processes. In this way, as for the identification of dehydration according to the tolerance ellipses of the reference population, the limits for "normal hydration" should be reviewed. Research investigating different levels of dehydration and their relationship with the new specific tolerance ellipses is needed in order to identify the limit of "normal hydration". Furthermore, research is needed related to the sensitivity of "classic" BIVA to each type of dehydration, as well as the behaviour of each one with regard to the tolerance ellipses. On the other hand, research investigating the relationship between the new specific tolerance ellipses and different sport performance levels is required. With regard to the body composition assessment, it should be further investigated the effect on the bioelectrical signal of the FM, fluid overload and cross-sectional areas of the body. Furthermore, future investigations should seek to clarify if BCM changes shown by "classic" BIVA mean actually BCM variations, different fluid distribution between compartments, or a combination of both. More research is needed with regard to the application of "specific" BIVA in the sports field. Comparisons of BIVA outcomes with validated body composition and fluid status assessment are to be undertaken to better define the basis for interpretation and application of this technique. These types of analyses should be undertaken in both laboratory and field conditions adjusted to the reality of sport. On the other hand, it is surprising to realise how few reliability studies in BIVA there are, this being a critical factor in establishing its practical application as a diagnostic tool.

With regard to the localised bioimpedance vector analysis, it seems necessary to standardise the distribution of the electrodes and generate muscle-specific ellipses in order to improve the reproducibility of bioelectrical measurements. This standardisation should consider the muscle length instead of the body height to normalise the bioelectrical values, since differences in the proportionality between subjects may lead to greater errors. Besides, the symmetry between limbs should be determined for each sport and discipline, particularly in relation with differences between dominant and non-dominant limbs and asymmetrical sports (e.g. jumps, throws, team sports, tennis). When speaking of localised assessment in injured muscles, further research is needed in order to establish ranges of alterations in bioelectrical vector outcomes, as well as the time course of injury recovery and return-to-play.

Regarding sports practice, PA and "classic" BIVA showed that the intense training changed functional and hydration parameters of the athletes [43]. It should be analysed if BCM and fluid content reflect the sport-specific adaptations of BM and composition. Furthermore, the utility of integrated evaluation of PA and BIVA to identify possible risks derived by different training loads in athletes should be investigated. Further research is also required to assess the relationship between BIVA and other body composition techniques.

Related tests in acute and long-term designs (e.g. muscle function, glycogen storage, haematological and biochemical markers, etc.) should be performed to correlate them with vector displacements, in order to understand better the cause of vector migration. In addition, vector changes at the medium term (< 7 days) should be investigated. Finally, it would be interesting to investigate whether the vector position is an indicator of different individual biological responses to the training load or if it is the result of optimised training activity and/or recovery strategy.

With regard to the technical requirements to perform valid measurements (see the S1 Appendix for more information), the bioimpedance assessment must be performed by using a phase-sensitive device at 50 kHz, in a room with neutral environment. The whole-body assessment has to be performed through the standard tetra-polar electrode distribution. On the other hand, the localised assessment needs a standardisation of the electrodes placement. The minimal distance between electrodes must be 5 cm and, in the case that is needed, the electrode which should be moved is the proximal one. Furthermore, before placing the electrodes, the skin must be prepared by shaving the electrode site to remove hair, rubbing with gel and cleaning with alcohol. Another important requirement is the use of appropriate contact electrodes (i.e. electrically neutral). For the assessment, the subject must be euhydrated, with no injuries or disease condition. The site of the electrodes should be changed in case that skin lesions are at the sight of the original electrodes location. The evaluation should be performed in fasting state (for at least 8 hours) and avoiding previous alcohol ingestion. Besides, the measurement should be performed once the bladder is voided and after at least 10 minutes of stabilisation. In longitudinal protocols with different measurements, the position of the electrodes has to be marked, in order to preserve the same location. Furthermore, the temperature of the skin should be controlled, in order to measure in the same conditions. The environmental characteristics should be identical between assessments. The measurement after exercise should be performed once the electrolytes of the skin have been removed with a shower and the skin temperature, cutaneous blood flow and bioelectrical parameters have stabilised to basal values. No food/drink should be consumed between measurements in the evaluation of acute variations after exercise. Nevertheless, in ecological protocols, where this condition is difficult to be followed, the quantity, moment and characteristics of the food/drink consumed should be registered. Furthermore, in ecological protocols, it should be taken into account that in the case that the measurement is performed < 1 hour after the food/drink intake, this ingestion will have a minimal effect on the impedance value. Thus, the type of exercise performed will determine the post-exercise stabilisation time and the moment at which the measurement can be made, which may be affected by the food/beverage intake during the exercise. On the other hand, with regard to the measurements in women, the menstrual cycle should be controlled and the comparison should be performed according to the cycle. Finally, the measurements should be performed at the same moment of the day, both for the comparison between subjects and for the intra-individual comparison between different assessments.

Limitations

The main limitations derived from the literature analysis about the use of BIVA in the sport context are: 1) the difficulty of controlling multiple sources of error that may influence the bioelectrical signal; 2) the lack of tests correlating the bioelectrical signal (vector) with other variables studied in the literature; 3) the limited scientific evidence explaining the bioelectrical behaviour of human tissues induced by exercise; 4) the formulation of possible explanations for the bioelectrical behaviour of human tissues induced by exercise with inappropriate methodologies (e.g. the use of estimated fluid volumes with BIA prediction models to discuss vector

variations); 5) the limited sensitivity of "classic" BIVA for the assessment of a) individual dehydration in exercise and b) two-compartment body composition; and 6) the scarcity of scientific information related to the use of BIVA in sport and exercise. Furthermore, we did not consider investigations in languages other than English, so an information bias might have existed.

Conclusions

The main aim of this systematic review was to summarise the current knowledge on the applications of BIVA in sport and exercise. Contexts such as body composition, hydration, and other physiological and clinical conditions in physically active and trained individuals were checked.

As explored, BIVA is a relatively new technique that has a potential in sport and exercise, yet largely unexplored, especially for soft-tissue injury assessment. Regarding the assessment of hydration status through the current BIVA point graph, this is not a valid method to identify dehydration in individual athletes and a new approach is needed. On the other hand, "classic BIVA" is inconsistent in the assessment of two-compartment body composition and the vector position of athletes with regard to the reference population seems controversial in many cases. This is possibly due, between other factors, to the no consideration of the effect of cross-sectional areas. "Specific" BIVA emerges as the key to overcome this limitation.

Proper testing procedures to control factors that may affect the bioelectrical signal, as well as valid and reliable phase-sensitive measuring devices and appropriate disposables, are key to obtain more valid and precise impedance measurements. Currently, the relationship between the bioelectrical signal and physiological adaptations induced by different types of exercise remain largely unresolved, especially in how the structure and function of the cell are altered and how these affect the behaviour of R, and in particular Xc. Therefore, future research on BIVA related to sport and exercise should focus on these challenges.

Supporting information

S1 Checklist. PRISMA checklist for the current study.
(DOC)

S1 Appendix. BIVA methodological features.
(DOCX)

Acknowledgments

Jorge Castizo-Olier was a pre-doctoral researcher supported by a grant from Generalitat de Catalunya, Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR; http://agaur.gencat.cat/ca/inici/) (2015FI_B00217). Furthermore, this study was conducted with the support of the National Institute of Physical Education of Catalonia (INEFC), University of Barcelona (UB). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors have declared that no competing interests exist.

Author Contributions

Conceptualization: Jorge Castizo-Olier, Alfredo Irurtia, Monèm Jemni, Ferran A. Rodríguez.

Data curation: Jorge Castizo-Olier.

Formal analysis: Jorge Castizo-Olier, Alfredo Irurtia, Ferran A. Rodríguez.

Investigation: Jorge Castizo-Olier, Alfredo Irurtia, Ferran A. Rodríguez.

Methodology: Jorge Castizo-Olier, Alfredo Irurtia, Ferran A. Rodríguez.

Project administration: Jorge Castizo-Olier.

Supervision: Alfredo Irurtia, Monèm Jemni, Ferran A. Rodríguez.

Writing – original draft: Jorge Castizo-Olier.

Writing – review & editing: Jorge Castizo-Olier, Alfredo Irurtia, Monèm Jemni, Marta Carrasco-Marginet, Raúl Fernández-García, Ferran A. Rodríguez.

References

1. Lukaski HC, Bolonchuk WW, Siders WA, Hall CB. Body composition assessment of athletes using bioelectrical impedance measurements. J Sports Med Phys Fitness. 1990; 30(4):434–40. PMID: 2079851
2. Segal KR. Use of bioelectrical impedance analysis measurements as an evaluation for participating in sports. Am J Clin Nutr. 1996; 64(3):469S–471S.
3. Kerr A, Slater G, Byrne N, Chaseling J. Validation of Bioelectrical Impedance Spectroscopy to Measure Total Body Water in Resistance-Trained Males. Int J Sport Nutr Exerc Metab. 2015; 25(5):494–503. https://doi.org/10.1123/ijsnem.2014-0188 PMID: 26011918
4. Buffa R, Mereu E, Comandini O, Ibanez ME, Marini E. Bioelectrical impedance vector analysis (BIVA) for the assessment of two-compartment body composition. Eur J Clin Nutr. 2014; 68(11):1234–40. https://doi.org/10.1038/ejcn.2014.170 PMID: 25139557
5. Moon JR. Body composition in athletes and sports nutrition: an examination of the bioimpedance analysis technique. Eur J Clin Nutr. 2013; 67(1):S54–9.
6. Loenneke JP, Wilson JM, Wray ME, Barnes JT, Kearney ML, Pujol TJ. The estimation of the fat free mass index in athletes. Asian J Sports Med. 2012; 3(3):200–3. PMID: 23012640
7. Papadopoulou SK, Gouvianaki A, Grammatikopoulou MG, Maraki Z, Pagkalos IG, Malliaropoulos N et al. Body Composition and Dietary Intake of Elite Cross-country Skiers Members of the Greek National Team. Asian J Sports Med. 2012; 3(4):257–66. PMID: 23342224
8. Fernandez-Elias VE, Martinez-Abellan A, Lopez-Gullon JM, Moran-Navarro R, Pallares JG, De la Cruz-Sanchez E et al. Validity of hydration non-invasive indices during the weightcutting and official weigh-in for Olympic combat sports. PloS one. 2014; 9(4):e95336. https://doi.org/10.1371/journal.pone.0095336 PMID: 24740242
9. Matias CN, Judice PB, Santos DA, Magalhaes JP, Minderico CS, Fields DA et al. Suitability of Bioelectrical Based Methods to Assess Water Compartments in Recreational and Elite Athletes. J Am Coll Nutr. 2016; 35(5):413–421. https://doi.org/10.1080/07315724.2015.1058198 PMID: 26934568
10. Yanovski SZ, Hubbard VS, Heymsfield SB, Lukaski HC. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement. Am J Clin Nutr. 1996; 64(3):524S–532S.
11. Norman K, Stobaus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis—clinical relevance and applicability of impedance parameters. Clin Nutr. 2012; 31(6):854–61. https://doi.org/10.1016/j.clnu.2012.05.008 PMID: 22698802
12. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM et al. Bioelectrical impedance analysis-part I: review of principles and methods. Clin Nutr. 2004; 23(5):1226–43. https://doi.org/10.1016/j.clnu.2004.06.004 PMID: 15380917
13. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gomez J et al. Bioelectrical impedance analysis-part II: utilization in clinical practice. Clin Nutr. 2004; 23(6):1430–53. https://doi.org/10.1016/j.clnu.2004.09.012 PMID: 15556267
14. Maughan RJ, Shirreffs SM. Hydrometry, Hydration Status, and Performance. In: Lukaski HC, editor. Body Composition: Health and Performance in Exercise and Sport. Florida: CRC Press, Taylor & Francis Group; 2017. pp. 49–68.
15. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 1986; 60(4):1327–32. https://doi.org/10.1152/jappl.1986.60.4.1327 PMID: 3700310
16. Lukaski HC. Applications of bioelectrical impedance analysis: a critical review. Basic Life Sci. 1990; 55:365–74. PMID: 2088294
17. Lukaski HC, Siders WA. Validity and accuracy of regional bioelectrical impedance devices to determine whole-body fatness. Nutrition. 2003; 19(10):851–7. PMID: 14559320

18. Lukaski HC. Evolution of bioimpedance: a circuitous journey from estimation of physiological function to assessment of body composition and a return to clinical research. Eur J Clin Nutr. 2013; 67(1):S2–9.
19. Oppliger RA, Nielsen DH, Shetler AC, Crowley ET, Albright JP. Body composition of collegiate football players: bioelectrical impedance and skinfolds compared to hydrostatic weighing. J Orthop Sports Phys Ther. 1992; 15(4):187–92. https://doi.org/10.2519/jospt.1992.15.4.187 PMID: 18781010
20. Piccoli A, Rossi B, Pillon L, Bucciante G. A new method for monitoring body fluid variation by bioimpedance analysis: the RXc graph. Kidney Int. 1994; 46(2):534–9. PMID: 7967368
21. Pillon L & Piccoli A. (2003). U.S. Patent Application No. 10/740,911.
22. Buffa R, Saragat B, Cabras S, Rinaldi AC, Marini E. Accuracy of specific BIVA for the assessment of body composition in the United States population. PloS one. 2013; 8(3):e58533. https://doi.org/10.1371/journal.pone.0058533 PMID: 23484033
23. Lukaski HC, Kyle UG, Kondrup J. Assessment of adult malnutrition and prognosis with bioelectrical impedance analysis: phase angle and impedance ratio. Curr Opin Clin Nutr Metab Care. 2017; 20(5):330–9. https://doi.org/10.1097/MCO.0000000000000387 PMID: 28548972
24. Buffa R, Saragat B, Succa V, Ruggiu R, Carboni L, Putzu PF et al. Elderly subjects with type 2 diabetes show altered tissue electrical properties. Nutrition. 2013; 29(1):132–7. https://doi.org/10.1016/j.nut.2012.05.007 PMID: 23010417
25. Castillo-Martínez LC-R E.; Orea-Tejeda A.; González Islas D. G.; Rodríguez García W. D.; Santillán Díaz C.; Gutiérrez Rodríguez A. E.; Vázquez Durán M.; Keirns Davies C. Cachexia assessed by bioimpedance vector analysis as a prognostic indicator in chronic stable heart failure patients. Nutrition. 2012; 28(9):886–91. https://doi.org/10.1016/j.nut.2011.11.024 PMID: 22480798
26. Haas V, Riedl A, Hofmann T, Nischan A, Burghardt R, Boschmann M et al. Bioimpedance and bioimpedance vector analysis in patients with anorexia nervosa. Eur Eat Disord Rev. 2012; 20(5):400–5. https://doi.org/10.1002/erv.1166 PMID: 22271391
27. Marini E, Buffa R, Saragat B, Coin A, Toffanello ED, Berton L et al. The potential of classic and specific bioelectrical impedance vector analysis for the assessment of sarcopenia and sarcopenic obesity. Clin Interv Aging. 2012; 7:585–91. https://doi.org/10.2147/CIA.S38488 PMID: 23269864
28. Norman K, Smoliner C, Kilbert A, Valentini L, Lochs H, Pirlich M. Disease-related malnutrition but not underweight by BMI is reflected by disturbed electric tissue properties in the bioelectrical impedance vector analysis. Br J Nutr. 2008; 100(3):590–5. https://doi.org/10.1017/S0007114508911545 PMID: 18234142
29. Saragat B, Buffa R, Mereu E, Succa V, Cabras S, Mereu R et al. Nutritional and psycho-functional status in elderly patients with Alzheimer's disease. J Nutr Health Aging. 2012; 16(3):231–6. PMID: 22456778
30. Walter-Kroker A, Kroker A, Mattiucci-Guehlke M, Glaab T. A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. Nutr J. 2011; 10(1):1.
31. Piccoli A. Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status. Kidney Int. 2004; 65(3):1050–63. https://doi.org/10.1111/j.1523-1755.2004.00467.x PMID: 14871426
32. Baldwin CE, Paratz JD, Bersten AD. Body composition analysis in critically ill survivors: a comparison of bioelectrical impedance spectroscopy devices. JPEN J Parenter Enteral Nutr. 2012; 36(3):306–15. https://doi.org/10.1177/0148607111433055 PMID: 22318964
33. Piccoli A, Brunani A, Savia G, Pillon L, Favaro E, Berselli M et al. Discriminating between body fat and fluid changes in the obese adult using bioimpedance vector analysis. Int J Obes. 1998; 22(2):97–104.
34. Savastano S, Belfiore A, Di Somma C, Mauriello C, Rossi A, Pizza G et al. Validity of bioelectrical impedance analysis to estimate body composition changes after bariatric surgery in premenopausal morbidly women. Obes Surg. 2010; 20(3):332–9. https://doi.org/10.1007/s11695-009-0006-5 PMID: 19862582
35. Lukaski HC, Moore M. Bioelectrical impedance assessment of wound healing. J Diabetes Sci Technol. 2012; 6(1):209–12. https://doi.org/10.1177/193229681200600126 PMID: 22401341
36. Nescolarde LY, J.; Medina, D.; Rodas, G.; Rosell-Ferrer, J. Assessment and follow-up of muscle injuries in athletes by bioimpedance: preliminary results. Conf Proc IEEE Eng Med Biol Soc. 2011;1137–40.
37. Nescolarde L, Yanguas J, Lukaski H, Alomar X, Rosell-Ferrer J, Rodas G. Localized bioimpedance to assess muscle injury. Physiol Meas. 2013; 34(2):237–45. https://doi.org/10.1088/0967-3334/34/2/237 PMID: 23354019
38. Lukaski HC, Hall CB, Siders WA. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 2007; 23(7–8):543–50. https://doi.org/10.1016/j.nut.2007.05.001 PMID: 17570642

39. Bronhara B, Piccoli A, Pereira JC. Fuzzy linguistic model for bioelectrical impedance vector analysis. Clin Nutr. 2012; 31(5):710–6. https://doi.org/10.1016/j.clnu.2012.02.014 PMID: 22445559

40. Barbosa-Silva MC, Barros AJ. Bioelectrical impedance analysis in clinical practice: a new perspective on its use beyond body composition equations. Curr Opin Clin Nutr Metab Care. 2005; 8(3):311–7. PMID: 15809535

41. Micheli ML, Pagani L, Marella M, Gulisano M, Piccoli A, Angelini F et al. Bioimpedance and impedance vector patterns as predictors of league level in male soccer players. Int J Sports Physiol Perform. 2014; 9(3):532–9. https://doi.org/10.1123/ijspp.2013-0119 PMID: 23881291

42. Piccoli A, Pastori G, Codognotto M, Paoli A. Equivalence of information from single frequency v. bioimpedance spectroscopy in bodybuilders. Br J Nutr. 2007; 97(1):182–92. https://doi.org/10.1017/S0007114507243077 PMID: 17217575

43. Koury JC, Trugo NMF, Torres AG. Phase Angle and Bioelectrical Impedance Vectors in Adolescent and Adult Male Athletes. Int J Sports Physiol Perform. 2014; 9(5):798–804. https://doi.org/10.1123/ijspp.2013-0397 PMID: 24414089

44. Bonuccelli A, Marzatico F, Stesina G, Stefanini L, Buonocore D, Rucci S et al. Bioelectrical impedance vector analysis (BIVA) to evaluate seasonal variations in body composition of elite soccer players. J Int Soc Sports Nutr. 2011; 8(1):P37.

