



UNIVERSITAT DE
BARCELONA

Evaluation of changes in global and muscular bioimpedance vector (BIVA) induced by physical exercise

Avaluació dels canvis en el vector de bioimpedància (BIVA)
global i muscular induïts per l'exercici físic

Álex Cebrián Ponce

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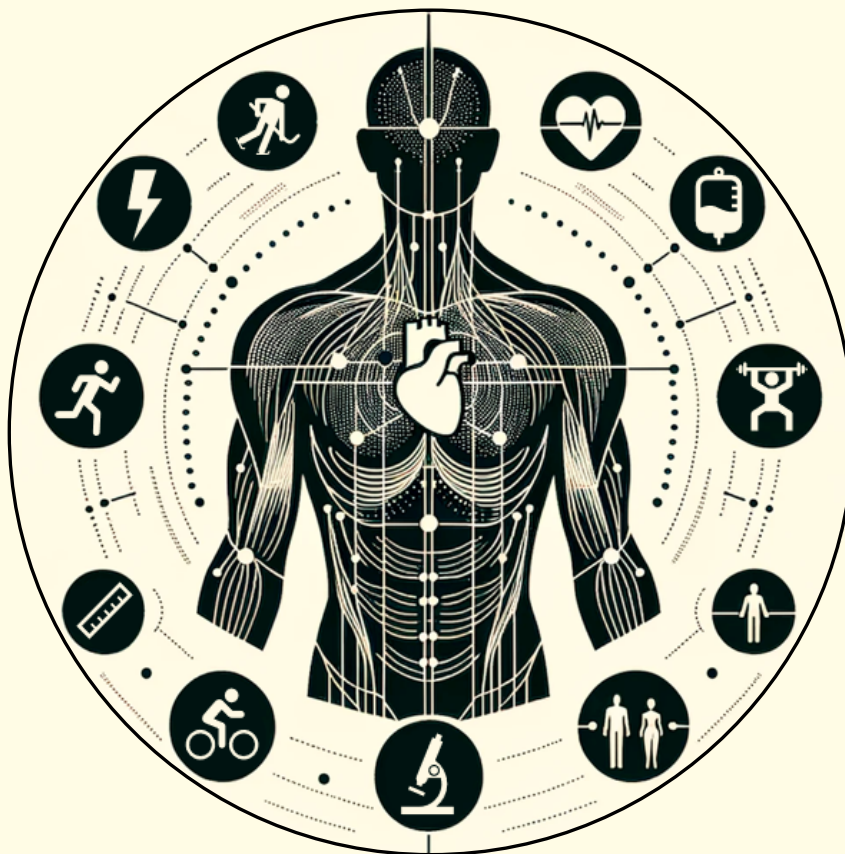
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Tesi Doctoral

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Álex Cebrián Ponce
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Centre de Barcelona

Programa de Doctorat

Activitat Física, Educació Física i Esport

Evaluation of changes in global and muscular bioimpedance
vector (BIVA) induced by physical exercise

Avaluació dels canvis en el vector de bioimpedància (BIVA)
global i muscular induïts per l'exercici físic

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Doctor per la Universitat de Barcelona amb menció internacional

Barcelona, 2024

Certificat de supervisió de la Tesi Doctoral

Dr. Alfredo Irurtia Amigó - INEFC Barcelona, Universitat de Barcelona.

Per mitjà d'aquest document, declaro:

La tesi doctoral titulada "**Avaluació dels canvis en el vector de bioimpedància (BIVA) global i muscular induïts per l'exercici físic**", presentada per l'Álex Cebrián Ponce per a l'adquisició del grau de Doctor amb Menció Internacional, és un compendi de publicacions científiques realitzades sota la meva supervisió.

Confirmo que el treball realitzat compleix amb tots els estàndards acadèmics i requisits establerts per a la seva defensa i lectura.

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La tesi doctoral titulada "**Avaluació dels canvis en el vector de bioimpedància (BIVA) global i muscular induïts per l'exercici físic**", presentada per l'Álex Cebrián Ponce per a l'adquisició del grau de Doctor amb Menció Internacional, és un compendi de publicacions científiques realitzades sota la meva supervisió.

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Per a constància de tot l'anterior, signo el present certificat.



Dr. Manuel Vicente Garnacho Castaño

Co-director de la Tesi Doctoral

Barcelona, 1 de març de 2024.

Ajut per a la formació Doctoral i Docent universitària

El candidat al doctorat compta amb el suport d'una ajuda de recerca dins del marc de les ajudes "Formació de Professorat Universitari" (FPU) [FPU20/00998]. Aquestes ajudes són part del Programa Estatal de Promoció del Talent i la seva Ocupabilitat en R+D+i, sota el Pla Estatal d'Investigació Científica, Tècnica i d'Innovació 2017-2020, segons l'Ordre CNU/692/2019 del 20 de juny.

Aquestes ajudes comprenen el finançament dels contractes predoctorals destinats a la formació investigadora en programes de doctorat per a la consecució del títol de Doctor/a i l'adquisició de competències docents universitàries en qualsevol àrea del coneixement científic, que facilitin la futura incorporació de nous doctors al sistema espanyol d'educació superior i d'investigació científica.

Álex Cebrián Ponce, 2024

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*Digues i ho oblido,
ensenya'm i ho recordo,
involucra'm i ho aprenc*

Benjamin Franklin

Agraïments

Aquesta tesi reflecteix les connexions i experiències compartides amb la meva família, amics i companys que han marcat el meu camí durant aquests quatre intensos anys. Cadascun de vosaltres m'ha inspirat i motivat a perseguir els meus somnis. Aquesta tesi és el fruit d'un esforç col·lectiu, i us estic profundament agraït. Gràcies a...

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Llistat de publicacions i comunicacions

La present tesi es fonamenta en les següents publicacions:

- I. **Cebrián-Ponce, Á.**, Irurtia, A., Carrasco-Marginet, M., Saco-Ledo, G., Girabent-Farrés, M., & Castizo-Olier, J. (2021). Electrical Impedance Myography in Health and Physical Exercise: A Systematic Review and Future Perspectives. *Frontiers in Physiology*, 12(September). <https://doi.org/10.3389/fphys.2021.740877>
- II. **Cebrián-Ponce, Á.**, Garnacho-Castaño, M. V., Castellano-Fàbrega, M., Castizo-Olier, J., Carrasco-Marginet, M., Serra-Paya, N., & Irurtia, A. (2022). Bioelectrical Impedance Vector and Creatine Phosphokinase Changes Induced by a High-Intensity Training Session in Rink Hockey Players. *Applied Sciences*, 12(2). <https://doi.org/10.3390/app12020751>
- III. **Cebrián-Ponce, Á.**, Irurtia, A., Castizo-Olier, J., Garnacho-Castaño, M. V., Espasa-Labrador, J., Noriega, Z., & Carrasco-Marginet, M. (2023). Bioelectrical, Anthropometric, and Hematological Analysis to Assess Body Fluids and Muscle Changes in Elite Cyclists during the Giro d'Italia. *Biology*, 12(450). <https://doi.org/10.3390/biology12030450>
- IV. **Cebrián-Ponce, Á.**, Marini, E., Stagi, S., Castizo-Olier, J., Carrasco-Marginet, M., Garnacho-Castaño, M. V., Noriega, Z., Espasa-Labrador, J., & Irurtia, A. (2023). Body fluids and muscle changes in trail runners of various distances. *PeerJ*, 1–17. <https://doi.org/10.7717/peerj.16563>

El marc teòric i la contextualització d'aquesta tesi doctoral s'arrelen en l'Estudi I, que consisteix en una revisió sistemàtica centrada en l'anàlisi vectorial de la impedància bioelèctrica aplicada al nivell muscular dins de l'àmbit esportiu i de la salut. D'altra banda, el component quasi-experimental de la tesi es materialitza a través dels Estudis II, III i IV, on s'analitzen els canvis del vector de bioimpedància induïts per l'exercici físic, abordant una gamma variada d'esports (hoquei patins, ciclisme i trail running), amb diversos contextos ecològics (entrenament i competició). A més, es distingeixen per la seva anàlisi de diferents co-paràmetres (càrrega d'entrenament i variables hematològiques). Aquesta aproximació multidimensional ofereix una perspectiva àmplia i detallada sobre la matèria del vector de bioimpedància i els seus canvis.

Aquest treball també compta amb el suport de diverses presentacions en conferències:

- V. **Cebrián-Ponce, Á.**, Garnacho-Castaño, M. V., Castizo-Olier, J., Irurtia, A., Carrasco-Marginet, M., & Serra-Paya, N. (2022). Cambios bioeléctricos en sujetos con claudicación intermitente tras 8 semanas de entrenamiento: estudio preliminar. Pòster presentat en el 8è congrés internacional de readaptació i prevenció de lesions en la activitat física i l'esport i 6è congrés internacional de salut i exercici físic. JAM Sports, 1-10 abril de 2022, en línia.
- VI. **Cebrián-Ponce, Á.**, Garnacho-Castaño, M. V., Castizo-Olier, J., Carrasco-Marginet, M., Espasa-Labrador, J., Noriega, Z., & Irurtia, A. (2022a). Muscle-Localized bioelectrical changes over a Giro d'Italia Pro race. Comunicació oral presentada en la 15th Baltic Sport Science Conference "Challenges and Solutions in Sport Science", ICSSPE, 28-29 d'abril de 2022, Lituània.
- VII. **Cebrián-Ponce, Á.**, Castizo-Olier, J., Garnacho-Castaño, M. V., Serra-Paya, N., Carrasco-Marginet, M., Espasa-Labrador, J., & Irurtia, A. (2022). Whole-body and muscle-localised bioelectrical impedance vector migration induced by a high-intensity rink hockey training session. Pòster presentat en el XVII Congreso Mundial de Cineantropometría, ISAK, 14-16 de juliol de 2022, Alacant, Espanya.
- VIII. **Cebrián-Ponce, Á.**, Castizo-Olier, J., Garnacho-Castaño, M. V., Serra-Paya, N., Carrasco-Marginet, M., Noriega, Z., & Irurtia, A. (2022). Inter-and intra-limb symmetry analysed by muscle-localised bioelectrical impedance vector analysis (ML-BIVA) in elite soccer players. Pòster presentat en el XVII Congreso Mundial de Cineantropometría, ISAK, 14-16 de juliol de 2022, Alacant, Espanya.
- IX. **Cebrián-Ponce, Á.**, Garnacho-Castaño, M. V., Castizo-Olier, J., Carrasco-Marginet, M., Espasa-Labrador, J., Noriega, Z., & Irurtia, A. (2022b). Whole-body and muscle-localized bioelectrical impedance vector migration over a Giro d'Italia pro race. Comunicació oral presentada en el European College of Sport Science, ECSS, 31 d'agost - 2 de setembre de 2022, Sevilla, Espanya.
- X. **Cebrián-Ponce, Á.**, Iglesias, X., Chaverri, D., Montraveta, J., Noriega, Z., Espasa-Labrador, J., Carrasco-Marginet, M., & Irurtia, A. (2022). Caracterización bioeléctrica de corredores de montaña experimentados. Comunicació oral

presentada en el I Congreso Iberoamericano de Antropometría Aplicada, RIBA², 21-22 d'octubre de 2022, Llatinoamèrica, España y Portugal (en línia).

- XI. Cebrián-Ponce, Á., & Irurtia, A. (2023).** Análisis de la fisiología muscular del deportista mediante el uso de la bioimpedancia. Comunicació oral presentada en la I Jornada de Tecnologías Aplicadas Al Deporte y a La Salud, UPC, 27 d'octubre de 2023, Terrassa, Espanya.
- XII. Cebrián-Ponce, Á., Marini, E., Stagi, S., Carrasco-Marginet, M., & Irurtia, A. (2024).** Cambios en los fluidos corporales y musculares en trail-runners. Pòster presentat en IV Congrés Anual Internacional d'Estudiants de Doctorat, Universitat Miguel Hernández d'Elx, 1-2 de febrer de 2024, Elx (en línia), Espanya.

Finalment, la present tesi doctoral s'enriqueix amb la inclusió d'altres estudis de recerca en els que he participat, aplicant el vector de bioimpedància des de perspectives alternatives, valorant facetes relacionades amb l'esport i la salut, proporcionant una visió més integral i holística sobre l'aplicabilitat de la bioimpedància i per tant, augmentant així la profunditat i l'abast d'aquesta tesi.

- XIII. Cebrián-Ponce, Á., Petri, C., Izzicupo, P., Micheli, M. L., Cortis, C., Fusco, A., Carrasco-Marginet, M., & Mascherini, G. (2023).** In-Season Longitudinal Hydration/Body Cell Mass Ratio Changes in Elite Rugby Players. *Sports*, 11(8). <https://doi.org/10.3390/sports11080142> [Annex 1]
- XIV. Mascherini, G., Corsi, M., Falconi, E., Cebrián-Ponce, Á., Checcucci, P., Pinazzi, A., Russo, D., Gitto, S., Sofi, F., & Stefani, L. (2024).** Unsupervised Exercise Intervention vs. Adherence to a Mediterranean Diet Alone : The Role of Bioelectrical Impedance Vector Analysis and Cardiovascular Performance in Liver-Transplanted Recipients. *Nutrients*, 16(190), 1–11. <https://doi.org/10.3390/nu16020190> [Annex 2]

Glossari

%FM	Percentatge de massa de greix
1RM	1 repetició màxima
A	Àrea de secció transversal
ADP	Pletismògraf per desplaçament d'aire
AQUA	<i>Anatomical Quality Assessment</i>
BCM	Massa cel·lular corporal
BIA	Anàlisi d'impedància bioelèctrica
BIS	Bioimpedància espectroscòpica
BIVA	Anàlisi del vector d'impedància bioelèctrica
BM	Massa corporal
BMI	Índex de massa corporal
CAT	Tomografia computada
CK	Creatina-fosfocinasa
D-EIM	Miografia d'impedància elèctrica dinàmica
DXA	Densitometria òssia de raigs X
ECM	Massa extracel·lular
ECW	Aigua extracel·lular
EIM	Miografia d'impedància elèctrica
FFM	Massa lliure de greix
FM	Massa de greix
Gràfics RXc	Gràfics de Resistència-Reactància
Hb	Hemoglobina
Hct	Hematòcrit
I	Intensitat del corrent elèctric
ICW	Aigua intracel·lular
ISAK	<i>International Society for the Advancement of Kinanthropometry</i>
L	Longitud del conductor (o longitud del segment corporal)
MID	Avaluació a meitat de la competició
ML-BIVA	Anàlisi del vector d'impedància bioelèctrica muscular
MRI	Ressonància magnètica

p	p-valor
PhA	Angle de fase
POST	Avaluació al finalitzar l'exercici
POST24	Avaluació a les 24 hores de finalitzar l'exercici
PRE	Avaluació abans de l'exercici
PRISMA	<i>Preferred Reporting Items for Systematic Reviews and Meta-Analyses</i>
R	Resistència
R/H	Resistència ajustada per l'estatura
R/L	Resistència ajustada per la longitud del segment
R_0	Resistència a freqüència zero
R_∞	Resistència a freqüència infinita
R_{esp}	Resistència específica
sEMG	Electromiografia de superfície
SENIAM	<i>Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles</i>
SFL	Capa de greix subcutània
T^2	Test quadrat d'Hotelling
TBW	Aigua corporal total
V	Tensió del corrent elèctric
WB-BIVA	Anàlisi del vector d'impedància bioelèctrica de tot el cos
X_c	Reactància
X_c/H	Reactància ajustada per l'estatura
X_c/L	Reactància ajustada per la longitud del segment
X_{Cesp}	Reactància específica
Z	Impedància
Z(R)	Z-score de resistència
Z(X_c)	Z-score de reactància
Z/H	Impedància ajustada per l'estatura
Z/L	Impedància ajustada per la longitud del segment
Z_{esp}	Impedància específica
% Δ	Valor percentual delta
ρ	Resistivitat específica
ω_c	Freqüència característica

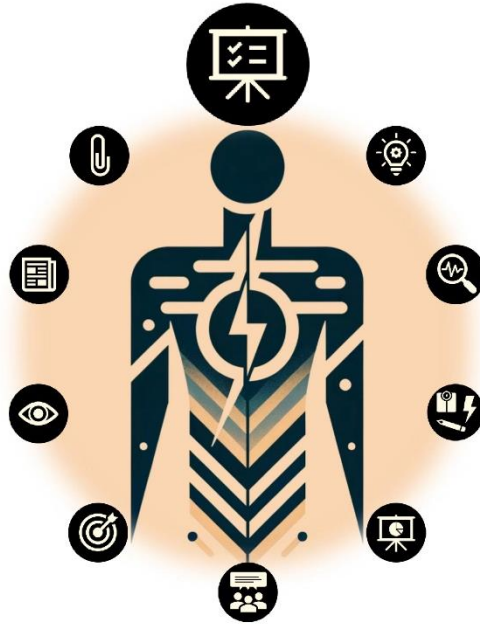
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RESUM

a. Resum (català)

La present tesi doctoral investiga l'aplicació de l'anàlisi del vector de la impedància bioelèctrica (BIVA) dins l'àmbit de les ciències de l'activitat física i l'esport. Aquesta recerca es centra en la utilització del mètode BIVA per a l'avaluació de la composició corporal i la gestió dels fluids corporals en esportistes. Contràriament a l'enfocament tradicional de la bioimpedància elèctrica, BIVA es distingeix per la seva habilitat proporcionant valoracions qualitatives i semi-quantitatives sense recórrer a equacions predictives, eludint així suposicions prèvies i potencials biaixos.

L'objectiu principal d'aquesta recerca és aprofundir en l'enteniment dels canvis induïts per l'exercici físic, els quals es poden observar mitjançant el vector de bioimpedància, tant a nivell global com a nivell muscular (ML-BIVA). Mitjançant aquesta perspectiva, es pretén aportar informació valuosa que contribueixi al desenvolupament de noves eines diagnòstiques i de seguiment específicament adaptades per a l'ús en el context esportiu.

Aquesta investigació integra quatre estudis científics. Inicialment, es realitza una revisió sistemàtica de la literatura existent sobre ML-BIVA (Estudi I), centrada en la seva aplicabilitat en el context de la salut i l'exercici físic. Els estudis següents, amb un enfocament quasi-experimental, exploren l'ús de BIVA (global i muscular) en diferents disciplines esportives: l'hoquei sobre patins (Estudi II), el ciclisme (Estudi III) i el trail running (Estudi IV). L'Estudi II es centra en la resposta dels paràmetres bioelèctrics, tant globals com musculars, a una sessió d'entrenament d'alta intensitat en jugadors d'hoquei patins. A més, busca correlacionar aquests canvis bioelèctrics amb els nivells de creatina-fosfocinasa (CK), per determinar el dany muscular post-exercici. L'Estudi III, avalua els canvis en els fluids corporals i musculars en ciclistes professionals al llarg del Giro d'Itàlia, mitjançant avaluacions antropomètriques, bioelèctriques i hematològiques, amb l'objectiu de trobar correlacions entre aquests indicadors. Finalment, l'Estudi IV investiga com les diferents distàncies en curses de trail running (14, 35 i 52 km) afecten els fluids corporals i musculars, aplicant mètodes antropomètrics, bioelèctrics i de CK.

Els resultats obtinguts de la recerca evidencien que BIVA representa una eina no invasiva, econòmica i eficaç, que facilita una monitorització precisa i en temps real de l'estat fisiològic dels esportistes. S'ha demostrat que les variacions observades a curt, mitjà i llarg termini en els paràmetres bioelèctrics (resistència, reactància i angle de fase) reflecteixen adaptacions en la distribució dels fluids i en la funcionalitat cel·lular posterior

a l'exercici físic. Això és particularment evident en processos com la rehidratació, la recuperació i els canvis en la composició corporal i la salut cel·lular. A més, aquestes adaptacions mostren una correlació amb millores en el rendiment esportiu, com per exemple en la força i la potència muscular.

En relació amb ML-BIVA, aquesta ofereix informació detallada essencial per a la creació d'estratègies personalitzades, tant en la recuperació de lesions com en l'elaboració de programes d'entrenament específics. L'avaluació de diversos grups musculars en múltiples contextos d'exercici destaca patrons que corroboren la seva capacitat per detectar canvis morfològics i fisiològics. Per a un ús eficaç de ML-BIVA, és crucial destacar la necessitat d'una estandardització rigorosa en el protocol d'avaluació. Aquesta estandardització és vital per augmentar la fiabilitat i la validesa de les mesures, i per assegurar una millor comparabilitat entre diferents investigacions.

Les futures línies d'investigació inclouen: ampliar bases de dades d'esportistes, realitzar estudis longitudinals, analitzar la interacció entre canvis bioelèctrics i paràmetres fisiològics, integrar noves tecnologies en l'avaluació esportiva i desenvolupar protocols estandarditzats per a ML-BIVA.

Paraules clau: bioimpedancia, composició corporal, esport, exercici físic, salut.

b. Abstract (english)

This doctoral thesis investigates the application of the bioelectrical impedance vector analysis (BIVA) within the field of physical activity and sports sciences. This research focuses on the use of the BIVA method for assessing body composition and body fluid management in athletes. Unlike the traditional approach of electrical bioimpedance, BIVA is distinguished by its ability to provide qualitative and semi-quantitative assessments without relying on predictive equations, thereby avoiding prior assumptions and potential biases.

The main objective of this research is to deepen the understanding of the changes induced by physical exercise, which can be observed through the bioimpedance vector, both at a whole-body level and at a muscular level (ML-BIVA). From this perspective, the aim is to provide valuable information that contributes to the development of new diagnostic tools and monitoring protocols, specifically adapted for the use in the sports context.

This investigation integrates four main scientific studies. Initially, a systematic review of the existing literature on ML-BIVA (Study I) is conducted, focusing on its applicability in the context of health and physical exercise. The subsequent studies, with a quasi-experimental approach, explore the use of BIVA (whole-body and muscular) in different sports disciplines: roller hockey (Study II), cycling (Study III), and trail running (Study IV). Study II focuses on the response of bioelectrical parameters, both whole-body and muscular, to a high-intensity training session in roller hockey players. Additionally, it seeks to correlate these bioelectrical changes with levels of creatine kinase (CK), to determine post-exercise muscle damage. Study III evaluates changes in body and muscle fluids in professional cyclists during the Giro d'Italia, using anthropometric, bioelectrical and hematological assessments, aiming to find correlations among these indicators. Finally, Study IV investigates how different distances in trail running races (14, 35, and 52 km) affect body and muscle fluids, applying anthropometric, bioelectrical, and CK methods.

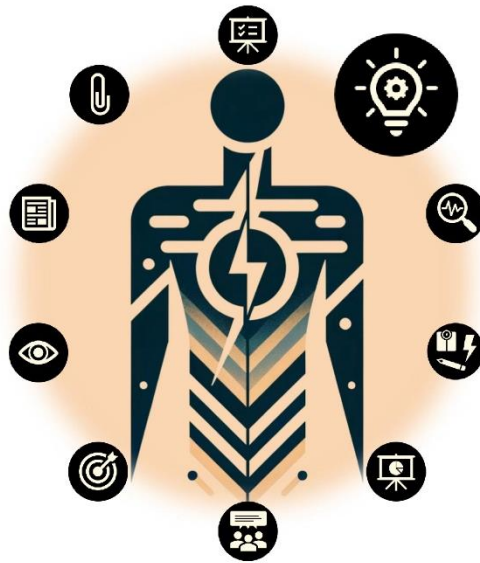
The results obtained from the research show that BIVA represents a non-invasive, economical and effective tool, facilitating accurate and real-time monitoring of athletes' physiological status. The observed short, medium and long-term variations in bioelectrical parameters (resistance, reactance and phase angle) reflect adaptations in fluid distribution and cellular functionality after physical exercise. This is particularly

evident in processes such as rehydration, recovery and changes in body composition and cellular health. Moreover, these adaptations demonstrate a correlation with improvements in athletic performance, such as strength and muscle power.

Regarding ML-BIVA, it provides essential detailed information to create personalized strategies, both in injury recovery and in the development of specific training programs. The evaluation of various muscle groups in multiple exercise contexts highlights patterns that corroborate its ability to detect morphological and physiological changes. For the effective use of ML-BIVA, it is crucial to emphasize the need for rigorous standardization in the evaluation protocol. This standardization is vital to increase the reliability and validity of the measurements and to ensure better comparability between different investigations.

Future research directions include: expanding athlete databases, conducting longitudinal studies, analyzing the interaction between bioelectrical changes and physiological parameters, integrating new technologies in sports evaluation and developing standardized protocols for ML-BIVA.

Keywords: bioimpedance, body composition, sport, physical exercise.



INTRODUCCIÓ

1. Introducció

1.1. Introducció a la bioimpedància

En el decurs dels darrers quaranta anys, l'anàlisi de la impedància bioelèctrica (BIA) s'ha establert fermament com una metodologia destacada per a l'anàlisi de la composició corporal (Ward, 2021). Aquesta tècnica, no invasiva i versàtil, s'aplica en una ampla gamma d'àmbits, incloent-hi l'avaluació de l'estat nutricional (Catapano et al., 2023; Moon, 2013), la distribució de fluids (Lukaski et al., 2019; Oppliger & Bartok, 2002) i la condició física, tant en el context esportiu com en el sanitari (De-Mateo-Silleras et al., 2018; Marra et al., 2020; Mascherini et al., 2017; Micheli et al., 2014; Petri et al., 2023). BIA és, per tant, una eina essencial en la monitorització continuada de la salut i el benestar, així com en la valoració de l'entrenament i el rendiment esportiu, encara que va ser desenvolupada per estimar quantitativament l'aigua total del cos (TBW), la massa lliure de greix (FFM) o la massa de greix (FM) (Ward & Brantlov, 2023), basant-se en un model multi-component del cos humà (Campa et al., 2021).

La història de l'ús del corrent elèctric per explorar teixits i revelar informació sobre la seva composició i funció parteix de diverses contribucions que formen la base de l'anàlisi de bioimpedància contemporània (Lukaski, 2013). La seqüència d'esdeveniments en la investigació de la bioimpedància va, des del desenvolupament de models biofísics per abordar aplicacions com l'estimació del flux sanguini pulsatiu fins a l'avaluació de la composició corporal (Kyle et al., 2004a, 2004b), àmbit on ha trobat la seva principal finalitat (Lukaski, 2013). La composició corporal engloba la totalitat dels components (cèl·lules, teixits i òrgans) que donen massa, forma i funció als éssers vius.

La bioimpedància, que es fonamenta en la llei d'Ohm (Kyle et al., 2004a), es basa en les diferències de conductivitat elèctrica entre els diversos teixits corporals, incloent músculs, ossos i vasos sanguinis (Jaffrin & Morel, 2008). Els teixits rics en aigua, com els músculs, presenten una millor conductivitat elèctrica que aquells amb menor contingut aquós, com el greix. Aquest contrast en la conductivitat facilita la distinció entre varietats de teixits i permet calcular la seva proporció i distribució dins del cos.

En el decurs dels anys, la bioimpedància ha avançat significativament en termes tecnològics, expandint així el seu abast a una àmplia varietat d'aplicacions. Aquestes

inclouen, des d'anàlisis detallats de la composició corporal, fins a avaluacions més específiques, com les mesures segmentals o musculars (Campa et al., 2021; Cebrián-Ponce et al., 2021). A més, s'apliquen diverses metodologies: per a la recopilació de dades quantitatives s'aplica BIA mitjançant l'ús d'algoritmes, mentre que per a l'examen qualitatiu dels paràmetres bioelèctrics en estat cru, s'analitza el vector de bioimpedància (BIVA) (Piccoli et al., 1994).