45. Bonuccelli A, Causarano A, Marzatico F, Catanese S, D'Urbano G, Beschi S et al. Innovative assessment of seasonal variations in body composition of elite soccer players with the integrated analysis DXA-BIVA. J Int Soc Sports Nutr. 2012; 9(1):P1.

46. Gatterer H, Schenk K, Ferrari P, Faulhaber M, Schopp E, Burtscher M. Changes in hydration status of soccer players competing in the 2008 European Championship. J Sports Med Phys Fitness. 2011; 51 (1):89–94. PMID: 21297568

47. Gatterer H, Schenk K, Laninschegg L, Schlemmer P, Lukaski H, Burtscher M. Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. PloS one. 2014; 9(10): e109729. https://doi.org/10.1371/journal.pone.0109729 PMID: 25279660

48. Mascherini G, Gatterer H, Lukaski H, Burtscher M, Galanti G. Changes in hydration, body-cell mass and endurance performance of professional soccer players through a competitive season. J Sports Med Phys Fitness. 2014; 55(7–8):749–55. PMID: 25303072

49. Piccoli A, Piazza P, Noventa D, Pillon L, Zaccaria M. A new method for monitoring hydration at high altitude by bioimpedance analysis. Med Sci Sports Exerc. 1996; 28(12):1517–22. PMID: 8970147

50. Reljic D, Hassler E, Jost J, Friedmann-Bette B. Rapid weight loss and the body fluid balance and hemoglobin mass of elite amateur boxers. J Athl Train. 2013; 48(1):109–17. https://doi.org/10.4085/1062-6050-48.1.05 PMID: 23672332

51. Antoni G, Marini E, Curreli N, Tuveri V, Comandini O, Cabras S et al. Energy expenditure in caving. PloS one. 2017; 12(2):e0170853. https://doi.org/10.1371/journal.pone.0170853 PMID: 28158208

52. Fukuda DH, Stout JR, Moon JR, Smith-Ryan AE, Kendall KL, Hoffman JR. Effects of resistance training on classic and specific bioelectrical impedance vector analysis in elderly women. Exp Gerontol. 2016; 74:9–12. https://doi.org/10.1016/j.exger.2015.12.002 PMID: 26657810

53. Meleleo D, Bartolomeo N, Cassano L, Nitti A, Susca G, Mastrototaro G et al. Evaluation of body composition with bioimpedence. A comparison between athletic and non-athletic children. Eur J Sport Sci. 2017; 17(6):710–9. https://doi.org/10.1080/17461391.2017.1291750 PMID: 28319679

54. Mascherini G, Petri C, Galanti G. Integrated total body composition and localized fat-free mass assessment. Sport Sci Health. 2015; 11(2):217–25.

55. Pollastri L, Lanfranconi F, Tredici G, Schenk K, Burtscher M, Gatterer H. Body fluid status and physical demand during the Giro d'Italia. Res Sports Med. 2016; 24(1):30–8. https://doi.org/10.1080/15438627.2015.1126273 PMID: 26942840

56. Pollastri L, Lanfranconi F, Tredici G, Burtscher M, Gatterer H. Body Water Status and Short-term Maximal Power Output during a Multistage Road Bicycle Race (Giro d'Italia 2014). Int J Sports Med. 2016; 37(4):329–33. https://doi.org/10.1055/s-0035-1565105 PMID: 26701829

57. Carrasco-Marginet M, Castizo-Olier J, Rodriguez-Zamora L, Iglesias X, Rodriguez FA, Chaverri D et al. Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. PloS one. 2017; 12(6):e0178819. https://doi.org/10.1371/journal.pone.0178819 PMID: 28591135

58. Silvestre R, Kraemer WJ, West C, Judelson DA. Body composition and physical performance during a National Collegiate Athletic Association Division I men's soccer season. J Strength Cond Res. 2006; 20 (4):962. https://doi.org/10.1519/R-18165.1 PMID: 17149986

59. Kalapotharakos V, Strimpakos N, Vithoulka I, Karvounidis C. Physiological characteristics of elite professional soccer teams of different ranking. J Sports Med Phys Fitness. 2006; 46(4):515. PMID: 17119514

60. Melchiorri G, Monteleone G, Andreoli A, Calla C, Sgroi M, De Lorenzo A. Body cell mass measured by bioelectrical impedance spectroscopy in professional football (soccer) players. J Sports Med Phys Fitness. 2007; 47(4):408. PMID: 18091679

61. Silvestre R, West C, Maresh CM, Kraemer WJ. Body composition and physical performance in men's soccer: a study of a National Collegiate Athletic Association Division I team. J Strength Cond Res. 2006; 20(1):177. https://doi.org/10.1519/R-17715.1 PMID: 16506863

62. Arnason A, Sigurdsson SB, Gudmundsson A, Holme I, Engebretsen L, Bahr R. Physical fitness, injuries, and team performance in soccer. Med Sci Sports Exerc. 2004; 36(2):278–85. https://doi.org/10.1249/01.MSS.0000113478.92945.CA PMID: 14767251

63. Cian C, Barraud P, Melin B, Raphel C. Effects of fluid ingestion on cognitive function after heat stress or exercise-induced dehydration. Int J Psychophysiol. 2001; 42(3):243–51. PMID: 11812391

64. Maughan R. Impact of mild dehydration on wellness and on exercise performance. Eur J Clin Nutr. 2003; 57(S2):S19.

65. Oppliger RA, Bartok C. Hydration Testing of Athletes. Sports Med. 2002; 32(15):959–71. PMID: 12457417

66. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PloS Med. 2009; 6(7):e1000097. https://doi.org/10.1371/journal.pmed.1000097 PMID: 19621072

67. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015; 4(1):1.

68. Heavens KR, Charkoudian N, O'Brien C, Kenefick RW, Cheuvront SN. Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the resistance-reactance-score graph method. Am J Clin Nutr. 2016; 103(3):724–9. https://doi.org/10.3945/ajcn.115.115352 PMID: 26843158

69. Lukaski HC, Piccoli A. Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions. In: Preedy RV, editor. Handbook of Anthropometry: Physical Measures of Human Form in Health and Disease. New York, NY: Springer New York; 2012. pp. 287–305.

70. Marini E, Sergi G, Succa V, Saragat B, Sarti S, Coin A, Manzato E, Buffa R. Efficacy of specific bioelectrical impedance vector analysis (BIVA) for assessing body composition in the elderly. J Nutr Health Aging. 2013; 17(6):515–21. https://doi.org/10.1007/s12603-012-0411-7 PMID: 23732547

71. Lukaski HC. Biological indexes considered in the derivation of the bioelectrical impedance analysis. Am J Clin Nutr. 1996; 64(3):397S–404S.

72. Chertow GM, Lowrie EG, Wilmore DW, Gonzalez J, Lew NL, Ling J et al. Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. J Am Soc Nephrol. 1995; 6(1):75–81. PMID: 7579073

73. O'Brien C, Young AJ, Sawka MN. Bioelectrical impedance to estimate changes in hydration status. Int J Sports Med. 2002; 23(5):361–6. https://doi.org/10.1055/s-2002-33145 PMID: 12165888

74. Andreoli A, Monteleone M, Van Loan M, Promenzio L, Tarantino U, De Lorenzo A. Effects of different sports on bone density and muscle mass in highly trained athletes. Med Sci Sports Exerc. 2001; 33(4):507–11. PMID: 11283423

75. Brumitt J, Cuddeford T. Current Concepts of Muscle and Tendon Adaptation to Strength and Conditioning. Int J Sports Phys Ther. 2015; 10(6):748. PMID: 26618057

76. Sperelakis N. Cell physiology source book: essentials of membrane biophysics. Elsevier; 2012.

77. Gerth WA, Montgomery LD, Wu Y-C. A computer-based bioelectrical impedance spectroscopic system for noninvasive assessment of compartmental fluid redistribution., Conf Proc Third Annual IEEE Symposium on Computer-Based Medical Systems. 1990;446–456.

78. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J Appl Physiol. 1997; 82(5):1542–58. https://doi.org/10.1152/jappl.1997.82.5.1542 PMID: 9134904

79. Matthie J, Withers P. The ambiguities of predicting total body water and body cell mass with a single frequency (50KHz) measurement of bioimpedance. J Am Soc Nephrol. 1995; 6(6):1682–5.

80. Lofgren B. The electrical impedance of a complex tissue and its relation to changes in volume and fluid distribution; a study on rat kidneys. Acta Physiol Scand Suppl. 1951; 81:1–51. PMID: 14837765

81. Saragat B, Buffa R, Mereu E, De Rui M, Coin A, Sergi G et al. Specific bioelectrical impedance vector reference values for assessing body composition in the Italian elderly. Exp Gerontol. 2014; 50(1):52–6.

STUDY II

PLOS ONE

RESEARCH ARTICLE

Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers

Marta Carrasco-Marginet[1], Jorge Castizo-Olier[1], Lara Rodríguez-Zamora[2,3], Xavier Iglesias[1], Ferran A. Rodríguez[1], Diego Chaverri[1], Daniel Brotons[4], Alfredo Irurtia[1]*

1 INEFC-Barcelona Sports Sciences Research Group, National Institute of Physical Education of Catalonia, Universitat de Barcelona, Barcelona, Spain, **2** Department of Health Sciences, Mid Sweden University, Östersund, Sweden, **3** Swedish Winter Sports Research Centre, Mid Sweden University, Östersund, Sweden, **4** Sport and Health Area of the Catalan Sport Council, Government of Catalonia, Barcelona, Spain

* airurtia@gencat.cat

Citation: Carrasco-Marginet M, Castizo-Olier J, Rodríguez-Zamora L, Iglesias X, Rodríguez FA, Chaverri D, et al. (2017) Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. PLoS ONE 12(6): e0178819. https://doi.org/10.1371/journal.pone.0178819

Editor: Tiago M. Barbosa, Nanyang Technological University, SINGAPORE

Received: February 14, 2017

Accepted: May 21, 2017

Published: June 7, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by research grants from Generalitat de Catalunya, Institut Català de les Dones (U-34/10) http://www20.gencat.cat/portal/site/icdones, and Agència de Gestió d'Ajuts Universitaris i de Recerca (AGUAR), (VCP/3346/2009) http://agaur.gencat.cat/es/inici/, and Consejo Superior de Deportes (001/UPB10/11) http://www.

Abstract

Purpose

The assessment of body hydration is a complex process, and no measurement is valid for all situations. Bioelectrical impedance vector analysis (BIVA) has emerged as a relatively novel technique for assessing hydration status in sports. We applied BIVA a) to determine hydration changes evoked by an intense synchronized swimming (SS) training session; b) to characterize the sample of young elite swimmers in relation with a nonathletic reference population; and c) to generate its 50%, 75% and 95% percentiles of the bioelectrical variables.

Methods

Forty-nine elite SS female swimmers of two age categories, comen (C_o: 13.9 ± 0.9 years, n = 34) and junior (J_r: 16.3 ± 0.6 years, n = 15), performed a long, high intensity training session. Body mass (BM) and bioelectrical variables (R, resistance; Xc, reactance; PA, phase angle; and Z, impedance module) were assessed pre- and post-training. BIVA was used to characterize 1) the distribution pattern of the bioelectrical vector (BIA vector) for both age groups, and 2) pre- to post-training BIA vector migration. Bioelectrical variables were also correlated with BM change values.

Results

Most swimmers were mostly located outside the 75% and some beyond the 95% percentile of the bioelectrical tolerance ellipses of the general population. The BIA vector showed statistically significant differences in both C_o ($T^2 = 134.7$, $p = 0.0001$) and J_r ($T^2 = 126.2$, $p < 0.001$). Both groups were also bioelectrically different ($T^2 = 17.6$, $p < 0.001$). After the training session, a decrease in BM ($p = 0.0001$) and an increase in BIA variables ($p = 0.01$) was observed. BIVA also showed a significant pre-post vector migration both in C_o ($T^2 = 82.1$;

csd.gob.es/. Marta Carrasco-Marginet was a predoctoral researcher also supported by AGAUR (VCP/3346/2009). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: BCM, Body cell mass; BIA, Bioelectrical impedance analysis; BMI, Body mass index; BIVA, Bioelectrical impedance vector analysis; ECW, Extracellular water; ECW:TBW, Extracellular water/total body water ratio; Fc, Characteristic frequency; FFM, Fat-free mass; FM, Fat mass; h, Body height; Hotelling's T^2, Test comparing mean group vectors; ICW, Intracellular water; Cm, Membrane capacitance; PA, Phase angle; R, Bioelectrical resistance (R/h if adjusted by height); RXc graph, R/h vs. Xc/h probabilistic plot; SD, Standard deviation; SF-BIA, Single-frequency bioelectrical impedance analysis; s-RPE, Session rating of pereceived exertion; SS, Synchronized swimmming; TBW, Total body water; Xc, Bioelectrical reactance (Xc/h if adjusted by height); Z, Bioelectrical impedance; Z vector, Vector yield by the RXc graph; $^{\circ}C_{core}$, $^{\circ}C_{hand}$, $^{\circ}C_{foot}$, Core and skin temperatures of the right hand and foot.

$p < 0.001$) and J_r ($T^2 = 41.8$; $p < 0.001$). No correlations were observed between BM changes and bioelectrical variables.

Conclusions

BIVA showed specific bioelectrical characteristics in young elite SS athletes. Considering the decrease in BM and the migration of the BIA vector, we conclude that the homeostatic hydration status of these young elite female swimmers was affected by the execution of intense training sessions. From a methodological perspective, BIVA appears to be sensitive enough to detect subtle hydration changes, but further research is needed to ensure its validity and reliability. Moreover, these findings highlight the importance of ensuring adequate fluid intake during training in young SS athletes.

Introduction

Since becoming part of the Olympic program in 1984, synchronized swimming has enjoyed a growing worldwide popularity. This highly technical sport combines aerobic and anaerobic endurance, flexibility, strength, power, acrobatics and performance skills, and choreography [1] requiring long hours of training to attain such broad attributes [2].

Most synchronized swimmers enter the sport as young girls at the recreational level, and by the age of 13–15 years, the more talented athletes start training and competing at a more intense level [3]. Elite swimmers tend to train 6 days per week with one day off, and training sessions usually last between 3 and 5 hours [2, 3] and are divided in two workouts per day with different content. For example, sport-specific skill training in the water could follow a pool session of swimming for aerobic fitness. A dry land training could occur later in the same day, consisting of flexibility, dry land drills, or a psychology session [3]. As a result, training demands at the elite level often result in high-volume—averaging approximately 40 h per week—and high-intensity training programs [2, 4].

Young athletes may experience fluid imbalances if some conditions are met, with possible consequences on their physical performance, cognitive performance and health maintenance [5–8].

Despite the high requirements at such a young age, information about fluid intake and hydration during the strenuous SS training is scarce. Female swimmers show low energy availability, especially in phases of intensified training performed before competition [9]. Findings highlight the importance of ensuring adequate fluid intake during synchronized swimming training to enable optimal performance. Nevertheless, it has been suggested that there is lower fluid replacement during pool sessions, possibly due to the limited drink breaks or because athletes try to avoid potential gastrointestinal discomfort if the exercise requires them to be upside down [10].

The assessment of body hydration is a dynamic and complex process, and no measurement is valid for all situations [11]. In this context, bioelectrical impedance vector analysis (BIVA) emerges as a relatively novel technique for assessing hydration status without algorithm-inherent errors or requiring assumptions such as constant tissue hydration [12, 13]. BIVA uses raw bioelectrical impedance parameters, i.e., resistance (R, the opposition to flow through intra- and extracellular ionic solutions) and reactance (Xc, additional opposition from the capacitance effect of cell membranes and tissue interfaces), standardized by height (h) to remove the

effect of conductor length, which yields a vector that is plotted in an RXc graph [14]. Overall, BIVA properties are especially interesting for hydration assessment in sports, during both competitions and training [15, 16].

The aim of this study was, first, to determine the hydration changes evoked during a synchronized swimming training session by focusing on changes of the whole-body impedance vector. Secondly, we compared the SS young elite sample with a reference nonathletic population and generated its 50%, 75% and 95% percentiles of the bioelectrical variables distribution, also known as tolerance ellipses. We hypothesized that the hydration status of the young swimmers would be altered by the long, intense training sessions and the barriers for an adequate fluid intake. In this line, these swimmers would be characterized by a specific distribution of BIVA variables when compared to the reference population.

Materials and methods

Participants

Eighty-four female SS athletes of two competitive categories, comen (C_o, n = 53) and junior (J_r, *n* = 31) swimmers, including the entire Spanish national junior team, were recruited for the study in March 2012. Thirty-five (C_o, n = 19; J_r, *n* = 16) did not meet inclusion criteria. Inclusion criteria were as follows: (1) to have competed at national and/or international level at least in the previous two years; (2) to not present injuries or any clinical condition at the time of the study; (3) to be in a postmenarcheal state with the ovarian cycle between days 5^{th} to 11^{th} [17]; (4) to not be under contraceptives or menstrual cycle pharmacological regulators treatment. Sample size was calculated to detect an effect size (ES) = 0.5, with an estimated sample standard deviation (SD) = 7.0, and a SD for changes = 0.7, requiring a minimum of 15 subjects per group. Power (P = 1 –β) was set at 0.80, and the confidence interval was α = 0.05. Forty-nine athletes were selected (C_o, n = 34; J_r, n = 15). All subjects voluntarily participated in the study and delivered written informed consent, with parental permission when needed. The study was conducted following the WMA Helsinki Declaration Statement [18] and approved by the Ethics Committee for Clinical Sport Research of Catalonia. The characteristics of the participants are shown in Table 1.