1.2. Principis fonamentals de la bioimpedància

L'origen de la història de la bioimpedància es pot relatar amb els estudis d'Hugo Fricke i Kenneth Stewart Cole. Fricke (1925), va ser un dels primers intel·lectuals a estudiar la conductivitat elèctrica de les cèl·lules sanguínies, establint un model per entendre la conductivitat elèctrica i la capacitat dels teixits. Més endavant, Cole (1968) va continuar avançant en aquesta línia de recerca, desenvolupant models de circuits equivalents i fonamentals per explicar el comportament bioelèctric de les membranes cel·lulars, que van ser claus per a la comprensió de la bioimpedància. Aquest model representa els fluids iònics extracel·lulars i intracel·lulars com resistors en paral·lel i la membrana cel·lular com un condensador (Figura 1).

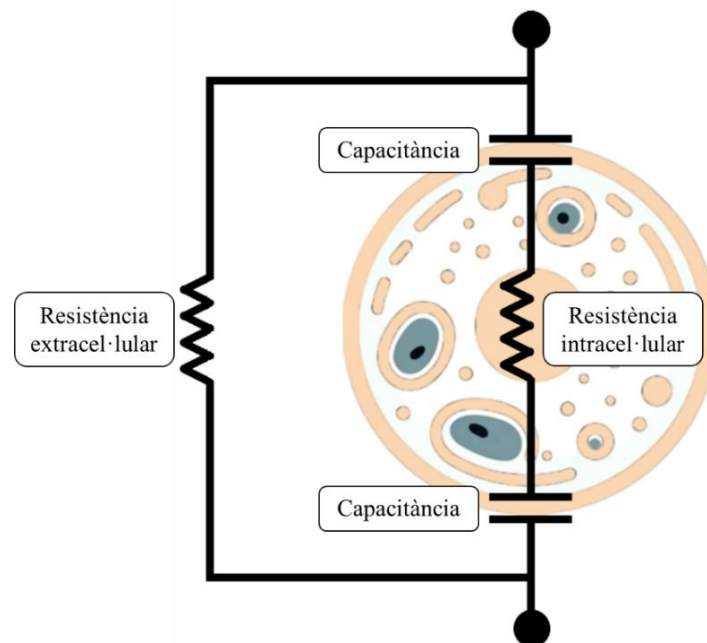


Figura 1. Circuit elèctric equivalent per descriure els components bioelèctrics d'una cèl·lula viva. Adaptat de Lukaski (2013).

La bioimpedància es fonamenta en la Llei d'Ohm (Ward & Brantlov, 2023), que explica la relació entre tensió (V), corrent (I) i resistència (R) en un conductor, resumida amb la

fórmula $V = I \times R$. Això indica que la V a través d'un conductor és el producte del I que hi circula multiplicat per la seva R . Així, en un material com un fil, la tensió observada depèn de la facilitat o dificultat amb què el corrent es mou a través d'ell. En conductes bioelèctrics amb estructura i composició uniformes, la impedància (Z) es relaciona amb la resistivitat específica del material (ρ), la longitud del conductor (L) i inversament amb l'àrea de secció transversal (A), segons la fórmula $Z = \rho (L / A)$ (Lukaski, 2013). Aquest model parteix de la idea que el conducte té una composició i una geometria constants (Lukaski & Talluri, 2021). En els cossos humans, aquesta uniformitat no es manté a causa de la complexitat i la varietat de l'estructura i la composició del nostre organisme (Figura 2) (Lukaski & Raymond-Pope, 2021).

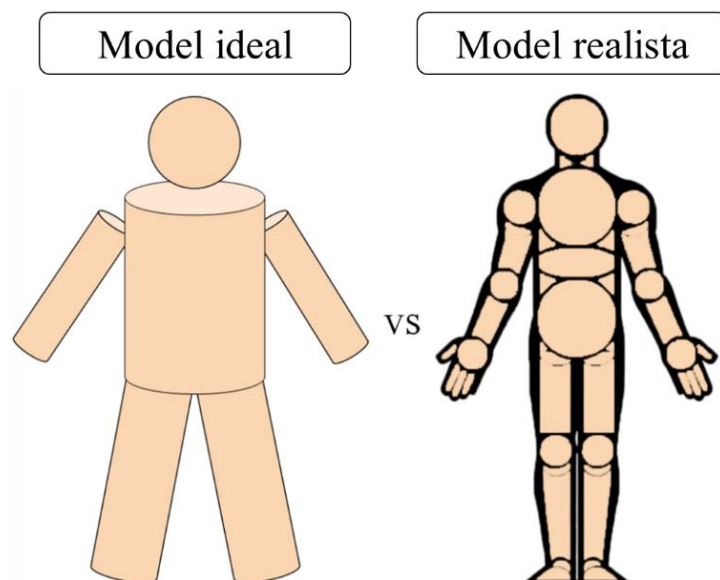


Figura 2. Comparació entre un model simètric ideal i la complexitat realista estructural del cos humà.

D'altra banda, la simetria teòrica del cos humà també es veu influenciada per la posició del cor i, per tant, de tot el sistema cardiovascular, essent més complex en l'hemicòs esquerre que en el dret (Marieb & Hoehn, 2018). Malgrat les limitacions del model en termes de composició homogènia i geometria constant, l'aplicació d'aquesta teoria amb corrent altern al cos humà representa una eina valuosa per analitzar les propietats estructurals i funcionals dels teixits biològics. Això permet una comprensió detallada dels processos biològics gràcies a l'anàlisi de les propietats elèctriques dels teixits.

Introduint els organismes en un circuit elèctric segur i controlat, es poden avaluar dos paràmetres clau: la R , que mesura l'oposició al pas de la corrent altern a través de solucions iòniques intra- i extra-cel·lulars, i la reactància (X_c), que mesura el component

capacitatiu de les interfícies tissulars, membranes cel·lulars i orgànuls (Lukaski, 2013). A partir d'aquests dos paràmetres, es pot calcular la magnitud impedància elèctrica ($|Z|$, però que denominarem com Z) amb la fórmula $Z = \sqrt{R^2 + X_c^2}$, que representa l'oposició total al pas d'un corrent elèctric. A més, també es pot calcular l'angle de fase (PhA), que es tracta de la transformació angular entre R i X_c i que és particularment rellevant en l'àmbit de l'exercici físic, com veurem en properes seccions d'aquesta tesi. Aquest PhA s'origina de la capacitància a causa de les membranes cel·lulars, causant que el corrent elèctric es retardi en relació amb la tensió, oferint una visió profunda sobre la relació entre l'energia emmagatzemada i alliberada degut a la X_c i l'energia dissipada a causa de la R (Norman et al., 2012; Sardinha, 2018).

En resum, aquest enfocament ens permet utilitzar aquests paràmetres (R , X_c , Z i PhA) com a indicadors crucials per avaluar diverses característiques biològiques, reflectint la dinàmica dels components reactius i resistius en un circuit de corrent altern, tant en l'àmbit biològic com l'elèctric (Lukaski, 1996). La Figura 3 mostra com es relacionen entre si la R , X_c , Z i PhA, oferint una visió geomètrica de la seva interacció.

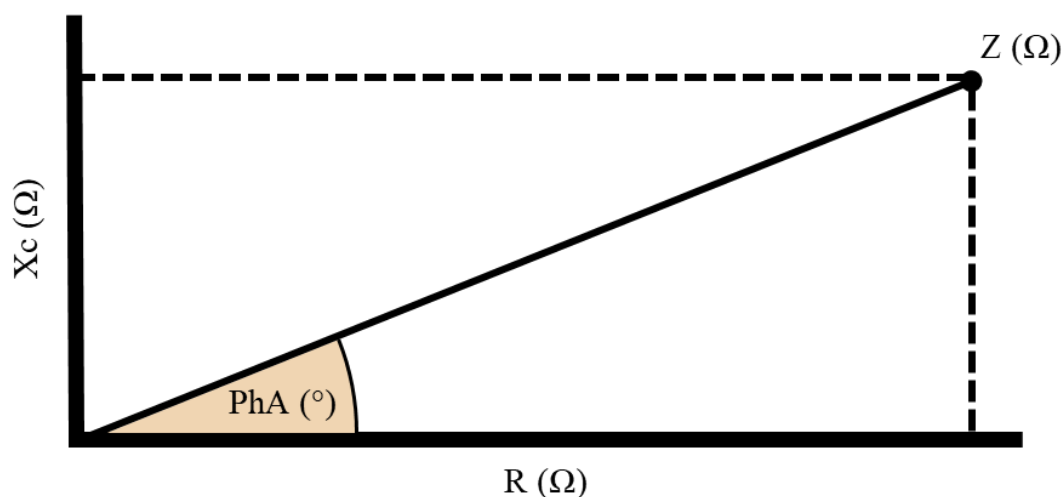


Figura 3. Relació geomètrica entre resistència, reactància, impedància i angle de fase en els éssers vius. PhA, angle de fase; R , resistència; X_c , reactància.

Entendre els fenòmens físics en els sistemes biològics és crucial per a les aplicacions en ciències de la salut i en l'anàlisi de la composició corporal, on la mesura de la impedància elèctrica proporciona dades importants sobre els diferents components del cos humà, tenint en compte la variabilitat en la conductivitat dels diversos teixits.

Quan un corrent elèctric altern transita a través del cos humà, aquest distribueix el seu camí entre els fluids extracel·lulars i intracel·lulars (Piccoli et al., 2007). Aquesta

distribució està influenciada per la freqüència del corrent (Figura 4) així com per les propietats elèctriques inherents a cada tipus de teixit (Kyle et al., 2004a). Per a entendre millor aquestes interaccions, sovint s'utilitzen models de circuits equivalents per representar les característiques elèctriques dels teixits biològics (Ward & Brantlov, 2023), com el que es troba representat a la Figura 1, el qual és el model més elemental i àmpliament acceptat. En aquest, el camí extracel·lular està simbolitzat per una R que es troba en paral·lel amb una altra R que representa el fluid intracel·lular, juntament amb un condensador que simula les membranes cel·lulars. La impedància d'aquest circuit equivalent a una determinada freqüència es calcula tenint en compte la resistència a freqüència zero (R_0), moment en el qual la impedància de la membrana és màxima, i la resistència a freqüència infinita (R_∞), situació en la qual les membranes actuen com perfectes conductors. A R_0 , es considera que el corrent transcorre exclusivament a través del component extracel·lular per l'alt valor de la impedància de la membrana. En contrast, a R_∞ , les membranes es comporten com a conductors ideals, permetent el pas lliure del corrent. Per freqüències intermitges, el corrent es distribueix proporcionalment entre les dues branques del circuit en paral·lel (Ward & Brantlov, 2023).

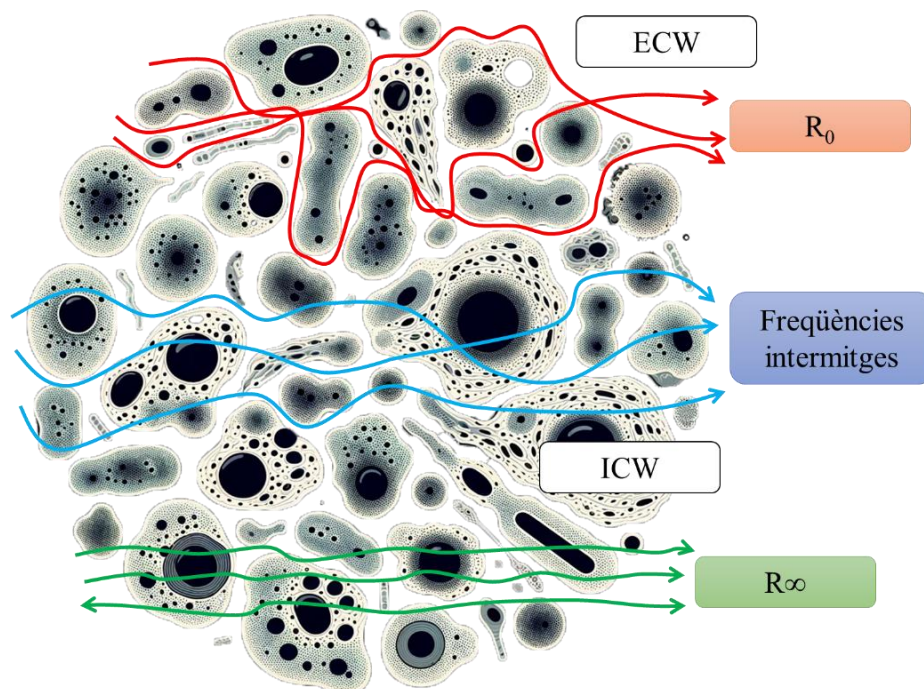


Figura 4. Corrent altern circulant pel teixit biològic depenent de la freqüència. Adaptat de Nescolarde et al. (2023). ECW, aigua extracel·lular; ICW, aigua intracel·lular; R_0 , resistència a freqüència zero; R_∞ , resistència a freqüència infinita.

El model de Cole (1968), representat a la Figura 5, descriu la interacció entre la freqüència del corrent elèctric i les propietats dielèctriques de les membranes cel·lulars. Segons

aquest model, l'augment de la freqüència provoca que el vector d'impedància segueixi una trajectòria semicircular en sentit antihorari. A un punt determinat d'aquest camí, conegut com la freqüència característica (ω_c), i normalment propera als 50 kHz (Piccoli, 2010), el PhA i X_c assoleixen els seus valors màxims. Aquest valor màxim de X_c reflecteix les propietats dielèctriques òptimes de les membranes cel·lulars (Lukaski et al., 1985), fent de ω_c la freqüència ideal per a les medicions de bioimpedància. Per aquest motiu, és comú que els dispositius de bioimpedància comercials treballin a 50 kHz, optimitzant així la seva eficàcia en la mesura de la composició corporal i altres paràmetres fisiològics.

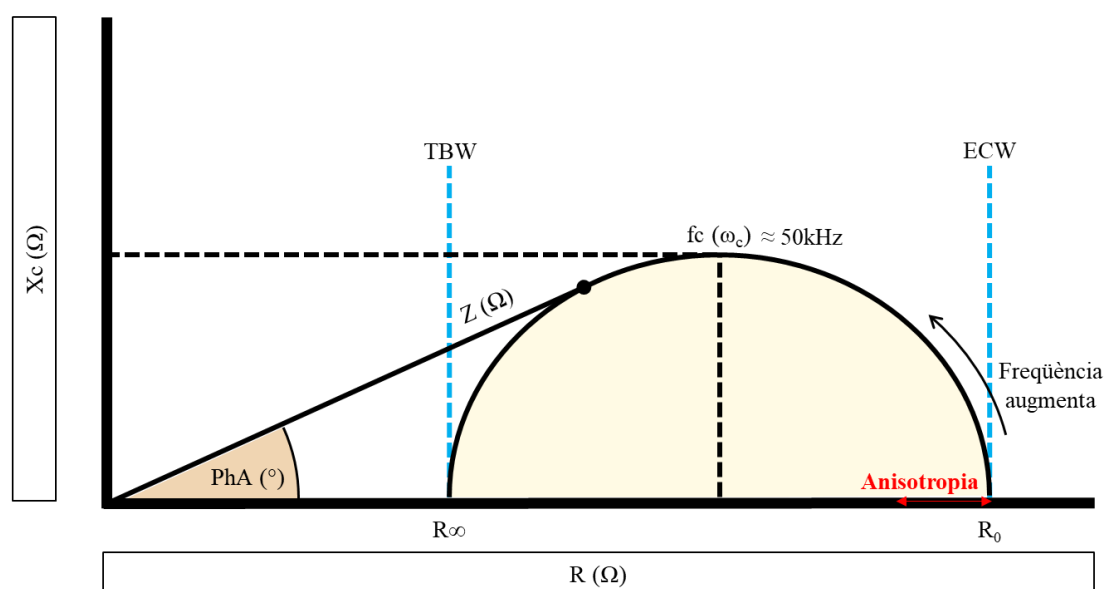


Figura 5. Dependència freqüencial de resistència i reactància en teixits biològics. Adaptat de Piccoli et al. (2007). ECW, aigua extracel·lular; PhA, angle de fase; R, resistència; R_0 , resistència a freqüència zero; R_∞ , resistència a freqüència infinita; TBW, aigua corporal total; X_c , reactància; Z, impedància; ω_c , freqüència característica.

Aquesta tecnologia permet als investigadors i professionals de la salut detectar canvis fins i tot mínims en els diferents components del cos. Gràcies a aquesta precisió, es pot no solament identificar la quantitat de FM i FFM, sinó també avaluar l'estat dels fluids corporals, incloent la TBW i els seus subcompartiments -l'aigua extracel·lular (ECW) i l'aigua intracel·lular (ICW)-, entre d'altres elements importants. Aquest tipus d'anàlisi detallat és útil per comprendre millor l'estat de salut general d'una persona, així com per ajustar tractaments o plans d'entrenament i de nutrició específics. Amb tot, l'aplicació d'aquestes mesures permet establir un vincle clar entre les teories científiques i les aplicacions pràctiques en el camp de l'exercici físic, oferint així una base sòlida per a la presa de decisions en matèria de salut i rendiment.

1.3. Composició corporal

1.3.1. Avaluació de la composició corporal en l'exercici físic

L'avaluació de la composició corporal és essencial per a professionals de la salut. Les avaluacions d'elements com la massa muscular, la FM o el teixit ossi, poden guiar el procés de millora, tant de la salut general com del rendiment esportiu (Thomas et al., 2016). D'aquesta manera, mesurar la composició corporal és cada vegada més rellevant, tant per identificar riscos de malalties com per avaluar l'impacte de l'exercici físic sobre el nostre organisme. Aquest enfoc integral ajuda a comprendre millor com l'estructura i la funció del cos afecten la salut, marcant el pas d'una simple descripció a un model més complet que combini la composició corporal amb la funcionalitat i la salut global de les persones (Lukaski, 2017).

Behnke et al. (1995) van ser els primers en observar que els esportistes professionals masculins eren classificats amb sobrepès segons les taules de massa corporal (BM) estàndard per estatura, encara que tenien nivells de condició física molt alts. Per tant, basar-se únicament en la BM o l'índex de massa corporal (BMI) no és suficient. Aquest descobriment va establir que amb l'avaluació de la composició corporal podien distingir cossos grans i musculosos de cossos grans i grassos.

Els factors ambientals i conductuals influeixen significativament la composició corporal, la salut, el rendiment i el risc de patir lesions (Figura 6). Altres factors com la capacitat metabòlica, l'habilitat, els atributs psicològics i la genètica també juguen un paper crucial en el rendiment. Per això, les avaluacions de la composició corporal haurien de complementar-se amb altres mètodes si l'objectiu és personalitzar els plans d'entrenament i nutricionals (Lukaski, 2017).

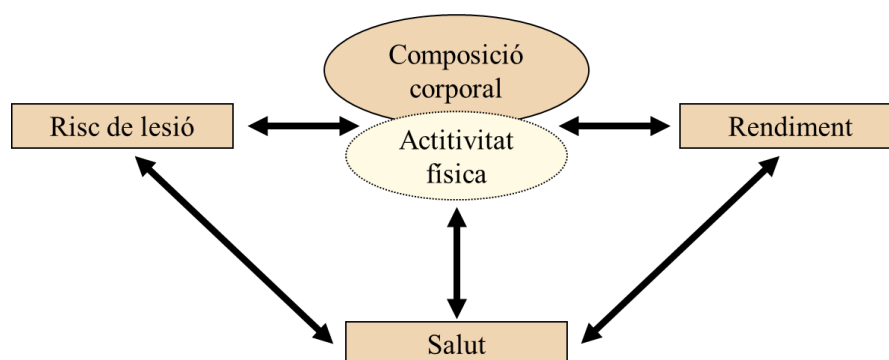


Figura 6. Model general de la interacció de l'activitat física i la composició corporal sobre la salut, el risc de lesions i el rendiment. Adaptat de Lukaski (2017).

Per exemple, l'acumulació excessiva de FM, es relaciona directament amb un augment en la morbiditat i mortalitat (Poirier et al., 2006), i aquesta es pot combatre mitjançant una estratègia combinada d'exercici físic regular i una dieta equilibrada. Per tant, avaluar la composició corporal esdevé una eina clau no solament per a monitorar l'estat de salut general, sinó també per a valorar l'èxit dels programes d'entrenament (Drinkwater et al., 2008). Aquesta millora en la composició corporal no només es pot traduir en beneficis evidents com en un increment del consum màxim d'oxigen (Högström et al., 2012), sinó també en un augment de la força màxima i la potència muscular (Granados et al., 2008; Silva et al., 2010, 2014). En moltes ocasions, el seguiment de la composició corporal no pretén obtenir estimacions precises, sinó més aviat enfocar-se en monitorar els canvis significatius al llarg del temps que es relacionen amb els paràmetres d'interès, ja sigui de salut com de rendiment (Lukaski, 2017).

1.3.2. Ús de la bioimpedancia per a l'anàlisi de la composició corporal

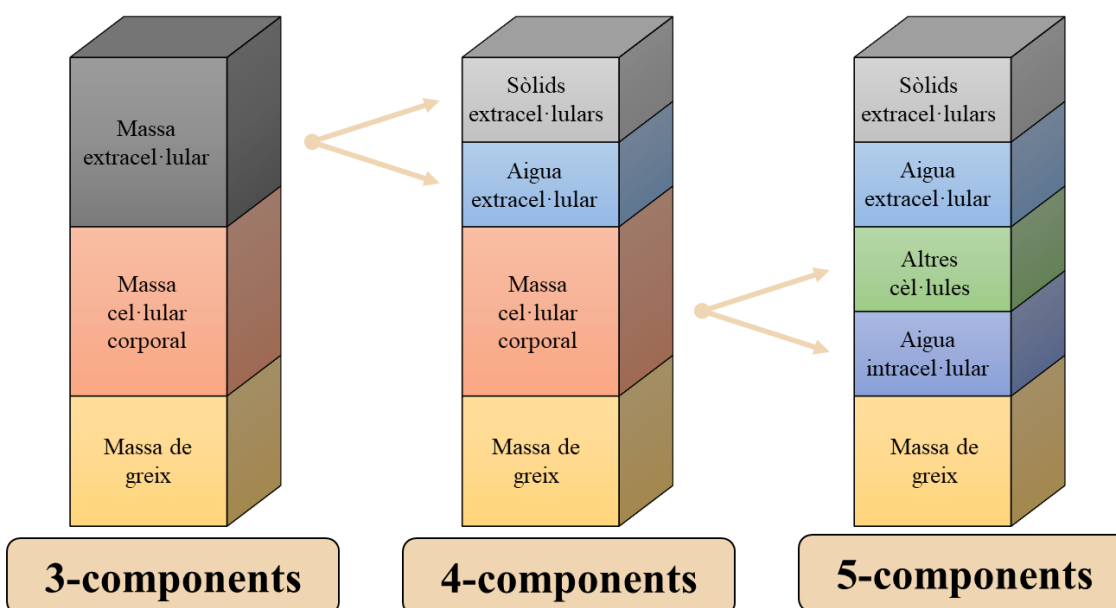
La diversitat en formes i dimensions corporals deriva de factors genètics i ambientals, incloent l'alimentació i l'exercici al llarg de la vida. Això s'observa en la composició corporal, que reflecteix aquestes influències i serveix com a indicador de l'estat nutricional i de salut. La composició corporal no només mostra la mida dels òrgans i teixits, sinó que també es basa en models que distingeixen entre funcions biològiques com la massa magra o el greix total (Ward, 2018). Les variacions en la composició corporal faciliten l'avaluació de l'estat nutricional i la funcionalitat del cos, aspectes essencials per la salut. A més, anàlisis repetits permeten veure l'evolució que experimenta cada component amb el temps, revelant els efectes de l'envelliment, condicions de salut o plans d'entrenament específics.

L'anàlisi de composició corporal pot ser aproximat en base a cinc nivells de complexitat progressiva, prenent en compte la BM com la combinació d'àtoms, molècules, cèl·lules, teixits i segments corporals diversos (Wang et al., 1992). L'avaluació adequada de la composició corporal implica considerar els paràmetres derivats de cada nivell de manera separada, de tal manera que la suma dels paràmetres dins d'un mateix nivell determini la BM (Taula 1). En cada nivell, es poden implementar diversos models segons la quantitat de components.

Taula 1. Nivells d'anàlisi i paràmetres de la composició corporal. Adaptat de Campa et al. (2021).

NIVELL	PARÀMETRES
ATÒMIC	Quantitat d'hidrogen, carboni, oxigen i altres àtoms
MOLECULAR	Massa de greix, massa lliure de greix (aigua corporal total, contingut mineral ossi)
CEL·LULAR	Cèl·lules adiposes, aigua intracel·lular i extracel·lular, massa cel·lular corporal
TISSULAR	Quantitat de teixit adipós, teixit magre suau, massa muscular
COS COMPLET	Massa total dels diferents segments corporals (cap, tronc, extremitats)

Per exemple, tal com es mostra a la Figura 7, el nivell cel·lular es pot dividir en models de 3, 4 o 5 components.

**Figura 7.** Model de 3, 4 i 5 components del nivell cel·lular. Adaptat de Campa et al. (2021)

Un dels models més aplicats tant en la pràctica clínica com en la recerca epidemiològica és el model de dos components (Rebato, 2021), en el qual el cos es divideix en FM i FFM. En aquests contextos, el model molecular de quatre components i el model tissular de tres components també són àmpliament emprats (Wang et al., 2002). Una vegada escollit el model de composició corporal més adequat al context específic, és pot escollir el mètode més apropiat per garantir la màxima precisió. Segons Campa et al. (2021) i Heymsfield et al. (2005), els mètodes considerats *gold* estàndard per a l'anàlisi de paràmetres clau en la composició corporal són els següents:

- **Contingut mineral ossi:** densitometria òssia de raigs X (DXA).
- **TBW:** tècniques de dilució de deuteri.
- **ECW:** tècniques de dilució de bromur.
- **ICW:** TBW – ECW.
- **FM:** pletismògraf per desplaçament d'aire (ADP) i hidrodensitometria. No obstant això, la fórmula més precisa es basa en el model de quatre compartiments amb l'ús de DXA, dilució per deuteri i ADP combinats.
- **FFM:** BM – FM.
- **Massa muscular:** ressonàncies magnètiques (MRI)
- **Teixit tou magre:** DXA

Com es pot veure a la Taula 2, les metodologies de mesura es distingeixen entre mètodes de mesura directes (quantifiquen el paràmetre de manera explícita), indirectes, (obtenen el paràmetre mitjançant l'aplicació d'algoritmes) i doblement indirectes (usen equacions de regressió validades amb informació obtinguda de mètodes indirectes) (Campa et al., 2021). Malgrat la prevalença i el reconeixement de la precisió dels mètodes indirectes per a l'estimació de paràmetres específics, no existeix un dispositiu o tècnica que pugui ser considerat com a *gold* estàndard. És més, en molts casos, els inconvenients d'aquestes tècniques (alt cost, complexitat i necessitat de personal altament qualificat) predominen sobre els seus avantatges (Ward, 2018), degut a factors com la precisió, la exactitud o la influència de l'experiència de l'operador, entre d'altres.

Taula 2. Tipus i mètodes per a l'anàlisi de la composició corporal. Adaptat de Campa et al. (2021).

TIPUS DE MÈTODE	MÈTODES D'ANÀLISI DE LA COMPOSICIÓ CORPORAL
DIRECTES	Autòpsia cadavèrica
INDIRECTES	Antropometria
	Hidrodensitometria
	DXA
	Densitometria
	Tomografia computada
	MRI
DOBLEMENT INDIRECTES	Equacions predictives basades en l'antropometria
	Equacions predictives basades en BIA

En canvi, BIA destaca per la seva naturalesa no invasiva, portabilitat, cost relativament baix i facilitat d'ús, convertint-se així en una opció creixentment popular (Kyle et al., 2004a, 2004b). A més, ofereix un equilibri òptim entre la dependència de l'operador i la precisió de les dades, superant algunes limitacions presents en altres mètodes (Figura 8).