Study design

This pre-post quasi-experimental study was both descriptive and correlational and aimed to approach the topic from an ecological perspective. The study analyzed the acute adaptations induced by synchronized swimming training session on body mass–BM (kg), bioelectrical vector variables [resistance (R, Ω), resistance adjusted by height (R/h, Ω/m), reactance (Xc, Ω), reactance adjusted by height (Xc/h, Ω/m), impedance module (Z, Ω), and phase angle (PA, °)] and the extracellular water/total body water ratio (ECW:TBW, %). In addition to these independent variables, several others were recorded to characterize the sample (Table 1) and the training (Table 2).

Procedures

The study was conducted two weeks before the Spanish National Synchronized Swimming Championship, within the 4-week precompetitive mesocycle. One training session was performed by each group on the same day. The protocol is chronologically summarized in Fig 1.

To attain a state of euhydration prior to BIA measurements [6], swimmers were required to abstain from caffeine, alcohol and exercise the day before the investigation [19]. They were also instructed to drink 3.0 L of fluid over 24 h (2.0 L to be consumed between 6:00 p.m. and

Table 1. Characteristics of participants.

		All swimmers (95% CI)	Comen (95% CI)	Junior (95% CI)	Unpaired t-test	
		n = 49	*n* = 34	*n* = 15	*t*	*p*
General						
	Age (years)	14.6 ± 1.4 (14.2–15.0)	13.9 ± 0.9 (13.6–14.2)	16.3 ± 0.6 (16.0–16.7)	-10.851	0.0001*
	Training (h/week)	19.4 ± 7.6 (17.4–21.8)	15.0 ± 2.7 (14.0–15.9)	30.0 ± 3.8 (28.0–32.1)	-15.911	0.0001*
	Practice (years)	6.9 ± 1.8 (6.4–7.4)	5.9 ± 1.1 (5.6–6.3)	9.1 ± 1.0 (8.6–9.7)	-9.980	0.0001*
Anthropometric						
	Height (cm)	163.3 ± 7.6 (161.1–165.4)	161.9 ± 8.2 (159.0–164.8)	166.3 ± 4.8 (163.7–169.0)	-1.943	0.058
	BM (kg)	49.1 ± 7.0 (47.1–51.2)	47.2 ± 7.0 (44.8–49.7)	53.5 ± 5.2 (50.6–56.3)	-3.103	0.003*
	BMI (kg/m^2)	18.4 ± 1.8 (17.9–18.9)	18.0 ± 1.9 (17.3–18.6)	19.3 ± 1.3 (18.6–20.0)	-2.514	0.015*
	Fat mass (%)	16.5 ± 4.4 (15.2–17.8)	15.6 ± 4.7 (13.9–17.2)	18.6 ± 2.6 (17.2–20.1)	-2.382	0.021*
	Muscle mass (%)	38.0 ± 4.7 (36.7–39.4)	37.7 ± 5.4 (35.8–39.6)	38.8 ± 2.6 (37.3–40.2)	-0.722	0.474
Bioelectrical						
	R/h (Ω/m)	319.7 ± 36.7 (309.1–330.2)	328.4 ± 38.8 (314.9–341.9)	299.9 ± 21.6 (287.9–311.9)	3.286	0.002*
	Xc/h (Ω/m)	39.9 ± 3.9 (38.7–41.0)	40.0 ± 4.5 (38.4–41.5)	39.6 ± 2.2 (38.4–40.8)	0.395	0.695
	PA (˚)	7.1 ± 0.5 (7.0–7.3)	7.0 ± 0.5 (6.8–7.1)	7.5 ± 0.4 (7.3–7.7)	-4.166	0.0001*

Values are mean ± SD; BM, body mass; BMI, body mass index; R, resistance; Xc, reactance; PA, phase angle; h, height; CI, 95% confidence interval

* significant differences between comen and junior swimmers (p < 0.05).

https://doi.org/10.1371/journal.pone.0178819.t001

10:00 p.m.) in addition to their habitual western dietary practices. From 10:00 p.m. until the start of the pre-test next morning, no further fluid or food intake was allowed [6]. From 7:00 a.m. to 8:00 a.m. the following day, after monitoring body and skin temperatures, pre-training measurements were performed in a thermoneutral room (25˚C) to obtain anthropometric (BM) and bioelectrical data (R, R/h, Xc, Xc/h, PA, Z, and ECW:TBW). Immediately after, participants consumed a standardized breakfast consisting of 1 cheese and ham sandwich, 1 plain yogurt, 1 banana, and 220 mL of natural orange juice [20]. At 10:00 a.m., all swimmers performed a category-specific training session in a 50-m indoor pool with 30 m available for use (water temperature: 25–26˚C). The characteristics of both training are shown in Table 2.

Fluid intake (H2O) during the training was monitored by a certified dietician. Swimmers were instructed to drink a similar amount of water in the middle and at the end of training. Pre- and post-training, BIA measurements were conducted after urination and defecation [21] to minimize the influence of food/fluid ingestion [22] and exercise [23]; pre-training measurements were conducted while fasting, and the post-training data were obtained within the first hour of recovery. Just after completing the training session, the rating of perceived exertion

Table 2. Characteristics of the training sessions.

	All swimmers (95% CI)	Comen (95% CI)	Junior (95% CI)	Unpaired t-test	
	n = 49	*n* = 34	*n* = 15	*t*	*p*
Duration (min)	167.6 ± 28.0 (159.6–175.7)	149.6 ± 3.3 (148.5–150.8)	208.4 ± 10.3 (202.7–214.1)	-21.695	0.001*
Internal training load					
RPE (a.u)	6.6 ± 0.5 (6.4–6.7)	6.4 ± 0.5 (6.3–6.6)	6.8 ± 0.6 (6.5–7.1)	-2.220	0.03*
Session–RPE	1102.4 ± 231.3 (1036.0–1168.9)	963.9 ± 78.5 (963.5–991.3)	1416 ± 129 (1344.8–1488.0)	-12.572	0.001*
Water intake (L)	0.6 ± 0.2 (0.5–0.6)	0.5 ± 0.2 (0.4–0.6)	0.7 ± 0.3 (0.5–0.8)	-2.177	0.04*

Values are mean ± SD; RPE, rating of perceived exertion (CR-10 scale); a.u, arbitrary units; CI, 95% confidence interval

* significant differences between comen and junior swimmers (p < 0.05).

https://doi.org/10.1371/journal.pone.0178819.t002

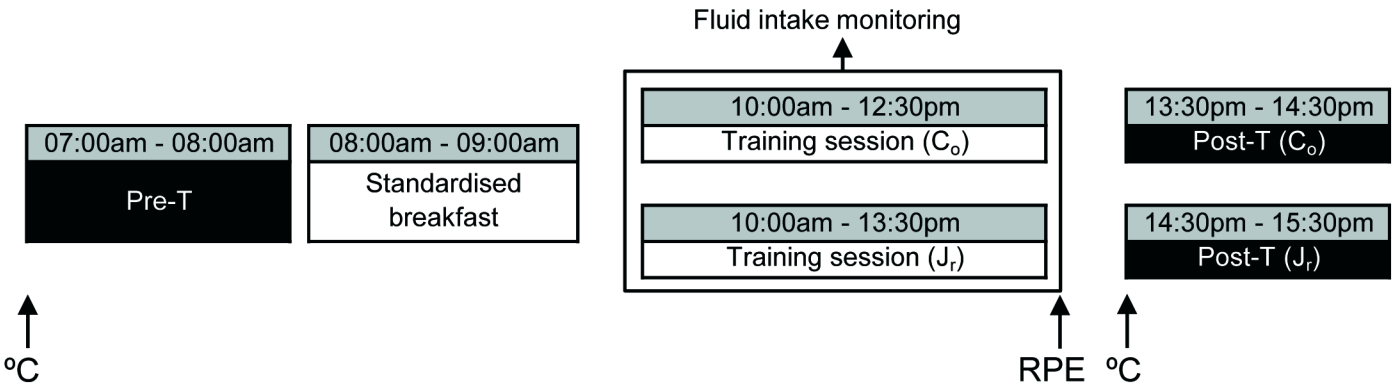

Fig 1. Study protocol. ˚C, body and skin temperature measurements; Pre-T, pre-training measurements; Post-T, post-training measurements; RPE, rating of perceived exertion; C_o, comen; J_r, junior.

https://doi.org/10.1371/journal.pone.0178819.g001

(RPE) was assessed using the Borg CR-10 scale [24]. Finally, after checking that body and skin temperatures were similar to those registered in the pre-training measurements, the post-training assessment was performed.

Anthropometric assessment. BM was measured to the nearest 0.05 kg using a calibrated weighing scale (Seca 710, Hamburg, Germany). Height (h) was measured to the nearest 1 mm using a telescopic stadiometer (Seca 220, Hamburg, Germany). Body mass index (BMI) was calculated as body mass / height2 (kg/m^2). Anthropometric measurements were taken according to the standard criteria of The International Society for the Advancement of Kinanthropometry [25].

Whole-body bioimpedance assessment. R and Xc were measured using a previously calibrated plethysmograph (Z-Metrix, BioparHom, Le Bourget-du-Lac, France) that emitted a 77 μA alternating sinusoidal current at seven operating frequencies (1, 5, 50, 150, 200, 250, and 325 kHz). The 50-kHz single frequency was selected for BIVA [26]; meanwhile, multi-frequency capabilities were used to estimate body composition–fat mass (FM) and muscle mass (MM), and the ECW:TBW was calculated by ECW/TBW•100. The device provides impedance values with an accuracy average error of 0.95 ± 1.58% and average repeatability errors of 0.55 ± 0.38% for all the frequency range (1 to 1000 kHz) [27]. Bioelectrical measurements were conducted under controlled conditions [14] through the standard whole-body, tetrapolar, distal BIA technique [28]. The anatomical sites for electrodes (Red Dot 2660–5, 3M Corporate Headquarters, St. Paul, MN, USA) were marked with a waterproof pen [29]. Bioelectrical measurements were repeated until they were stable to within 1 Ω (usually up to three times within an interval of 20–30 s). The average value was used in calculations [21].

Regarding the BIVA method, the correlation between R and Xc determines the ellipsoidal form of the bivariate probability distributions (confidence intervals for average vectors and tolerance for individual vectors). The vector direction is defined as the phase angle (PA) and is the geometric relationship between R and Xc. PA has been validated as an indicator of cellular health [12, 28] and has been interpreted as an index of fluid distribution between the intracellular and extracellular compartments [30], showing an inverse correlation with the ECW:TBW [31]. On the other hand, the length of the vector indicates hydration status from fluid overload (decreased resistance, short vector) to exsiccosis (increased resistance, longer vector), and a sideways migration of the vector due to low or high reactance indicates a decrease or increase in the dielectric mass (membranes and tissue interfaces) of soft tissues [32]. The individual vector can be ranked on the RXc point graph with regard to tolerance ellipses representing 50%, 75% and 95% according to the values of a reference population [14]. A comparison

between the mean vectors of different samples with the 95% confidence ellipses can be performed on the RXc mean graph. Furthermore, the mean vector displacement of a group with the 95% confidence ellipse pre- to post-intervention was plotted on the RXc paired graph [33].

Temperature assessment. Core ($^{\circ}C_{core}$) and skin temperatures of the right hand ($^{\circ}C_{hand}$) and foot ($^{\circ}C_{foot}$) were measured using thermistors connected to a data logger (Squirrel 2010, Grant Instruments Ltd, Cambridge, UK). All swimmers were instructed to take a cold shower (as cold as tolerable) for 10–15 minutes post-training, in order to reduce cutaneous blood flow and temperature and remove accumulated electrolytes [34]. Skin temperature, as a surrogate for cutaneous blood flow [35], was measured just before BIA measurements; this verified the return to temperatures close to the pre-training values ($p < 0.05$): Pre-$^{\circ}C_{core}$: 36.8 ± 0.2˚C vs. Post-$^{\circ}C_{core}$: 37.2 ± 0.3˚C; Pre-$^{\circ}C_{hand}$: 29.6 ± 0.8˚C vs. Post-$^{\circ}C_{hand}$: 29.2 ± 1.1˚C; Pre-$^{\circ}C_{foot}$: 29.0 ± 1.2˚C vs. Post-$^{\circ}C_{foot}$: 28.6 ± 1.0˚C. Ambient air temperature and relative humidity in the indoor pool area were also controlled (27.5 ± 0.5˚C and 64.5 ± 1.5%, respectively).

Internal training load assessment. The individual session-RPE (s-RPE) was chosen for rating the perceived exertion during training [36]. The CR-10 RPE scale [24] was shown to the swimmers immediately after the training was completed. Scores were computed by multiplying the duration of the training by the relative RPE values. One week before the study, all participants were assessed repeatedly during at least 3 training to disclose learning effects and to improve the consistency of the measurements [37].

Statistical analysis

Descriptive statistics (mean, SD) were calculated for each independent variable and age category. Once the data were tested for normality (Shapiro-Wilks test), differences in anthropometric (BM) and bioelectrical variables (R, Xc, R/h, Xc/h, PA and Z) between pre- and post-training were analyzed by the Student's paired *t*-test. The Student's unpaired *t*-test was used to analyze group differences between age categories. Whole-body bioimpedance vectors were analyzed by the RXc graph method [14] using the BIVA software [38]. Each swimmer was plotted in the tolerance ellipses (50%, 75% and 95%) of the 14- to 15-year-old healthy female Italian reference population [39] as this was the reference population closest in age to our sample. The BIVA mean graph was performed to compare whole-body vectors of C_o vs. J_r groups, and each SS group vs. the reference population. The BIVA paired graph was used to analyze pre- to post-training changes in the vectors of C_o and J_r. To examine the magnitude of pre-post ratio changes in anthropometric and bioelectrical variables, delta values (Δ, % of pre) were calculated. To estimate the relevance of these changes, relative ES were calculated using Cohen's d. According to Cohen [40], ES was defined as small, $d \leq 0.2$; medium, $d \leq 0.5$; and large, $d \leq 0.8$. Pearson's correlation coefficient was used to determine possible statistical associations between a) PA vs. chronological age and PA vs. the ECW:TBW; and b) ΔBM vs. BIA vector variables (ΔR/h, ΔXc/h, ΔPA, ΔZ). A paired one-sample Hotelling's T^2 test was used to analyze pre- to post-training changes in the vector through the 95% confidence ellipses. A two-sample Hotelling's T^2 test was used to determine the BIA vector differences between C_o and J_r and between each SS group vs. the reference population. $P < 0.05$ was considered significant.

Results

Determinants of BIA vector distribution pattern in synchronized swimmers

The BIVA point graph (Fig 2) indicated that swimmers fell mostly outside the 75% tolerance ellipse regardless of age or competition level; in many cases, they were outside the 95%

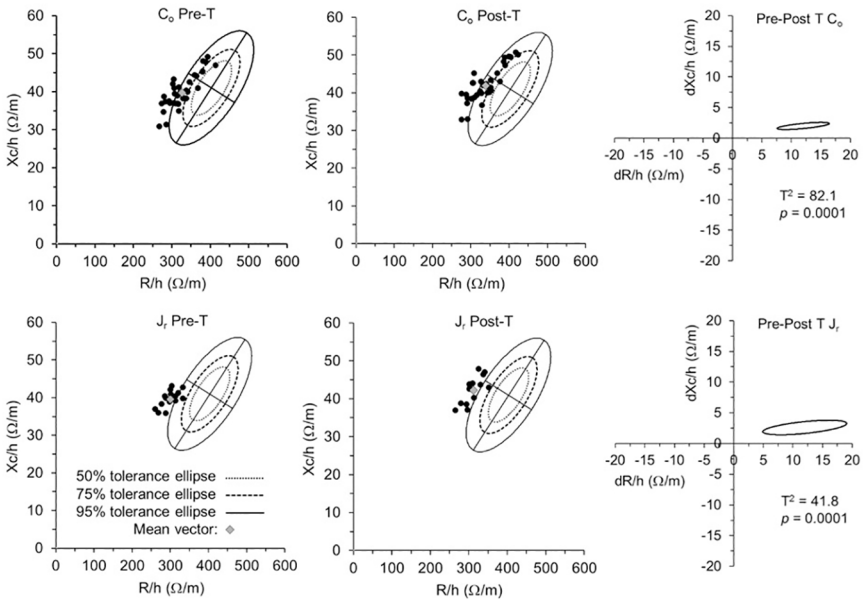

Fig 2. BIVA patterns before and after training. On the left side, scattergrams of the C_o and J_r individual (as well as the mean) impedance vectors, plotted on the 50%, 75%, and 95% tolerance ellipses of the corresponding healthy female reference population [39] are displayed both for pre- and post-training (Pre-T and Post-T, respectively). On the right side, mean vector displacements of C_o and J_r from pre- to post-training are shown. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance; T^2, Hotelling's T^2 test; *p*-value (significance at $p < 0.05$).

https://doi.org/10.1371/journal.pone.0178819.g002

tolerance ellipse, denoting a higher density of body cell mass (BCM) than the reference population. Differences in the BIA vector in comparison with the reference population were found for C_o ($T^2 = 134.7$, $p = 0.0001$) and J_r ($T^2 = 126.2$, $p < 0.001$), as well as between both groups of SS swimmers ($T^2 = 17.6$, $p < 0.001$) (Fig 3).

Fig 4 shows the 50%, 75% and 95% tolerance ellipses corresponding to the whole SS sample (C_o and J_r together): R/h = 319.7 ± 36.7 Ω/m; Xc/h = 39.9 ± 3.9 Ω/m; $r = 0.78$.

Pre-post differences

The BIA vector migration (Fig 2) was characterized by an increase in R/h and Xc/h, indicating mild dehydration after training both in C_o ($T^2 = 82.1$) and J_r ($T^2 = 41.8$) ($p < 0.001$). This was paralleled by a decrease in BM in both groups of swimmers ($p = 0.0001$) as shown in Table 3. In contrast, all bioelectrical variables significantly increased (Table 3).

BIVA correlations

A positive correlation ($r = 0.45$, $p = 0.001$) was found between PA and chronological age in the whole SS sample. Additionally, PA was negatively related ($r = -0.91$; $p < 0.001$) to the ECW: TBW. No correlations were observed between bioelectrical pre to post changes in relation to BM.