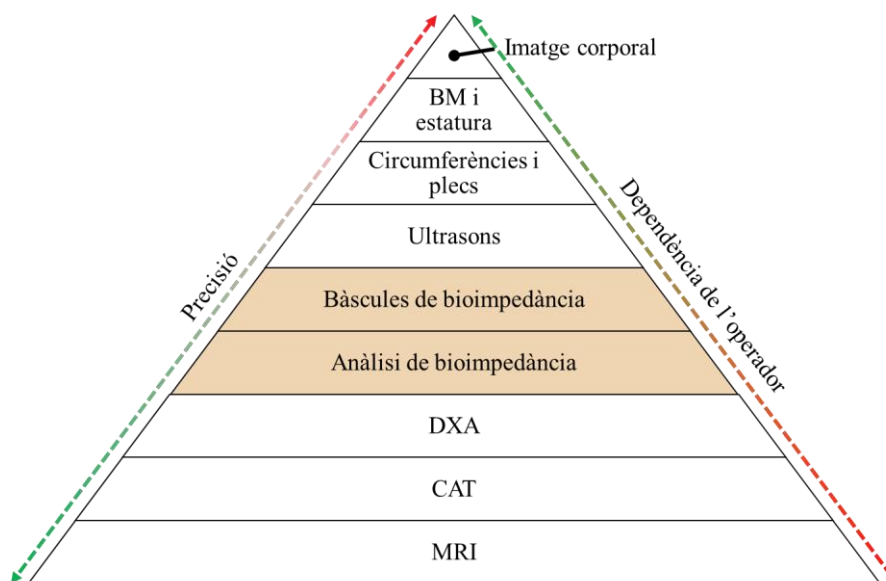


Figura 8. Gradació de precisió i dependència operativa en tecnologies d'avaluació de la composició corporal. BM, massa corporal; CAT, tomografia computada; DXA, densitometria òssia de raigs X; MRI, ressonància magnètica.

BIA és un mètode doblement indirecte basat en un model de 2 components, és a dir, divideix el cos humà en dos components principals: FM i la FFM (Ellis, 2000). Aquesta distinció es basa en la diferència en la conductivitat elèctrica entre els teixits grassos i els no grassos, on els primers són menys conductius a causa del seu menor contingut d'aigua i electròlits. Per contra, la FFM, que inclou músculs, ossos, òrgans i altres teixits no adiposos, té un alt contingut d'aigua i electròlits, fent-la més conductiva al corrent elèctric aplicat durant una mesura de BIA (Kyle et al., 2004a).

Malgrat que el model de dos components ofereix una eina valuosa per a l'avaluació de la composició corporal, aquest presenta certes limitacions (Sergi et al., 2017). L'assumpció que la composició corporal es pot dividir de manera tan simplificada no reflecteix adequadament la variabilitat inter i intra individual en la distribució del teixit adipós i el teixit no adipós, així com les diferències en la hidratació dels teixits. Aquestes limitacions poden comprometre la precisió de les estimacions, especialment en poblacions amb característiques corporals particulars, com ara els esportistes d'alt rendiment o les persones amb obesitat mòrbida. No obstant això, s'han desenvolupat models de múltiples

components que permeten una avaluació individualitzada dels diversos components de la FFM (Sun et al., 2003). Aquests models resulten especialment adequats quan es comparen grups en els quals la composició de la FFM varia, per exemple, entre nens, adults i gent gran, així com entre dones i homes, proporcionant així una eina més precisa per a l'anàlisi de la composició corporal en diverses poblacions (Rebato, 2021).

1.4. Metodologies en l'anàlisi de la bioimpedància

Cal tenir en compte que les diferències en els resultats obtinguts amb la bioimpedància poden variar, depenent dels dispositius utilitzats com dels diferents procediments que s'apliquin. Actualment, hi ha una gran varietat de mètodes i tecnologies per mesurar la bioimpedància disponibles en el mercat, cadascuna amb les seves particularitats i aplicacions. Entendre aquestes diferències és essencial per obtenir resultats precisos i interpretar-los correctament. La selecció del mètode i dispositiu més adequats depèn de factors com el tipus d'informació que es desitja obtenir, el lloc i moment de la mesura, així com de les característiques del subjecte a mesurar.

La Figura 9 presenta un diagrama que resumeix aquesta secció sobre les diverses metodologies i aparells de bioimpedància.

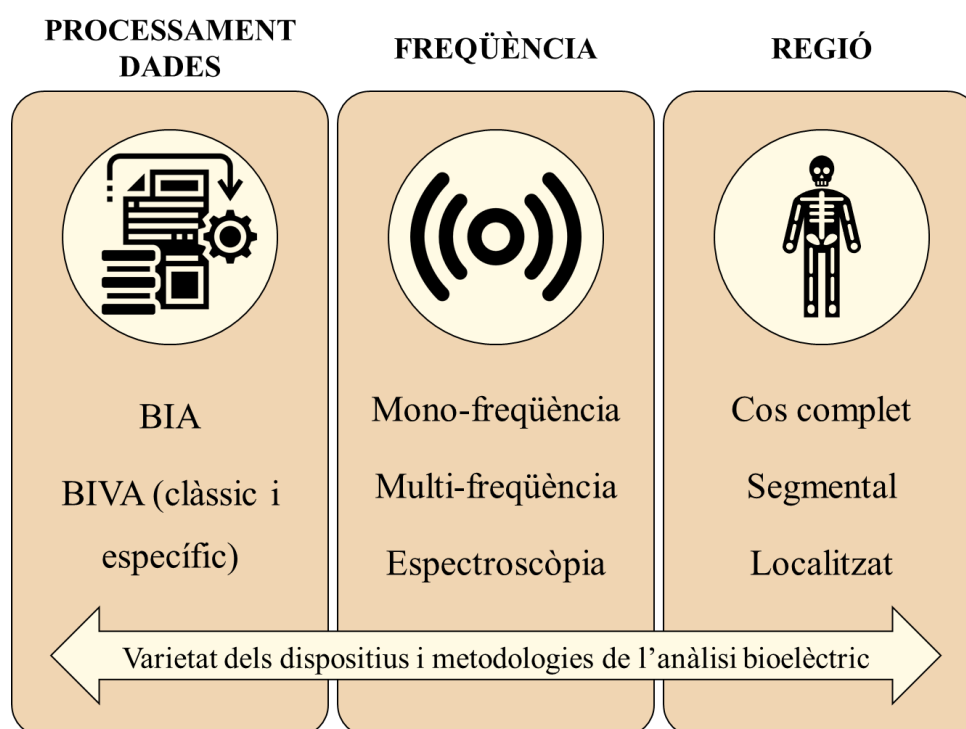


Figura 9. Dispositius i metodologies de l'anàlisi de bioimpedància elèctrica. Adaptat de Ward (2019). BIA, anàlisi d'impedància bioelèctrica; BIVA, anàlisi del vector d'impedància bioelèctrica.

1.4.1. Segons el tipus d'anàlisi

Els diferents tipus d'anàlisi de bioimpedància fan referència a com es recullen i es tracten les dades bioelèctriques recollides pels dispositius, és a dir, la R , la X_c , la Z i el PhA .

Anàlisi d'impedància bioelèctrica (BIA)

Com s'ha explicat anteriorment, BIA no mesura directament la composició corporal, sinó que registra la resposta elèctrica del cos (R i X_c , de les quals se'n deriven la Z i PhA). Aquesta resposta es tradueix en una avaluació quantitativa dels diferents components corporals quan s'utilitzen algorismes específics, que s'elaboren en funció de perfils poblacionals variats, com ara sexe, edat o esportistes, però poden presentar un cert marge d'error (Esco et al., 2017; Hartmann-Nunes et al., 2020). Els errors en la interpretació poden ser atribuïts a suposicions com la presumpció que els teixits mantenen una hidratació constant o que existeix una isotropia muscular uniforme en tot el cos (Moon, 2013; Norman et al., 2012). Aquests algorismes s'utilitzen per quantificar de manera absoluta i relativa els diferents paràmetres de la composició corporal, gràcies a les diferents propietats conductives de cada teixit biològic (Figura 10).

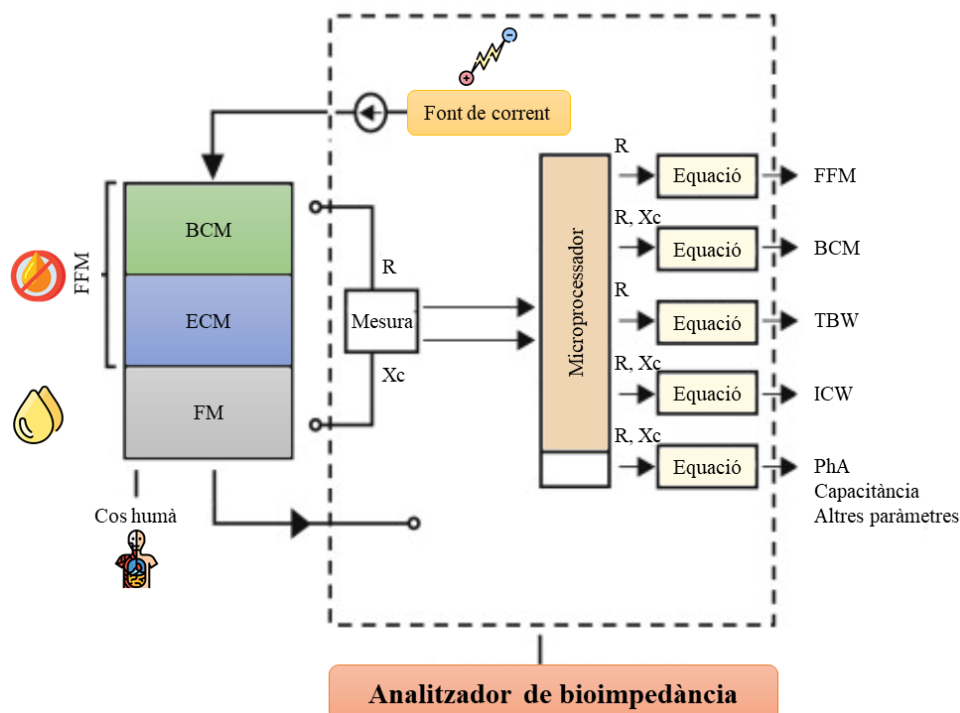


Figura 10. Diagrama de blocs de l'anàlisi de bioimpedància. Adaptat de Pupim et al. (2013). BCM, massa cel·lular corporal; ECM, massa extracel·lular; FFM, massa lliure de greix; FM, massa de greix; ICW, aigua intracel·lular; R , resistència; TBW, aigua corporal total; X_c , reactància.

Els dispositius BIA poden proporcionar una estimació quantitativa dels paràmetres de la composició corporal utilitzant equacions predictives establertes pel fabricant, o bé proporcionant dades brutes de R i X_c per ser inserides en fórmules específiques a discreció de l'operador. La majoria d'aquestes fórmules s'han desenvolupat basant-se en mitjanes poblacionals, la qual cosa pot conduir a resultats menys precisos quan s'apliquen a individus amb característiques diferents de les de la població de referència (Kyle et al., 2004a). Això pot incrementar l'error en les prediccions, ja que els coeficients i estimacions utilitzats poden no ser sempre aplicables a tots els individus. Aquesta limitació subratlla que les mesures obtingudes amb tècniques d'impedància poden no ser tan precises com els mètodes de referència utilitzats per a la seva validació, per la qual cosa és crucial ajustar les expectatives i ser cautelosos en la interpretació dels resultats. Cada dispositiu BIA, depenent del fabricant, té característiques específiques, i per tant, és important no barrejar dades amb equacions de diferents dispositius.

Anàlisi del vector d'impedància bioelèctrica (BIVA)

BIVA és una metodologia proposada per Piccoli et al. (1994) que van revolucionar la manera d'examinar la composició corporal. A diferència de BIA, que mesura els components del cos de manera quantitativa, BIVA es centra en una avaluació qualitativa i semi-quantitativa de la massa cel·lular corporal (BCM) i de la distribució de fluids, a través de l'anàlisi del vector de bioimpedància sense fer ús d'equacions predictives. Aquest enfocament evita suposicions prèvies relacionades amb la morfologia corporal, la constant d'hidratació o les característiques elèctriques de les membranes cel·lulars, evitant biaixos potencials derivats dels ajustos de regressió.

Sovint, BIVA emprava una configuració tetrapolar no invasiva per a la col·locació dels elèctrodes, coneguda com a configuració "*whole-body*" (WB-BIVA), global o costat dret (Figura 11), que es sol realitzar a 50 kHz, ja que és la freqüència amb millor relació senyal-soroll (Piccoli, 2010). Aquesta disposició implica l'ús de quatre elèctrodes: dos injectors i dos detectors. Els elèctrodes injectors es col·loquen a la superfície dorsal de la mà dreta (a prop de l'articulació metacarpofalàngica del tercer dit) i del peu (a prop de l'articulació metatarsfalàngica del tercer dit). Els elèctrodes detectors es situen proximalment a 5-6 cm de distància dels injectors, evitant la interacció entre els camps elèctrics. Generalment s'utilitzen elèctrodes de gel de baixa impedància Ag/AgCl, per

obtenir resultats fiables, ja que el tipus d'elèctrode pot tenir un impacte significatiu en els valors obtinguts.

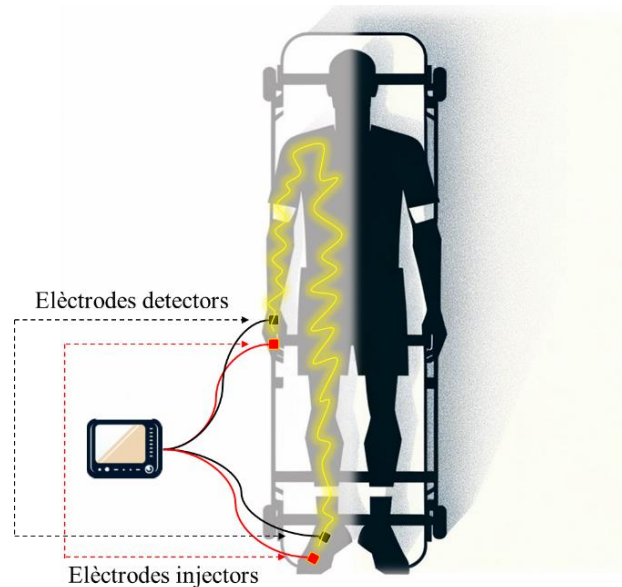


Figura 11. Col·locació estàndard dels elèctrodes per l'anàlisi BIVA de tot el cos.

Encara que a la Figura 11 s'il·lustra la configuració estàndard més comuna, la singularitat de BIVA no es troba en la disposició específica dels elèctrodes en si mateixa, ja que diferents configuracions poden ser igualment vàlides. El que realment distingeix BIVA és la manera en què es processen i analitzen les dades bioelèctriques obtingudes (R i X_c) sense l'ús d'equacions. Aquest enfocament analític és el que confereix a BIVA la seva robustesa i fiabilitat en l'avaluació de la composició corporal i de la distribució de fluids (Castizo-Olier, Irurtia, et al., 2018).

Per a realitzar els anàlisis, els valors de R i X_c s'ajusten en funció de l'estatura dels individus (R/H i X_c/H), i consegüentment, la impedància ajustada (Z/H), i es projecten en gràfics de Resistència-Reactància (RX_c). Aquests gràfics es relacionen amb el·lipses de tolerància per a l'anàlisi intra-grup, o amb el·lipses de confiança per a inter-grups. Per aquest motiu, s'han dissenyat diverses metodologies enfocades en la representació gràfica i l'elaboració d'anàlisis de dades bioelèctriques. Aquestes tècniques es poden realitzar mitjançant el programari BIVA, el qual va ser desenvolupat per Piccoli i Pastori (2002). Aquestes són, basat en Castizo-Olier (2018):

Gràfic de punts (*RX_c point graph*)

El gràfic de punts mostra una distribució bivariada amb regions el·líptiques que serveixen per avaluar la TBW i el percentatge de massa de greix (%FM). Aquest gràfic permet

visualitzar la projecció dels vectors d'impedància bioelèctrica, tant a nivell individual com grupal, dins d'el·lipses de tolerància que representen percentatges del 50, 75 i 95% d'una població de referència determinada. Per exemple, a la Figura 12 es pot observar la projecció dels vectors d'una mostra de 9 ciclistes masculins sobre una població de referència masculina d'adults sans (Piccoli et al., 1994), la qual ha estat àmpliament utilitzada.

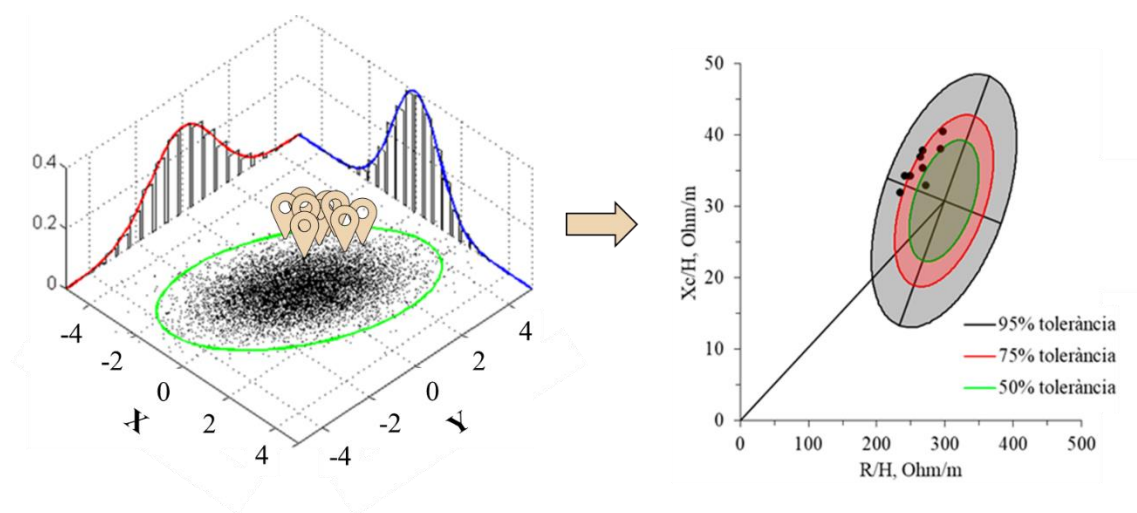


Figura 12. Gràfic de punts de 9 ciclistes en relació a una població de referència. Adaptat de Piccoli et al. (1994). R/H, resistència ajustada per l'estatura; Xc/H, reactància ajustada per l'estatura.

Mitjançant aquest tipus de gràfics i utilitzant poblacions de referència específiques, es pot dur a terme una caracterització dels individus en funció de si es troben dins dels percentils 50, 75, 90 o fora del 90% de l'el·lipse de tolerància. A més, es pot realitzar una caracterització segons el quadrant en el qual es troben, determinat per les coordenades R i Xc, i que forma els paràmetres Z i PhA. En la població de referència proporcionada per Piccoli (1995), els subjectes atlètics solen situar-se al quadrant superior esquerre, els subjectes més magres al quadrant superior dret, els subjectes obesos al quadrant inferior esquerre i els subjectes caquètics al quadrant inferior dret (Figura 13), encara que com veurem més endavant, no sempre s'ajusta estrictament a aquesta norma (Buffa et al., 2014). A més de caracteritzar els individus, aquest gràfic també permet analitzar les variacions al llarg del temps observant els canvis en el vector (Figura 13). Les alteracions observades en l'eix major del gràfic indiquen canvis en la TBW: un vector més curt s'associa amb una TBW major, mentre que un vector més llarg indica una TBW menor. Paral·lelament, els desplaçaments del vector al llarg de l'eix menor de les el·lipses indiquen canvis en la BCM i en la proporció ECW/ICW. Un desplaçament cap a l'esquerra

suggeriria un augment del PhA, de la BCM i una reducció en la proporció ECW/ICW; en canvi, un desplaçament cap a la dreta indicaria una disminució del PhA, de la BCM i un augment en la proporció ECW/ICW. Cal destacar que una interpretació precisa d'aquests gràfics requereix l'anàlisi conjunt de les variables Z i PhA (Lukaski, 2017).

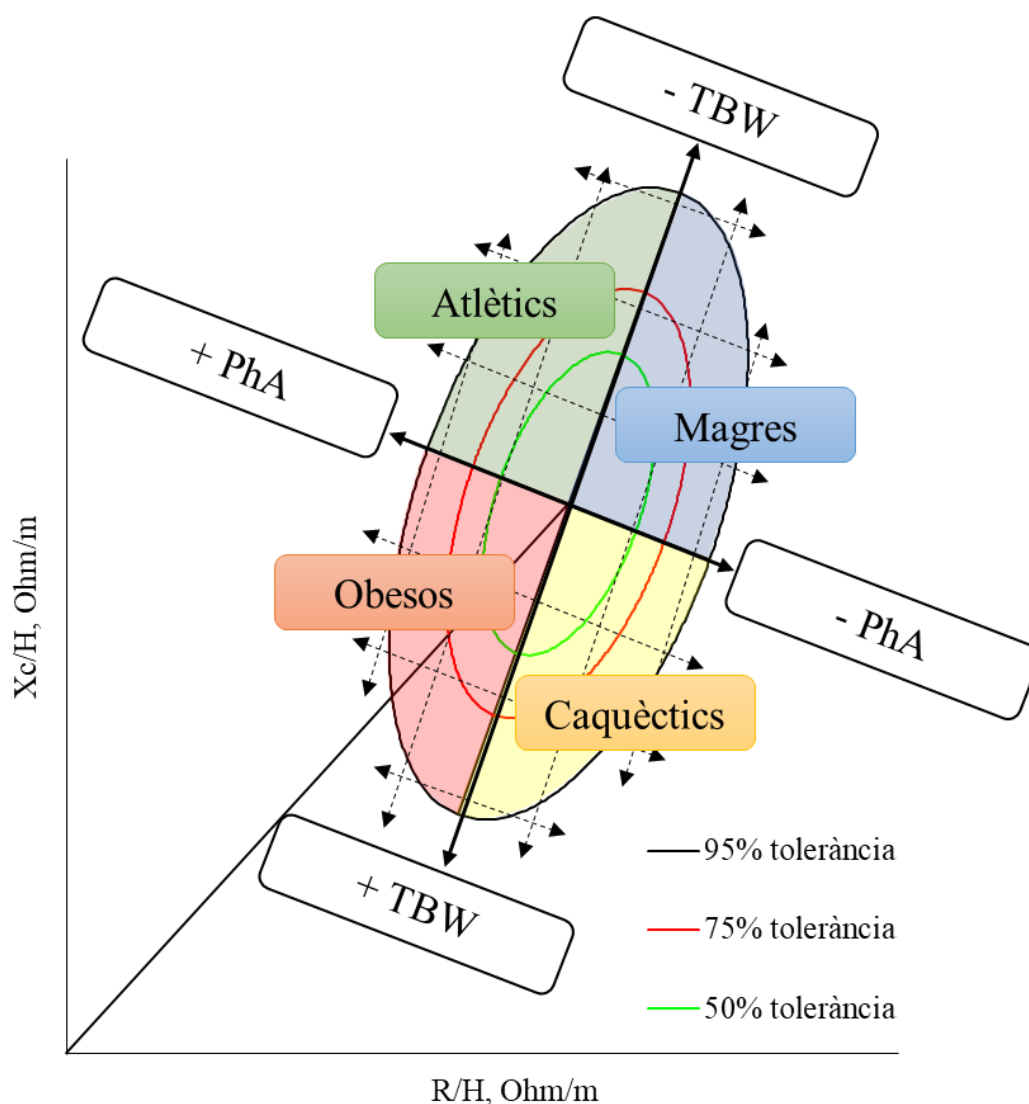


Figura 13. Quadrants de l'el·lipse de tolerància i variacions del vector. Basat en Campa et al. (2021). PhA, angle de fase; R/H, resistència ajustada per l'estatura; TBW, aigua corporal total; Xc/H, reactància ajustada per l'estatura.

Gràfic de punts Z (*RXc score graph*)

En convertir els components del vector en puntuacions bivariades Z, les mesures poden ser comparades amb les de diferents poblacions fent ús del gràfic de punts Z (Figura 14A). Això és útil per determinar quina és la diferència, en termes de desviacions estàndard, entre esportistes o grups. A més, aquesta transformació en valors Z facilita la comparació de dades bioelèctriques obtingudes amb dispositius diferents.

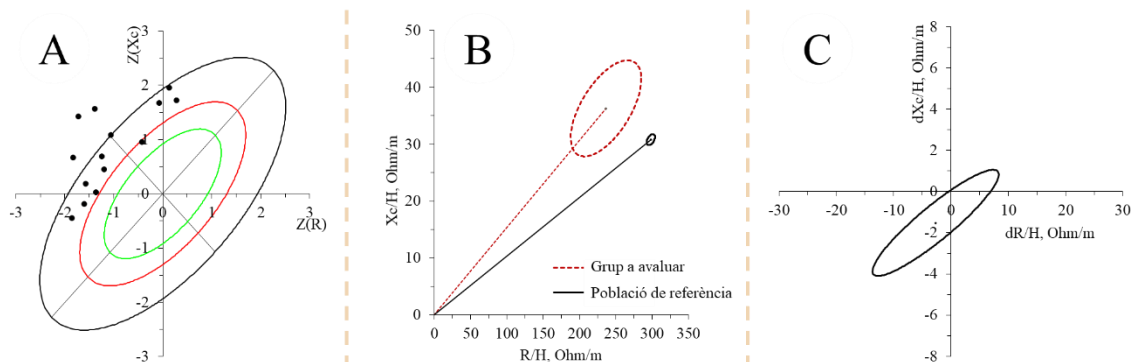


Figura 14. Exemples de Gràfics RXc. A: gràfic de punts; B: gràfic de valor mitjà; C: gràfic aparellat. R/H, resistència ajustada per l'estatura; Xc/H, reactància ajustada per l'estatura; Z(R), z-score de resistència; Z(Xc), z-score de reactància.

Gràfic de valor mitjà (RXc mean graph)

Permet fer la representació del vector mitjà dins del 95% de l'el·lipse de confiança per a diferents grups de subjectes amb mostres no aparellades (Figura 14B). Aquesta metodologia és molt útil per fer comparacions visuals i estadístiques entre diversos grups, proporcionant una comprensió clara de les diferències i similituds dins d'aquests conjunts de dades.

Gràfic aparellat (RXc paired graph)

La variació del vector d'un grup de subjectes pot ser representada amb l'el·lipse de confiança del 95% utilitzant aquest gràfic per a mostres aparellades (Figura 14C). Aquesta eina resulta ser particularment útil per a l'anàlisi comparativa en estudis amb intervencions o tractaments d'un grup.

Angle de fase (PhA)

El PhA, és un indicador bioelèctric fonamental per obtenir informació rellevant d'aspectes com la salut o la composició corporal (Marini et al., 2020; Sardinha & Rosa, 2023; Ward & Brantlov, 2023). Aquest paràmetre que habitualment es valora a la freqüència de 50 kHz (encara que a qualsevol freqüència existeix un PhA), s'obté a partir de la relació entre R i Xc (Figura 3), permetent un anàlisi sense dependre de mesures com l'estatura o la BM.

Essencialment, el PhA ofereix una finestra a la salut de les cèl·lules, reflectint tant la seva quantitat com la qualitat, així com l'estat de la membrana cel·lular. Diversos factors com l'edat, el sexe, la distribució de líquids, la massa muscular i el BMI, poden influir en els

valors de PhA (Figura 15) (Gonzalez & Heymsfield, 2021). Un valor baix de PhA pot ser indicatiu de problemes a nivell cel·lular, conduint a conseqüències com reducció en la força muscular (Souza et al., 2017), deteriorament de la salut general (Zanforlini et al., 2019) i un augment en les taxes d'hospitalització i mortalitat (Buter et al., 2018; Santana et al., 2018). En canvi, un valor alt de PhA suggereix una BMC més sana (Yamada et al., 2019), i està associat amb una millor salut general (Campa et al., 2018; Ribeiro et al., 2017) i rendiment (Campa, Thomas, et al., 2022; Hetherington-Rauth et al., 2021; Matias et al., 2022). El PhA també proporciona informació sobre la relació entre l'ECW i ICW, ja que existeix una correlació inversa entre el PhA i la ràtio ECW/ICW (Marini et al., 2020).



Figura 15. Factors que determinen l'angle de fase. Adaptat de Gonzalez & Heymsfield (2021).

En conclusió, BIVA s'ha establert com una eina potent en la valoració de la composició corporal i de la distribució de fluids, destacant-se per la seva facilitat d'ús i la capacitat de proporcionar informació valuosa sense la necessitat de recórrer a equacions predictives que poden portar a errors.

BIVA específica

La metodologia BIVA descrita a la secció anterior, on es normalitzen les dades en funció de l'estatura, també rep el nom de BIVA clàssica. Aquesta aproximació es pot veure afectada per l'efecte geomètric de les àrees transversals del cos, ja que segons la llei d'Ohm, la R no només és proporcional a la longitud del segment, sinó també és inversament proporcional a l'àrea transversal d'aquests. Aquesta situació implica que la normalització per estatura en BIVA clàssica no elimina completament l'efecte del volum (Saragat et al., 2014). En la Figura 16, es pot apreciar que per a una mateixa estatura, el

volum dels segments corporals pot variar significativament amb diferents seccions transversals, afectant els valors bioelèctrics.

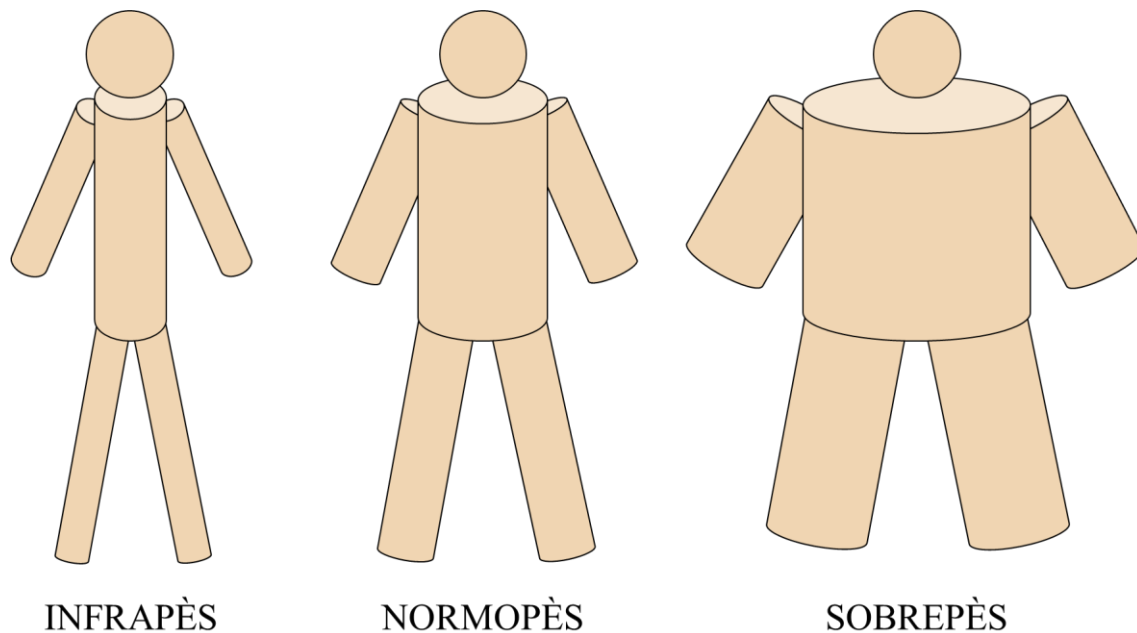


Figura 16. Diferències volumètriques dels segments corporals en funció de la massa corporal.

BIVA clàssica mostra una alta sensibilitat a mesures absolutes com el TBW, però és menys eficaç en detectar variacions en continguts relatius dels compartiments corporals, com el %FM (Marini et al., 2012). Un exemple d'aquesta limitació es manifesta en el quadrant d'obesitat de BIVA clàssica, on es relacionen vectors curts amb grans circumferències corporals, associacions que poden conduir a una interpretació incorrecta en el cas d'individus atlètics que, malgrat tenir una elevada FFM, poden ser erròniament classificats com a obesos a causa de les seves dimensions corporals elevades (Buffa et al., 2014).

Per superar aquestes limitacions, s'ha desenvolupat BIVA específica (Marini et al., 2013), una variant metodològica que, mantenint l'enfocament empíric de BIVA clàssica, normalitza els valors bioelèctrics en funció de la longitud del conductor i de les seves àrees transversal, en aquest cas les dels braços, cames i tronc.

Aquest enfocament varia la interpretació de la R , X_c i Z específiques (R_{esp} , $X_{c_{esp}}$ i Z_{esp}), mentre que el PhA no es veu afectat per la correcció. En BIVA específica, els desplaçaments del vector en l'eix major de les el·lipses de tolerància indiquen variacions en el %FM, amb valors més alts cap al pol superior dret. Per contra, l'eix menor proporciona informació similar a BIVA clàssica (Figura 17).

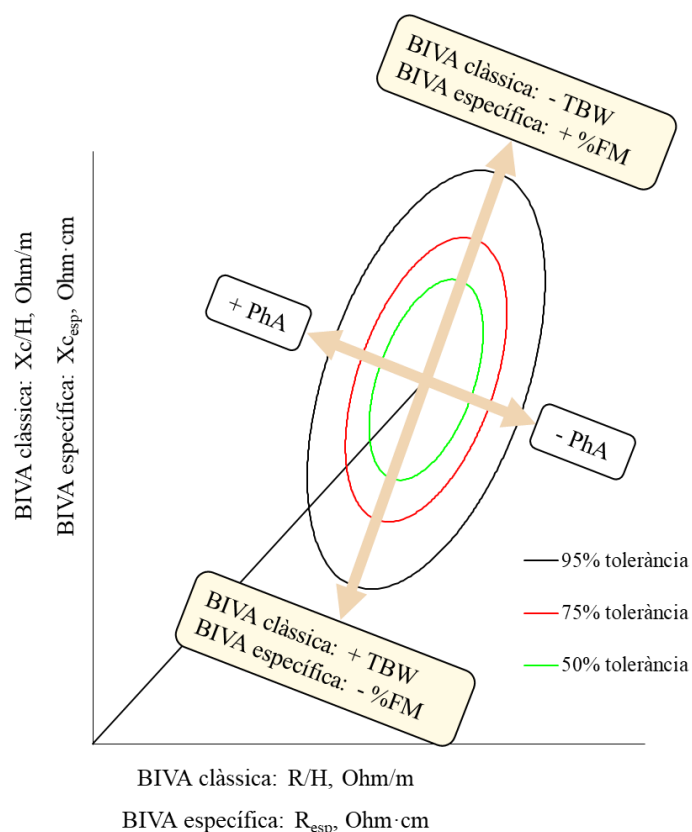


Figura 17. Interpretacions de BIVA clàssica i BIVA específica. Adaptat de Campa et al. (2021). PhA, angle de fase; R/H, resistència ajustada per l'estatura; R_{esp} , resistència específica; TBW, aigua corporal total; Xc/H , reactància ajustada per l'estatura; Xc_{esp} , reactància específica; %FM, percentatge de massa de greix.

1.4.2. Segons la freqüència del senyal

El comportament de la membrana cel·lular com a condensador varia a diferents freqüències del senyal, repercutint directament en els valors bioelèctrics mesurats i, per tant, en la interpretació de les dades (Piccoli et al., 2007). Generalment, es parla de tres categories principals d'analitzadors d'impedància bioelèctrica: d'una única freqüència, multi-freqüència i de bioimpedància espectroscòpica.

Dispositius mono-freqüència

Els dispositius mono-freqüència són àmpliament reconeguts per la seva eficiència i simplicitat, operant generalment a una freqüència de 50 kHz amb un model tetrapolar estàndard (Figura 11). Aquesta tecnologia és particularment utilitzada pel seu equilibri entre fiabilitat, accessibilitat i economia, convertint-la en la preferida per a una àmplia gamma d'estudis en contextos nutricionals, d'exercici físic i per establir valors de referència en diferents poblacions. A 50 kHz el corrent elèctric travessa els fluids dins i

fora de les cèl·lules, la qual cosa significa que les avaluacions són el resultat d'una combinació de l'aigua present tant a l'interior com a l'exterior de les cèl·lules (Kyle et al., 2004a).

No obstant això, els dispositius mono-freqüència no estan exempts de limitacions. Una de les més significatives és l'assumpció que fa del cos humà com a cilindre geomètric i simètric, i amb una composició homogènia. Com van suggerir Ellis et al. (1999), seria ideal considerar cinc cilindres diferents que representin les quatre extremitats i el tronc. Aproximacions com BIVA específica aborden aquesta limitació. Un altre repte amb aquests dispositius és que molts no són fase-sensitius, és a dir, que no proporcionen els valors de X_c , restringint o eliminant directament les opcions per seleccionar equacions específiques actuals o realitzar l'anàlisi de BIVA. A més, la falta de transparència per part dels fabricants respecte a les fórmules utilitzades per avaluar la composició corporal és una preocupació addicional que pot limitar la utilitat d'aquests dispositius en determinades aplicacions clíniques i de recerca.

Dispositius multi-freqüència

Els dispositius multi-freqüència generen corrents alterns a diverses combinacions de freqüències, incloent típicament almenys una freqüència baixa (<5 kHz), diverses de nivell mitjà (50, 100, 200 kHz) i una o més a freqüència alta (500 kHz - 1 MHz) (Lukaski, 2017). A diferència dels dispositius mono-freqüència, aquests permeten diferenciar entre les estimacions de ECW i ICW (Earthman, 2015; Kyle et al., 2004a). Aquesta tecnologia més costosa i més avançada facilita la diferenciació de com les diverses freqüències de corrent interactuen amb els teixits corporals, proporcionant informació més detallada sobre la composició corporal.

En quant al tractament de dades, els dispositius multi-freqüència segueixen un mètode similar als mono-freqüència, emprant equacions predictives o l'anàlisi vectorial per a la interpretació dels resultats. Tot i així, molts dispositius multi-freqüència recorren de forma única a la freqüència de 50 kHz per a les seves estimacions.

Aquesta tecnologia no està exempta de limitacions en la seva aplicació, especialment degut a la naturalesa anisotròpica dels teixits humans (Ward & Brantlov, 2023), característica que fa que el model de bioimpedància sigui més teòric que pràctic. Fins ara, s'han demostrat pocs avantatges significatius dels dispositius multi-freqüència envers als

mono-freqüència, posant en dubte la seva superioritat en certes aplicacions pràctiques (Kyle et al., 2004a, 2004b).

Dispositius espectroscòpics

La bioimpedància espectroscòpica (BIS) és un mètode avançat en l'àmbit de l'avaluació de la composició corporal, aplicant un espectre extens de freqüències que va des de les molt baixes (1–5 kHz) fins a les molt altes (1000–1200 kHz) (Earthman, 2015). L'aplicació d'un corrent elèctric dins d'aquest espectre de freqüències genera un perfil semicircular discontinu, que permet traçar el diagrama de Cole (Figura 5) (Lukaski, 2013). Els valors obtinguts del diagrama de Cole (R_0 i R_∞) són fonamentals per a la formulació d'equacions de predicció (Kyle et al., 2004a). Aquest procés facilita la identificació de la ω_c on la informació relativa a les membranes cel·lulars es manifesta amb major claredat, facilitant així una estimació tant de la ICW com de la ECW per separat. Tal com ocorre amb els dispositius multi-freqüència, l'anisotropia dels teixits musculars fa que el model teòric sigui menys aplicable en la pràctica.

La precisió dels mètodes de bioimpedància és excel·lent (Lukaski, 2017), mostrant una variabilitat del 1-2% per als mono- i multifreqüència i del 2-3% per a la BIS (Earthman, 2015). És important subratllar que l'exactitud associada a qualsevol mètode està influenciada per diverses condicions: adequació del model predictiu, congruència entre la mostra i la població d'origen del model, fiabilitat de les premisses subjacents a la bioimpedància (com una hidratació uniforme i una geometria corporal estandarditzada), etc. (Kyle et al., 2004b).

1.4.3. Segons la regió

Tal com s'ha indicat, el procediment habitual per a la mesura de la bioimpedància consisteix en situar els elèctrodes des del peu fins a la mà del costat dret del cos, amb el subjecte en decúbit supí (Figura 11). No obstant això, aquesta configuració pot experimentar variacions depenent de la tecnologia o de l'àrea corporal objecte d'estudi.

Cos complet

Per a l'anàlisi del cos complet, els dispositius de bioimpedància poden variar en la distribució dels elèctrodes i en les posicions, oferint quatre distribucions principals (Figura 18): mà-a-mà, peu-a-peu, peu-a-mà i segmental directa (Campa et al., 2021).

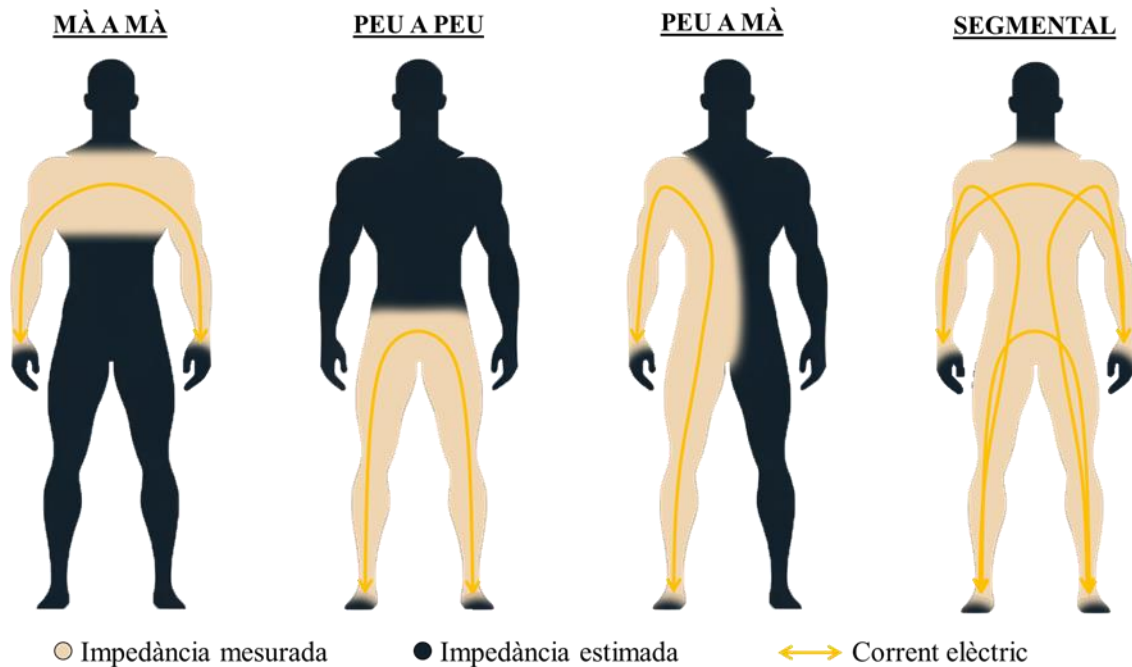


Figura 18. Distribucions per l'anàlisi de la composició corporal. Adaptat de Campa et al. (2021).

La tecnologia mà-a-mà es centra en la mesura de la impedància del cos superior, utilitzant algoritmes especialitzats per estimar les dades de la part inferior. D'igual forma la tecnologia peu-a-peu es focalitza en la part inferior del cos, estimant la impedància de la part superior. La tecnologia peu-a-mà mesura la impedància del costat dret del cos, i estima la informació del costat esquerre. Així doncs, depenent del tipus de dispositiu utilitzat, es mesura una part específica del cos, mentre que les dades de les altres zones s'estimen. A diferència d'aquestes, la tecnologia segmental ofereix una visió global, capturant la impedància de tot el cos directament, sense recórrer a estimacions. No obstant això, aquesta tecnologia també permet (depenent del dispositiu utilitzat) analitzar de manera individualitzada cada una de les extremitats, proporcionant així informació detallada i específica de cada segment del cos. Aquestes suposicions es donen en el cas de BIA, però en BIVA no és necessari realitzar cap estimació.

La diversitat en els mètodes de bioimpedància, que inclouen des de plataformes de bàscula amb elèctrodes integrats fins a elèctrodes manuals o de pinça, així com la varietat en els tipus, quantitat i posicions dels elèctrodes, pot afectar significativament les dades recollides. Aquesta variabilitat subratlla la importància d'escollir acuradament tant la tecnologia específica com la tècnica de col·locació dels elèctrodes.

Localitzada

La bioimpedància localitzada es basa en la col·locació dels elèctrodes en regions anatòmiques d'interès, realitzant una investigació detallada i focalitzada de les propietats bioelèctriques del teixit en qüestió (Lukaski & Moore, 2012).

Quan aquest anàlisi es centra en un múscul específic (Figura 19), aquesta metodologia es denomina bioimpedància muscular localitzada (ML-BIVA) o miografia d'impedància elèctrica (EIM) (Rutkove, 2009). Aquesta tècnica permet una exploració exhaustiva de l'estructura i la funcionalitat muscular, utilitzant generalment una disposició lineal de quatre elèctrodes sobre el múscul, encara que existeixen altres disposicions (Cebrián-Ponce et al., 2021). Aquest tipus d'anàlisi es duu a terme sense dependre d'equacions de predicció, ja sigui per a un anàlisi exploratori o per fer un seguiment al llarg del temps.

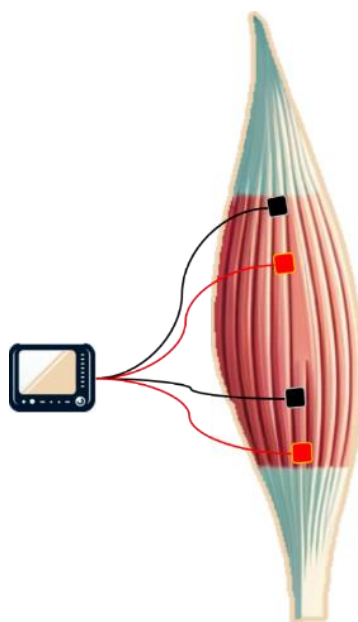


Figura 19. Exemple de col·locació dels elèctrodes de la bioimpedància muscular localitzada.

La selecció acurada de la mida dels elèctrodes i l'ajust de les distàncies entre ells és essencial per evitar interaccions o per no incidir en altres grups musculars, ja que cal tenir en consideració que l'amplitud de penetració del corrent augmenta directament amb la distància entre els elèctrodes (Rutkove et al., 2017). Per això, un dels reptes més significatius en aquest camp és aconseguir un acord unànime sobre distribució òptima dels elèctrodes (Jafarpoor et al., 2013; Sanchez et al., 2016). Per garantir l'eficàcia del mètode, és essencial col·locar els elèctrodes de forma precisa i homogènia en totes les ocasions, especialment en el marc d'estudis longitudinals, on el repte és preservar una col·locació uniforme dels elèctrodes a través de múltiples mesures. Això subratlla la

importància crítica de desenvolupar estàndards i protocols detallats que assegurin la màxima reproductibilitat i fiabilitat en els resultats obtinguts.

La mida i forma del múscul, així com l'orientació de les fibres, a més d'altres elements anatòmics no musculars, com la grandària de l'extremitat en qüestió o la presència de teixits cutanis i de greix subcutani, tenen un paper clau en l'impacte sobre les dades recollides (Jafarpoor et al., 2013; Sanchez et al., 2021).

El camp d'aplicació de la ML-BIVA s'ha expandit recentment, centrant-se principalment com a eina diagnòstica i de seguiment de malalties neuromusculars per la seva capacitat per detectar variacions significatives en la composició i l'arquitectura muscular (Rutkove, 2009; Rutkove & Sanchez, 2019). Aquesta sensibilitat es manifesta en la detecció de desajusts com ara l'alteració de l'equilibri iònic i la integritat de la membrana cel·lular, repercutint de manera directa sobre les característiques electrofisiològiques del teixit (Sanchez et al., 2021). Així, ML-BIVA emergeix com a recurs diagnòstic clau en el maneig de trastorns com l'esclerosi lateral amiotròfica (Tarulli et al., 2009) o la distròfia muscular de Duchenne (Rutkove et al., 2014).

En quant a la investigació relacionada amb l'exercici físic, tot i ser prometedora, es troba encara en una etapa inicial de desenvolupament. Això inclou la detecció i seguiment de lesions fins al retorn a l'activitat esportiva (Nescolarde et al., 2023), així com la monitorització de la càrrega interna d'entrenament mitjançant l'anàlisi dels canvis fisiològics en el múscul (Cebrián-Ponce et al., 2022; Cebrián-Ponce, Irurtia, et al., 2023; Cebrián-Ponce, Marini, et al., 2023).

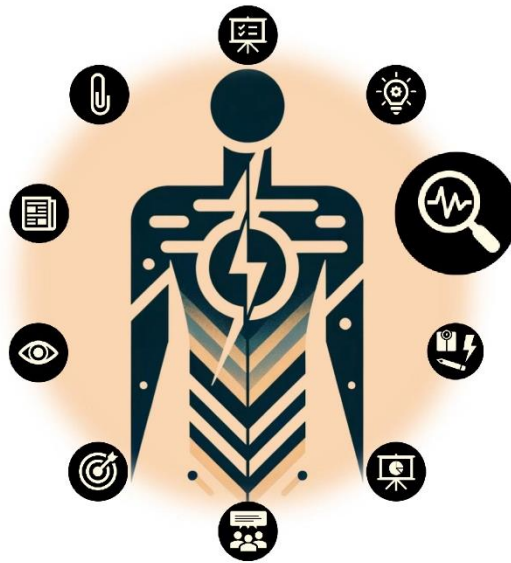
1.5. Protocol de la bioimpedància

L'adherència a un protocol meticulós i consistent és essencial per assegurar l'exactitud i la fiabilitat de les dades. A continuació, es detallen algunes pautes clau proporcionades per Earthman, (2015), encara que aquestes es poden modificar segons l'objecte d'estudi:

1. **Regulació de la ingesta alimentària i hídrica, i activitat física:** es suggereix fer les mesures en dejú, evitant l'alcohol, la cafeïna i l'activitat física, almenys 8 hores abans de l'avaluació (idealment pel matí). Pot haver-hi flexibilitat en aquests requeriments, però és crucial registrar l'hora de la mesura per assegurar consistència en les avaluacions següents.

2. **Gestió de fluids/sòlids en tercers espais:** aspectes com l'orina o el volum fecal, malgrat no ser directament mesurats, poden influir la BM total.
3. **Preparació cutània:** és imprescindible que la zona de contacte amb els elèctrodes estigui neta i lliure de qualsevol tipus de crema o impuresa.
4. **Ajust i calibració de l'equip de mesura:** la verificació quotidiana dels instruments assegura la precisió dels instruments de bioimpedància.
5. **Posició i entorn:** la postura del cos ha de ser horitzontal, mantenint el cos en decúbit supí (Figura 11), facilitant així una distribució homogènia dels fluids corporals. Situar al subjecte en una superfície que no sigui de metall i amb una distància mínima d'un metre de qualsevol aparell electrònic o magnètic.
6. **Selecció i aplicació dels elèctrodes:** la certificació d'aquests components per a la seva utilització és crucial, així com la seva correcta col·locació, que hauria de ser uniforme en totes les sessions.
7. **Distribució dels elèctrodes:** Si es fa servir la distribució tetrapolar (Figura 11), es deu mesurar sempre al mateix costat del cos (generalment el dret). En casos d'amputació o atrofia, s'avalua el costat no afectat. Es deu mantenir la coherència en el costat i en la col·locació dels elèctrodes per a futurs seguiments.
8. **Durada de l'anàlisi:** un interval d'1 a 5 minuts resulta ser el més idoni per a la recopilació de dades, variant segons la tecnologia i les dimensions del participant. Evitar mantenir la posició per més de 10 minuts.
9. **Control del medi ambient:** la temperatura ambient hauria de romandre entre els 20 i els 28 °C per evitar distorsions en les mesures degudes a variacions en la circulació sanguínia perifèrica.
10. **Consideracions respecte al cicle menstrual:** es recomana anotar el cicle menstrual i mantenir una consistència en el moment de realitzar les mesures de seguiment.

A més, és crucial considerar la influència de la temperatura corporal en les mesures de bioimpedància, ja que variacions significatives, com les causades per febre o exercici físic poden distorsionar els resultats. Segons Di Iorio et al. (1999), la febre pot disminuir els valors de R fins a un 10%, mentre que en el context de l'exercici físic, Campa, Gatterer, et al. (2019) van examinar com una dutxa freda de 10 minuts després de l'exercici físic ajuda a estabilitzar les lectures BIA més ràpidament en comparació amb no dutxar-se, permetent una avaluació més precisa dels canvis hídrics post-exercici.



JUSTIFICACIÓ I OBJECTIUS

2. Justificació i objectius

2.1. Justificació

La relació entre l'exercici físic i els seus efectes sobre la composició corporal, l'equilibri hidroelèctric i la conservació de la integritat muscular és un àmbit d'estudi crucial, tant per a la millora de la salut i del rendiment esportiu, com per a la prevenció i tractament de lesions (Lukaski, 2017; Lukaski & Raymond-Pope, 2021; Marini & Toselli, 2021). En aquest context, BIVA es presenta com una eina prometedora, capaç d'aportar informació valuosa sobre aquests canvis de manera no invasiva, econòmica i en temps real (Castizo-Olier, Irurtia, et al., 2018). La seva habilitat per proporcionar informació detallada sobre la composició corporal i l'estat hidroelèctric sense la necessitat de processos complexos o invasius la converteix en una eina particularment atractiva per a la recerca i la pràctica aplicada en els àmbits de la medicina esportiva i de les ciències de l'esport (Campa et al., 2021; Campa, Gobbo, et al., 2022; Campa, Matias, Gatterer, et al., 2019).

Malgrat les potencials aplicacions de BIVA en l'àmbit esportiu, la investigació referent al seu ús per a analitzar els efectes de l'exercici físic sobre la composició corporal, l'equilibri hidroelèctric i l'estat cel·lular encara no està plenament desenvolupada (Castizo-Olier, 2018). Existeix una notable falta d'estudis específics que abordin l'aplicabilitat del mètode BIVA (especialment en la variant ML-BIVA), en contextos relacionats amb els canvis bioelèctrics induïts per l'exercici físic (Freeborn et al., 2020). Aquesta escassetat d'investigacions restringeix la comprensió de la utilització òptima de BIVA, destacant la necessitat urgent d'estudis ben estructurats i rigorosos que puguin definir els efectes de diversos tipus i intensitats d'exercici físic sobre els paràmetres mesurats per BIVA. Aquesta investigació no només amplia el coneixement sobre BIVA, sinó que també millora les estratègies d'intervenció basades en l'exercici, oferint una base més sòlida per futures investigacions.

2.2. Objectiu principal

Aquesta tesi doctoral té com a fonament investigar com l'exercici físic indueix canvis en BIVA, tant a nivell global com muscular. Per això, l'objectiu principal és detectar i avaluar els canvis en la composició corporal, la dinàmica hidroelèctrica i la integritat de les cèl·lules musculars provocats per diferents tipus i intensitats d'exercici, proporcionant

així una base sòlida per al desenvolupament de mètodes diagnòstics i de seguiment en l'àmbit de la salut i de l'exercici físic.

2.3. Objectius específics

Estudi I

L'objectiu d'aquesta revisió sistemàtica és doble: primerament, busca resumir els coneixements actuals sobre l'ús de ML-BIVA com a eina diagnòstica en els àmbits de la salut i de l'exercici físic. En segon lloc, pretén orientar les perspectives futures en aquest camp i suggerir una agenda de recerca, donat que els estudis en aquesta àrea encara són limitats i heterogenis..