Discussion

This study showed that synchronized swimmers experienced a modest level of dehydration after an intense training session (BM loss ~0.6–0.8% BM) that was detected by BIVA. In addition, we report a specific BIA vector distribution in these young elite SS swimmers in comparison with a healthy, nonathletic reference population of similar age. In fact, this is the first

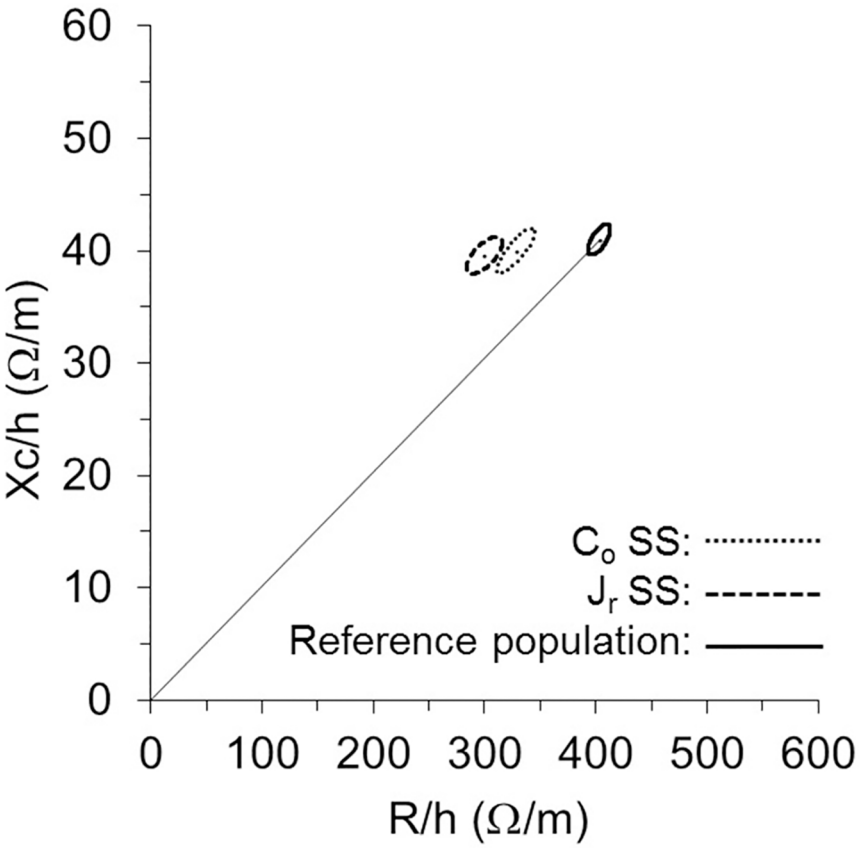

Fig 3. RXc mean graph. The 95% confidence ellipses for the mean impedance vectors of C_o (dotted line ellipse), J_r (dark dashed line ellipse) and the healthy female reference population (solid line ellipse with vector) [39] are shown. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance; C_o, comen; J_r, junior; SS, synchronized swimmers.

https://doi.org/10.1371/journal.pone.0178819.g003

time that specific reference distribution ellipses in a female sporting group is being reported (Fig 4).

BIVA allows for an analysis of both the homeostatic state and possible BIA vector migration, arising from any variation in body fluid [12, 26]. Nowadays, BIVA is a widely used technique in medicine as a valid tool in the assessment of different physiological states and clinical conditions in which euhydration is frequently altered, such as renal disease [41], critically ill patients [42], pulmonary disease [43], heart failure [44], gastrointestinal disease [45], and pregnancy and postpartum [46]. Its properties are especially interesting for hydration assessments in both the training process and competitive sporting events [16, 47].

Nevertheless, in protocols measuring parameters before and after exercise to analyze acute vector shifts, certain factors that may generate errors in the bioelectrical signal should be controlled in order to provide accurate and reliable results, including: skin preparation [48]; previous hydration status [49]; previous consumption of food or beverage [35, 50, 51]; body position and posture during measurements [21, 51, 52]; electrode impedance [53], position and placement modification [51, 52]; time of body fluid stabilization [54, 55]; variations in cutaneous blood flow and temperature [35, 52]; skin electrolyte accumulation produced by physical exercise [35]; reproducibility of bioelectrical measurements influenced by biological intra-day [56] and inter-day variations [50]; environmental conditions [21, 52]; menstrual cycle [17, 57]; and injury condition [58].

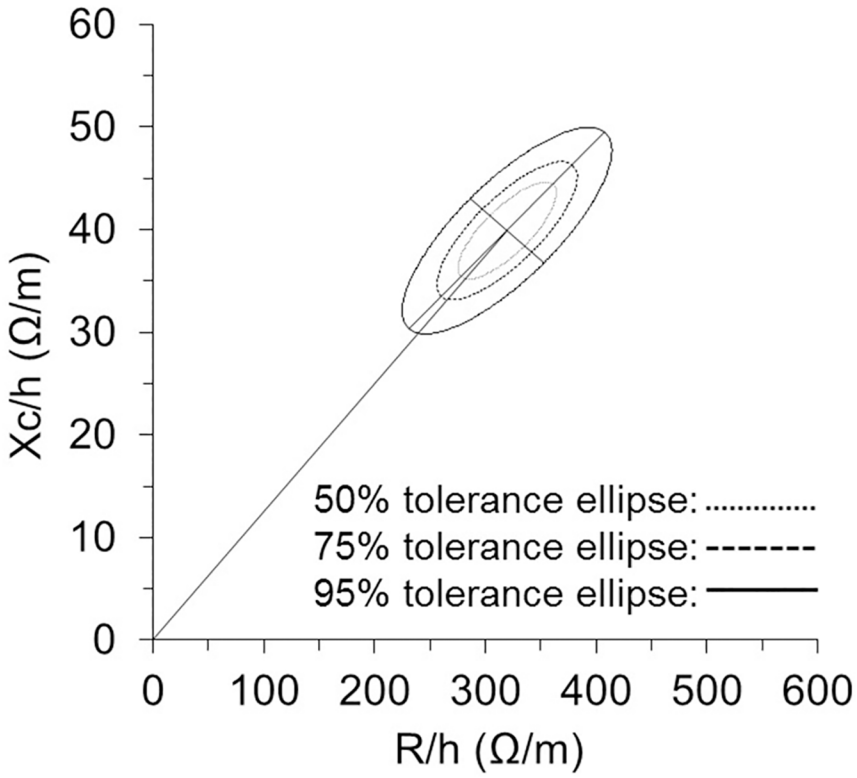

Fig 4. Tolerance ellipses. 50%, 75%, and 95% tolerance ellipses generated of the entire group of synchronized swimmers. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance.

https://doi.org/10.1371/journal.pone.0178819.g004

Despite the ecological design of this research, the study protocol attempted to control these factors. As mentioned above, ingestion of a meal or beverage has an influence on Z, which may decrease over a 2-to 4-h period after a meal, generally representing a change of < 3% in Z values [22]. Therefore, in our study, post-exercise BIVA measurements could have been influenced by breakfast and water intake in the middle of the training session, possibly underestimating Z values by ~9–10 Ω. The amount of water intake at the end of the training should not have affected the BIVA measurements because the recent ingestion of a meal or beverage (< 1 h from the ingestion to BIA measurements) appears to be "electrically silent" and to have a minimal effect on whole- body Z [59]. With regard to the temperature control, it is known that every 1.0˚C increase in the skin can lead to a decrease in R of up to ~11% [60]. Possible pre-post BIA differences related to environmental and cutaneous temperatures of the swimmers were controlled, accepting increases or decreases lower than 1˚C as, in this range of values, differences in Z appear not to be significant [61]. Finally, it should be noted that the study sample was composed of female athletes. Thus, to minimize the body fluid fluctuations caused by the effect of female hormonal kinetics [17] and consequent changes in performance ability [62], international recommendations were followed [28, 57]. Thus, those swimmers who were in a premenstrual phase (luteal or secretory phase) or who were taking contraceptives and/or menstrual cycle pharmacologic regulators were excluded.

Table 3. Anthropometric and bioelectrical parameters before (Pre) and after (Post) training.

		Pre (95% CI)	Post (95% CI)	Δ-value	Paired t-test	*p*-value	Cohen's d
				%	*t*	*p*	*d*
Comen (n = 34)							
Anthropometric							
	BM (kg)	47.2 ± 7.0 (44.8–49.7)	46.9 ± 7.0 (44.5–49.3)	-0.8 ± 0.6	8.081	0.0001*	0.20[a]
Bioelectrical							
	R (Ω)	529.5 ± 46.1 (513.4–545.6)	548.8 ± 48.5 (531.9–565.7)	3.7 ± 3.0	-7.251	0.0001*	0.39[b]
	Xc (Ω)	64.4 ± 5.1 (62.7–66.2)	67.7 ± 5.0 (66.0–69.5)	5.2 ± 3.3	-9.193	0.0001*	0.67[c]
	R/h (Ω/m)	328.4 ± 38.8 (314.9–341.9)	340.5 ± 41.0 (326.1–354.8)	3.7 ± 3.0	-7.104	0.0001*	0.30[b]
	Xc/h (Ω/m)	40.0 ± 4.5 (38.4–41.5)	42.0 ± 4.6 (40.4–43.6)	5.2 ± 3.3	-8.905	0.0001*	0.43[b]
	PA (Ω)	7.0 ± 0.5 (6.8–7.1)	7.1 ± 0.5 (6.9–7.2)	1.5 ± 2.5	-2.863	0.007*	0.20[a]
	Z (Ω/m)	330.9 ± 38.9 (317.2–344.4)	343.1 ± 41.2 (328.7–357.4)	3.7 ± 3.0	-7.178	0.0001*	0.20[a]
	r (R/h, Xc/h)	0.84	0.84	—	—	—	
Junior (n = 15)							
Anthropometric							
	BM (kg)	53.5 ± 5.2 (50.6–56.3)	53.2 ± 5.1 (50.3–56.0)	-0.6 ± 0.4	4.634	0.0001*	0.17[a]
Bioelectrical							
	R (Ω)	498.5 ± 35.1 (479.1–518.0)	518.5 ± 38.9 (497.0–540.1)	4.0 ± 3.3	-4.870	0.0001*	0.53[c]
	Xc (Ω)	65.8 ± 2.9 (64.2–67.4)	70.2 ± 4.8 (67.5–72.8)	6.6 ± 3.9	-6.447	0.0001*	0.82[c]
	R/h (Ω/m)	299.9 ± 21.6 (287.9–311.9)	311.9 ± 23.4 (298.9–324.9)	4.0 ± 3.3	-4.864	0.0001*	0.53[c]
	Xc/h (Ω/m)	39.6 ± 2.2 (38.4–40.8)	42.2 ± 3.4 (40.3–44.1)	6.6 ± 3.9	-6.352	0.0001*	0.62[c]
	PA (Ω)	7.5 ± 0.4 (7.3–7.8)	7.7 ± 0.4 (7.5–7.9)	2.4 ± 3.3	-2.909	0.011*	0.45[b]
	Z (Ω/m)	302.5 ± 21.7 (290.5–314.5)	314.8 ± 23.5 (301.7–327.8)	4.1 ± 3.3	-4.928	0.0001*	0.51[c]
	r (R/h, Xc/h)	0.66	0.76	—	—	—	

Values are the mean ± standard deviation; BM, body mass; R, resistance; Xc, reactance; h, height; PA, phase angle; Z, impedance vector module; r, Pearson correlation coefficient between R/h and Xc/h; %Δ, percent differences Pre to Post; CI, 95% confidence interval

*significant differences between Pre and Post, p-value < 0.05 (paired t-test Pre vs. Post); a, small effect size (≤ 0.2); b, medium effect size (d ≤ 0.5); c, large effect size (d ≤ 0.8).

https://doi.org/10.1371/journal.pone.0178819.t003

BIA vector changes evoked by training

This study is the first to use BIVA to characterize variations in hydration status in young SS athletes evoked by training. RXc paired graphs showed significant vector changes after exercise in both groups (Fig 2), which were interpreted as mild dehydration (average loss <1% BM) [63]. Nevertheless, no correlation was observed between changes in BM and BIA vector migration. This could be due to the fluid intake of the athletes during training, which was maintained due to the ecological study design. A recent investigation in which no food/fluid intake was allowed found similar results with exercise-induced dehydration [29]. The researchers noted that this could be influenced by inadequate criteria for stable bioelectrical impedance measurements or by exercise-related factors, such as sweat rate, respiratory water loss and oxidative water production, that may lead to BM loss without an effective net negative fluid balance [64]. However, these results differ from other studies [65, 66] that found a significant relationship between changes in bioelectrical values and BM induced passively and/or chronically. It is possible that greater changes could have been observed if the swimmers had not ingested fluids during the long and intense training (Table 2). Nevertheless, no significant relationships were found with BM in the present study.

Only two studies in the literature have investigated short-term vector changes induced by exercise. Collodel et al. [67] did not find differences in R and Xc—and thus in vector position—after an incremental maximal cycle ergometer test (of an unspecified duration) performed by healthy sedentary subjects and moderately trained subjects, although both the BM and the hematocrit experienced significant changes. Nevertheless, two possible limitations could have influenced their bioelectrical results: the post-exercise measurement was performed 5 min after finishing the test; thus, some previously mentioned sources of error may have influenced the bioelectrical signal [35]. Furthermore, an RXc mean graph was reported for pre-post analysis, instead of an RXc paired graph. The type of Hotelling's T^2 test chosen should have also been clarified because the RXc paired graph and the paired one-sample Hotelling's T^2 test are the appropriate analyses in this case, and they may have given different results. Conversely, Gatterer et al. [29] analyzed the short-term bioelectrical changes in well-trained subjects after 1 h of a self-rated intensity cycle ergometer test in the heat (environmental chamber). The authors reported findings similar to those of the present study, observing increased resistance and reactance, as well as a vector migration, after exercise in the heat.

In our study, vector migration along the major axis due to increased R/h and Xc/h indicates fluid loss (Fig 2), as the length of the vector is inversely related to TBW [13]. Resistance is pure opposition of the conductor to the flow of current [52]. Therefore, the significantly increased resistance experienced by the swimmers reflects the decreased body fluids [35], which is supported by the decrease in BM, and is also probably followed—which we cannot prove—by changes in electrolyte concentration [66]. With regard to the reactance, Gatterer et al. [29] suggested that the increased reactance after exercise could indicate fluid shifts between intra- and extracellular compartments. Xc maintains a relationship with cell membrane capacitance (Cm), which is affected by the size, thickness and composition of the cell membranes [68]. Alterations such as fluid shifts between compartments induced by physical activity modify the characteristics of the muscle cells. As suggested, the cell membrane becomes thinner as the cell swells and Cm increases, and the opposite occurs when the cell shrinks [69], thus affecting Xc. Furthermore, as proposed by De Lorenzo et al. [70], variations in fluid distribution would modify the characteristic frequency (Fc)—i.e., the frequency at which Xc is maximal). Because Xc is highly dependent on the relationship between the frequency of measurement and Fc, changes in Fc evoke great variations in Xc at 50 kHz, simply because this frequency is a fixed point on the changing impedance locus [71]. However, De Lorenzo and colleagues' hypothesis should be considered with caution because it refers to Hanai's model, which relays on assumptions such as spherical cells shape. Nevertheless, the meaning of Xc behavior after exercise remains to be clarified. In this regard, consideration of the Xc as an indicator of dielectric mass (membranes and tissue interfaces) of soft tissues [52] should be reviewed, as it may not be applicable in this type of protocols. It should be noted that despite the fact that vector changes after fluid removal and overload (e.g., the wet–dry cycle of dialysis) as a non-physiological process is clinically well-established [13], every dehydration process induced by physical exercise is a consequence of several physiological adaptations whose relationship with the vector behavior is scarcely explored, especially at the cellular level and considering the kinetics of Xc.

Because BIVA appears to be sensitive to body water adaptations evoked by high intensity SS training, it could help to assess hydration variations in real time and could also substitute the current hydration biomarkers that require a mobile laboratory. Nevertheless, we are still far from confirming BIVA as a valid and reliable biomarker of hydration status. Its progressive use as a complementary measure to hematological hydration indicators will allow us to parameterize its values and demonstrate its real possibilities in the near future.

Determinants of BIA vector distribution pattern in synchronized swimmers

The individual anthropometric dimensions, such as weight and height, determine the body's bioelectrical properties [52]. Although bioelectrical variables are normalized for height, BMI calculation enables better contextualization of the sample. The results of the BMI in both groups (Table 1)—underweight and normal weight in C_o and J_r swimmers, respectively—seem to be coherent with the great physiological demands of this sport [4]. These anthropometric characteristics are necessary to understand that the bioelectrical signal will also be specifically related to each sport, sex and age [15]. The comparison of BIA vector distribution values of SS athletes with that of other sports practitioners is difficult due to the absence of values for female athletes and differences in age and gender with regard to the only study that, to our knowledge, has provided a characterization of a sport-specific population, i.e. male soccer players [47]. Nevertheless, a comparison between SS and reference populations or between both groups of swimmers according to their age can be discussed.

Characterization of synchronized swimmers. In the present group of swimmers, PA variation was positively correlated with age, following a trend similar to that of the general athletic population of the same sex and age, in accordance with Koury et al. [15]. This positive correlation in athletes is in agreement with the increase in metabolic tissues during biological maturation [39]. Mean and individual Z vectors (Fig 2) were found to be displaced to the left and mostly scattered outside the 75% tolerance ellipse (in many cases, outside the 95% tolerance ellipse) on the RXc graphs when swimmers were compared to the reference nonathletic Italian female population of similar age [39]. Furthermore, with increasing age and performance level of the athletes (Fig 3), a displacement to the left was also observed, due to a decrease in the R/h component in the absence of a difference in the Xc/h component. Other studies [15, 29, 47] have also reported vectors of sport samples shifted to the left when compared to their reference populations, which might reflect the specific adaptations of body composition in different sports [72]. Additionally, vectors shifted to the left have been reported with increasing age [15] and performance level [47] in sport samples. It remains to be investigated whether the differences are the result of vector displacement due to biological maturation, to the specific training process or a combination of both.

Athletes generally possess increased soft tissue mass and differing fluid content compared to the sedentary population [72]. Total body fluid is affected by factors such as training [73]. Trained athletes have a greater amount of body fluid and different fluid distribution between the intracellular and extracellular compartments. This can be because of their larger muscle mass, increased plasma volume and muscle glycogen reserves [8, 35], which could increase water transport into the muscle [74] and fluid-regulating hormone adaptations (i.e., aldosterone and sensitivity) [75].

As suggested, both the increased BCM in SS indicated by the BIA vector and the vector differences due to decreased R/h with similar Xc/h values could reflect different intracellular water (ICW) content. On a related note, and according to Chertow et al. [31], a negative relationship was found between the ECW:TBW and PA in the present study. Because SS showed a greater PA, the greater ICW content of the swimmers compared to the reference population—as well as J_r compared to C_o—is likely due to the hypertrophy of the muscle fibers [47]. Additionally, the greater PA could also reflect better cell function [12].

Thus, the present findings highlight the need for specific new tolerance ellipses for the SS sporting population (Fig 4). These ellipses might be useful for interpretation of individual vectors and for defining target regions of impedance vectors for lower-level SS athletes. Nevertheless, further studies should increase the sample size and analyze different performance levels;

this will help determine whether specific training activity may induce vector migration to the side in the higher level swimmers, as well as the utility of the tolerance ellipses for monitoring hydration status and performance state.

The main limitation of the present study, in addition to those previously mentioned for the sake of text fluency, is the previously mentioned ecological constraints of the protocol, which may have caused an attenuation of the bioelectrical changes after training. Additionally, with regard to the comparison of the SS sample to the reference population, no tolerance ellipses of the healthy reference population have been published for this specific age range. Thus, this study used the tolerance ellipses of the healthy reference population closest in age.

In conclusion, BIVA appears to be sensitive to hydration changes evoked by high intensity SS training, regardless of age and performance level. Moreover, the present study showed that SS swimmers are characterized by a specific distribution of BIVA parameters when compared to a healthy nonathletic reference population. Furthermore, BIVA also showed differences between swimmers of different age and performance level. This is the first time that specific tolerance ellipses in a female sport group are being reported.

The use of BIVA as an indicator of dehydration in sport practice is clearly an emerging research area. Beyond the need for further validation of this methodology, especially in pre- to post-exercise designs, generation of new ellipses according to each sport, age, sex, race and sport level is needed in order to establish useful and comparable reference values for the field of sport sciences.

Supporting information

S1 Dataset. Study database.
(XLSX)

Acknowledgments

Our gratitude to the synchronized swimming clubs CN Kallipolis, CN Granollers and AD Sincro Retiro, and to the Esplugues de Llobregat Technification Center (Catalan Sports Council, Government of Catalonia) for permitting data release for scientific purposes. We are also indebted to the athletes who participated in the study.

Author Contributions

Conceptualization: AI MC-M XI FAR.

Data curation: MC-M AI XI FAR.

Formal analysis: AI MC-M JC-O DB FAR.

Funding acquisition: XI DB.

Investigation: MC-M LR-Z DC AI XI FAR.

Methodology: AI MC-M XI FAR.

Project administration: AI.

Resources: XI FAR.

Supervision: AI DB XI FAR LR-Z.

Validation: AI DB XI FAR.

Visualization: MC-M JC-O AI LR-Z FAR.

Writing – original draft: MC-M JC-O AI LR-Z FAR.

Writing – review & editing: AI DB XI FAR.

References

1. Jamnik V. An evaluation of the physiological response to competitive synchronized swimming and the physiological characteristics of elite synchronized swimmers: Toronto, ON: York University; 1987.
2. Mountjoy M. Injuries and medical issues in synchronized Olympic sports. Curr Sports Med Rep. 2009; 8 (5):255–61. https://doi.org/10.1249/JSR.0b013e3181b84a09 PMID: 19741353
3. Mountjoy M. The basics of synchronized swimming and its injuries. Clin Sports Med. 1999; 18(2):321–36. PMID: 10230568
4. Rodríguez-Zamora L, Iglesias X, Barrero A, Chaverri D, Erola P, Rodríguez FA. Physiological responses in relation to performance during competition in elite synchronized swimmers. PLoS One. 2012; 7(11):e49098. https://doi.org/10.1371/journal.pone.0049098 PMID: 23155452
5. Bar-Or O, Dotan R, Inbar O, Rotshtein A, Zonder H. Voluntary hypohydration in 10-to 12-year-old boys. J Appl Physiol (1985). 1980; 48(1):104–8.
6. Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN. Mechanisms of aerobic performance impairment with heat stress and dehydration. J Appl Physiol (1985). 2010; 109(6):1989–95.
7. Montain SJ, Coyle EF. Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. J Appl Physiol (1985). 1992; 73(4):1340–50.
8. Meyer F, Volterman KA, Timmons BW, Wilk B. Fluid balance and dehydration in the young athlete assessment considerations and effects on health and performance. Am J Lifestyle Med. 2012; 6 (6):489–501.
9. Schaal K, Tiollier E, Le Meur Y, Casazza G, Hausswirth C. Elite synchronized swimmers display decreased energy availability during intensified training. Scand J Med Sci Sports. 2016. https://doi.org/10.1111/sms.12716
10. Lundy B. Nutrition for synchronized swimming: a review. Int J Sport Nutr Exerc Metab. 2011; 21:436–45. PMID: 21904005
11. McGarvey J, Thompson J, Hanna C, Noakes TD, Stewart J, Speedy D. Sensitivity and specificity of clinical signs for assessment of dehydration in endurance athletes. Br J Sports Med. 2010; 44(10):716–9. https://doi.org/10.1136/bjsm.2008.053249 PMID: 18981042
12. Norman K, Stobäus N, Pirlich M, Bosy-Westphal A. Bioelectrical phase angle and impedance vector analysis—Clinical relevance and applicability of impedance parameters. Clin Nutr. 2012; 31(6):854–61. https://doi.org/10.1016/j.clnu.2012.05.008 PMID: 22698802
13. Lukaski HC, Piccoli A. Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions. In: Preedy V, editor. Handbook of Anthropometry. London: Springer; 2012. p. 287–305.
14. Piccoli A, Rossi B, Pillon L, Bucciante G. A new method for monitoring body fluid variation by bioimpedance analysis: the RXc graph. Kidney Int. 1994; 46(2):534–9. PMID: 7967368
15. Koury J, Trugo N, Torres A. Phase angle and bioelectrical impedance vectors in adolescent and adult male athletes. Int J Sports Physiol Perform. 2014; 9(5):798–804. https://doi.org/10.1123/ijspp.2013-0397 PMID: 24414089
16. Mascherini G, Gatterer H, Lukaski H, Burtscher M, Galanti G. Changes in hydration, body-cell mass and endurance performance of professional soccer players through a competitive season. J Sports Med Phys Fitness. 2015; 55(7–8):749–55. PMID: 25303072
17. Gleichauf C, Roe D. The menstrual cycle's effect on the reliability of bioimpedance measurements for assessing body composition. Am J Clin Nutr. 1989; 50(5):903–7. PMID: 2816797
18. WMA. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310(20):2191–4. https://doi.org/10.1001/jama.2013.281053 PMID: 24141714
19. Fortes MB, Diment BC, Di Felice U, Walsh NP. Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. Appl Physiol Nutr Metab. 2012; 37(5):850–9. https://doi.org/10.1139/h2012-054 PMID: 22686429
20. Aranceta J, Serra-Majem L, Ribas L, Perez-Rodrigo C. Breakfast consumption in Spanish children and young people. Public Health Nutr. 2001; 4(6a):1439–44. PMID: 11918497

21. Rush E, Crowley J, Freitas I, Luke A. Validity of hand-to-foot measurement of bioimpedance: standing compared with lying position. Obesity. 2006; 14(2):252–7. https://doi.org/10.1038/oby.2006.32 PMID: 16571850

22. Kushner R, Gudivaka R, Schoeller D. Clinical characteristics influencing bioelectrical impedance analysis measurements. Am J Clin Nutr. 1996; 64(3):423S–7S.

23. Lukaski HC, Bolonchuk WW, Siders WA, Hall CB. Body composition assessment of athletes using bioelectrical impedance measurements. J Sports Med Phys Fitness. 1990; 30(4):434–40. PMID: 2079851

24. Borg G, Hassmén P, Lagerström M. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. Eur J Appl Physiol Occup Physiol. 1987; 56(6):679–85. PMID: 3678222

25. Stewart A, Marfell-Jones M, Olds T, de Ridder H. International standards for anthropometric assessment. Murcia, Spain: International Society for the Advancement of Kinanthropometry. 2011.

26. Piccoli A. Bioelectric impedance measurement for fluid status assessment. Contrib Nephrol. 2010; 164:143–52. https://doi.org/10.1159/000313727 PMID: 20428000

27. Moreno MV, Ribbe-Cornet E, Rebeyrol J, Vannicatte A, Krief L. Evaluation of a new impedancemeter to independently measure extracellular, intracellular and total body water volumes: application to the measurement of hydration. Med Biol Eng Comput. 2015; 53(10):989–99. https://doi.org/10.1007/s11517-015-1305-8 PMID: 26036775

28. Yanovski SZ, Hubbard VS, Heymsfield SB, Lukaski HC. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health technology assessment conference statement. Am J Clin Nutr. 1996; 64(3):524S–32S.

29. Gatterer H, Schenk K, Laninschegg L, Schlemmer P, Lukaski H, Burtscher M. Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. PLoS One. 2014; 9(10): e109729. https://doi.org/10.1371/journal.pone.0109729 PMID: 25279660

30. Goovaerts H, Faes TJ, De Valk-De Roo G, Ten Bolscher M, Netelenbosch J, Van der Vijgh W, et al. Extra-cellular volume estimation by electrical impedance-phase measurement or curve fitting: a comparative study. Physiol Meas. 1998; 19(4):517. PMID: 9863677

31. Chertow GM, Lowrie EG, Wilmore DW, Gonzalez J, Lew NL, Ling J, et al. Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. J Am Soc Nephrol. 1995; 6 (1):75–81. PMID: 7579073

32. Piccoli A. Whole body-single frequency bioimpedance. Contrib Nephrol. 2005; 149:150–61. https://doi.org/10.1159/000085478 PMID: 15876839

33. Pillon L, Piccoli A, inventors. RXc graph and RXc Z-score graph methods. United States patent application US 10/740,911. 2003 Dec 18.

34. Peiffer JJ, Abbiss CR, Nosaka K, Peake JM, Laursen PB. Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. J Sci Med Sport. 2009; 12 (1):91–6. https://doi.org/10.1016/j.jsams.2007.10.011 PMID: 18083634

35. O'Brien C, Young A, Sawka M. Bioelectrical impedance to estimate changes in hydration status. Int J Sports Med. 2002; 23(5):361–6. https://doi.org/10.1055/s-2002-33145 PMID: 12165888

36. Foster C, Daines E, Hector L, Snyder AC, Welsh R. Athletic performance in relation to training load. Wis Med J. 1996; 95(6):370–4. PMID: 8693756

37. Psycharakis SG. A longitudinal analysis on the validity and reliability of ratings of perceived exertion for elite swimmers. J Strength Cond Res. 2011; 25(2):420–6. https://doi.org/10.1519/JSC.0b013e3181bff58c PMID: 20351574

38. Piccoli A, Pastori G. BIVA software. Padova: Department of Medical and Surgical Sciences, University of Padova, Italy. 2002.

39. De Palo T, Messina G, Edefonti A, Perfumo F, Pisanello L, Peruzzi L, et al. Normal values of the bioelectrical impedance vector in childhood and puberty. Nutrition. 2000; 16(6):417–24. PMID: 10869896

40. Cohen J. A power primer. Psychol Bull. 1992; 112(1):155–9. PMID: 19565683

41. Piccoli A. Bioelectric impedance vector distribution in peritoneal dialysis patients with different hydration status. Kidney Int. 2004; 65(3):1050–63. https://doi.org/10.1111/j.1523-1755.2004.00467.x PMID: 14871426

42. Baldwin CE, Paratz JD, Bersten AD. Body composition analysis in critically ill survivors: a comparison of bioelectrical impedance spectroscopy devices. JPEN J Parenter Enteral Nutr. 2012; 36(3):306–15. https://doi.org/10.1177/0148607111433055 PMID: 22318964

43. Walter-Kroker A, Kroker A, Mattiucci-Guehlke M, Glaab T. A practical guide to bioelectrical impedance analysis using the example of chronic obstructive pulmonary disease. Nutr J. 2011; 10(1):1.

44. Castillo-Martinez L, Colin-Ramirez E, Orea-Tejeda A, Gonzalez Islas DG, Rodriguez Garcia WD, Santillan Diaz C, et al. Cachexia assessed by bioimpedance vector analysis as a prognostic indicator in

chronic stable heart failure patients. Nutrition. 2012; 28(9):886–91. https://doi.org/10.1016/j.nut.2011.11.024 PMID: 22480798

45. Norman K, Smoliner C, Kilbert A, Valentini L, Lochs H, Pirlich M. Disease-related malnutrition but not underweight by BMI is reflected by disturbed electric tissue properties in the bioelectrical impedance vector analysis. Br J Nutr. 2008; 100(3):590–5. https://doi.org/10.1017/S0007114508911545 PMID: 18234142

46. Lukaski HC, Hall CB, Siders WA. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 2007; 23(7–8):543–50. https://doi.org/10.1016/j.nut.2007.05.001 PMID: 17570642

47. Micheli ML, Pagani L, Marella M, Gulisano M, Piccoli A, Angelini F, et al. Bioimpedance and Impedance Vector Patterns as Predictors of League Level in Male Soccer Players. Int J Sports Physiol Perform. 2014; 9(3):532–9. https://doi.org/10.1123/ijspp.2013-0119 PMID: 23881291

48. Laferriere P, Lemaire ED, Chan AD. Surface electromyographic signals using dry electrodes. IEEE Trans Instrum Meas. 2011; 60(10):3259–68.

49. Berneis K, Keller U. Bioelectrical impedance analysis during acute changes of extracellular osmolality in man. Clin Nutr. 2000; 19(5):361–6. https://doi.org/10.1054/clnu.2000.0133 PMID: 11031076

50. Deurenberg P, Weststrate JA, Paymans I, Van der Kooy K. Factors affecting bioelectrical impedance measurements in humans. Eur J Clin Nutr. 1988; 42(12):1017–22. PMID: 3234328

51. Gualdi-Russo E, Toselli S. Influence of various factors on the measurement of multifrequency bioimpedance. Homo. 2002; 53(1):1–16. PMID: 12365353

52. Lukaski HC. Biological indexes considered in the derivation of the bioelectrical impedance analysis. Am J Clin Nutr. 1996; 64(3):397S–404S.

53. Nescolarde L, Lukaski H, De Lorenzo A, de-Mateo-Silleras B, Redondo-Del-Rio MP, Camina-Martin MA. Different displacement of bioimpedance vector due to Ag/AgCl electrode effect. Eur J Clin Nutr. 2016; 70(12):1401–7. https://doi.org/10.1038/ejcn.2016.121 PMID: 27380885

54. Slinde F, Bark A, Jansson J, Rossander-Hulthén L. Bioelectrical impedance variation in healthy subjects during 12 h in the supine position. Clin Nutr. 2003; 22(2):153–7. PMID: 12706132

55. Zhu F, Schneditz D, Wang E, Levin NW. Dynamics of segmental extracellular volumes during changes in body position by bioimpedance analysis. J Appl Physiol (1985). 1998; 85(2):497–504.

56. Rodríguez G, Moreno LA, Sarría A, Fleta J, Bueno M. Assessment of nutritional status and body composition in children using physical anthropometry and bioelectrical impedance: influence of diurnal variations. J Pediatr Gastroenterol Nutr. 2000; 30(3):305–9. PMID: 10749416

57. Lusseveld E, Peters ETJ, Deurenberg P. Multifrequency bioelectrical impedance as a measure of differences in body water distribution. Ann Nutr Metab. 1993; 37(1):44–51. PMID: 8470872

58. Nescolarde L, Yanguas J, Lukaski H, Alomar X, Rosell-Ferrer J, Rodas G. Effects of muscle injury severity on localized bioimpedance measurements. Physiol Meas. 2015; 36(1):27–42. https://doi.org/10.1088/0967-3334/36/1/27 PMID: 25500910

59. Evans W, McClagish H, Trudgett C. Factors affecting the in vivo precision of bioelectrical impedance analysis. Appl Radiat Isot. 1998; 49(5):485–7.

60. Caton J, Mole P, Adams W, Heustis D. Body composition analysis by bioelectrical impedance: effect of skin temperature. Med Sci Sports Exerc. 1988; 20(5):489–91. PMID: 3193865

61. Liang M, Norris S. Effects of skin blood flow and temperature on bioelectric impedance after exercise. Med Sci Sports Exerc. 1993; 25(11):1231–9. PMID: 8289609

62. McKee JE, Cameron N. Bioelectrical impedance changes during the menstrual cycle. Am J Hum Biol. 1997; 9(2):155–61.

63. Armstrong L, Rosenberg I, Armstrong L, Manz F, Dal Canton A, Barclay D, et al. Hydration assessment techniques. Nutr Rev. 2005; 63(6 II):S40–S54. https://doi.org/10.1301/nr.2005.jun.S40-S54

64. Maughan RJ, Shirreffs SM, Leiper JB. Errors in the estimation of hydration status from changes in body mass. J Sports Sci. 2007; 25(7):797–804. https://doi.org/10.1080/02640410600875143 PMID: 17454547

65. Gatterer H, Wille M, Faulhaber M, Lukaski H, Melmer A, Ebenbichler C, et al. Association between body water status and acute mountain sickness. PLoS One. 2013; 8(8):e73185. https://doi.org/10.1371/journal.pone.0073185 PMID: 24013267

66. Piccoli A, Piazza P, Noventa D, Pillon L, Zaccaria M. A new method for monitoring hydration at high altitude by bioimpedance analysis. Med Sci Sports Exerc. 1996; 28(12):1517–22. PMID: 8970147

67. Collodel L, Favretto G, Teodori T, Caenaro G, Mordacchini M, Stritoni P, et al. Use of bioelectrical impedance analysis for monitoring fluid shift during maximal aerobic exercise. Med Sport. 1997; 50 (2):197–202.

68. Sperelakis N. Origin of resting membrane potentials. In: Sperelakis N, editor. Cell physiology source book: essentials of membrane biophysics. London: Academic Press; 2012. p. 123.

69. Gerth WA, Montgomery LD, Wu Y-C, editors. A computer-based bioelectrical impedance spectroscopic system for noninvasive assessment of compartmental fluid redistribution. Third IEEE Symposium on Computer-Based Medical Systems. 1990:446–453.

70. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J Appl Physiol (1985). 1997; 82(5):1542–58.