Estudi II

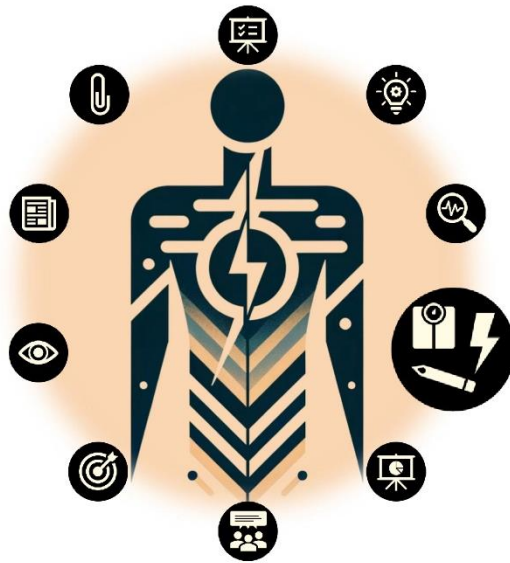
Analitzar la cinètica dels paràmetres de BIVA (tant globals com musculars) induïda per una sessió d'entrenament d'alta intensitat en jugadors d'hoquei sobre patins. Això inclou la valoració de la pèrdua de fluids, els efectes fisiològics de l'entrenament i la integritat cel·lular del múscul. Addicionalment, l'estudi tracta de correlacionar els canvis d'aquests paràmetres bioelèctrics amb els canvis en la creatina-fosfocinasa (CK), considerant-los com a possibles indicadors de dany muscular després de l'exercici.

Estudi III

Analitzar els canvis en els fluids corporals i musculars induïts en ciclistes professionals durant el transcurs del Giro d'Itàlia, utilitzant avaluacions antropomètriques, bioelèctriques (globals i musculars) i hematològiques, per buscar correlacions entre aquests paràmetres i BIVA. A més, es compararan les característiques bioelèctriques d'aquests ciclistes d'elit amb les d'una població de referència d'adults masculins sans.

Estudi IV

Analitzar els canvis en els fluids corporals i musculars provocats per diferents perfils de curses de trail running (14, 35 i 52 km), utilitzant avaluacions antropomètriques, bioelèctriques (globals i musculars) i de CK, com a estudi pioner en l'ús de BIVA en aquest context esportiu.



MATERIALS I MÈTODES

3. Materials i mètodes

3.1. Estructura de la tesi

La present tesi doctoral es compon de quatre estudis científics, cadascun amb els objectius especificats en la secció prèvia, emprant diverses estratègies metodològiques. El primer pas va ser una revisió sistemàtica que aprofundeix en ML-BIVA aplicada a la salut i l'exercici físic, on s'analitza l'evidència científica existent. Aquesta revisió, degut a la notable heterogeneïtat de les dades, no inclou meta-anàlisi. Posteriorment, es van dur a terme tres estudis quasi-experimentals per avaluar l'ús de BIVA (global i muscular) en una varietat de disciplines esportives (hoquei sobre patins, ciclisme i trail running), realitzant-se en diferents contextos ecològics i considerant co-paràmetres de diversa índole. La Taula 3 ofereix una visió esquemàtica i detallada de les quatre publicacions que formen la tesi, proporcionant una perspectiva integral de l'obra.

3.2. Participants

En el marc d'aquesta tesi doctoral, els participants es distribueixen de la següent manera en els quatre estudis: l'**Estudi I**, com a revisió sistemàtica, no implica directament la participació de subjectes, però analitza dades de 607 subjectes en 18 estudis seleccionats -a més d'una quantitat de subjectes no especificats d'un altre estudi-, cobrint un ampli espectre d'edats i condicions físiques en homes i dones. L'**Estudi II** es va realitzar amb 7 jugadors masculins professionals d'hoquei sobre patins, amb una edat mitjana de 26.3 ± 5.4 anys, una estatura de 177.0 ± 6.2 cm, un BM de 79.0 ± 9.8 kg i un BMI de 25.1 ± 2.1 kg/m². Els jugadors havien de ser majors de 18 anys, sense lesions ni condicions clíniques, ni estar prenent medicaments durant l'estudi. L'**Estudi III** va incloure 9 ciclistes masculins professionals del Cannondale Pro-Cycling Team que van participar en el Giro d'Itàlia 2013, amb una edat mitjana de 27.9 ± 2.4 anys, una estatura de 181.4 ± 6.1 cm, un BM de 70.5 ± 6.1 kg i un BMI de 21.4 ± 0.4 kg/m². Tots estaven amb una bona salut segons l'avaluació del metge de l'equip i no consumien substàncies que poguessin afectar l'equilibri de fluids. Finalment, l'**Estudi IV** va comprendre 92 corredors/es de trail running, 55 homes amb una edat mitjana de 36.8 ± 7.2 anys, una estatura de 176.2 ± 7.0 cm, un BM de 73.2 ± 8.6 kg i un BMI de 23.6 ± 2.3 kg/m²; i 37 dones amb una edat mitjana de 35.2 ± 8.5 anys, una estatura de 164.7 ± 6.1 cm, un BM de 59.0 ± 8.0 kg i un BMI de 21.7 ± 2.4 kg/m², que van participar en la Volta a la Cerdanya Ultrafons 2013.

Els participants, lliures de lesions o condicions clíniques, van escollir entre curses de 14, 35 i 52 km, segons el seu nivell de preparació i aptitud física.

3.3. Disseny

Es va adoptar una diversitat de metodologies d'investigació per explorar les dimensions globals i musculars de BIVA en una varietat de contextos esportius i de salut. La revisió sistemàtica de l'**Estudi I** va seguir les directrius PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) de Moher et al. (2009), seleccionant només estudis que fan servir dispositius de bioimpedància en músculs de forma específica. Mitjançant l'ús d'*Anatomical Quality Assessment* (AQUA) d'Henry et al. (2017), es va avaluar el risc de biaix, assegurant la qualitat dels estudis inclosos. L'**Estudi II**, es va enfocar en jugadors d'hoquei patins, adoptant un disseny quasi-experimental, descriptiu i correlacional. Aquest estudi va examinar les adaptacions agudes en paràmetres antropomètrics i bioelèctrics, tant globals com localitzats (al quàdriceps), així com en biomarcadors com la CK, després d'una sessió d'entrenament intens. Les avaluacions es van realitzar en tres moments diferents: abans, immediatament després i 24 hores post-exercici, fent un anàlisi detallat de les respostes fisiològiques. En l'**Estudi III**, també amb un enfoc quasi-experimental, descriptiu i correlacional, va examinar a ciclistes d'elit durant el Giro d'Itàlia, una competició de 23 dies. Es van realitzar mesures bioelèctriques globals i localitzades (al quàdriceps, isquiotibials i tríceps sural), antropomètrics i hematològics. Les avaluacions es van realitzar en tres moments diferents: abans, a meitat, i després de la competició, capturant així les adaptacions fisiològiques a aquesta prova d'alta exigència i oferint una perspectiva enriquidora sobre l'impacte d'aquestes curses en la vessant bioelèctrica. Finalment, l'**Estudi IV**, va seguir també un disseny quasi-experimental, descriptiu i correlacional en corredors de curses de muntanya de diverses distàncies (14, 32 i 52 km). Es van realitzar mesures bioelèctriques globals i localitzades (als quàdriceps, isquiotibials i tríceps sural), a més d'antropomètriques i de nivells de CK, abans i després de les curses, proporcionant dades valuoses sobre l'impacte d'aquest tipus d'esforç i les diferents respostes bioelèctriques de cada múscul.

3.4. Material

Es van emprar materials específics en cadascun dels estudis per garantir la precisió i la fiabilitat dels resultats. Per a l'**Estudi I**, no es va requerir material específic, ja que es

tracta d'una revisió sistemàtica. No obstant, es van incloure només estudis que fan servir dispositius de bioimpedància fase-sensitius a 50 kHz. En l'**Estudi II**, es van utilitzar els següents materials antropomètrics: un tallímetre (Seca 220®, Birmingham, Regne Unit) per a mesurar l'estatura; una bàscula (Seca 710®, Birmingham, Regne Unit) per a la BM; i una cinta antropomètrica Lufkin Executive (Lufkin, Texas, Estats Units) per a la col·locació dels elèctrodes i mesura dels perímetres. Per a la bioimpedància es va fer servir un analitzador BIA 101 Anniversary Sport Edition (Akern Srl, Florència, Itàlia) que emet un corrent sinusoidal altern a 50 kHz, amb elèctrodes Red Dot™ 2660-5 (3M Corporate Headquarters, Minnesota, Estat Unit). En l'**Estudi III**, es van utilitzar els mateixos materials antropomètrics que en l'Estudi II, mentre que per les mesures bioelèctriques es va fer servir un dispositiu multifreqüència fase-sensitiu DS Medica (Human-Im PLUS Impedance meter unit 80C32, Milà, Itàlia). Per als tests hematològics, es van fer servir tubs evacuats (BD Vacutainer Systems, Becton-Dickinson, Nova Jersey, Estats Units) i l'anàlisi hematològic es va realitzar amb un instrument Sysmex XE 2100 (Sysmex, Kobe, Japó). En l'**Estudi IV**, els materials antropomètrics i bioelèctrics utilitzats van ser els mateixos que en l'Estudi II. En els tres estudis quasi-experimentals, tota l'anàlisi bioquímica es va realitzar utilitzant el dispositiu Advia 2400 (Siemens Medical Solutions Diagnostics, Nova Jersey, Estats Units).

3.5. Disposició bioelèctrica

Es van aplicar protocols específics per a la disposició bioelèctrica dels elèctrodes, amb enfocaments globals i musculars. L'**Estudi I**, com a revisió sistemàtica, no va requerir d'una disposició bioelèctrica pròpia, però va incloure només estudis emprant ML-BIVA. En la resta d'estudis, WB-BIVA es va utilitzar de forma consistent mitjançant la distribució estàndard (Figura 11). En quant a ML-BIVA, l'**Estudi II** va analitzar el quàdriceps, seguint les directrius de *Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles* (SENIAM) d'Hermens et al. (2000) i de la *International Society for the Advancement of Kinanthropometry* (ISAK) de Stewart et al. (2011) per a la col·locació dels elèctrodes. Es va marcar un punt mitjà entre l'espina ilíaca anterosuperior i el pol superior de la ròtula, amb els elèctrodes detectors col·locats a una tercera part de l'origen i inserció del múscul, i els injectors a 5 cm dels detectors, distalment al centre del múscul. Pel que fa a l'**Estudi III** i **Estudi IV**, es va seguir el mateix protocol per a l'anàlisi de tres grups musculars. En el quàdriceps, els elèctrodes injectors es van col·locar a 5 cm

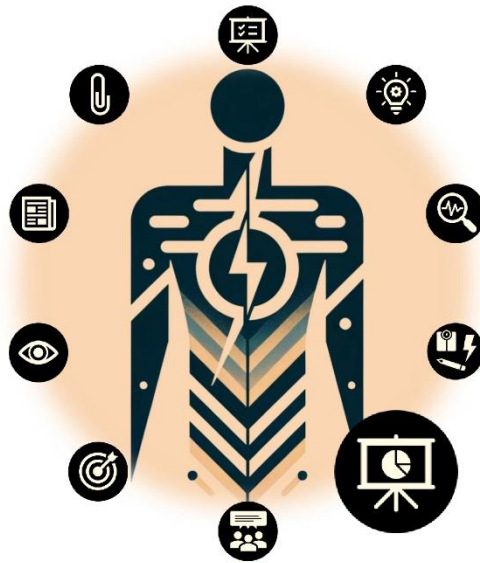
tant de l'espina ilíaca anterosuperior com del pol superior de la ròtula. Per als isquiotibials, es van situar a 5 cm de la tuberositat isquiatàtica i del buit popliti. En el tríceps sural, els elèctrodes injectors es van col·locar a 5 cm del buit popliti i de la línia intermaleolar. Els elèctrodes detectors, en tots els casos, es van posicionar a 5 cm dels injectors, proximalment al centre del múscul analitzat.

3.6. Anàlisi estadística

Es va implementar una varietat de tècniques i mètodes estadístics, adaptant-se específicament a les característiques i necessitats de cada estudi. En l'**Estudi I**, al tractar-se d'una revisió sistemàtica sense meta-anàlisi, no va requerir anàlisi estadística. Per a la resta d'estudis, es va establir un format comú on les dades es presenten com a mitjana $\pm$ desviació estàndard, amb un nivell de significació fixat a $p < 0.05$. Els programaris SPSS (versió 21) i BIVA Software (Piccoli & Pastori, 2002) es van utilitzar per a l'anàlisi de les dades i per a la creació de gràfics RXc. En l'**Estudi II**, es va aplicar el test de Shapiro–Wilks per verificar la normalitat de les distribucions, i el test de Friedman per analitzar les diferències en les variables, recorrent al test de Wilcoxon per a identificar diferències significatives. La magnitud dels canvis es va calcular com valors percentuals delta ($\% \Delta$), i la correlació entre aquests canvis i els nivells de CK es va avaluar amb el coeficient de Spearman. Es van utilitzar gràfiques RXc i el test T^2 d'Hotelling per a l'anàlisi vectorial bioelèctrica. Per l'**Estudi III**, també es va emprar el test de Shapiro–Wilks per a la normalitat i el test de Friedman per a identificar diferències en les variables al llarg del temps, amb l'ús del test de Wilcoxon quan es trobaven diferències. Es van expressar els canvis com $\% \Delta$, utilitzant el coeficient de Spearman per a detectar associacions entre canvis hematològics i bioelèctrics. Les gràfiques RXc i el test T^2 d'Hotelling van servir per a l'anàlisi dels canvis bioelèctrics en el transcurs de la competició. Finalment, en l'**Estudi IV**, després de la comprovació de la normalitat amb el test de Shapiro–Wilks, es van avaluar les diferències entre els valors previs i posteriors a les curses amb el t-test de Student per a distribucions paramètriques i el test de Wilcoxon per a no paramètriques. La magnitud dels canvis es va expressar també com $\% \Delta$. El coeficient de correlació de Pearson es va utilitzar per identificar associacions entre canvis bioelèctrics i $\% \Delta$ de CK, així com entre el temps de cursa i paràmetres bioelèctrics. El test T^2 d'Hotelling va servir per a comparar les diferències bioelèctriques entre ambdós moments.

Taula 3. Visió esquemàtica de les quatre publicacions que integren la tesi doctoral.

	Publicacions Estudi I	Estudi II	Estudi III	Estudi IV
OBJECTIUS	Resumir el coneixement existent de ML-BIVA en salut i exercici. Esbossar perspectives futures	Analitzar BIVA en jugadors d'hoquei patins en un entrenament d'alta intensitat. Buscar correlacions bioelèctriques i bioquímiques	Analitzar BIVA en ciclistes d'elit durant una competició del Giro d'Itàlia. Buscar correlacions bioelèctriques i hematològiques	Analitzar BIVA en corredors de trail running de diferents distàncies. Buscar correlacions bioelèctriques i bioquímiques
PARTICIPANTS	No aplica al ser una revisió sistemàtica. Però inclou almenys 609 subjectes de diferents edats	7 jugadors d'hoquei masculins, mitjana d'edat 26,3 anys	9 ciclistes professionals masculins, mitjana d'edat 27,9 anys	92 corredors de trail (55 homes i 37 dones) de diferents edats, majors de 18 anys
DISSENY	Revisió sistemàtica segons PRISMA, 19 estudis seleccionats revisats amb AQUA	Quasi-experimental en hoquei. Mesures pre i post-entrenament	Quasi-experimental en ciclisme. Mesures pre, durant i post-competició	Quasi-experimental en trail running. Mesures pre i post-cursa
MATERIAL	No aplica al ser una revisió sistemàtica. Inclou només estudis de bioimpedància a 50 kHz	Tallímetre Seca 220, bàscula Seca 710, cinta Lufkin, analitzador BIA 101ASE, analitzador bioquímic Advia 2400	Tallímetre Seca 220, bàscula Seca 710, cinta Lufkin, analitzador BIA DS Medica, analitzador bioquímic Advia 2400, tubs BD Vacutainer, Sysmex XE 2100	Tallímetre Seca 220, bàscula Seca 710, cinta Lufkin, analitzador BIA 101ASE, analitzador bioquímic Advia 2400
DISPOSICIÓ BIOELÈCTRICA	No aplica al ser una revisió sistemàtica, però només inclou estudis amb ML-BIVA	WB-BIVA, ML-BIVA (quàdriceps)	WB-BIVA, ML-BIVA (quàdriceps, isquiotibials, tríceps sural)	WB-BIVA, ML-BIVA (quàdriceps, isquiotibials, tríceps sural)
ANÀLISI ESTADÍSTICA	No aplica al ser una revisió sistemàtica sense meta-anàlisi	Shapiro-Wilks, Friedman, Wilcoxon, Spearman, gràfiques RXc, T ² Hotelling	Shapiro-Wilks, Friedman, Wilcoxon, Spearman, gràfiques, RXc T ² Hotelling	Shapiro-Wilks, t-student, Wilcoxon, Pearson, gràfiques RXc, T ² Hotelling



PUBLICACIONES

4. Publicacions

Estudi I

Article publicat en *Frontiers in Physiology* (2021):

JIF del JCR SCIE/SSCI 4.755	Índex SJR 1.126
Quartil JCR SCIE/SSCI Q1	Quartil SJR Q1
Categoria JCR SCIE/SSCI Physiology	Categoria SJR Physiology (medical)

Electrical Impedance Myography in Health and Physical Exercise: A Systematic Review and Future Perspectives

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Electrical Impedance Myography in Health and Physical Exercise: A Systematic Review and Future Perspectives

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Background: Electrical impedance myography (EIM) is a non-invasive method that provides information about muscle health and changes that occur within it. EIM is based on the analysis of three impedance variables: resistance, reactance, and the phase angle. This systematic review of the literature provides a deeper insight into the scope and range of applications of EIM in health and physical exercise. The main goal of this work was to systematically review the studies on the applications of EIM in health and physical exercise in order to summarize the current knowledge on this method and outline future perspectives in this growing area, including a proposal for a research agenda. Furthermore, some basic assessment principles are provided.

Methods: Systematic literature searches on PubMed, Scopus, SPORTDiscus and Web of Science up to September 2020 were conducted on any empirical investigations using localized bioimpedance devices to perform EIM within health and physical exercise contexts. The search included healthy individuals, elite soccer players with skeletal muscle injury, and subjects with primary sarcopenia. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist was used to develop the systematic review protocol. The quality and risk of bias of the studies included were assessed with the AQUA tool.

Results: Nineteen eligible original articles were included in this review, which were separated into three tables according to the nature of the study. The first table includes six studies on the bioelectrical characterization of muscle. The second table includes five studies analyzing muscle changes in injured elite soccer players. The third table includes studies on the short-, medium-, and long-term bioelectrical adaptations to physical exercise.

Conclusions: EIM has been used for the evaluation of the muscle condition in the clinical field over the last few years, especially in different neuromuscular diseases. It can

also play an important role in other contexts as an alternative to complex and expensive methods such as magnetic resonance imaging. However, further research is needed. The main step in establishing EIM as a valid tool in the scientific field is to standardize the protocol for performing impedance assessments.

Keywords: muscle, physiology, bioimpedance, bioelectrical vector, sport

INTRODUCTION

Bioimpedance assessment carried out on a particular part of the human body is known as localized bioimpedance analysis (L-BIA). If the specific area analyzed is a muscle or a group of muscles, it is known either as muscle-localized bioimpedance analysis or electrical impedance myography (EIM), a term that was first introduced in 2002 (Rutkove et al., 2002), but had already been studied before (Elleby et al., 1990). EIM is a non-invasive, painless assessment method that quantifies the passive electrical behavior of muscle (Rutkove, 2009). It involves the application of an electrical current and the measurement of the voltage across the biological tissue. It has been proposed that EIM can provide detailed physiological data on the composition and structure of muscle (Sanchez and Rutkove, 2017b), depending on factors such as cell populations, cell volumes, cell membrane integrity, and intra-/extracellular fluids of the tissue (Freeborn et al., 2020). EIM provides a set of quantitative parameters, usually: resistance (R), the major resistance to the current through intra- and extracellular ionic fluids; reactance (Xc), the additional resistance due to capacitive elements such as cell membranes, tissue interfaces, and non-ionic substances; impedance (Z), the resistance of the tissues to the electric current flow; and the phase angle (PhA), the geometric relationship between R and Xc (Castizo-Olier et al., 2018). This set of parameters might be useful in the assessment of muscle health and other outcomes.

To date, the main purpose of EIM has been to evaluate neuromuscular diseases, more specifically to assess disease severity, progression over time, and response to therapy; however, it has not been used to establish an initial diagnosis (Sanchez and Rutkove, 2017a). EIM is based on the idea that conductivity and permittivity in a diseased muscle are altered (Foster and Schwan, 1989) and these alterations in the muscle can cause changes in the voltage generated. There are several studies showing that a diseased muscle has different impedance values compared to healthy muscle, for instance, in individuals with neurogenic disorders such as amyotrophic lateral sclerosis (Tarulli et al., 2009), spinal muscular atrophy (Rutkove et al., 2010), and radiculopathy (Spieker et al., 2013) or in those with muscular dystrophies such as Duchenne muscular

dystrophy (Rutkove et al., 2014) and facioscapulohumeral muscular dystrophy (Statland et al., 2016). In general, a diseased muscle presents significantly decreased R, Xc, and PhA values, mainly due to muscle fiber atrophy, connective tissue accretion, fat infiltration, and edema (Rutkove et al., 2008; Nescolarde et al., 2020). However, depending on the disease, there may be some parameters that are more useful than others. Thus, future investigations should focus on detecting which parameter is more useful in each condition. This article does not go into detail about the applicability of EIM in neuromuscular diseases since there are recent reviews that address this topic (Sanchez and Rutkove, 2017a,b; Rutkove and Sanchez, 2019).

EIM has also been used to investigate age-related changes in the muscle in sarcopenia, skeletal muscle injuries, and adaptations generated by physical exercise within the muscle.

Sarcopenia is a widespread and progressive disease of the skeletal muscle, characterized by decreased muscle strength, muscle mass, and functional ability, which is often directly related to advanced age (Thompson, 2009; Narici and De Boer, 2011). This condition affects ~25% of individuals aged over 70 years and 30–50% of individuals aged over 80 (Baumgartner et al., 1998), but these prevalence rates may change depending on the cut-off points established for the muscle assessments (Masanés et al., 2017). The most common methods to evaluate sarcopenia are a combination of muscle mass and muscle strength measurements, and radiological imaging (Pillard et al., 2011; Cruz-Jentoft and Sayer, 2019). However, strength measurements require subjective elements well-beyond the properties of muscle tissue itself such as motivation or willingness to participate (Kortman et al., 2013), while radiological imaging is very costly, difficult to apply, and with a great invasiveness (Pahor et al., 2009). For this reason, a quick evaluation method of the muscle condition in sarcopenia is needed to facilitate individual patient care and clinical research. Subjects with sarcopenia present a lower number and smaller size of muscle fibers. Thus, differences in the impedance characteristics of the muscle might be expected (Aaron et al., 2006; Rutkove et al., 2008; de-Mateo-Silleras et al., 2018), supporting the potential application of EIM and overcoming the limitations of other methods. Sarcopenia might be classified into two categories. If the primary cause of sarcopenia is aging, it is considered to be “primary sarcopenia.” If there is another factor other than aging, like a systemic disease, it is considered to be “secondary sarcopenia” (Cruz-Jentoft et al., 2019). Other intrinsic factors that can play an important role in the localized bioimpedance analysis are gender (Aaron et al., 2006; Tarulli et al., 2007; Kortman et al., 2013; Mascherini et al., 2017; de-Mateo-Silleras et al., 2018) and body composition (Narayananwami et al., 2012; Baidya and Ahad, 2016).

Abbreviations: BIA, bioelectrical impedance analysis; BIVA, bioelectrical impedance vector analysis; D-EIM, dynamic electrical impedance myography; EIM, electrical impedance myography; HEA, handheld electrode array; L-BIA, localized bioelectrical impedance analysis; MRI, magnetic resonance imaging; MVIC, maximum voluntary isometric contraction; PhA, phase angle; R, bioelectrical resistance; RTP, return to play; SFL, subcutaneous fat layer; Xc, bioelectrical reactance; 1RM, one-repetition maximum.

A skeletal muscle injury occurs when a force applied to a muscle causes structural damage such as contusions, elongations or strains of different grades, depending on the severity of the injury (Maffulli et al., 2014). These types of injuries are the most common in sports, where they are a major challenge in primary care and sports medicine (Baoge et al., 2012) with regards to their prevention, the determination of the best time for the return to play (RTP) and the reduction of the re-injury rate (Nescolarde et al., 2013). Some longitudinal studies using EIM have been performed with injured elite male soccer players in the last decade (Nescolarde et al., 2013, 2015, 2017, 2020; Francavilla et al., 2015). In general, the impedance values of the injured muscle are decreased and gradually recover over time during the process of healing. This means that EIM might be a practical non-invasive tool for assessing muscle health quality in physical exercise and sports (Sanchez and Rutkove, 2017b).

There are several investigations using bioelectrical impedance analysis (BIA) in physical exercise and sports. However, most of them have used whole-body BIA and there are only a few applying localized bioimpedance analysis (Castizo-Olier et al., 2018). These studies can be separated into three groups according to the classification of Castizo-Olier et al. (2018). The first group includes short-term investigations (<24 h) with the main purpose of analyzing impedance values during the execution of an exercise (Shiffman et al., 2003; Li L. et al., 2016; Fu and Freeborn, 2018; Freeborn and Fu, 2019; Huang et al., 2020), also known as dynamic electrical impedance myography (D-EIM), a term first coined in 2003 (Shiffman et al., 2003). The second group contains medium-term investigations (≥ 24 h and <7 days) that aim to assess muscle responses in the days following an exercise protocol (Elleby et al., 1990; Freeborn et al., 2020). The third group includes long-term investigations (≥ 7 days) analyzing localized chronic adaptations to exercise; however, there is only one study inside this category (Mascherini et al., 2015). While short-term analysis with EIM may be useful in understanding what happens inside a muscle when performing different exercise protocols, medium-, and long-term analyses may be useful in evaluating how training affects muscle during recovery in the days after the exercise has been performed. Therefore, this could help in controlling the training load.

EIM is a relatively new tool for muscle assessment and although there are several studies investigating the topic, it is a technology that is far from being considered as consolidated. For this reason, the assessment technique as well as its applicability in health and physical exercise need to be further studied. If EIM wants to reach its full potential, theoretical, engineering, and clinical improvements are needed (Sanchez and Rutkove, 2017a). To date, the main point of interest regarding EIM has been in studies on neuromuscular diseases, but it may have great potential in other areas, for example, in the recognition of injury and its follow-up during recovery until the RTP, as well as in the tracking of the training load based on physiological changes in the muscle without the use of expensive and complex technology that is not accessible to everyone. Therefore, the increase in the number of publications regarding EIM in physical exercise, sports, and health seems justified in order to investigate the applicability of EIM for assessments in real time and in a precise, accurate, reliable, non-invasive, portable, inexpensive, safe, and

simple way. In addition, since studies in these fields are still scarce and very heterogeneous, a compilation of current knowledge is needed to suggest a research agenda.

This systematic review aims to summarize the current knowledge on the use of EIM as a diagnostic tool for muscle adaptations in muscle health and physical exercise. Beyond that, this review attempts to outline future perspectives in this field and suggest a research agenda.

Lastly, this review will also report some basic assessment principles, since there is a variety of ways of using EIM and there are certain aspects that must be taken into account before performing the assessment, like the device, materials, electrode materials and arrangement, frequency, and factors that could affect the results (Tarulli et al., 2007; Kortman et al., 2013; Sung et al., 2013; Mascherini et al., 2015, 2017).

METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied to undertake the present review (Moher et al., 2009). The PRISMA checklist was also used to develop the systematic review protocol (Moher et al., 2015). The checklist is reported in **Supplementary Table 1**.

Eligibility Criteria

This study reviewed and analyzed both descriptive and analytic empirical studies that used bioimpedance devices to perform assessments in exercise, sports, or health. Only studies where the assessment was performed on the muscle itself were selected. Articles that used EIM in healthy sedentary people, subjects with primary sarcopenia, physically active individuals, and athletes of all levels were eligible for review. The following studies were included: (a) studies with muscle-localized bioimpedance measurements at 50 kHz, discarding those that analyzed wounds or burns or were performed with animals; (b) investigations on muscle bioelectrical and/or physiological properties, discarding those on BIA technology and its reliability and validity, localized bioimpedance techniques, or reproducibility of the measurements; (c) investigations involving healthy subjects, subjects with skeletal muscle injuries or subjects with primary sarcopenia, excluding those involving subjects with any other type of acute or chronic disease, as well as subjects with secondary sarcopenia; (d) studies published in a peer-reviewed journal; and (e) studies published in the English language. No restrictions in terms of country, time frame, or age of the subjects were considered.

Information Sources

The present review searched the following databases: PubMed, Scopus, SPORTDiscus and Web of Science. The last update was conducted in mid-September 2020.

Search Strategy

The title, abstract and keyword fields were searched in each of the aforementioned databases using the following search terms and syntax: [(“EIM” OR “electrical impedance”) AND (“muscle” OR “myography”)] OR [(locali* OR “regional”) AND

("bioimpedance" OR "bioelectrical" OR "BIA" OR "BIVA"). This syntax was the same for the four databases used. No data restrictions were applied. However, only studies published in the English language were selected.

Study Records

Records were exported from the electronic databases to a reference management software (Mendeley Desktop 1.19.4) and duplicated references were removed.

After removing the duplicates, the eligible articles were screened by two investigators (ÁC-P and GS-L), with disagreement settled by consensus. An initial screening of the titles and abstracts was performed to check the eligibility criteria. Differences in study eligibility were compared and deviations were discussed with a third investigator (AI) until consensus was reached. When a paper could not be rejected with certainty, it was included in the eligible papers for full-text evaluation. The articles were then assessed for eligibility through a full-text screening and those meeting the established criteria were included in the review. The reference lists of the 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 the reasons for their exclusion were recorded and codified. Each selected article was reviewed for information on the (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, and condition); (5) BIA characteristics (device employed, extrapolated frequency, electrode arrangement, brand of the electrodes used, and their localization); and (6) results, conclusions, and reported limitations. Furthermore, the nomenclature of each study was respected according to their original articles. All this information was organized and compiled into tables.

Data Extraction and Prioritization

Full texts were reviewed for the following variables: bioelectrical resistance (R , R/h), reactance (X_c , X_c/h), and the PhA of the muscle selected, as well as the electrode placement to assess bioelectrical values. Bioelectrical measures and directly derived parameters were considered the main outcome, independently of study design. Only data at a frequency of 50 kHz from healthy subjects, subjects with muscle injury, or subjects with primary sarcopenia were analyzed. Bioimpedance analyses different from the muscle-localized one were also obviated.

Risk of Bias Assessment of the Studies Included

The quality of all the included studies was assessed with the AQUA tool (Henry et al., 2017). This tool is designed to assess the quality and reliability of studies by addressing the risk of bias as "high," "low," or "unclear," using five key domains: (1) characterization of the study target and subject; (2) design of the research; (3) characteristics of the methodology; (4) descriptive anatomy; and (5) reporting of outcomes. If a study did not meet the recommended guidelines ascribed to each domain, it

was classified as being at "high" risk of bias within the confines of that specific category. If the domain met the recommended guidelines, it was marked as having a "low" risk of bias. "Unclear" was used in the cases of insufficient or vague data. Two of the authors (ÁC-P and GS-L) screened the articles and assessed the risk of bias for each of the five domains. Disagreement was resolved by consensus or third-party adjudication (AI).

RESULTS

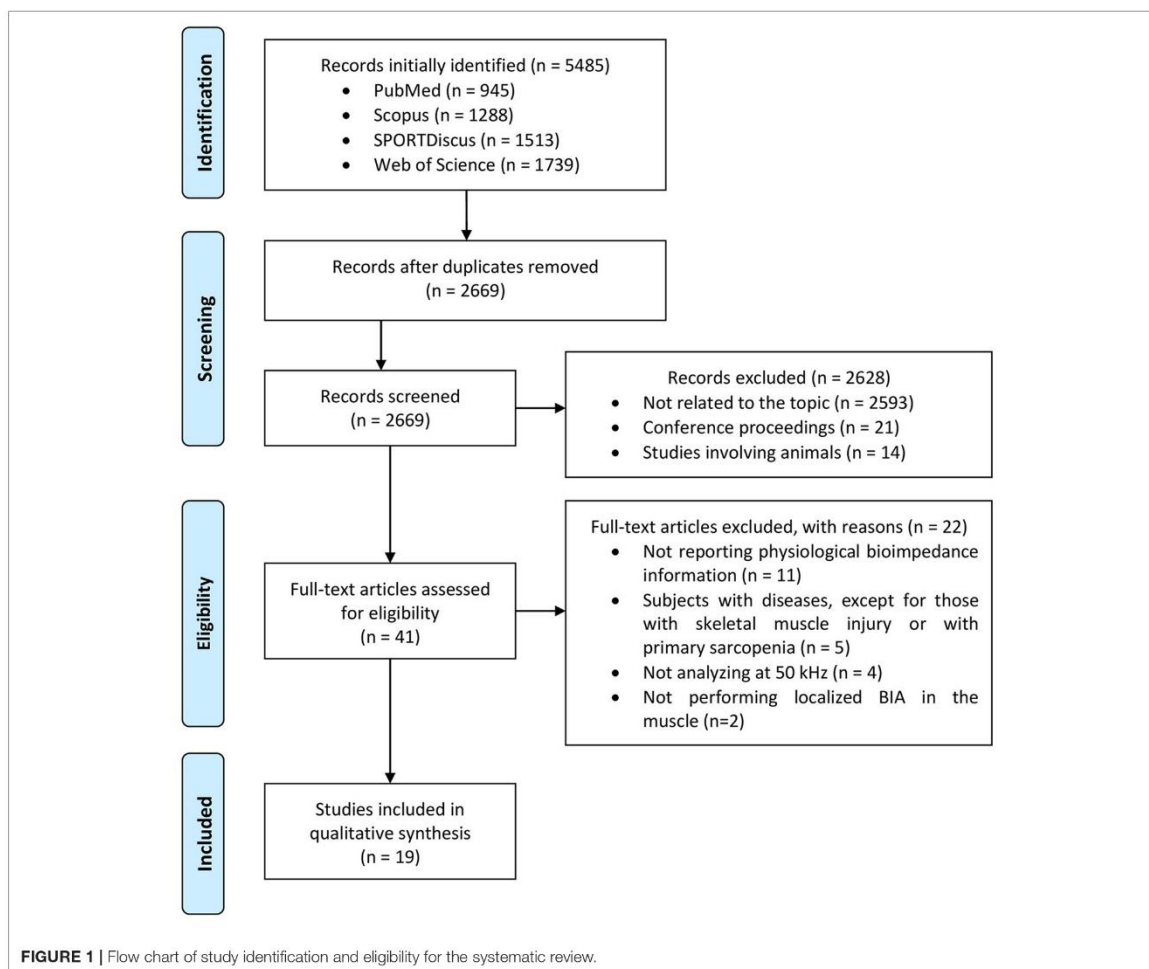
Results of the Search

Figure 1 displays the flow chart of study identification and eligibility for the systematic review. The search term provided 5,485 results, which were reduced to 2,669 after the removal of duplicates. Of the 2,669 records identified, 41 were selected after screening titles, abstracts and keywords, discarding those not related to the topic such as studies on a topic with little or no relationship at all, those not using localized bioimpedance, those involving subjects that are not healthy, or a combination of all of them. Conference proceedings and studies involving animals were also excluded. After full-text evaluation, 19 studies matched the selection criteria and were included in the qualitative synthesis analysis. These are summarized in Tables 1–3. The other 22 studies were excluded for different reasons: not reporting physiological bioimpedance information and instead focusing on technological or methodological aspects, not analyzing at 50 kHz, using subjects with diseases, or not performing localized BIA. An important aspect is that the inclusion criteria included studies on healthy subjects, those with skeletal muscle injury and those with primary sarcopenia. The publication date ranged from 1990 to 2020, but only one study was published before 2003 (Elleby et al., 1990) and only five before 2013. Therefore, the interest in this field of study has increased in the last decade, unlike the studies on neuromuscular diseases that have been studied for longer.

Participants and Characteristics of the Studies

A total number of 607 subjects were involved in the studies included in the review, in addition to the unspecified number of participants in the study of Nescolarde et al. (2015), who only indicated 21 muscle injuries. Seven studies were carried out with elite soccer athletes, of which two involved non-injured subjects (Mascherini et al., 2015, 2017) and five involved subjects with skeletal muscle injury (Nescolarde et al., 2013, 2015, 2017, 2020; Francavilla et al., 2015). Despite all the studies including healthy subjects, one of them compared their data with those of a preliminary study in patients with neuromuscular disease in one section of the article (Shiffman et al., 2003), while another included healthy subjects alongside data from healthy control subjects of a previous study focused on neuromuscular disorders, excluding those with disorders (Aaron et al., 2006). No age restrictions were made. All the studies analyzed males and thirteen of them also included females.

The 19 selected studies were classified according to the characteristics of the study objectives. Table 1 is composed of six studies characterizing muscle values according to age (Aaron



et al., 2006; Rutkove et al., 2008; de-Mateo-Silleras et al., 2018), gender (Mascherini et al., 2017), or subcutaneous fat layer (SFL) thickness (Tarulli et al., 2007; Sung et al., 2013). **Table 2** includes another five studies reporting changes in EIM parameters during injury and recovery in athletes with muscle injuries (Nescolarde et al., 2013, 2015, 2017, 2020; Francavilla et al., 2015). The combination of these two tables may be used to establish some interpretations regarding bioimpedance parameters and muscle health. **Table 3** consists of eight studies focusing on changes in EIM values during or after an exercise protocol, with five investigating short-term adaptations (Shiffman et al., 2003; Li L. et al., 2016; Fu and Freeborn, 2018; Freeborn and Fu, 2019; Huang et al., 2020), two studying medium-term changes (Elleby et al., 1990; Freeborn et al., 2020), and one looking at long-term changes (Mascherini et al., 2015). It is worth mentioning that the variability between the studies is large, which makes it difficult to compare them to obtain clear results. For this reason, the

studies were classified in such a way that they were as similar as possible.

Bioelectrical Measures

All the studies performed localized bioimpedance measurements. However, three of them also included whole-body electrode distribution (Mascherini et al., 2015, 2017; de-Mateo-Silleras et al., 2018). Thirteen studies analyzed the muscles of the lower body and seven studies assessed the muscles of the upper body. All the studies involving subjects with muscle injuries performed the assessments in the lower body. Thirteen investigations used single-frequency impedance devices (50 kHz). The other six studies used multi-frequency bioimpedance analyzers, but also focusing on 50 kHz (Sung et al., 2013; Li L. et al., 2016; Fu and Freeborn, 2018; Freeborn and Fu, 2019; Freeborn et al., 2020; Huang et al., 2020). Furthermore, two of these studies used a

TABLE 1 | Reviewed EIM studies on the bioelectrical characterization of muscle.

Study	Aim	Design	Type of analysis	Subjects			BIA			Electrodes used	Electrode arrangement	Results	Conclusions	Reported limitations		
				N	Sex	Age	Condition	Device	Fr						Distribution	Localized muscle
Aaron et al. (2006)	To analyze EIM values in people of different ages	Cross-sectional plus longitudinal measurements up to 4.5 years after the initial assessment of 4 subjects	Intra-group analysis. Comparison between a preliminary study of patients with various ND	100	56 F; 44 M	18–90	Healthy subjects, with data on some of them obtained from a preliminary study	R/L Model 101-A	50 kHz	Localized	Quadriceps, tibialis anterior	019-400500; Viasonics Healthcare/ Nicolet Biomedical	Quadriceps: the initial electrode (V1) was placed 10 cm proximal to the patella with V2–V8 placed 2.5 cm apart in a proximal line along the thigh, anterior; Tibialis anterior: V6 was placed 2.5 cm distal to the midpoint between the bottom of the patella and the fibular head, with V5–V1 placed 2.5 cm apart in a line down the belly of the muscle along the anterior foreleg	PhA decreased with increasing age, especially after 60 years of age. The correlation was stronger in M and effort-independent ($r^2 = 0.68$ for M, 0.52 for F; tibialis anterior: $r^2 = 0.74$ for M, 0.38 for F; $p < 0.001$ throughout)	Greater reductions in the PhA with increasing age. EIM has potential and is a simple and effort-independent test of muscle health in the elderly	NR
Tarulli et al. (2007)	To determine the impact of SFL thickness on EIM	Cross-sectional	Intra-group and inter-group analysis. Comparison between a wide variety of SFL thicknesses measured by ultrasound	62	29 F; 33 M	52.2 ± 20.6	Healthy	R/L systems BIA-101A	50 kHz	Localized	Quadriceps	019-768500, Viasonics Healthcare/ Nicolet Biomedical and 019-435500, Viasonics Healthcare/ Nicolet Biomedical	I pair: attached to the dorsum of the feet. V pair: over the quadriceps in a standard array with 2.5 cm between the electrodes. Starting from 10 cm proximal to the patella	A significant but weak positive correlation ($r^2 = 0.14$, $p < 0.05$) was seen between age and SFL thickness and SFL thickness in F, but not in M. A strong negative correlation between age and PhA was observed for both M ($r^2 = 0.48$, $p < 0.01$) and F ($r^2 = 0.68$, $p < 0.01$). In multiple regression analysis, age but not SFL thickness was found to have a significant association with PhA	SFL did not contribute substantially to the PhA measured by linear-EIM healthy subjects were analyzed	Some unavoidable inaccuracy in measurements of the SFL thickness with ultrasound. Only healthy subjects were analyzed

(Continued)

TABLE 1 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA			Electrode arrangement	Results	Conclusions	Reported limitations		
				N	Sex	Age	Condition	Device	Fr					Distribution	Localized muscle
Rutkove et al. (2008)	To create a reference set of EIM values in healthy subjects	Cross-sectional	Inter-group analysis. Establishment of reference values	87	46 F; 41 M	52.4 (18.9–90.8)	Healthy	RJL Model 101-A	50 kHz	Localized	Biceps brachii, forearm flexors, quadriceps, tibialis anterior, medial gastrocnemius	For each muscle, the V pair was placed along the long axis of the limb, and the I pair was placed at a distance from tibialis anterior upper extremity muscle measurements, the I pair was placed on the palms of both hands, and for each lower extremity muscle they were placed on the dorsum of both feet	The reference PhA values for 20, 30, 40, 50, 60, 70 and 80 years, respectively, were: methodology	PhA decreased with age. Results could be altered depending on measurement methodology	NR
Sung et al. (2013)	To identify the EIM parameter least impacted by SFL thickness when using an HEA	Cross-sectional	Inter-group analysis. Comparison between a variety of skin-SFL thicknesses measured by ultrasound	18	9 F; 9 M	50.7 (19–83)	Healthy	Imp SFB7 (HEA)	50 kHz	Localized	Medial gastrocnemius	Inner electrodes were 3 cm apart and the outer electrodes were 1.5 cm peripheral to that over the center of the muscle belly	For both R and PhA, a strong relationship with subcutaneous fat layer thickness was observed ($r^2 = 0.64$ and $r^2 = 0.70$, respectively; $p < 0.001$ for both). By contrast, for Xc, the relationship was not significant ($r^2 = 0.07$, $p = 0.30$)	Unlike R and PhA, Xc was smaller in magnitude than R, thus potentially making it a noisier measure, which could have contributed to the lack of a significant relationship with SFL thickness. Possible error measuring SFL thickness	

TABLE 1 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA		Results	Conclusions	Reported limitations					
				N	Sex	Age	Condition	Device				Fr	Distribution	Localized muscle	Electrodes used	Electrode arrangement
Mascherini et al. (2017)	To compare body composition between sexes using L-BIA in elite soccer athletes	Cross-sectional	Intra-group and inter-group analysis. Comparison between sexes	36	18 F; 18 M	F: 26.2 ± 2.4; M: 26.9 ± 2.5	Healthy elite soccer athletes	BIA-101 ASE, Akern/RLL Systems	50 kHz	Whole-body and localized	Quadriceps, hamstrings, and calves	Ag/AgCl (NR)	Quadriceps: 5 and 10 cm distally from the anterior inferior iliac spine and proximally from the superior pole of the patella. $p = 0.0001$; (b) F reversed for the Xc Hamstring: 5 and 10 cm distally from the ischiatric tuberosity and proximal from the popliteal line. Calves: 5 and 10 cm distally from the popliteal line and 15 and 10 cm from the posterior intermalleolar line	Significant differences between (a) F vs. M quadriceps, hamstrings, and calves, these differences were reversed for the Xc values vs. calves ($p = 0.0001$); (c) F and M hamstrings vs. calves ($p = 0.001$); and (d) F quadriceps vs. hamstrings ($p = 0.02$). There were no significant differences between the quadriceps and hamstrings in the M sample ($p = 0.57$)	Significant differences in the thighs between the sexes, while in the calves, these differences were reversed for the Xc values	Absence of BIA data for F athletes
de-Mateo-Silleras et al. (2018)	To assess the association between whole-body and calf impedance vectors and muscle mass and strength in a group of elderly individuals	Cross-sectional	Intra-group analysis. Comparison between muscle health, whole-body BVA and localized BVA	113	59 F; 53 M	79.8	All types of elderly people	BIA-101 ASE, Akern/RLL Systems	50 kHz	Whole-body and localized	Calves	NR	Two measuring (ES1 and ES2) and two injecting electrodes (E1 and E2) were placed on the lateral side of the right leg. The ES1 electrode was placed at the maximum circumference of the calf; ES2 was placed 10 cm distal to ES1. Injecting electrode E1 was placed with R ES1, and E2 was placed 5 cm distal to ES2	Possible associations were analyzed between BIA values obtained using whole-body BVA and those from localized BVA in the assessment of body composition in the elderly	Localized BVA of NR	NR

N, number of subjects; BIA, bioelectrical impedance analysis; BIVA, bioelectrical impedance vector analysis; EIM, electrical impedance myography; F, female; Fr, frequency; HEA, handheld electrode array; I, current injection electrodes; L-BIA, localized bioelectrical impedance analysis; M, males; NR, not reported; PhA, phase angle; R, resistance; SFL, subcutaneous fat layer; V, voltage sensing electrodes; Xc, reactance.

TABLE 2 | EIM studies analyzing skeletal muscle injuries in elite soccer athletes.

Study	Aim	Design	Type of analysis	Subjects			BIA			Results	Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr				Distribution	Localized muscle	Electrodes used	Electrode arrangement
Nescolarde et al. (2013)	To report the effects of the severity of muscle injury and recovery in L-BIA variables	Longitudinal. From injury until RTP	Intra-individual analysis. Comparison with MRI results as gold-standard	3	M	21 ± 5	Injured elite soccer athletes (hamstrings and calves)	BIA-101 ASE, AkernRLL Systems	50 kHz	Localized	Hamstrings and calves	3MRed DotAdult Solid Gel 2239	Calf: 5 cm (I pair) and 10 cm (V pair) distally from the popliteal line and proximal from the posterior intermalleolar line, grade I (11.9, 23.5, and 12.1%); Xc and PhA hamstrings: 5 cm (V pair) proximally and distally to the point of maximum pain, the I pair adjacent to the others	Compared to non-injury values, R, Xc, and PhA decreased with increasing muscle injury severity: fluid accumulation, grade I (11.9, 23.5, and 12.1%); Xc and PhA hamstrings: 5 cm (V pair) proximally and distally to the point of maximum pain, the I pair adjacent to the others	R, Xc, and PhA decreased with injury severity. Decreases in R reflected localized muscle injury severity, while reductions in fluid accumulation, grade I (11.9, 23.5, and 12.1%); Xc and PhA hamstrings: 5 cm (V pair) proximally and distally to the point of maximum pain, the I pair adjacent to the others	Excessive inter-observer variability
Francavilla et al. (2015)	To report L-BIA changes during the course of muscle injury healing	Longitudinal. From injury until RTP	Intra-individual analysis. Comparison between different measurements after injury with baseline values pre-injury	1	M	24	Injured elite soccer athlete (long head biceps of the leg, grade II)	BIA-101 ASE, AkernRLL Systems	50 kHz	Localized	Hamstring	Biartodes, Akern	I pair over the third metatarsal of both feet and the V pair over the muscle	Marked decrease in R, Xc, and PhA over the 12 days following the injury, especially on the 4th day. R: -11.2%; Xc: -52.9%; PhA: -46.5%	R, Xc, and PhA decreased during injury and returned to baseline levels once the injury healed. Decrease in R reflected localized fluid accumulation, while the decrease in Xc and PhA reflected a disruption in cell membrane integrity and cell density during healing	The daily activities of the player posed numerous limits on the routine use of L-BIA

(Continued)

TABLE 2 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA		Results		Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr	Distribution			Localized muscle	Electrodes used	Electrode arrangement	
Nescolarde et al. (2015)	To identify the pattern of change in L-BIA values according to injury severity	Cross-sectional. Measurements made 24 h after injury	Intra-individual and intra-group analysis. Comparison with MRI results as gold-standard	21	M	NR	Injured elite soccer athletes (hamstrings and calves)	BIA-101 ASE, AxernRJL Systems	50 kHz	Localized	Quadriceps, hamstrings, and adductors	Ag/AgCl (COVIDEN Ref. 31050522)	V pair: 5 cm proximally and distally from the center of the injury. I pair: close to the others. In the vastus intermedius injury (10.4, 17.5, and 9.0%), showing a pattern (1 case), V pair: grade II (18.4, 32.9, and 16.6%), severity of the and distally from the center of the injury. I pair: close to the others. In the adductors, the electrodes were placed in a transverse position, placing the V pair 5 cm medially and laterally from the center of the injury and the I pair close to the others	Compared to non-injury values, R, Xc, and PhA decreased with increasing injury severity. The most significant change was evidenced in Xc 24 h after injury, showing a pattern in line with the grade II (18.4, 32.9, and 16.6%), severity of the and distally from the center of the injury. I pair: close to the others. In the adductors, the electrodes were placed in a transverse position, placing the V pair 5 cm medially and laterally from the center of the injury and the I pair close to the others	R, Xc, and PhA decreased with increasing injury severity. The most significant change was evidenced in Xc 24 h after injury, showing a pattern in line with the grade II (18.4, 32.9, and 16.6%), severity of the and distally from the center of the injury. I pair: close to the others. In the adductors, the electrodes were placed in a transverse position, placing the V pair 5 cm medially and laterally from the center of the injury and the I pair close to the others	NR
				11												
Nescolarde et al. (2017)	To correlate injury severity through L-BIA parameters	Longitudinal. Measurements made 24 h after injury and the day of RTP	Intra-individual and intra-group analysis. Comparison with MRI results as gold-standard	18	M	20-28	Injured elite soccer athletes (hamstrings and calves)	BIA-101 ASE, AxernRJL Systems	50 kHz	Localized	Quadriceps, hamstrings, adductors, and calves	Ag/AgCl (COVIDEN Ref. 31050522)	V pair: 5 cm proximally and distally from the center of the injury. I pair: close to the others. In the vastus intermedius, the pair was placed 10 cm proximally and distally to the center of the injury and the I pair close to the others	Compared to non-injury values, R, Xc, and PhA decreased with increasing injury severity. There were significant differences in R, grade I (10.2, 13.4, and 3.2%), between grade I and grade II with no gap (12.8, 23.5, and 11.2%), and between grade II and grade I with gap and 20.5%),	After grouping the data according to the muscle gap (by MRI examination), there were significant differences in R, between grade I and grade II with gap, as well as between grade II and grade I with gap and grade II with no gap	NR
				22												

(Continued)

TABLE 2 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA		Results	Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device				Fr	Distribution	Localized muscle	Electrodes used
Nescolarde et al. (2020)	To evaluate by L-BIA different muscle and tendinous injuries 24 h after injury and at their return to play	Cross-sectional. 24 h after injury	Intra-individual and intra-group analysis. Comparison with MRI results as gold-standard	32 (37 muscle injuries)	M	23.5 ± 1.5	Injured elite soccer athletes (hamstrings and calves)	BIA-101 ASE, AkernFULL Systems (P-S)	50 kHz	Localized	NR	Ag/AgCl (COVIDIEN Ref. 31050522)	V pair: 5 cm proximally and distally from the center of the injury. Xc and PhA in I pair: close to the both MTJ and others. In injuries, MFJ as well as in the different located near the bone detector, the grades of MTJ V pair was placed 10 cm proximally and distally from the center of the injury, and the I pair close to the others	Significant decrease ($p < 0.01$) in R _i , center of the injury. Xc and PhA in I pair: close to the both MTJ and others. In injuries, MFJ as well as in the different located near the bone detector, the grades of MTJ V pair was placed 10 cm proximally and distally from the center of the injury, and the I pair close to the others	L-BIA was a complementary method to MRI, the study should have extended to other professional football teams to increase the sample size and also should have included female athletes

N, number of subjects; BIA, bioelectrical impedance analysis; EIM, electrical impedance myography; Fr, frequency; I, current injection electrodes; L-BIA, localized bioelectrical impedance analysis; M, males; MFJ, myofascial junction; MRI, magnetic resonance imaging; MTJ, myotendinous junction; NR, not reported; PhA, phase angle; R_i, resistance; RTP, return to play; V, voltage sensing electrodes; Xc, reactance.

TABLE 3 | EIM studies analyzing adaptations to physical exercise.