71. Lofgren B. The electrical impedance of a complex tissue and its relation to changes in volume and fluid distribution; a study on rat kidneys. Acta Physiol Scand Suppl. 1951; 81:1–51. PMID: 14837765

72. Andreoli A, Monteleone M, Van Loan M, Promenzio L, Tarantino U, De Lorenzo A. Effects of different sports on bone density and muscle mass in highly trained athletes. Med Sci Sports Exerc. 2001; 33 (4):507–11. PMID: 11283423

73. Convertino VA. Blood volume: its adaptation to endurance training. Med Sci Sports Exerc. 1991; 23 (12):1338–48. PMID: 1798375

74. Sawka MN. Physiological consequences of hypohydration: exercise performance and thermoregulation. Med Sci Sports Exerc. 1992; 24(6):657–70. PMID: 1602938

75. Fellmann N. Hormonal and plasma volume alterations following endurance exercise. Sports Med. 1992; 13(1):37–49. PMID: 1553454

STUDY III

©Journal of Sports Science and Medicine (2018) **17,** 571-579
http://www.jssm.org

Research article

Bioelectrical Impedance Vector Analysis (BIVA) and Body Mass Changes in an Ultra-Endurance Triathlon Event

Jorge Castizo-Olier [1,2], Marta Carrasco-Marginet [1,2], Alex Roy [1], Diego Chaverri [1,2], Xavier Iglesias [1,2], Carla Pérez-Chirinos [1], Ferran Rodríguez [1,2] and Alfredo Irurtia [1,2]✉
[1] Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
[2] INEFC-Barcelona Sport Sciences Research Group, Barcelona, Spain

Abstract
This study aimed to provide the first description of the whole-body bioimpedance vector of nine non-professional triathletes, and to assess body mass (BM) and vector variations evoked by an ultra-endurance triathlon event. Anthropometric and bioelectrical assessments were performed before (PRE), after (POST), and 48 hours following the race (POST48h). Bioimpedance vector analysis (BIVA) showed triathletes' vectors placed to the left of the major axis and mostly outside the 50% tolerance ellipse of the reference population. Vector migration in POST indicated dehydration, paralleled by a decrease in BM ($p = 0.0001$). Increased hydration status from POST to POST48h was suggested by a reversed vector migration and increased BM ($p = 0.0001$). Compared to PRE, POST48h values reflected fluid retention by changes in BIVA, while BM was still lower ($p = 0.0001$). Racing time was positively related to basal resistance -R/h- ($r = 0.68$; $p = 0.04$) and bioimpedance -Z/h- ($r = 0.68$; $p = 0.045$). Besides, basal R/h and Z/h were positively related to PRE-to-POST changes of R/h and Z/h ($r = 0.80$; $p = 0.009$). PRE-to-POST changes of R/h and Z/h were positively related to racing time ($r = 0.80$, $p = 0.01$) and internal workload ($r = 0.80$, $p \leq 0.02$). Notwithstanding the lack of significant correlation between BM and bioelectrical parameters, the vector's behavior was explained from a multifactorial perspective (including BM variations) by using multiple regression analysis. On the other hand, BM changes were not related to racing time, internal workload or energy deficit (ranges: $r = -0.46$ to 0.65; $p = 0.06$ to 0.98). In conclusion, these triathletes exhibit a specific bioelectrical distribution. Furthermore, vector migration was consistent with fluid loss induced by the event. Finally, vector analysis seems to provide additional information about hydration changes 48h after the event in comparison with BM alone.

Key words: Body composition; hydration; bioimpedance; BIVA; performance; triathlon.

Introduction

Ultra-endurance triathlon (UET) combines three disciplines (3.8-km swimming, 180-km cycling, 42.2-km running) and involves from 8 to 17 hours of competition depending on the fitness level and efficiency rates of the triathlete (Laursen and Rhodes, 2001). During a UET, dehydration and glycogen depletion are the main causes of metabolic fatigue, whereas gastrointestinal problems, hyperthermia and hyponatremia are potential threats to the triathletes' health (Jeukendrup et al., 2005).

Dehydration decreases endurance performance (Cheuvront and Kenefick, 2014) and increases the injury risk (Oppliger and Bartok, 2002). Furthermore, it is an important factor in race completion in ultra-endurance events (Knechtle et al., 2015). However, the lack of a 'gold-standard' marker of hydration status must be emphasized. The assessment of body hydration status is a dynamic and complex process and no method is valid for all situations (Armstrong, 2007). Finding a method which is sensitive to the type (intra- or extra-cellular) and magnitude of dehydration is necessary (Cheuvront and Kenefick, 2017).

A common method to assess dehydration in endurance athletes has been pre- and post-exercise body mass (BM) control (McGarvey et al., 2010). Ultra-endurance athletes may suffer great BM losses (Hew-Butler et al., 2007; Laursen et al., 2006; Sharwood et al., 2004), principally due to the sweat rate (Cheuvront and Kenefick, 2017). Other possible sources are the respiratory and urinary/fecal water losses (Cheuvront and Kenefick, 2017).

Literature supports that reductions in total BM of ≥ 2% generate negative effects on the endurance performance of the athletes (Cheuvront and Kenefick, 2014; McDermott et al., 2017). However, well-trained ultra-triathletes should expect to lose about 3% of their BM without any adverse consequences (Laursen et al., 2006). Therefore, despite the fact that measuring BM variation provides a simple estimate of post-race hydration status in athletes (Maughan et al., 2007; McGarvey et al., 2010), it is not always a reliable measure. Furthermore, it may give rise to misleading results since, for instance, a significant loss of BM may be observed without an effective hypohydration resulting (Cheuvront and Kenefick, 2017; Maughan et al., 2007).

In this regard, the bioelectrical impedance vector analysis (BIVA) emerges as a technique to assess hydration status with no inherent errors of bioimpedance equations or requirements for biological assumptions such as the constant tissue hydration (Lukaski and Piccoli, 2012; Norman et al., 2012). The method is used in the clinical context for the analysis of both homeostatic state and possible vector variations resulting from modifications in body fluid status (Norman et al., 2012; Piccoli, 2010). In the exercise context, as mentioned in Heavens et al. (2016), directional changes in vector values from serial measurements are consistent with fluid loss according to the theory (Piccoli et al., 1994; Piccoli et al., 2002). Therefore, since any vector change is a function of extracellular water –ECW- changes (Segal et al., 1991) because a 50 kHz current does not penetrate cells (De Lorenzo et al., 1997), a potential strength of BIVA would be to afford insight into ECW dehydration

Received: 24 May 2018 / Accepted: 05 September 2018 / Published (online): 01 December 2018

(Heavens et al., 2016). Moreover, it might help to provide additional information about hydration changes in ultra-endurance events than, for instance, BM loss alone. Thus, due to the already mentioned deleterious effects of dehydration and to the potential strengths of the method, BIVA is especially interesting for hydration assessment in both sport training and competitive event (Carrasco-Marginet et al., 2017; Koury et al., 2014).

In this way, this study aimed at providing the first description of the whole-body bioelectrical impedance vector in a group of ultra-endurance triathletes, and to assess the variation in the BM and the bioimpedance vector of the athletes evoked during a UET event. We hypothesized that a specific distribution of BIVA would be found in the triathletes when compared to the reference population, and that the BM and the directional changes of the vector in this type of events would be consistent with a decrease in body fluids, according to the literature.

Methods

Participants

An advertisement was placed on the triathlon race webpage to recruit non-professional male triathletes. The inclusion criteria were to train at least 10 h per week and the participation in a minimum of one UET during the past 3 years.

Sample size was calculated based on a potential increase of the impedance vector module (Z) of 4% based on our previous observations in synchronized swimmers after a high-intensity training session (Carrasco-Marginet et al., 2017) as the only available estimate for short-term (< 24 hours) bioelectrical changes (Castizo-Olier et al., 2018). Seven athletes per intervention group were required (two-tailed $\alpha = 0.05$, $\beta = 0.80$, effect size = 0.95, change SD = 2.5).

Nine experienced, well-trained, non-professional ultra-endurance male triathletes volunteered for the study [mean ± SD: age 36.6 ± 5.5 years; body mass (BM) 76.0 ± 6.9 kg; height 1.75 ± 0.06 m; body mass index (BMI) 24.8 ± 2.0 kg/m^2; $\dot{V}O_{2max}$ = 66.3 ± 4.3 ml/kg/min]. The participants had an average of 10 ± 3 years of experience in UET and ultra-endurance events, and they had been training regularly for approximately 14–20 hours per week for at least three years. All participants passed a medical examination before the race and gave their informed written consent prior to their participation. The study was performed following the Helsinki Declaration Statement and was approved by the Ethics Committee for Clinical Sport Research of Catalonia.

Study design

The participants completed a UET race, specifically, the "Extreme Man Salou–Costa Daurada Triathlon", composed of three segments consisting of a 3.8 km swim, 180 km cycle with a positive elevation over 2600 m and a 42.2 km marathon run. The mean (range) ambient temperature was 26 °C (13 - 30 °C), the water temperature was 21 °C (20.8 - 21.2 °C) and the relative humidity was 77% (64% - 94%). The mean wind speed was 1.3 m/s (range 0.3 – 5.0 m/s). All the triathletes undertook the tests measurements designed for the study at three time points: before the race (PRE), after finishing the race (POST) and 48h after POST measurements (POST48h). Detailed information about the study design, race characteristics and procedures related to the performance variables analyzed in the present study (racing time, internal workload and energy deficit) can be consulted in a previously published article (Barrero et al., 2014).

Procedures

Anthropometric and bioelectrical variables were obtained by the same trained investigator in a thermally neutral room (25.0 ± 1.0ºC).

Anthropometric assessment: Anthropometric measurements were performed according to the standard criteria of The International Society for the Advancement of Kinanthropometry (ISAK) (Stewart et al., 2011). Body height (h) was assessed to the nearest 1 mm using a telescopic stadiometer (Seca 220®, Hamburg, Germany). BM was measured to the nearest 0.05 kg using a calibrated weighing scale (Seca 710®, Hamburg, Germany). BMI (kg/m^2) was calculated as body mass / height^2. The circumferences of the left and right thigh –C_{LT} and C_{RT}, respectively- (taken at mid-thigh) and the left and right calf –C_{LC} and C_{RC}, respectively- (taken at the greater perimeter of the calf) were measured to the nearest 1 mm using an anthropometric tape (Lufkin Executive®, Lufkin, USA), in order to evaluate possible variations between the different time points. This is important since the whole-body impedance can be significantly reduced if a lower limb affected by swelling is in the same side as the electrodes (Codognotto et al., 2008).

Whole-Body Bioimpedance assessment: BIVA uses raw bioelectrical impedance parameters, i.e., resistance ("R", the opposition to flow through intra- and extracellular ionic solutions) and reactance ("Xc", additional opposition from the capacitance effect of cell membranes and tissue interfaces), standardized by height in order to remove the effect of conductor length, yielding a vector, which is plotted in an RXc graph (Piccoli et al., 1994). The vector direction (PA) is the geometric relationship between R and Xc. PA is a validated indicator of cellular health (Norman et al., 2012; Yanovski et al., 1996) and has been interpreted as an indicator of fluid distribution between intra- and extracellular compartments (Goovaerts et al., 1998), reporting an inverse correlation with the ECW - total body water (TBW) ratio (Chertow et al., 1995). The length of the vector states hydration status from fluid overload (short vector) to exsiccosis (longer vector), and lateral migration of the vector projects a decrease or increase in the dielectric mass (membranes and tissue interfaces) of soft tissues (Piccoli, 2005). Individual vectors can be normalized to Z scores and classified on the RXc score graph, according to the tolerance ellipses (50%, 75% and 95%) of a reference population, independently of the bioimpedance analyzer used (Piccoli et al., 2002). Individuals positioned within the 50% tolerance ellipses, according to the literature (Lukaski, 2013; Lukaski and Piccoli, 2012) are considered "normally hydrated".

In the present study, R and Xc were measured by a previously calibrated multifrequency bioimpedance

analyser (Z-Métrix®, BioparHom®, Bourget du Lac, France) that emitted 77 μA alternating sinusoidal current at different frequencies (1 to 325 kHz). The device provides impedance values with an accuracy characterized by an average error of 0.95% ± 1.58% and an average repeatability errors of 0.55% ± 0.38% for all the frequency range (Moreno, 2015). The 50-kHz frequency was selected for BIVA (Piccoli, 2010). The bioimpedance module [$Z = \sqrt{(R^2 + Xc^2)}$] and phase angle [$PA = \arctan(Xc/R) \cdot (180/\pi)$] were derived from the bioelectrical raw parameters. Triathletes were tested under controlled conditions through the standard whole-body, tetrapolar, distal BIA technique (Yanovski et al., 1996). All the participants arrived to the measurements after voiding their bladder and rectum (Rush et al., 2006). Triathletes were tested in PRE and POST48h in euhydration state according to a standardized 8-point urine color chart –PRE: 1.7 ± 0.7; POST48h: 1.8 ± 0.8- (Armstrong et al., 1994). Before placing the electrodes (Red Dot™, 3M Corporate Headquarters, St. Paul, MN, USA), the skin was prepared by shaving the electrode site to remove hair, rubbing with gel and cleaning with alcohol in order to reduce possible interferences in the assessment (Hermens et al., 1999). A waterproof pen was used to mark the anatomical sites for electrodes, in order to preserve the same location, due to the influence of the electrode placement modification in the bioelectrical outputs (Gualdi-Russo and Toselli, 2002). Just before the bioimpedance measurements, core and skin temperatures were registered. PRE and POST48h bioimpedance assessments were performed after 10 minutes of stabilization (Slinde et al., 2003). Measurements were repeated until they were stable to within 1 Ω (usually up to three times within an interval of 20-30 s) and the average value was used in calculations. POST measurements were perfomed once the core and skin temperatures were close to the basal values (± 1 ºC), 35 ± 5 min after finishing the race. Before measurements, participants were instructed to take a cold shower (as cold as tolerable) for 10 minutes post-race, in order to reduce cutaneous blood flow and temperature and remove accumulated electrolytes (Peiffer et al., 2009).

Temperature assessment: An increase in the skin temperature can lead to an important decrease in R (Caton et al., 1988). Temperature variations within the range of 1 ºC seem not to significantly affect the impedance (Liang and Norris, 1993) and greater differences must be avoided. Therefore, in the present study, core ($ºC_{core}$) and skin temperatures of the right hand ($ºC_{hand}$) and foot ($ºC_{foot}$) were measured using thermistors connected to a data logger (Squirrel 2010, Grant Instruments Ltd, Cambridge, UK).

Internal workload assessment: To estimate the total workload of exercise performed by each participant in the race, the training impulse (TRIMP) method was used.

Energy deficit assessment: All the wraps and bottles of each participant were collected in order to calculate the energy intake during the race. The energy expenditure during the race was estimated through three different individualized equations (one for each segment), derived from preliminary exercise tests. Finally, the energy deficit was calculated as energy intake minus energy expenditure.

Statistical analysis

Descriptive statistics for each independent variable were calculated. After testing each variable for the normality of the distribution (Shapiro-Wilks test), differences in anthropometric (BM and circumferences of the thigh and calf), temperature and bioelectrical data (R, Xc, Z, R/h, Xc/h, Z/h and PA) PRE, POST and POST48h were analyzed through a repeated-measures one-way ANOVA (RM-ANOVA). Post-hoc analyses were performed using the Bonferroni correction and the *p*-value was adjusted at $p_{adj} = 0.017$. The magnitude of ratio changes was computed as delta percent values (Δ%). Effect sizes (ES) were calculated using Cohen's d and defined as small, $d \leq 0.2$; medium, $d \leq 0.5$; and large, $d \leq 0.8$. Pearson's correlation coefficient was applied to determine possible associations between: a) bioelectrical baseline values (PRE; POST; POST48h) vs. bioelectrical delta values (PRE-to-POST; POST-to-POST48h; and PRE-to-POST48h); b) bioelectrical data (baseline and delta values) vs. racing time, internal workload, energy deficit and BM delta values; c) BM delta values vs. racing time, internal workload, energy deficit. A multiple linear regression analysis was performed in order to explain the changes at the same time points of each bioelectrical (dependent) variable in relation to performance (independent) variables: racing time, internal workload, energy deficit, and BM changes. The adjusted square multiple regression coefficient ($r_{m}^{2}{}_{adj}$) was used to quantify the goodness-of-fit of the model. To add information to the multivariate analysis, the process was further applied in reverse, alternating the role between dependent and independent variables (i.e. the analysis was performed to explain the performance variables results in relation to the changes of each bioelectrical variable). Whole-body individual bioimpedance vectors were analyzed by the standard, reference RXc score graph (Piccoli et al., 2002), according to the healthy, Italian reference population (Piccoli et al., 1995). The RXc mean graph was performed to compare the whole-body mean vector of triathletes vs. the reference population. A two-sample Hotelling's T^2 test was used to determine the vector differences between triathletes vs. the reference population. $P < 0.05$ was considered significant.

Results

The triathletes of the present study completed the race in 752 ± 70 min, the estimated internal workload was 1055 ± 172 arbitrary units, and the energy deficit was 30.5 ± 5.5 MJ (7283 ± 1321 kcal).

All bioelectrical variables significantly increased post-race and decreased at POST48h (Table 1), except PA. No statistically significant differences were found for lower-limbs circumferences in any time points (Table 1). The same was observed for the temperature differences: PRE-to-POST $ºC_{core}$: 0.0 ± 1.2 %; PRE-to-POST48h $ºC_{core}$: -0.3 ± 1.3 %; PRE-to-POST $ºC_{hand}$: 0.1 ± 1.9 %; PRE-to-POST48h $ºC_{hand}$: 0.4 ± 2.4 %; PRE-to-POST $ºC_{foot}$: -0.3 ± 2.4 %; PRE-to-POST48h $ºC_{foot}$: 0.2 ± 2.1 %; $p > 0.05$.

The BIVA score graph (Figure 1A) showed that the triathletes' vectors fell mostly outside the 50% tolerance ellipse and occupied a position more to the left of the

major axis compared with the reference population, indicating a higher density of body cell mass (BCM) than the reference population.

The BIVA mean graph (Figure 2) also showed the mean triathletes' vector shifted to the left and upwards (T^2 = 18.6; p = 0.0001), and therefore with greater PA, in comparison with the reference population.

The BIA vector migration was characterized by an increase in R/h and Xc/h (p = 0.001), indicating dehydration after the race (Figure 1A). This was in agreement with the observed loss of BM (p = 0.0001).

On the other hand, increased BM and decreased bioelectrical values (p = 0.0001) were observed from POST to POST48h. When PRE to POST48h were compared, BM values were still significantly lower than at baseline. In turn, bioelectrical values were found to be significantly lower compared with PRE values, also identified by BIVA (Figures 1B).

A positive relationship was found between racing time and basal R/h (r = 0.68; p = 0.04) and Z/h (r = 0.68; p = 0.045). Besides, basal R/h and Z/h were highly and positively related to PRE-to-POST bioelectrical changes of R/h and Z/h (r = 0.8; p = 0.009). PRE-to-POST bioelectrical changes of R/h and Z/h were also highly and positively related to racing time (r = 0.8, p = 0.01) and TRIMP (r = 0.8, p ≤ 0.02). Finally, no statistically significant correlation was observed between bioelectrical changes in relation to BM. Furthermore, no statistically significant correlation was observed between BM changes in the different time points and racing time, internal workload and energy deficit (ranges: r = - 0.46 to 0.65; p = 0.06 to 0.98).

On the other hand, multiple linear regression analysis revealed that an increase in R/h and Z/h after the race was explained by a larger decrease in BM, greater TRIMP, and a slower racing time and ($r_m^2{}_{adj}$ = 0.88) (Table 2). On the other hand, the racing time was found to be moderately explained by an increase in Z/h at POST ($r_m^2{}_{adj}$ = 0.58).

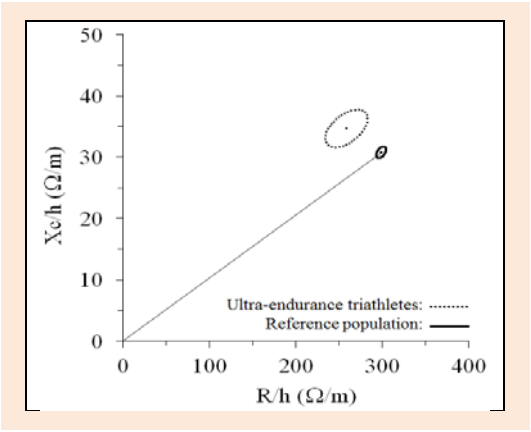

Figure 2. RXc mean graph. The 95% confidence ellipses for the mean impedance vectors of triathletes (dotted line ellipse) and the healthy male reference population (solid line ellipse with vector) (Piccoli et al., 1995) are shown. R/h, height-adjusted resistance; Xc/h, height-adjusted reactance.

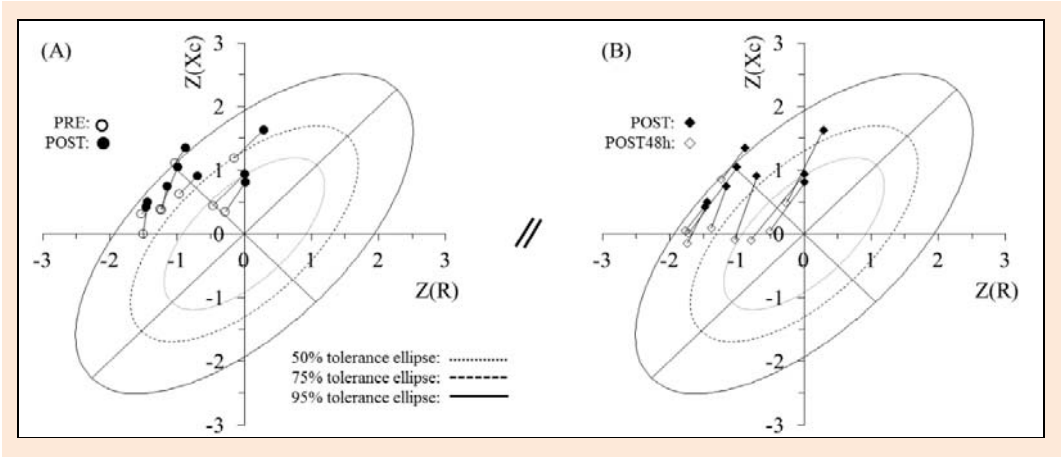

Figure 1. Individual vector score values for the RXc score graph with 50%, 75%, and 95% tolerance ellipses are plotted for: A) PRE- to POST-race, B) POST- to POST48h-race. Z(R), resistance Z score; Z(Xc), reactance Z score.

Discussion

Bioelectrical patterns in the ultra-endurance triathletes

When triathletes were compared to the healthy reference population, individual vectors were scattered mostly outside the 50% tolerance ellipse, positioned to the left of the major axis (Figure 1A). According to the urine color analysis, participants arrived to PRE in euhydration state. However, only two of them were plotted inside the 50% tolerance ellipse (Figure 1A). Since similar findings have already been observed in other sport samples (Campa and Toselli, 2018; Carrasco-Marginet et al., 2017; Gatterer et al., 2014; Giorgi et al., 2018; Koury et al., 2014; Micheli et al., 2014), this could reflect the specific body composition of athletes, characterized by greater soft tissue mass and different fluid content (Andreoli et al., 2001). Furthermore, the greater PA of the triathletes observed in this study (Figure 2) could indicate better cell function (Norman et al., 2012) and differing fluid distribution (i.e., increased intracellular water content) (Chertow et al., 1995), likely due to the hypertrophy of muscle fibers (Micheli et al., 2014). On the other hand, a significantly positive relationship was found between basal R/h (and therefore, Z/h) and racing time in the triathletes. This implies that the vector of triathletes who had better performance in the race (and, presumably, higher performance levels pre-race) would be displaced to the left, due to lower R/h values. Accordingly, it was observed that triathletes who registered lower basal R/h (and Z/h), racing time and internal workload showed smaller changes in these parameters after the race,

Table 1. Anthropometric and bioelectrical variables before (PRE), after (POST), and 48 hours after the race (POST48h).

				Δ-value (%)			Cohen's d		
	PRE	POST	POST48h	PRE–POST	POST–POST48h	PRE–POST48h	PRE–POST	POST–POST48h	PRE–POST48h
Anthropometric									
BM (kg)	76.0 (6.9)	72.1 (6.1)	75.0 (6.3)	-5.0 (0.9)*	4.0 (0.9)*	-1.3 (1.1)*	3.9‡	-4.3‡	1.1‡
C_{RT} (mm)	54.0 (1.7)	53.3 (1.9)	53.9 (1.7)	-1.3 (1.7)	1.1 (1.8)	-0.2 (0.3)	0.8‡	-0.6†	0.8‡
C_{LT} (mm)	54.2 (1.8)	53.5 (2.0)	54.1 (1.7)	-1.2 (1.7)	1.1 (1.7)	-0.2 (0.3)	0.7†	-0.6†	0.5†
C_{RC} (mm)	38.1 (1.3)	37.7 (1.5)	38.0 (1.3)	-1.1 (1.6)	0.9 (1.6)	-0.3 (0.4)	0.7†	-0.6†	0.8‡
C_{LC} (mm)	38.3 (1.3)	37.8 (1.5)	38.2 (1.2)	-1.2 (1.6)	1.0 (1.8)	-0.2 (0.4)	0.7†	-0.5†	0.6†
Bioelectrical									
R (Ω)	452.6 (45.8)	470.0 (56.6)	435.2 (46.4)	3.7 (2.3)*	-7.2 (2.9)*	-3.9 (2.1)*	-1.4‡	2.2‡	1.9‡
Xc (Ω)	60.6 (4.4)	65.6 (4.6)	55.5 (3.7)	8.3 (3.7)*	-15.2 (4.5)*	-8.2 (4.8)*	-2.3‡	3.0‡	1.7‡
Z (Ω)	456.6 (45.8)	474.6 (56.5)	438.8 (46.2)	3.8 (2.3)*	-7.4 (2.9)*	-3.9 (2.0)*	-1.5‡	2.2‡	2.0‡
R/h (Ω/m)	258.4 (22.4)	268.3 (28.3)	248.6 (24.0)	3.7 (2.3)*	-7.2 (2.9)*	-3.9 (2.1)*	-1.5‡	2.3‡	1.9‡
Xc/h (Ω/m)	34.7 (2.8)	37.5 (2.8)	31.8 (2.4)	8.3 (3.7)*	-15.2 (4.5)*	-8.2 (4.8)*	-2.3‡	3.0‡	1.7‡
Z/h (Ω/m)	260.8 (22.4)	270.9 (28.3)	250.7 (23.9)	3.8 (2.3)*	-7.4 (2.9)*	-3.9 (2.0)*	-1.5‡	2.3‡	2.0‡
PA (Ω)	7.7 (0.5)	8.0 (0.7)	7.3 (0.7)	3.7 (4.9)	-8.7 (4.9)*	-5.4 (6.4)	-0.8‡	1.7‡	0.9‡
r (R/h, Xc/h)	0.52	0.65	0.27	–	–	–	–	–	–

Values are mean ± SD (95% CI); BM, body mass; C_{RT}, circumference of the right thigh; C_{LT}, circumference of the left thigh; C_{RC}, circumference of the right calf; C_{LC}, circumference of the left calf; R, resistance; Xc, reactance; Z, impedance vector module; h, body height; PA, phase angle; r, Pearson's correlation coefficient between R/h and Xc/h; time-point differences**:** *RM-ANOVA, repeated measures analysis of variance with Bonferroni post-hoc test**,** significance at $p_{adj} < 0.017$; †, medium effect size ($d \leq 0.5$); ‡, large effect size ($d \leq 0.8$).

Table 2. Multiple linear regression analysis of bioelectrical, anthropometric and performance parameters before (PRE) and after the race (POST).

		r_m^2		ANOVA				SE_E
Dependent Variables	**Explanatory equations**	**Exact**	**Adjusted**	**F**	df_1	df_2	p	
$R/h_{PRE\text{-}POST}$ (Δ%)	-28.40 - (1.14 · $BM_{PRE\text{-}POST}$) + (0.021 · time) + (0.010 · TRIMP)	0.92	0.88	19.95	3	5	0.003	0.80
$Z/h_{PRE\text{-}POST}$ (Δ%)	-28.62 - (1.17 · $BM_{PRE\text{-}POST}$) + (0.022 · time) + (0.010 · TRIMP)	0.93	0.88	29.42	3	5	0.003	0.79
Racing time (min)	660.3 + (24.4 · $Z/h_{PRE\text{-}POST}$)	0.63	0.58	11.914	1	7	0.01	45.7

F_{in} ($p \leq 0.05$), F_{out} ($p \geq 0.10$). R, resistance; Z, impedance module; h, body height; BM, body mass; TRIMP, training impulse; time, racing time; r_m^2, multiple regression coefficient squared; SE_E, standard error of estimation.

experiencing lower decreases of body fluids. Vectors shifted to the left have been already reported with performance level in different sport samples (Carrasco-Marginet et al., 2017; Giorgi et al., 2018; Micheli et al., 2014), probably as a result of the specific training process. This particularly relevant finding highlights the need of further research regarding this matter, since the application of a non-invasive technique could eventually help to discriminate between performance levels of athletes according to the position of their vectors.

BM and bioelectrical changes evoked by UET

Regarding the changes induced by UET, the event evoked a mild-to-moderate dehydration, according to the average BM loss of ~5% (McDermott et al., 2017). Furthermore, individual vectors' migration along the major axis was observed due to an increase in R and Xc (Figure 1A), which is consistent with fluid loss according to the literature. To our knowledge, only three studies have applied BIVA to analyze short-term vector changes induced by exercise. The articles revealed similar findings to those of the present study, reporting increased R and Xc after exercise, as well as a vector migration (Carrasco-Marginet et al., 2017; Gatterer et al., 2014, Heavens et al., 2016).

Since R is the opposition of the conductor to the flow of current, a significant increase in these values would indicate a decrease in body fluids (O'Brien et al., 2002), which was supported by the significant decrease in BM.

With regard to reactance, the increased Xc after exercise has been suggested as an indicator of fluid shifts between intra- and extracellular compartments (Gatterer et al., 2014). Nevertheless, the meaning of Xc after performing exercise remains to be clarified. As mentioned in Castizo-Olier et al. (2018), multiple factors may affect Xc values (e.g. size, thickness, composition and distance between cell membranes; fluid distribution and characteristic frequency variations; ...) and further research should focus on this parameter in the exercise context.

In relation to the vector analysis, however, it is important to highlight that although directional changes in vector values from serial measurements seem consistent with fluid loss, BIVA is not currently able to identify type and magnitude of fluid loss. This is probably because the range of "normal hydration" comprised by the ellipses is wider than a hydration status/change considered as "dehydration" through other methodologies (Heavens et al., 2016). As mentioned in Heavens et al. (2016), the analysis of the vector length could be the key for serial measurements of hydration status.

On the other hand, although significant differences were found after the race, no correlation was observed between changes in BM and BIA variations in any situation. The lack of correlation found PRE-to-POST has been also depicted in Gatterer et al. (2014) and Carrasco-Marginet et al. (2017). The authors related the absence of correlation with a decrease in BM without an effective net negative

fluid balance as a result of exercise-related factors such as sweat rate, respiratory water loss and oxidative water production (Maughan et al., 2007).

The multiple regression analysis allows the study of the bioelectrical vector's behavior from a multifactorial perspective. Thus, while BM does not correlate with bioelectrical parameters as an isolated variable, it is selected as an explanatory factor in the multivariate model (Table 2). When the bioelectrical parameters were analyzed as dependent variables, the changes observed in R/h and Z/h after the race were significantly explained by the behavior of BM, the racing time and the estimated internal workload, which makes sense. When the process was further applied in reverse in order to add information to the multivariate analysis, alternating the role between dependent and independent variables, the racing time was significantly explained by the behavior of Z/h from PRE-to-POST. In the event that, in the future, this methodology could be validated, it could be a possible indicator of the training/competition load. In addition, using the multivariate analysis, some variables (e.g., race time) could be estimated analyzing the behavior of other parameters, with a certain degree of error. Therefore, this type of analysis should be taken into consideration both in the interpretation of certain variables related to vector behavior and in future studies about this topic. However, the limited sample analyzed in the present study forces us to be cautious in drawing robust conclusions.

In relation with PRE-to-POST48h changes, the individual vectors' migration along the major axis due to significant decreases in R and Xc (Figure 1B) indicates fluid accumulation (Lukaski and Piccoli, 2012), while BM values were still significantly lower than at baseline. Triathletes followed individual uncontrolled POST-to-POST48h recovery strategies (nutrition, hydration, physical activity, environmental conditions, etc.). However, the other mentioned factors that could affect the accuracy and reliability of BM and BIA measurements were controlled, being PRE and POST48h on equal terms. Thus, while BM alone does not detect a return to basal conditions, significant bioelectrical decreases below basal values were observed, indicating fluid retention in comparison with PRE. This highlights the potential advantage of BIVA in providing additional information about hydration changes in comparison with BM alone. However, it is important to clarify that neither BM nor BIVA can really identify what happened to TBW. As already mentioned, BIVA reflects changes in ECW estimates only. Thus, ICW may still be reduced (captured by BM at this time point) with a migration of fluids to the ECW space.

Fluid retention has been already reported in ultra-endurance events (Knechtle et al., 2008a; Knechtle et al., 2008b; Knechtle et al., 2009). As explained by Knechtle et al. (2009), although the reasons and mechanisms for the fluid increase are still unknown, it could be due to several factors. One explanation for this fluid retention could be an increase in plasma volume due to a higher activity of aldosterone and antidiuretic hormone (Neumayr et al., 2005). Moreover, protein catabolism with consequent fluid shifts (hypoproteinemic edema) might occur in an ultra-endurance effort (Lehmann et al., 1995). Another possible factor for the observed fluid expansion could be the increased plasma protein concentration inducing an increase in plasma oncotic pressure (Maughan et al., 1985; Mischler et al., 2003). On the other hand, the increase of body fluids could also be a result of the impairment of renal function due to the rhabdomyolysis that may occur in ultra-endurance events (Kim et al., 2007; Skenderi et al., 2006; Uberoi et al., 1991), although in general, acute renal failure in an ultra-endurance event is very rare (MacSearraigh et al., 1979). Finally, regarding the putative factors explaining the fluid increase 48 hours after the race, the already mentioned individual uncontrolled POST-to-POST48h recovery strategies may have affected these findings. Therefore, the identification of fluid retention after ultra-endurance events through BIVA should be further studied together with tests investigating the aforementioned mechanisms, in order to analyze possible associations.

Regarding the behavior of Xc from PRE-to-POST48h, the decreased values could indicate a reduction in soft tissues, since Xc is proposed as an indicator of dielectric mass (membranes and tissue interfaces) in soft tissues (Lukaski, 1996). As already mentioned, there is evidence of muscle damage after prolonged exercise. Significant modifications in markers of muscle damage and inflammation two days after the end of the event have been reported (Carmona et al., 2015; Millet et al., 2011; Overgaard et al., 2002). Further studies should investigate the relationship between the behavior of Xc and muscle damage biomarkers after completing this type of events. It would be interesting to analyze the validity of this parameter (obtained from a minimally invasive method) in order to control the muscle disruption recovery after exercise. Nevertheless, taking into account the aforementioned limitation of not controlling individual recovery strategies, it is difficult at present to elucidate the reasons why this behavior occurs due to the already mentioned problems in the interpretation of Xc when the fluid distribution changes.

Limitations of the study

In protocols measuring acute vector changes (before and after exercise), some factors should be controlled due to their influence in the bioelectrical signal in order to avoid measurement errors and provide accurate and reliable results (Castizo-Olier et al., 2018). The present study attempted to control all these factors. Nevertheless, in order to respect an ecological design and due to the characteristics of the sport event, the free consumption of food and beverage was allowed. The ingestion of food or beverages has an influence on Z, which may decrease over a 2- to 4-h period after a meal, generally representing a change of < 3% in Z values (Kushner et al., 1996). The ultra-endurance triathlon race started at 6:00 a.m. and lasted for many hours (~12.5 h on average). Therefore, each participant had her/his own nutritional strategies before competition (e.g., usually they have breakfast 2-3 hours before the race), which we could not control. This could have influence the bioelectrical values obtained pre-race. Furthermore, due to the free consumption of food and beverage during the race, an underestimation of ~14 Ω in Z values could have occurred in the post-exercise bioelectrical measurements. However, the amount of water intake at the end of the race

should not have affected these measurements because the recent ingestion of a meal or beverage (< 1 h from the ingestion to bioelectrical measurements) appears to be "electrically silent" and to have a minimal effect on whole-body Z (Evans et al., 1998).

The ecological design of the study itself implied also certain limitations. The post-race measurement was notably the most difficult assessment because of the multiple factors that must be controlled due to their influence on the bioelectrical signal at a moment in which athletes are extremely tired and less motivated (after exercising for more than 12 h). Moreover, the time at which the fluid/food intakes were performed during the race was not registered. On the other hand, there were no records of each individual's recovery strategies. Although the sample size was previously estimated (see Participants) and post-tests power calculation was within the expected values, underpowered sample size cannot be completely discarded, which limit the possibility of reaching stronger inferences, especially in the multiple regression models. Finally, no specific triathlon tolerance ellipses exist yet to allow a more enriching analysis.

Conclusion

A specific bioelectrical distribution was found in the present group of triathletes in comparison with the healthy, general reference population. This justifies the need to create specific tolerance ellipses that could discriminate triathletes of different performance level, age, sex, race, etc. Furthermore, the relationship between basal vector position of triathletes and performance variables should be further investigated, since it could help to discriminate between performance levels of athletes according to the position of their vectors. Although BIVA is not currently able to identify type and magnitude of fluid loss, vector migration appears to be consistent with fluid loss induced by a UET event. Furthermore, reversed vector displacement beyond the basal position 48 h after the race is consistent with fluids recovery, while BM did not return to PRE values. This highlights the potential advantage of BIVA in better informing about hydration changes in comparison with BM alone. Furthermore, the bioelectrical analysis seems to be consistent to muscle disruption in the recovery period after completing this type of events. Although with the utmost caution, there seems to be a relationship between the behavior of the bioelectrical vector and certain performance parameters. The multivariate analysis may help to better understand the bioelectrical vector's behavior pre- to post-exercise. BIVA may be helpful in assessing hydration changes in real time and could also complement the current hydration biomarkers that require a mobile laboratory. Nonetheless, further research must investigate the applicability of BIVA as a valid and reliable biomarker of hydration status, especially regarding the behavior of Xc after exercise.