Study	Aim	Design	Type of analysis	Subjects			BIA			Results		Conclusions	Reported limitations
				N	Sex	Age	Condition	Device	Fr	Distribution	Localized muscle		
Elleby et al. (1990)	To analyze if localized impedance measurements could be used to show muscle changes following prolonged intensive exercise	Longitudinal. Medium-term adaptations. Pre-, intra-, post-race (21 km)	Intra-individual analysis. Comparison between measurements that were made for 5 days before the race, immediately after the race, 2 and 6 h after the race, and then daily for 5 days	4	1 F; 3 M	27–47	Healthy	NR	50 kHz	Localized	Calf and thigh	NR	NR
Shiffman et al. (2003)	To perform EIM during an isometric contraction	Longitudinal. Short-term adaptations	Intra-individual and inter-group analysis. Comparison with a preliminary study of patients with various ND	6	3 F; 3 M	19–70	Healthy individuals plus subjects with disorders obtained from a preliminary study	R/L systems with BIA-101A	50 kHz	Localized	Anterior forearm	NR	NR

(Continued)

TABLE 3 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA		Results		Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr	Distribution			Localized muscle	Electrodes used	Electrode arrangement	
Mascherini et al. (2015)	To report changes in localized BIVA after performing a soccer training program	Longitudinal. Long-term adaptations. 50 days between measurements	Intra-group analysis. Comparison between values from pre-season and from the first day of the championships	59	M	22.5 ± 5.6	Healthy elite soccer athlete	BIA-101 ASE, Akern/VLL Systems	50 kHz	Whole-body and localized	Quadriceps, hamstrings and calves	Biartodes, Akern	Quadriceps: 5 and 10 cm distally from the anterior inferior iliac spine and proximally from the superior pole of the patella. 15.96 Ω/m; Hamstrings: 5 quadriceps and 10 cm distally from the ischialc tuberosity and proximal from the popliteal line. Calves: 5 and 10 cm distally from the popliteal line and 15 and 10 cm from the posterior intermalleolar line	Values of R/L decreased significantly (hamstrings, 118.01 ± 19.63 vs. 114.49 ± 15.96 Ω/m; quadriceps, 120.80 ± 16.84 vs. 114.71 ± 15.79 Ω/m; calf, 209.57 ± 27.67 vs. 199.72 ± 25.58 Ω/m). There was a greater reduction of R/L in the quadriceps than the hamstrings. This was reflected in an increase in PhA in the quadriceps (13.11 ± 2.13 vs. 13.08 ± 2.25°). However, the greatest muscle modifications were in the calves. Indeed, Xc/L also decreased significantly (50.57 ± 7.39 vs. 48.51 ± 6.97 Ω/m)	The fat did not undergo quantitative changes, but only a change in distribution. Lower limb muscles showed an increase in the amount of water that was more significant than in the whole body assessment. The assessment of body segments under more stress by physical training provided more specific information than the whole body assessment in athletes	Data not valid for non-elite athletes

(Continued)

TABLE 3 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA			Results	Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr				Distribution	Localized muscle	Electrodes used	Electrode arrangement
L.J.L. et al. (2016)	To assess changes in EIM at different levels of MVC	Longitudinal. Short-term adaptations	Intra-individual and inter-group analysis. Comparison between different levels of MVC	19	11 F; 8 M	35 ± 10	Healthy	EIM103 System, Skulpt (HEA)	50, 100kHz	Localized	Biceps brachii	HEA	Longitudinal with the muscle fibers over the center of the muscle belly	R increased 10.1 and 9.2% at the higher contractions (60% MVC and MVC). Significant differences in R between the lower contraction (20% MVC) and the fatigue task (higher contraction and there were significant levels (6.5% increase at 60% MVC and 4.6% increase at MVC). No significant changes in R between rest and lower contraction level (20% MVC). In contrast to R, there were no significant alterations in muscle changes in Xc during isometric contraction. For the fatigue effects, R changed significantly during the four different testing conditions	R increased 10.1 and 9.2% at the higher contractions (60% MVC and MVC). Significant differences in R between the lower contraction (20% MVC) and the fatigue task (higher contraction and there were significant levels (6.5% increase at 60% MVC and 4.6% increase at MVC). No significant changes in R between rest and lower contraction level (20% MVC). These changes might be related to the modest alterations in muscle architecture during a contraction the fatigue effects, R changed significantly during the four different testing conditions	NR
Fu and Freeborn (2018)	To investigate changes in EIM during a fatiguing isotonic protocol	Longitudinal. Short-term adaptations	Intra-individual, inter-group and inter-group analysis. Comparison between two different protocols of bicep curl repetitions at either 60 or 75% 1RM until failure	18	3 F; 15 M	22.2 ± 3.2	Healthy	Keysight E4900A	10, 50, and 100kHz	Localized	Biceps brachii	Au/AgCl (Kendall 133)	I pair on the lateral side of the bicep, 14 cm apart, with the V pair placed 4.67 cm apart	There were significant differences ($p < 0.05$) between the pre- and post-fatigue R and Xc within groups due to the exercise protocol. There were no significant differences ($p < 0.05$) in the R and Xc between the groups at different intensities and no injury differences ($p < 0.05$) of changes between the left and right biceps in both groups	The level of exercise intensity did not significantly affect the changes in electrical impedance of the biceps tissue between the two intensity groups, which is important in monitoring changes as a R and Xc between result of fatigue the groups at different exercise-related intensities and no injury differences ($p < 0.05$) of changes between the left and right biceps in both groups	May be influenced by the subjective reporting of fatigue

(Continued)

(Continued)

Study	Aim	Design	Type of analysis	Subjects			BIA			Results	Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr				Distribution	Localized muscle	Electrodes used	Electrode arrangement
Freeborn and Fu (2019)	To investigate changes throughout a fatiguing exercise protocol	Longitudinal, Short-term adaptations	Intra-individual, intra-group and inter-group analysis. Comparison between two different protocols of 10 sets of bicep curl repetitions at either 60 or 75% 1RM	17	3F; 14M	22.2 ± 3.2	Healthy	Keysight E4990A	10, 50, and 100 kHz	Localized	Biceps brachii (Kendall 133)	Ag/AgCl	I pair on the lateral side of the bicep, 14 cm apart, with the V pair placed 4.67 cm apart	At 50 kHz, R decreased 5.6 and 6.6% in the 60% 1RM group, and 5.8 and 6.3% in the 75% 1RM group. Similar trends were observed at 50 and 100 kHz for Xc and PhA. These results indicated that the electrical impedance of tissues may be an effective monitor in quantifying the changes that occur in localized tissues, including muscle swelling and damage	Significant decrease in R, Xc, and PhA compared to the pre-fatigue measures, with the significant R changes occurring at earlier timepoints than Xc and PhA. These results indicated that the electrical impedance of tissues may be an effective monitor in quantifying the changes that occur in localized tissues, including muscle swelling and damage	Not directly measuring the swelling, important in quantifying the relationship with R
				6	5F; 1M	20–25	Healthy	Keysight E4990A	10, 50, and 100 kHz	Localized	Biceps brachii (Kendall 133)	Ag/AgCl	I pair over the biceps brachii 15 cm apart with the V pair placed 6 cm apart. Largest circumference and 96h post-exercise radius of the biceps was the midpoint	Statistically significant differences between the pre-exercise and 72-h ($p < 0.001$) and 96h post-exercise ($p < 0.001$) R-values at 50 kHz. Significant differences occurring at the same pre-exercise and 72-h ($p < 0.001$) and 96-h post-exercise that EIM is sensitive to the changes within the muscle with the potential to quantify exercise-induced changes	The self-reported pain was only collected for the exercised bicep. No direct measure of tissue damage was obtained	

TABLE 3 | Continued

Study	Aim	Design	Type of analysis	Subjects			BIA			Results	Conclusions	Reported limitations				
				N	Sex	Age	Condition	Device	Fr				Distribution	Localized muscle	Electrodes used	Electrode arrangement
Huang et al. (2020)	To evaluate local muscle adaptations fatigue status via changes in EIM	Short-term	Intra-individual analysis. Comparison between dynamic contraction EIM values at different intensities	8	4 F; 4 M	NR	Healthy	Rigol DG4162 signal generator; Agilent 1141A differential probe, and Agilent MSO7054A oscilloscope	1 kHz—1 MHz; Focus on 50 kHz	Localized	Biceps brachii	Physiotherapy electrodes, 40 × 10 mm-Shanghai Litu Medical Equipment Co., Ltd	Symmetrically along the direction of the biceps brachii muscle belly as the center. Electrode configuration optimized via a finite element model	R decreased by almost 8 Ω from the completely relaxed muscle to semi-fatigue point exhaustion, which was the same trend as that for 4 Ω. With the decrease in R, the median frequency approaching 8 Ω, muscle fatigue reached its limit, surface electromyography decreasing more quickly with heavier loads. Relationship with a decrease of the median frequency obtained via sEMG	Subjects were considered to reach the relaxed muscle to semi-fatigue point exhaustion, which was the same trend as that for 4 Ω. With the decrease in R, the median frequency approaching 8 Ω, muscle fatigue reached its limit, surface electromyography decreasing more quickly with heavier loads. Relationship with a decrease of the median frequency obtained via sEMG	Only evaluated R, not Xc nor PhA

N, number of subjects; BIA, bioelectrical impedance analysis; BIVA, bioelectrical impedance vector analysis; BMI, body mass index; EIM, electrical impedance myography; F, female; Fr, frequency; HEA, handheld electrode array; I, current injection electrodes; L, length; M, males; MVIC, maximum voluntary isometric contraction; ND, neuromuscular diseases; NR, not reported; PhA, phase angle; R, resistance; RM, repetition maximum; sEMG, surface electromyography; V, voltage sensing electrodes; Xc, reactance; ρ , resistivity.

handheld electrode array (HEA) to measure bioelectrical values (Sung et al., 2013; Li L. et al., 2016).

Quality Assessment of the Studies Included

A quality assessment of the studies is presented in **Table 4** and **Figure 2**. The majority of the studies included in this review presented a “high” risk of bias in three domains. The high risk of bias in the objectives and study characteristics domain was mainly due to the small sample size. To determine the risk in this domain, we first focused on whether the authors carried out a study with the ideal sample size and if they complied with it. If not, we established a size of at least 30 subjects for the study to be considered as having a “low” risk of bias, since this sample size is considered large enough to assume normality in the absence of a power analysis (Pandis, 2015). The second domain, study design, also presented a “high” risk of bias in more than half of the studies due to the differences in the placement of the electrodes, since we considered that the appropriate way to perform EIM is through 4 electrodes placed correctly on a specific muscle as this is the most common electrode configuration (Rutkove, 2009). Any other method, such as an HEA or linear-EIM (array of voltage electrodes), was considered as having a “high” risk of bias when establishing appropriate comparisons using the same methodology. The methodology characterization domain was the most affected by a “high” risk of bias, since there was almost no information on the measures taken to reduce inter- and intra-observer variability. The fourth and fifth domains presented a clear “low” risk of bias as the anatomical descriptions of the muscles measured were appropriate, as well as the statistical results. It should be noted that there has been an improvement in the quality of the studies over the years.

DISCUSSION

Despite EIM being mainly applied in the evaluation of neuromuscular disorders, we discuss its other applications of interest related to health and physical exercise in this review.

We consider that this technique still has much scope for improvement for use as a reference. However, what is undeniable is that it does have the capacity to assess muscle health, providing a quantitative index of the muscle condition (Rutkove and Sanchez, 2019). However, it is still not clear how to interpret the results in all the cases. The great advantages that this technology presents over others make it worthwhile to continue researching so that it can be accessible to everyone.

Muscle Characterization

The impedance values of the muscle may change according to intrinsic factors such as age, gender or the skin-SFL thickness.

Aaron et al. (2006) found a reduction in PhA values with increasing age, which declined more sharply after 60 years. Despite these changes being significant for both genders, the correlation between the decreased PhA and age was more remarkable in men (quadriceps: $r^2 = 0.68$ for men and 0.52 for women; tibialis anterior: $r^2 = 0.74$ for men and 0.38 for women; $p < 0.001$). In the same study, four subjects aged over 75 years

TABLE 4 | Quality assessment of the included studies with the AQUA tool.

Studies	Risk of bias				
	Objective(s) and study characteristics	Study design	Methodology characterization	Descriptive anatomy	Reporting of results
Elleby et al. (1990)	High	High	High	High	High
Shiffman et al. (2003)	High	High	Low	Low	Unclear
Aaron et al. (2006)	Low	High	High	Low	Low
Tarulli et al. (2007)	Low	High	High	Low	Low
Rutkove et al. (2008)	Low	High	High	Low	Low
Nescolarde et al. (2013)	High	Low	High	Low	Low
Sung et al. (2013)	High	High	High	Low	Low
Francavilla et al. (2015)	High	High	High	Low	Low
Mascherini et al. (2015)	Low	Low	High	Low	Low
Nescolarde et al. (2015)	High	Low	Low	Low	Low
Li L. et al. (2016)	High	High	High	Low	Low
Mascherini et al. (2017)	Low	High	High	Low	Low
Nescolarde et al. (2017)	High	Low	Low	Low	Low
de-Mateo-Silleras et al. (2018)	Low	Low	High	Low	High
Fu and Freeborn (2018)	High	Low	Low	Low	Low
Freeborn and Fu (2019)	High	Low	High	Low	Low
Freeborn et al. (2020)	High	Low	Unclear	Low	Low
Huang et al. (2020)	High	Low	High	Low	Low
Nescolarde et al. (2020)	Low	Low	Low	Unclear	Low

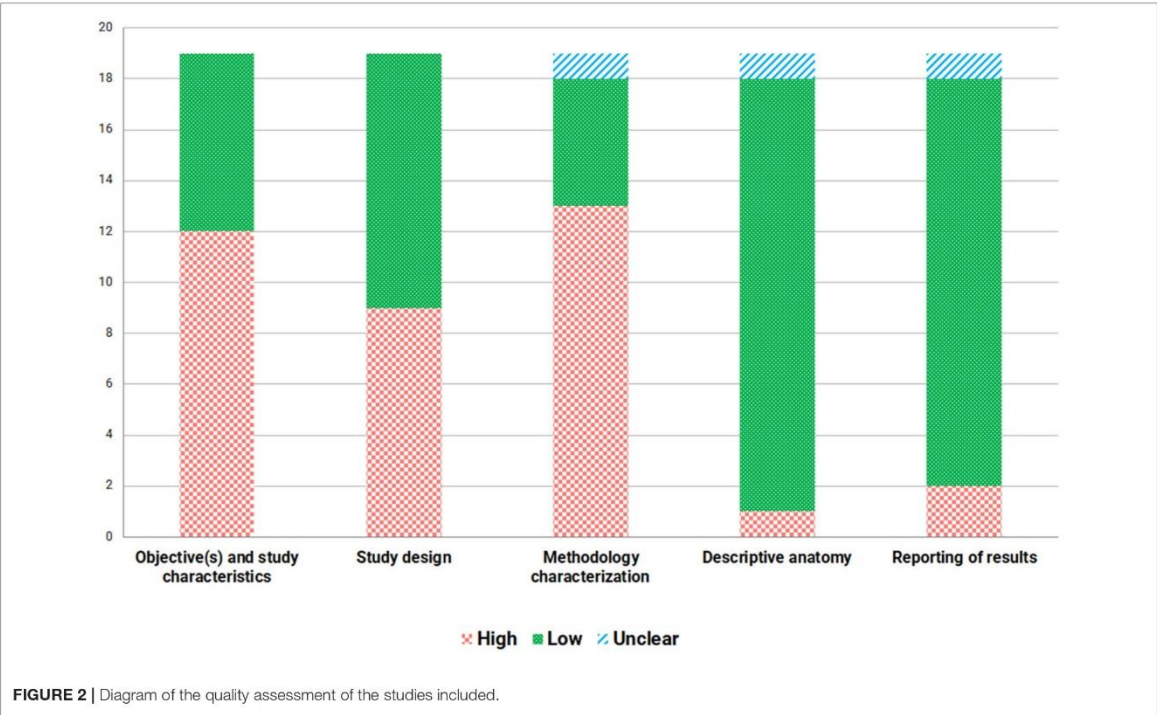


FIGURE 2 | Diagram of the quality assessment of the studies included.

repeated the test over a minimum of 3 years. Although the decline was not linear, the slope was between -0.3 and -0.6° per year, exhibiting the age dependence of the PhA. This is an important factor for EIM to measure inherent muscle qualities, avoiding any difficulties associated with contraction and central activation, which is especially of interest in studies on the elderly. It should be noted that the study used a linear electrode array arrangement instead of the classic tetrapolar electrode placement. However, this method also presents excellent test-retest reproducibility (Rutkove et al., 2006). The study did not report the R and Xc values.

Rutkove et al. (2008), to create a set of normal reference values for the PhA of different muscles (the biceps brachii, forearm flexors, quadriceps, tibialis anterior, and medial gastrocnemius), analyzed 87 healthy subjects aged 18.9 to 90.8 years. Again, a clear relationship was found between the PhA and age, with the PhA decreasing with increasing age in all five groups of muscles. The decrease of the PhA seemed to be more pronounced in the muscles of the lower extremities, which is consistent with the fact that loss of muscle mass and strength due to age (primary sarcopenia) occurs especially in the lower body (Janssen et al., 2000). The R and Xc data were not provided. Regarding the electrode arrangement, a pair of voltage electrodes was placed along the long axis of the limb of the specific muscle, while a pair of current electrodes was placed on the dorsum of the foot or palm of the hand regardless of whether the muscle was of the upper or lower body. Therefore, not all the electrodes were located over the muscles of interest.

Janssen et al. (2002) and de-Mateo-Silleras et al. (2018) established a relationship with tolerance ellipses between body composition (through whole-body BIA) and loss of muscle mass and strength in elderly subjects. Localized bioelectrical impedance vector analysis (BIVA) of the calf muscles only distinguishes subjects with loss of muscle mass. Therefore, by using whole-body BIA, de-Mateo-Silleras et al. (2018) could not correlate with a reduced maximum grip strength, which is considered a useful marker in monitoring functional autonomy and the risk of falls (Hairi et al., 2010; Sallinen et al., 2010). Localized BIVA might be an alternative to BIA/BIVA in the cases where the latter techniques cannot be performed for some reason (e.g., prosthesis, pacemakers, individuals with edemas, or dehydration).

Regarding gender, Mascherini et al. (2017) showed significant differences in the lower limbs between athletes of the same age and competitive level through localized BIVA. Males presented significantly lower R-values than females in the quadriceps (107.6 ± 11.9 vs. 134.4 ± 15.0 , $p = 0.0001$), hamstrings (104.9 ± 11.4 vs. 118.8 ± 16.4 , $p = 0.006$), and calves (182.1 ± 18.2 vs. 225.9 ± 27.7 , $p = 0.0001$). They also showed significantly higher Xc and PhA values in all three muscles, except for the calves, which were higher in females.

As previously mentioned, the presence of fat is a major aspect to consider when measuring impedance parameters. Baidya and Ahad (2016) observed reductions in the R and Xc values with increasing muscle thickness, and increases in the R and Xc values with increasing SFL thickness. Fat tends to increase with increasing age, even in healthy elderly adults (Bai et al., 2018),

resulting in changes in the EIM variables. Therefore, this should be taken into consideration when performing assessments. Tarulli et al. (2007) used ultrasound to evaluate SFL thickness in 62 healthy subjects, finding a weak positive correlation between age and SFL thickness in women ($r^2 = 0.14$, $p < 0.05$) but not in men, as well as a strong negative correlation between age and the PhA for both men ($r^2 = 0.48$, $p < 0.01$) and women ($r^2 = 0.68$, $p < 0.01$). However, no significant association was found between the PhA and SFL thickness. As in the study of Aaron et al. (2006), the measurement was not performed using the tetrapolar arrangement, but with linear-EIM, placing eight voltage electrodes in a standard array over the quadriceps, while the current electrodes were attached to the dorsum of the feet. However, the measurement of SFL thickness with ultrasound was made only between the voltage electrodes four and five rather than the entire array, which may vary along the length of the muscle. Furthermore, no data on the R and Xc values were provided.

By contrast, Sung et al. (2013) showed a great impact of SFL thickness on both the R ($r^2 = 0.64$; $p < 0.001$) and PhA values ($r^2 = 0.70$; $p < 0.001$), but not on Xc values ($r^2 = 0.07$; $p = 0.30$), which remained unaltered. However, the major difference regarding the previous study was the assessment technique, as Sung et al. (2013) used an HEA to measure the EIM parameters, unlike Tarulli et al. (2007), who used linear-EIM. An HEA may be less sensitive because the current penetrates only the superficial area of the muscle (Jafarpoor et al., 2013; Rutkove et al., 2017). We need to consider that the current will always take the path of least resistance and if the electrodes are close, the measured voltages will be influenced by the SFL thickness, leading to the evaluation of a mix of muscle and fat. Furthermore, the electrode arrangement of an HEA is always the same, with no variation in the distance between the inner and outer electrodes, which is a relevant point. The correct placement of the electrodes along the muscle belly is important, since small alterations may imply very significant modifications of the results (Jafarpoor et al., 2013; Sanchez et al., 2016b; Rutkove et al., 2017), making little sense to use the same distribution for small and large muscles. For that reason, it is important to highlight the need for choosing the appropriate electrical impedance assessment method, taking care when comparing studies with different techniques since the results may change considerably from one method to another. In this case, an HEA could lead to errors in subjects with higher SFL thickness, as Rutkove et al. (2017) indicated. Baidya and Ahad (2016) also suggested that the injection electrodes should be at the far end of the muscle group, while sensor electrodes should be closer to each other to eliminate variations caused by SFL or muscle thickness.

Taking all these points into consideration, we clearly see that the impedance parameters are altered by factors such as age, gender, fat, and the muscle area analyzed. Furthermore, EIM can be used to assess muscle health beyond neuromuscular diseases. However, the main problem in this section is the great inconsistency between the studies when it comes to performing assessments due to the use of very different techniques, making it very difficult to compare them and make relevant conclusions.

Injury Follow-Up

There is a series of studies that deal with the impedance changes occurring in a muscle when it gets injured. Considering that localized bioimpedance analysis can assess soft tissue hydration and cell membrane integrity, it is to be expected that some impedance parameters may change when a muscle injury occurs, as well as during the whole process of healing, due to changes in the distribution of fluids and in the cell structure (Järvinen et al., 2005).

Nescolarde et al. (2013) was the first to study this topic, performing tetrapolar measurements of the quadriceps, hamstrings, and calves in a soccer team during one season. During the season, three injuries of different severity (grade I, grade II, and grade III) were analyzed and classified with magnetic resonance imaging (MRI). They found that the *R*, *X_c*, and *PhA* values decreased with increasing severity of the injury and gradually recovered over time as the injury healed. The percentage of decrease in the *R*, *X_c*, and *PhA* values were -23.1 , -45.1 , and -27.6% , respectively, in the grade III injury and -11.9 , -23.5 , and -12.1% , respectively, in the grade I injury with regards to the contralateral non-injured side. This pattern of changes during recovery was similar to that obtained with the L-BIA of wound healing (Lukaski and Moore, 2012). The authors indicated that the changes in the *R*-values were due to fluid accumulation in the injured area, while the decreases in the *X_c* and *PhA* values were the result of disruption to cell membrane integrity and injury. Despite the fact that the sample size was too small for it to be significant, a clear relationship between the decrease in bioelectrical values and injury was seen that would merit further investigation.

Following the same protocol, Nescolarde et al. (2015) built on their previous study (Nescolarde et al., 2013), showing very similar results, but this time the most significant change was evidenced in the *X_c* value 24 h after injury ($p < 0.001$), with a decrease of -17.5% in grade I injuries (eleven injuries), -32.9% in grade II injuries (eight injuries), and -52.9% in grade III injuries (three injuries). This pattern was in line with the severity of the injury, while variations in the *R* (-10.4 , -18.4 , and -14.1% for grade I, II, and III injuries, respectively), and *PhA* (-9 , -16.6 , and -43.1% for grade I, II, and III injuries, respectively), were not as indicative. The major difference from their previous study was a larger sample size, although it was not very large, which provided more statistically significant data. A limitation of the study was that the total number of subjects was not reported, with the total number of injuries provided instead. There was a possibility that one player could have suffered more than one injury, which we cannot know.

In a case report of a soccer player with a grade II injury (proximal tendon of the long head of the femoral biceps) from Francavilla et al. (2015), serial measurements were performed until the RTP. Despite the electrode array being different to that of previous studies (Nescolarde et al., 2013, 2015) given that the current injection electrodes were placed on the feet and not on the muscle of interest, the results were very similar 24 h post-injury. However, the greatest changes were observed on the fourth day post-injury, when the *PhA* and *X_c* values reached -51.2 and -47.1% , respectively. After that, both substantially increased until the RTP (day 39 post-injury), reaching values

similar to those of non-injured players, except for the *R*-values. Both Nescolarde et al. (2015) and Francavilla et al. (2015) agreed that the *R* is not as indicative as the other parameters since it seems to be strongly influenced by the position of the subject and physical activities. Furthermore, according to previous studies using MRI criteria (Schneider-Kolsky et al., 2006; Reurink et al., 2014), the authors indicated that the presence of edema in the injured muscle did not correlate with the grade of the injury. Thus, *R* is the least suitable parameter in the management of a muscle injury, unlike the *X_c* and *PhA*.

Valle et al. (2017) proposed a new classification of muscle injuries in which the muscle gap plays an important role as an indicator of injury severity. However, this classification does not always allow the identification of injuries by MRI. Nescolarde et al. (2017) proposed L-BIA using tetrapolar assessment to overcome this limitation, since they found that the severity of the injury correlated with the muscle gap according to the RTP values. They noted that a greater injury severity resulted in larger decreases in the *R*, *X_c*, and *PhA*, whereas the presence and severity of muscle disruption adversely affected the time to RTP ($p < 0.0001$). In agreement with previous studies, the authors indicated that L-BIA can accurately predict the time to RTP according to the impedance values of the non-injured limb.

Based on another type of classification of muscle injuries (Pollock et al., 2016) and considering the histoarchitectural approach to skeletal muscle injury (Balius et al., 2020), Nescolarde et al. (2020) classified 37 muscle injuries into 4 different grades based on the severity and nature of the injury (tendinous, myotendinous junction, or myofascial junction injuries), using MRI and ultrasound assessment. They made a cross-sectional analysis measuring injured subjects through the tetrapolar technique. Again, significant decreases ($p < 0.01$) in the *R*, *X_c*, and *PhA* values were found 24 h after injury in both the myotendinous junction (-10.7 ± 6.3 , -20 ± 10.1 , and -9.9 ± 7 , respectively), and myofascial junction (-18.7 ± 5.2 , -33.7 ± 8.4 , and -17 ± 8.4 , respectively), as well as in the different grades of myotendinous junction injuries. The greater changes were observed in the *X_c* ($p < 0.001$). Regarding the days to RTP, there was a statistically significant difference among the three different grades of myotendinous junction injuries ($p < 0.001$). Therefore, L-BIA is a useful technique for assessing muscle injuries.

One aspect to highlight is that in contrast to the previous section on muscle characterization, almost all the studies on injury, except for that of Francavilla et al. (2015), worked with very similar protocols involving the same authors. They used subjects with similar characteristics, the same devices and materials, the same assessment method, and the same electrode placement, with the protocol improved each time. This emphasizes the importance of standardizing protocols for future research on different EIM applications in order to make more reliable comparisons between studies and to move forward with this technique.

In conclusion, EIM is a practical method for characterizing the severity of muscle injuries and monitoring follow-up until the RTP. *X_c* and *PhA* values abruptly decrease when an injury occurs, increasing linearly and consistently during the healing phase. In these studies, different classifications of muscle injuries were used and in all of them, EIM was able to identify the severity of the

injury. One of the major problems in sports medicine is reinjury (Orchard and Best, 2002). EIM could play an important role in minimizing its risk in athletes or sport entities that cannot afford alternative and more expensive methods like MRI and ultrasound (Rutkove, 2020). However, more research is needed, especially studies with larger sample sizes, investigations analyzing different sports, and longitudinal studies monitoring the follow-up from injury to full recovery.

Adaptations Generated by Physical Exercise

Acute and chronic physical exercise induces some morphological and physiological adaptations within the muscle and these changes in muscle properties can be quantified by EIM. There are some studies analyzing this topic and they can be classified into three groups based on what they are investigating: (1) short-term adaptations; (2) medium-term adaptations; and (3) long-term adaptations.

Short-Term Adaptations

The majority of the studies included in this review investigated the short-term adaptations to physical exercise. The first study using D-EIM in healthy subjects by Shiffman et al. (2003) assessed the anterior forearm muscles using a tetrapolar electrode arrangement in six healthy subjects. The evaluation was performed during the execution of a maximum voluntary isometric contraction (MVIC) of the finger flexor muscles, using a dynamometer to measure grip strength. The R and Xc values (PhA data were not provided) increased exponentially during the exercise, regardless of whether the force was generated abruptly or gradually. Both parameters decreased once the exercise stopped, although they did not return to baseline values. Furthermore, the differences were accumulative between sets of the same exercise. The Xc values remained above basal levels after stopping the exercise, while the R-values were either below or above that of the resting condition. Despite no statistical analysis in the study, this technique was sensitive to physiological changes that occurred when a force was applied and to the accumulated fatigue between sets. It is worth mentioning that these results were also compared with those of subjects with neuromuscular diseases from a preliminary study [as cited in Shiffman et al. (2003)], providing different bioimpedance behaviors. Therefore, it is important to check the health of the subject before assessment.

In the same vein, Li L. et al. (2016) assessed impedance changes in 19 healthy volunteers at different levels of isometric muscle contraction until task failure (20% of the MVIC, 60% of the MVIC, and the MVIC). The muscle analyzed was the biceps brachii and the assessment was performed using an HEA multifrequency device at 50 and 100 kHz. The study also observed substantial changes during the different stages of the exercise. This time, the most significant parameter was the R, which significantly increased during exercise, especially at higher intensity contractions (60% of the MVIC and MVIC). Right after relaxing the muscle, R significantly decreased and remained above resting values ($p = 0.014$). During the post-fatigue resting period, this parameter continued to decrease ($p = 0.011$).