Acknowledgements

This work was supported by the Government of Catalonia, Agency for Management of University and Research Grants (AGAUR) under Grant 2015 FI_B 00217. The study was conducted with the support of the National Institute of Physical Education of Catalonia (INEFC), Government of Catalonia. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors have no conflicts of interest to declare. All experiments comply with the current laws of the country.

References

Andreoli, A., Monteleone, M., Van Loan, M., Promenzio, L., Tarantino, U. and De Lorenzo, A. (2001) Effects of different sports on bone density and muscle mass in highly trained athletes. *Medicine and Science in Sports and Exercise* **33**, 507-511.

Armstrong, L.E. (2007) Assessing hydration status: the elusive gold standard. *Journal of the American College of Nutrition* **26**, 575S-584S.

Armstrong, L.E., Maresh, C.M., Castellani, J.W., Bergeron, M.F., Kenefick, R.W., LaGasse, K.E., Riebe, D. (1994) Urinary indices of hydration status. *International Journal of Sport Nutrition* **4**, 265-279.

Barrero, A., Erola, P. and Bescós, R. (2014) Energy balance of triathletes during an ultra-endurance event. *Nutrients* **7**, 209-222.

Campa, F. and Toselli, S. (2018) Bioimpedance Vector Analysis of Élite, Sub-Élite and Low-Level Male Volleyball Players. *International Journal of Sports Physiology and Performance* 1-13.

Carmona, G., Roca, E., Guerrero, M., Cussó, R., Irurtia, A., Nescolarde, L., Brotons, D., Bedini, J.L. and Cadefau, J.A. (2015) Sarcomere disruptions of slow fiber resulting from mountain ultramarathon. *International Journal of Sports Physiology and Performance* **10**, 1041-1047.

Carrasco-Marginet, M., Castizo-Olier, J., Rodríguez-Zamora, L., Iglesias, X., Rodríguez, F. A., Chaverri, D., Brotons, D. and Irurtia, A. (2017) Bioelectrical impedance vector analysis (BIVA) for measuring the hydration status in young elite synchronized swimmers. *PLoS One* **12**, e0178819.

Castizo-Olier, J., Irurtia, A., Jemni, M., Carrasco-Marginet, M., Fernández-García, R. and Rodríguez, F.A. (2018) Bioelectrical impedance vector analysis (BIVA) in sport and exercise: systematic review and future perspectives. *PLoS One*, in press.

Caton, J.R., Mole, P.A., Adams, W.C., Heustis, D.S. (1988) Body composition analysis by bioelectrical impedance: effect of skin temperature. *Medicine and Science in Sports and Exercise* 20, 489-491.

Chertow, G.M., Lowrie, E.G., Wilmore, D.W., Gonzalez, J., Lew, N.L., Ling, J., Leboff, M.S., Gottlieb, M.N., Huang, W. and Zebrowski, B. (1995) Nutritional assessment with bioelectrical impedance analysis in maintenance hemodialysis patients. *Journal of the American Society of Nephrology* **6**, 75-81.

Cheuvront, S.N., Kenefick, R.W. (2014) Dehydration: physiology, assessment, and performance effects. *Comprehensive Physiology* **4**, 257-285.

Cheuvront, S.N., Kenefick, R.W. (2017) CORP: Improving the status quo for measuring whole body sweat losses. *Journal of Applied Physiology* **123**, 632-636.

Codognotto, M., Piazza, M., Frigatti, P., Piccoli, A. (2008) Influence of localized edema on whole-body and segmental bioelectrical impedance. *Nutrition* **24**, 569-574.

De Lorenzo, A., Andreoli, A., Matthie, J. and Withers, P. (1997) Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. *Journal of Applied Physiology* **82**, 1542-1558.

Evans, W., McClagish, H. and Trudgett, C. (1998) Factors affecting the in vivo precision of bioelectrical impedance analysis. *Applied Radiation and Isotopes* **49**, 485-487.

Gatterer, H., Schenk, K., Laninschegg, L., Schlemmer, P., Lukaski, H. and Burtscher, M. (2014) Bioimpedance identifies body fluid loss after exercise in the heat: a pilot study with body cooling. *PLoS One* **9**, e109729.

Giorgi, A., Vicini, M., Pollastri, L., Lombardi, E., Magni, E., Andreazzoli, A., Orsini, M., Bonifazi, M., Lukaski, H. and Gatterer, H. (2018) Bioimpedance patterns and bioelectrical impedance vector analysis (BIVA) of road cyclists. *Journal of Sports Sciences* 1-6.

Goovaerts, H., Faes, T.J., De Valk-De Roo, G., Ten Bolscher, M., Netelenbosch, J., Van der Vijgh, W. and Heethaar, R. (1998) Extra-cellular volume estimation by electrical impedance-phase measurement or curve fitting: a comparative study. *Physiological Measurement* **19**, 517.

Gualdi-Russo, E., Toselli, S. (2002) Influence of various factors on the

measurement of multifrequency bioimpedance. *Homo* **53**, 1-16.
Heavens, K.R., Charkoudian, N., O'Brien, C., Kenefick, R.W., Cheuvront, S.N. (2016) Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the resistance-reactance–score graph method. *The American Journal of Clinical Nutrition* **103**, 724-729.
Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., Disselhorst-Klug, C., Hägg, G. (1999) European recommendations for surface electromyography. *Roessingh Research and Development* **8**, 13-54.
Hew-Butler, T., Collins, M., Bosch, A., Sharwood, K., Wilson, G., Armstrong, M., Jennings, C., Swart, J. and Noakes, T. (2007) Maintenance of plasma volume and serum sodium concentration despite body weight loss in ironman triathletes. *Clinical Journal of Sport Medicine* **17**, 116-122.
Jeukendrup, A.E., Jentjens, R. L. and Moseley, L. (2005) Nutritional considerations in triathlon. *Sports Medicine* **35**, 163-181.
Kim, H.J., Lee, Y.H. and Kim, C.K. (2007) Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *European Journal of Applied Physiology* **99**, 443-447.
Knechtle, B., Duff, B., Schulze, I. and Kohler, G. (2008a) A multi-stage ultra-endurance run over 1,200 km leads to a continuous accumulation of total body water. *Journal of Sports Science and Medicine* **7**, 357.
Knechtle, B., Fraire, O.S., Andonie, J. and Kohler, G. (2008b) Effect of a multistage ultra-endurance triathlon on body composition: World Challenge Deca Iron Triathlon 2006. *British Journal of Sports Medicine* **42**, 121-125.
Knechtle, B., Wirth, A., Knechtle, P. and Rosemann, T. (2009) Increase of total body water with decrease of body mass while running 100 km nonstop—formation of edema? *Research Quarterly for Exercise and Sport* **80**, 593-603.
Knechtle, B., Zingg, M.A., Rosemann, T., Stiefel, M., Rüst, C.A. (2015) What predicts performance in ultra-triathlon races?–a comparison between Ironman distance triathlon and ultra-triathlon. *Open Access Journal of Sports Medicine* **6**, 149-159.
Koury, J., Trugo, N. and Torres, A. (2014) Phase angle and bioelectrical impedance vectors in adolescent and adult male athletes. *International Journal of Sports Physiology and Performance* **9**, 798-804.
Kushner, R., Gudivaka, R. and Schoeller, D. (1996) Clinical characteristics influencing bioelectrical impedance analysis measurements. *The American Journal of Clinical Nutrition* **64**, 423S-427S.
Laursen, P.B. and Rhodes, E.C. (2001) Factors affecting performance in an ultraendurance triathlon. *Sports Medine* **31**, 195-209.
Laursen, P.B., Suriano, R., Quod, M.J., Lee, H., Abbiss, C.R., Nosaka, K., Martin, D.T. and Bishop, D. (2006) Core temperature and hydration status during an Ironman triathlon. *British Journal of Sports Medicine* **40**, 320-325.
Lehmann, M., Huonker, M., Dimeo, F., Heinz, N., Gastmann, U., Treis, N., Steinacker, J., Keul, J., Kajewski, R. and Häussinger, D. (1995) Serum amino acid concentrations in nine athletes before and after the 1993 Colmar ultra triathlon. *International Journal of Sports Medicine* **16**, 155-159.
Liang, M.T., Norris, S. (1993) Effects of skin blood flow and temperature on bioelectric impedance after exercise. *Medicine and Science in Sports and Exercise* 25, 1231-1239.
Lukaski, H.C. (1996) Biological indexes considered in the derivation of the bioelectrical impedance analysis. *The American Journal of Clinical Nutrition* **64**, 397S-404S.
Lukaski, H.C. (2013) Evolution of bioimpedance: a circuitous journey from estimation of physiological function to assessment of body composition and a return to clinical research. *European Journal of Clinical Nutrition* **67**, S2.
Lukaski, H.C. and Piccoli, A. (2012) Bioelectrical impedance vector analysis for assessment of hydration in physiological states and clinical conditions. In: *Handbook of Anthropometry*. Ed; Preedy, V. London: Springer. 287-305.
MacSearraigh, E.T., Kallmeyer, J.C. and Schiff, H.B. (1979) Acute renal failure in marathon runners. *Nephron* **24**, 236-240.
Matthie, J. and Withers, P. (1995) The ambiguities of predicting total body water and body cell mass with a single frequency (50KHz) measurement of bioimpedance. *Journal of the American Society of Nephrology* **6**, 1682-1685.
Maughan, R., Whiting, P. and Davidson, R. (1985) Estimation of plasma volume changes during marathon running. *British Journal of Sports Medicine* **19**, 138-141.
Maughan, R.J., Shirreffs, S.M. and Leiper, J.B. (2007) Errors in the estimation of hydration status from changes in body mass. *Journal of Sports Sciences* **25**, 797-804.
McDermott, B.P., Anderson, S.A., Armstrong, L.E., Casa, D.J., Cheuvront, S.N., Cooper, L., Kenney, W.L., O'Connor, F.G., Roberts, W.O. (2017) National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. *Journal of Athletic Training* **52**, 877-895.
McGarvey, J., Thompson, J., Hanna, C., Noakes, T. D., Stewart, J. and Speedy, D. (2010) Sensitivity and specificity of clinical signs for assessment of dehydration in endurance athletes. *British Journal of Sports Medicine* **44**, 716–719.
Micheli, M.L., Pagani, L., Marella, M., Gulisano, M., Piccoli, A., Angelini, F., Burtscher, M. and Gatterer, H. (2014) Bioimpedance and Impedance Vector Patterns as Predictors of League Level in Male Soccer Players. *International Journal of Sports Physiology and Performance* **9**, 532-539.
Millet, G.Y., Tomazin, K., Verges, S., Vincent, C., Bonnefoy, R., Boisson, R.-C., Gergelé, L., Féasson, L. and Martin, V. (2011) Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One* **6**, e17059.
Mischler, I., Boirie, Y., Gachon, P., Pialoux, V., Mounier, R., Rousset, P., Coudert, J. and Fellmann, N. (2003) Human albumin synthesis is increased by an ultra-endurance trial. *Medicine and Science in Sports and Exercise* **35**, 75-81.
Moreno, M.V., Ribbe-Cornet, E., Rebeyrol, J., Vannicatte, A. and Krief, L. (2015) Evaluation of a new impedancemeter to independently measure extracellular, intracellular and total body water volumes: application to the measurement of hydration. *Medical and Biological Engineering and Computing* **53**, 989-999.
Neumayr, G., Pfister, R., Hoertnagl, H., Mitterbauer, G., Prokop, W. and Joannidis, M. (2005) Renal function and plasma volume following ultramarathon cycling. *International Journal of Sports Medicine* **26**, 2-8.
Norman, K., Stobäus, N., Pirlich, M. and Bosy-Westphal, A. (2012) Bioelectrical phase angle and impedance vector analysis - Clinical relevance and applicability of impedance parameters. *Clinical Nutrition* **31**, 854-861.
O'Brien, C., Young, A. and Sawka, M. (2002) Bioelectrical impedance to estimate changes in hydration status. *International Journal of Sports Medicine* **23**, 361-366.
Oppliger, R.A., Bartok, C. (2002) Hydration testing of athletes. *Sports Medicine* **32**, 959-971.
Overgaard, K., Lindstrøm, T., Ingemann-Hansen, T. and Clausen, T. (2002) Membrane leakage and increased content of Na+-K+ pumps and Ca2+ in human muscle after a 100-km run. *Journal of Applied Physiology* **92**, 1891-1898.
Peiffer, J.J., Abbiss, C.R., Nosaka, K., Peake, J.M., Laursen, P.B. (2009) Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. *Journal of Science and Medicine in Sport* **12**, 91-96.
Piccoli, A. (2005) Whole body-single frequency bioimpedance. *Contributions to Nephrology* **149**, 150-161.
Piccoli, A. (2010) Bioelectric impedance measurement for fluid status assessment. *Contributions to Nephrology* **164**, 143-152.
Piccoli, A., Nigrelli, S., Caberlotto, A., Bottazzo, S., Rossi, B., Pillon, L. and Maggiore, Q. (1995) Bivariate normal values of the bioelectrical impedance vector in adult and elderly populations. *The American Journal of Clinical Nutrition* **61**, 269-270.
Piccoli, A., Pillon, L., Dumler, F. (2002). Impedance vector distribution by sex, race, body mass index, and age in the United States: standard reference intervals as bivariate Z scores. *Nutrition* **18**, 153-167.
Piccoli, A., Rossi, B., Pillon, L. and Bucciante, G. (1994) A new method for monitoring body fluid variation by bioimpedance analysis: the RXc graph. *Kidney International* **46**, 534-539.
Rush, E.C., Crowley, J., Freitas, I.F., Amy, L. (2006) Validity of hand-to-foot measurement of bioimpedance: standing compared with lying position. *Obesity* **14**, 252-57.
Segal, K.R., Burastero, S., Chun, A., Coronel, P., Pierson Jr, R.N., Wang, J. (1991) Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. *The American Journal of Clinical Nutrition* **54**, 26-29.
Sharwood, K., Collins, M., Goedecke, J., Wilson, G. and Noakes, T. (2004) Weight changes, medical complications, and performance during an Ironman triathlon. *British Journal of Sports Medicine* **38**, 718-724.

Skenderi, K.P., Kavouras, S.A., Anastasiou, C.A., Yiannakouris, N. and Matalas, A. (2006) Exertional rhabdomyolysis during a 246-km continuous running race. *Medicine and Science in Sports and Exercise* **38**, 1054-1057.

Slinde, F., Bark, A., Jansson, J., Rossander-Hulthén, L. (2003) Bioelectrical impedance variation in healthy subjects during 12 h in the supine position. *Clinical Nutrition* **22**, 153-157.

Stewart, A., Marfell-Jones, M., Olds, T. and de Ridder, H. (2011) *International standards for anthropometric assessment*. Portsmouth: International Society for the Advancement of Kinanthropometry.

Uberoi, H., Dugal, J., Kasthuri, A., Kolhe, V., Kumar, A. and Cruz, S. (1991) Acute renal failure in severe exertional rhabdomyolysis. *The Journal of the Association of Physicians of India* **39**, 677-679.

Yanovski, S.Z., Hubbard, V.S., Heymsfield, S.B. and Lukaski, H.C. (1996) Bioelectrical impedance analysis in body composition measurement: National institutes of health technology assessment conference statement. *The American Journal of Clinical Nutrition* **64**, 524S-532S.

Key points

- BIVA shows a specific bioelectrical distribution in a group of triathletes in comparison with the healthy, general reference population.
- Although BIVA is not currently able to identify type and magnitude of fluid loss, vector migration appears to be consistent with fluid loss induced by a UET event and with fluid retention 48 h after the race.
- BIVA seems to provide additional information about hydration changes 48 h after the UET event compared with BM alone. However, it is important to highlight that neither BM nor BIVA can really identify what happened to TBW. BIVA reflect changes in ECW estimates only. Thus, ICW may still be reduced (captured by BM 48h post-race) with a migration of fluids to the ECW space.
- This method could be sensitive to different performance levels between triathletes and to muscle disruption 48 hours after completing a UET event.

AUTHOR BIOGRAPHY

Jorge CASTIZO-OLIER
Employment
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, MSc, PhD candidate
Research interests
Body composition, bioimpedance, sports and exercise physiology
E-mail: j.castizo9@gmail.com

Marta CARRASCO-MARGINET
Employment
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, MSc, PhD
Research interests
Dietetics and sports nutrition
E-mail: martacm8@gmail.com

Alex ROY
Employment
Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, MSc, PhD candidate
Research interests
Triathlon, trail running, aquatic, cyclic and intermittent sports physiology, bioimpedance
E-mail: alex.roy.torner@gmail.com

Diego CHAVERRI
Employment
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, MSc, PhD
Research interests
Swimming, cycling and triathlon physiology and bioenergetics
E-mail: chaverri.diego@gmail.com

Xavier IGLESIAS
Employment
Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, PhD
Research interests
Fencing, bioenergetics and observational methodology in the field of sport
E-mail: xiglesias@gmail.com

Carla PÉREZ-CHIRINOS
Employment
Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, MSc, PhD candidate
Research interests
Bioimpedance, body composition, muscle injuries
E-mail: carla.perez.chirinos@gmail.com

Ferran A. RODRÍGUEZ
Employment
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
MD, PhD
Research interests
Aquatic, cyclic and intermittent sports physiology and bioenergetics, environmental physiology and medicine
E-mail: farodriguez@gencat.cat

Alfredo IRURTIA
Employment
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Barcelona, Spain
Degree
BSc, PhD
Research interests
Gymnastics, body composition, sports and exercise physiology
E-mail: airurtia@gencat.cat

✉ **Alfredo Irurtia**
INEFC-Barcelona Sport Sciences Research Group, Institut Nacional d'Educació Física de Catalunya (INEFC), Universitat de Barcelona (UB), Avinguda de l'Estadi, 12-22, 08038, Barcelona, Spain

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA) IN EXERCISE AND SPORTS PRACTICE

Jorge Castizo Olier

Institut Nacional d'Educació Física de Catalunya (INEFC) — Universitat de Barcelona (UB)
j.castizo9@gmail.com

BIOELECTRICAL IMPEDANCE VECTOR ANALYSIS (BIVA)
IN EXERCISE AND SPORTS PRACTICE

Jorge Castizo Olier