However, this reduction was not significant compared to the resting values ($p > 0.05$). Regarding the Xc values, changes were not significant at contraction or in the resting period. There was a significant correlation between the time to muscle failure and variation in R-values right after relaxation ($r^2 = 0.3123$; $p = 0.0159$). The authors suggested that the kinetics of R were due to confounding changes in architectural and metabolic factors within the contracting muscle.

Fu and Freeborn (2018) measured the biceps brachii performing an isotonic bicep curl exercise (joint angle and muscle length change while the tension remains constant) at different intensities until muscle failure. The load of the first group was set at 60% of the previously assessed one-repetition maximum (1RM), while the load of the second group was set at 75% of the 1RM. They used the tetrapolar impedance technique at 10, 50, and 100 kHz. At 50 kHz, there were significant differences in the R and Xc values (PhA data were not provided) between pre- and post-task in both groups ($p < 0.05$), but no significant differences between the two groups at different intensities, unlike the results of Li L. et al. (2016). Therefore, under the isotonic protocol applied in the study, although EIM seemed sensitive to pre-post changes, it did not seem to be able to detect differences between these intensities of exercise. However, the protocol used in the study was completely different to that in Li L. et al. (2016). Firstly, the electrode arrangement was different and, secondly, Li L. et al. (2016) used a single execution of an isometric exercise until task failure while Fu and Freeborn (2018) used multiple repetitions of an isotonic bicep curl exercise.

Freeborn and Fu (2019) analyzed 17 healthy subjects performing 10 sets of a fatiguing protocol of bicep curl repetitions until muscle failure at two different intensities: 60% of the 1RM vs. 75% of the 1RM. Impedance values were measured on the biceps brachii using the tetrapolar arrangement before the exercise and immediately after each set. Similar to the previously mentioned studies, the R, Xc, and PhA values tended to decrease at 50 kHz, with these reductions accumulating through the sets. As seen in Fu and Freeborn (2018), similar patterns were observed at both intensities, with R the first parameter to indicate a significant change (in the third set for 60% of the 1RM and in the fourth set for 75% of the 1RM). The authors suggested that R decreased due to the more localized fluid volume generated by muscle swelling, although they did not quantify it. This theory is based on the results of Yasuda et al. (2015), which showed increases in biceps brachii thickness after multiple sets of a bicep curl exercise, with muscle swelling increasing with every additional set. Furthermore, they also indicated that the Xc values may not be as affected by muscle swelling and fluid accumulation as the R-values and that it may serve as a marker of muscle health, fatigue, and injury. Another point to consider is that the authors did not indicate how the 1RM was assessed.

Finally, Huang et al. (2020) measured R-values (they did not report Xc or PhA data) at different intensities (20, 40, and 60% of the MVIC) of dynamic contractions in 8 healthy subjects. They employed a different bioimpedance device from the ones applied in the other studies, using a signal generator, an oscilloscope, a differential probe, and four electrodes linearly placed on the biceps brachii. Despite the use of a multifrequency

device, the authors focused only on the outcome at 50 kHz. The study established a 3D model of the arm to optimize electrode configuration using a simulation method. Furthermore, surface electromyography was performed at the same time to verify the use of EIM as an indicator of muscle fatigue. The results showed a marked decrease at all three intensities, averaging a reduction of $7.99 \pm 1.9 \Omega$ when comparing the *R*-values of fatigued vs. non-fatigued muscle. There were some consistencies when comparing *R* with the median frequency parameter obtained via surface electromyography. Thus, EIM can be used as an evaluator index of muscle fatigue. Ultimately, the decrease in *R* is affected by different load levels, declining faster as the load increases.

Medium-Term Adaptations

Regarding the studies on medium-term adaptations, Freeborn et al. (2020) also measured localized impedance changes in the biceps brachii using a multifrequency device and arranging the four electrodes linearly over the muscle. Six subjects performed an exhausting, eccentric, bicep curl exercise (90% of the 1RM) and were measured at different time points (pre-exercise and 24, 48, 72, and 96 h post-exercise). At 50 kHz, the *R* and *Xc* values decreased from pre- to post-measurements, with the largest reductions observed at 72 h and 96 h post-exercise. The statistically significant differences in both *R* and *Xc* values occurred at the same timepoints as that of the maximum post-exercise muscle swelling, in accordance with previous studies (Li L. et al., 2016; Fu and Freeborn, 2018; Freeborn and Fu, 2019). This indicates that EIM is sensitive to the muscle swelling induced by exercise (Shiffman et al., 2003; Li L. et al., 2016; Freeborn and Fu, 2019). On the other hand, changes in *Xc* values were similar to those observed in severe injuries (Nescolarde et al., 2013, 2015, 2017, 2020). Information on the PhA values and how 1RM was assessed was not provided.

Elleby et al. (1990) used localized electrical impedance to assess muscle (calves and thighs) changes induced by a half marathon in four healthy subjects. Assessments were undertaken on each of the 5 days before the race, immediately after the race (2 and 6 h after the race finished), and on each of the 5 days post-race. Therefore, the analysis of exercise adaptations encompassed 6 days plus the previous 5 days. Despite the tetrapolar 50-kHz measurements, the assessment technique was different (Brown et al., 1988) and only the resistivity [real part of the complex-valued impedance per unit length and per cross-sectional area (Faes et al., 1999)] of the whole muscles was analyzed. As mentioned by the authors, the impedance of a limb is mainly resistive at 50 kHz. They measured the longitudinal and transverse resistivity of the limb, as well as the limb circumference and the hematocrit. Transverse resistivity was found to be increased immediately after the race (7%) and the values returned to baseline levels the next day. However, no changes were observed in longitudinal resistivity. Despite the fact that the limb circumference and hematocrit were consistent with dehydration and the values returned to the pre-race levels on the day following the race, the origin of the small resistivity changes was difficult to explain. It should be noted that the study presented a high risk of bias according to the AQUA tool. Therefore, their results should be interpreted with caution.

Long-Term Adaptations

The study of Mascherini et al. (2015) was the only one analyzing long-term muscle impedance adaptations, evaluating elite soccer players during a pre-season training period of 50 days. They compared whole-body and localized (tetrapolar assessments at the quadriceps, hamstrings, and calves) BIA values. At the end of the training period, all three muscles showed significantly decreased *R*-values. The PhA increased only in the quadriceps, while the *Xc* decreased in the calves. Thus, the extensor muscles showed major changes. Whole-body analysis revealed an increased amount of water, especially in the lower limbs. This could explain the reduction in the *R* in all the muscle groups.

Summary of Bioelectrical Adaptations to Physical Exercise

In conclusion, few studies on adaptations to exercise are currently present in the scientific literature. However, from those reviewed, we conclude that localized bioimpedance is sensitive to muscle changes occurring during exercise. Therefore, considering that more research is needed, this technique may be useful, easy to use, and a real-time tool to non-invasively quantify changes caused by exercise, muscle fatigue, and recovery.

All investigations on short-term adaptations showed a clear relationship between changes in impedance values and D-EIM during or after exercise. *R*-values appeared to be the most significant parameter, possibly because of the multiple mechanisms that take place inside the muscle as acute adaptations to exercise, for example, an increase in blood flow, vasodilation, or metabolites in the tissues (Freeborn et al., 2020). In accordance with previous studies on human subjects (Rutkove, 2009) and animal models (Sanchez et al., 2016a), *R*-values increased during contractions, as well as the *Xc*, and decreased after relaxation. Furthermore, this decrease accumulated over the different sets of exercise with increasing fatigue. *R* seemed to be the most affected, its most significant change coinciding with the time point of maximum muscle swelling. The load level also appeared to affect the behavior of the impedance values, and this is important because short-term studies did not use loads greater than 75% of 1RM, unlike medium-term studies. The similarity between the changes in *Xc* in a healthy muscle performing an exercise compared to an injured muscle makes it worthwhile to continue investigating this further. Regarding the PhA, there is very little information, since most studies did not report the data. Furthermore, L-BIA seemed to be appropriate in assessing hydration and cell mass in a specific body area, which may help to reduce the risk factors for injury, such as dehydration, and prevent deterioration in physical performance (Nédélec et al., 2012). One limitation of the studies looking at short-term changes was that all of them were carried out in the biceps brachii. Therefore, the results could not be extrapolated to the rest of the body given the morphological and physiological differences between the biceps brachii and the other muscles. The studies on medium-term and long-term adaptations did not provide enough information to establish relevant conclusions due to the scarcity of the investigations and the low quality of some of the studies. Furthermore, the assessment technique was not uniform across the studies, making it difficult to compare their results. Therefore, more research is needed on this.

More studies applying different training protocols and observation periods, analyzing different muscles, and using larger sample sizes are needed. Despite the fact that the present article did not aim to review the topic, it should be noted that impedance adaptations evoked by exercise are different in healthy subjects compared to people with neuromuscular diseases (Shiffman et al., 2003), which has also been reported in studies with mice (Sanchez et al., 2016a).

The Principles of EIM

As already shown, electrical bioimpedance assessments can be performed using different types of technology and techniques and it is applicable in different contexts. This must be considered to minimize errors and obtain the most accurate and reliable measurements. Some of the factors that may affect results in localized measurements are: the anatomical characteristics of the muscle itself; the devices and materials used to measure bioimpedance; the design of the assessment technique; the characteristics and arrangement of the electrodes; the device frequency; and the preparation of the subject.

The most common way of performing EIM is using 4 electrodes linearly arranged over the muscle at a single frequency of 50 kHz. However, this was not the only protocol followed in the reviewed studies, which showed important inconsistencies even in the mentioned protocol. It is difficult to compare the results of investigations applying different techniques, considering that a small change in the measurement can significantly affect the results (Jafarpoor et al., 2013; Sanchez et al., 2016b; Rutkove et al., 2017). For this reason, it is imperative to standardize the assessment protocol. In this way, it would be interesting to develop (or even use) a methodology like the SENIAM Project, which is a surface electromyography methodology (Hermens et al., 2000) that tries to overcome the same issue, standardizing electrode placement on each muscle.

As far as material electrodes are concerned, the most common is the Ag/AgCl electrode, despite the presence of others such as dry electrodes, textile electrodes, and physiotherapy electrodes. These alternatives have their strengths and weaknesses and they can be used in specific occasions if the study design requires so (Sanchez and Rutkove, 2017a). A newer alternative is the microneedle electrode array (Li Z. et al., 2016), which has the advantage of being much more suitable for assessing small muscles and not being affected by surrounding tissues, unlike that observed with surface electrodes. However, it is more invasive than superficial electrodes.

Regarding electrode placement, an alternative used in some of the studies included in this review was an HEA. An HEA facilitates and speeds up bioimpedance assessments since the four electrodes are fixed in an array that is easy to maneuver. However, the major limitation is the close proximity of the electrodes, which may significantly affect the results because of the presence of subcutaneous tissue (Kortman et al., 2013; Sung et al., 2013; Li L. et al., 2016). An HEA is less sensitive than other methods since the electrical current penetrates only the superficial area of the muscle (Jafarpoor et al., 2013; Rutkove et al., 2017). Therefore, the great advantage of having the electrodes in a fixed position has an important drawback in that the electrode

arrangement cannot be adapted to the anatomical characteristics of the muscle being assessed. This is important because the greater the separation between the electrodes, the deeper the current penetrates. Therefore, if this electrode arrangement is used, the surrounding tissues (e.g., skin, SFL, and bone) will have less impact on the assessments. However, the blood volume in the vessels and arteries may have an effect (Sanchez et al., 2016b). Hence, the big question should be where to place the different electrodes over the muscle to ensure maximum reliability. To establish the optimal electrode configuration, some studies used a finite element model based on different equations to obtain a geometrical model of the segment analyzed (Jafarpoor et al., 2013; Baidya and Ahad, 2016; Sanchez et al., 2016b; Rutkove et al., 2017), moving the electrodes to different places throughout the muscle (Jafarpoor et al., 2013; Sanchez et al., 2016b) until an appropriate location was found that improved the efficiency and fully exploited the capacity of EIM to assess the muscle. The choice of the device frequency is also important because the results may completely change. Although some of the studies used multifrequency devices, the present review only analyzed results at 50 kHz because this is the closest frequency to the one at which the X_c presents a greater value (De Lorenzo et al., 1997). However, it is also interesting to measure in the kHz-MHz range to obtain complete impedance information of the underlying structures and muscle properties. In fact, some investigations performing multifrequency analysis suggested that this may be more sensitive in the assessment of diseased muscles and their progression over time (Rutkove et al., 2012; Schwartz et al., 2015). It is also necessary to use phase-sensitive devices, since non-phase-sensitive instruments cannot measure X_c and the proper application of BIA needs the R , X_c and PhA parameters (Castizo-Olier et al., 2018). There is an advantage in using the PhA compared to the other bioelectrical parameters as it does not need to be standardized by the length of the muscle. Before measuring, some considerations must be taken into account (Castizo-Olier et al., 2018): the skin must be prepared by shaving off hair, rubbing with gel, and cleaning with alcohol; the subject must be euhydrated, with no injury or disease conditions unless that is the study objective, in a fasting state for at least 8 h, with no recent alcohol ingestion, a voided bladder, and at least 10 min of stabilization; electrode placement should be marked in longitudinal studies to preserve the same location of electrode placement and to control the temperature of the skin; the different assessments must be performed under the same conditions and environmental characteristics and, if possible, at the same time of day (to make intra-individual and inter-individual comparisons); if measurements are performed after exercise, the subject must take a shower to remove the electrolytes off the skin and reduce the skin temperature, cutaneous blood flow, and BIA parameters to basal levels; food and drink should be avoided between measurements in the evaluation of acute variations after exercise, unless that is not possible, in which case this must be registered and it should be taken into account that the ingestion may have a minimal effect if the measurement is performed <1 h after the intake; the type of exercise must be taken into consideration since post-exercise stabilization and the moment at which the measurement is performed may vary; and

the menstrual cycle must be controlled for to make comparisons according to the phase.

The choice of an appropriate technique may depend on the study design, the muscle analyzed, the characteristics of the subjects, and costs. There will always be sources of error that can affect the results, although investigators must focus their efforts on minimizing these. For this reason, it is urgent to develop a common protocol for the entire scientific community interested in this topic so that EIM can be set in a more reliable scenario.

Prospective Research Applications and Research Agenda

The application of EIM covers areas of interest other than the evaluation of neuromuscular disorders. However, EIM has not reached its full potential and, therefore, cannot be considered a fully validated instrument. For this reason, it must be further studied and improved, both at a theoretical and technological level.

Impedance values in muscles are affected by several inherent characteristics of the subjects. The clearest one is that the PhA tends to decrease with increasing age, indicating that it can be a sensitive tool for assessing muscle deterioration. Regarding gender, the reviewed articles suggested that there could be impedance differences between males and females. Furthermore, body composition may play an important role since a greater fat mass can alter bioelectrical results, especially when using certain techniques (e.g., HEA).

With regards to skeletal muscle injuries, the results are very encouraging, since a very significant relationship has been identified between injury severity and impedance values, especially for the Xc and PhA, as they are the main indicators of cell health and integrity. However, all these studies focused on the same type of subjects. Therefore, future investigations should consist of longitudinal protocols with a larger sample size, analyzing different sports, and also including female athletes.

As far as exercise is concerned, we can only make statements about short-term adaptations since the studies on medium- and long-term adaptations were scarce and of a low quality. In the studies analyzing short-term adaptations with D-EIM, the most significant parameter seemed to be R, which increased during contraction and decreased during recovery. The Xc also showed the same trends, although to a lesser extent. However, the results urge further study on the topic in order to obtain a quantitative variable to assess training load and fatigue using a non-expensive and real-time method.

As stated throughout this review, the main focus in the continued development of this method should be on standardizing a protocol that allows the assessment of any person, thereby enabling the comparison of future studies in a much more valid and reliable way. The SENIAM Project could be a protocol to follow, with the necessary modifications made to EIM.

LIMITATIONS

The main limitations of analyzing the literature on the use of EIM in health and physical exercise were: (1) the inconsistency

between the EIM assessment techniques, with different protocols and devices used; (2) the difficulty in controlling multiple sources of error that may influence the bioelectrical signal; (3) the scarcity of scientific information regarding EIM not related to the study of neuromuscular diseases; (4) the lack of analysis of some impedance parameters that could be of interest; and (5) the limited scientific evidence explaining the bioelectrical behavior of human tissues induced by exercise.

CONCLUSIONS

The use of EIM has grown in recent years, along with the quality of the studies. The increased interest in this technique comes mainly from the clinical field, although it may be useful in other contexts, as shown in the present review. EIM is easy to use and quick to perform, which can be applied at any time and in any place. Furthermore, it is not expensive and can be applied to a great variety of superficial muscles. Although not yet fully defined, EIM is sensitive to the muscular adaptations produced by physical exercise and sports. Therefore, it could become a great tool to control the load in athletes, as well as in the recovery process after a muscle injury. However, more research is needed on this and for EIM to be applied as a tool with a greater scientific use given that EIM has some advantages that other methods lack. For this, the first step must be the standardization of an appropriate protocol.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ÁC-P, AI, JC-O, and GS-L: conceptualization. ÁC-P and MC-M: data curation. ÁC-P, AI, and MG-F: formal analysis. ÁC-P, AI, and MC-M: investigation. ÁC-P, AI, GS-L, and MG-F: methodology. ÁC-P: project administration and writing and original draft. AI, JC-O, and GS-L: supervision. ÁC-P, AI, JC-O, and MC-M: writing and review & editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2021.740877/full#supplementary-material>

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Article

Bioelectrical Impedance Vector and Creatine Phosphokinase Changes Induced by a High-Intensity Training Session in Rink Hockey Players

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Abstract: This study aimed to analyze anthropometric and whole-body/muscle-localized bioelectrical impedance vector analysis (BIVA) adaptations and their relation to creatine kinase (CK) as a biomarker of muscle damage in a group of seven male players in the maximum category of professional rink hockey. There were three checkpoint assessments in relation to a high-intensity training session: pre-session (PRE), post-session (POST), and 24 h-post-session (POST24H). The resistance, reactance, and impedance module were adjusted by height (R/h, Xc/h, and Z/h, respectively). The Wilcoxon signed-rank test was used to compare the data at baseline and follow-up, while Spearman correlation was used to explore the relationship between CK and the rest of the parameters. The results registered a decrease in body mass at POST ($p = 0.03$) and a reestablishment at POST24H ($p = 0.02$). Whole-body BIVA registered a significant increase in R/h between PRE-to-POST ($p = 0.02$) and returned to baseline values at POST24H ($p = 0.02$), which was expected since this parameter is related to hydration processes. Muscle-localized BIVA in the rectus femoris muscle showed an increase in both Xc/h and phase angle in POST ($p = 0.04$ and $p = 0.03$, respectively) and a decrease in Xc/h at POST24H ($p = 0.02$). CK correlated with R/h in the rectus femoris at all the checkpoints (PRE-to-POST: $r = 0.75$, $p = 0.05$; PRE-to-POST24H: $r = 0.81$, $p = 0.03$; POST-to-POST24H: $r = 0.82$, $p = 0.02$). Our results indicate that BIVA is a sensitive methodology to assess general and muscle-localized hydration induced by a high-intensity training session in rink hockey players. A correlation between BIVA and CK was also reported.

Keywords: BIA; BIVA; creatine phosphokinase; rectus femoris; hydration status; muscle damage; phase angle; reactance; resistance; hockey

1. Introduction

Rink hockey, also known as roller hockey, is a sport in which two teams of five players (four players and a goalkeeper) use curve-shaped wooden sticks to try to score more goals than the opponent with a rubber ball on a rectangular closed pitch. Each match consists of two periods of 25 min each, separated by a resting period of 15 min [1].

The contribution of the scientific literature regarding the physiological and bioenergetic knowledge of this sport is scarce [2,3] compared to other popular sports. However, it is well known that the character or specificity of physical effort in rink hockey is highly complex,

mainly because the technical and tactical actions are subject to a significantly higher rate than in other sports [4], requiring both high intensity short and long-duration efforts [2] without being able to fully recover [3].

This type of training generates physiological changes in the players, producing an adaptation according to the stimulus and the workload. An adequate control of this workload is important since it allows for the optimization of sports performance, reduces the risk of injury, and prevents overtraining. In this regard, creatine phosphokinase (CK) can be described as a marker that assesses the responses provoked by exercise, since it is considered a marker of muscle damage in the sports field [5]. It has been reported that an increase in serum CK concentrations combined with a reduced exercise tolerance is a critical overtraining marker [6,7]. Strenuous exercise damages the skeletal muscle cell structure, causing a substantial increase in total CK in blood in the following hours and days after the exercise is performed [6,8]. Trained subjects present higher resting values of CK compared to untrained subjects [9] and subjects with a higher fat percentage reach higher CK levels [10]. Interestingly, serum CK levels in the hours and days following a high-intensity session in professional rink hockey players are unknown.

Bioelectrical impedance analysis (BIA) is as a relatively new technique based on the opposition of the body tissues to the flow of an introduced electrical current. BIA is capable of assessing the state of body composition, nutrition, hydration, and cellular integrity in an economic, easy, fast, and non-invasive way, through the use of different multiple regression equations [11,12]. The resulting bioimpedance parameters are the resistance (R, the major resistance to the current through intra- and extracellular ionic fluids), reactance (Xc, the additional opposition due to the capacitive elements, such as cell membranes, tissue interfaces, and non-ionic substances), impedance (Z, the tissues' opposition to the electric current flow), and phase angle (PhA, the geometric relationship between R and Xc) [13]. However, the great limitation of this technique is that its accuracy is compromised because the regression equations derived from a population group may not match the targeted specific group, and assumptions such as constant tissue hydration or constant tissue isotropy are not frequently suitable [14,15].

In order to overcome the mentioned limitations of BIA, bioelectrical impedance vector analysis (BIVA) emerged, solving these problems by using raw impedance parameters instead of using the regression equations. BIVA can be performed through different protocols depending on the distribution and the number of the electrodes [16]. To date, the reference method is the foot-to-hand tetrapolar electrode arrangement at a 50 kHz single frequency to measure whole-body BIVA [11,12]. Low frequencies (<5 kHz) cannot penetrate the cell membrane, while high frequency (>200 kHz) current can flow through intra- and extracellular compartments. However, there is a poor reproducibility in both low and high frequencies; hence, the most commonly used frequency is 50 kHz, which is an intermediate frequency [16]. Whole-body BIVA has been studied in different sports, which has allowed for the elucidation of the body composition characteristics of each specific sport [13]. Furthermore, this knowledge of body composition has been suggested to discriminate athletes of different performance levels [17].

If the four electrodes are located on a specific muscle, it can be named both as muscle-localized BIVA (ML-BIVA) and electrical impedance myography (EIM). There is less consensus about the exact location of the electrodes regarding ML-BIVA, since the scientific literature is scarce and heterogeneous. ML-BIVA has mainly been studied in medicine in a wide range of different clinical conditions [18,19]. However, in recent years there has been growing interest in its applicability in the high-performance sports field [19], and in the assessment and follow-up of muscle injuries [20–24]. The most analyzed muscles are the quadriceps, hamstrings, and calves, as they are long muscles that allow an easier assessment than others. The information provided by this method could help technical and medical staff to make decisions about their athletes.

Unlike BIA, BIVA does not provide a quantitative evaluation of the outcomes; thus, making a single measurement may be inefficient, since one of the main strengths of BIVA

is to analyze how the vector changes over a given period of time and under previously established circumstances, e.g., comparing impedance values before and after intense physical exercise. Another interesting aspect of BIVA is its ability to make a comparison of bioelectrical data, without the need to perform a PRE-to-POST comparison, based on specific sectors of the population that may be different (e.g., healthy adult reference population) or similar than the subject under analysis. Furthermore, BIVA may be of great use in the assessment of body composition, considering that this issue is important since the physical stress imposed during exercise may lead to body composition alterations causing a poorer performance [25]. In addition, dehydration can also impair performance [26,27] and increase the risk of injury [28], thus monitoring body fluid can assist in prescribing water intake and mitigating dehydration effects. Lastly, one of the main interests regarding BIVA and sport lies in the analysis of how the impedance parameters correlate to other indicators that also serve as exercise response markers. Some of the pioneers of this possible application were Nescolarde et al. [29], who reported that whole-body vector displacement could be correlated with some biomarkers of renal damage at 24–48 h post-race but not with CK.

Thus, this study aimed to analyze the kinetics of BIVA parameters (global and muscle-localized), which can provide information about the degree of dehydration, the physiological training effects, and the cellular integrity of muscle induced by a session of high-intensity training in rink hockey players. Furthermore, we also aimed to analyze if some of these BIVA parameters are correlated to CK changes, since both are markers of adaptation to exercise that may indicate that muscle damage has occurred after training. In this sense, it is now recognized that the evaluation of body water is a dynamic and complex process, and that no measure is valid for all situations [30,31]. It is for this reason that BIVA emerges as a precise, accurate, reliable, non-invasive, portable, cheap, and safe method that can easily be used to assess hydration status in real time [32]. These properties are particularly interesting for the evaluation of sport for both the training process as well as in competitive events. The present study analyses anthropometric adaptations, bioelectrical data (global and localized), and their relation to the behavior of CK, beforehand, afterwards, and 24 h after a high-intensity rink hockey training session.

2. Materials and Methods

2.1. Participants

This study was conducted on 7 top-level male professional rink hockey players from the same team throughout the same season (mean $\pm$ SD: age 26.3 ± 5.4 years; height 177.0 ± 6.2 cm; body mass (BM) 79.0 ± 9.8 kg; body mass index (BMI) 25.1 ± 2.1 kg/m²). All players voluntarily participated in the study and delivered written informed consent prior to their participation. The inclusion criteria were as follows: (1) participants must be older than 18 years old; (2) must not present injuries or any clinical condition at the time of the study; and (3) are not taking any medication at the time of the study.

The study was conducted following the Helsinki Declaration Statement [33]. The protocol and procedures were approved by the Ethics Committee for Clinical Sport Research of Catalonia (Ethical Approval Code: 19/CEICGC/2020).

2.2. 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 a rink hockey training session on anthropometric variables, bioelectrical whole-body and muscle-localized vector displacement (resistance (R , Ω), resistance adjusted by height (R/h , Ω/m), reactance (X_c , Ω), reactance adjusted by height (X_c/h , Ω/m), impedance module (Z , Ω), impedance module adjusted by height (Z/h , Ω/m), and phase angle (PhA , $^\circ$)), and other markers related to adaptations to exercise (CK, rate of perceived exertion, and HR) that were obtained from the same subjects at three checkpoints: before the training session (PRE), after finishing the training session

(POST), and 24 h after POST measurements (POST24H). Using the resistance (Ω) and reactance (Ω), we derived the module bioimpedance (Ω) ($|Z| = \sqrt{R^2 + X_c^2}$) and the phase angle ($^\circ$), since it is the geometric relationship between these two parameters in degrees ($PhA = \tan^{-1} \frac{X_c}{R} \times \frac{180^\circ}{\pi}$).

2.3. Procedures

From just before the first assessment, the subjects were not allowed to urinate or eat solid food until the end of the second assessment, and fluid intake was monitored during training. In addition, the players had not trained during the previous 48 h. The players were measured wearing only underwear. Initially, the measurements of size, weight, and body temperature were performed.

After the measurement of the anthropometric, bioelectrical, and hematological values, all the players together took part in a training session, which consisted of a warming up phase, a block of technical and tactical tasks with skates at a high intensity, and a cooling down phase, for a total time of approximately 2.5 h (Table 1). The specific details of the training session can be found in the Supplementary Material. Two more assessments were performed under the same conditions, the first after finishing the training and the other 24 h later.

The heart rate (HR) of each player throughout 2.5 h training session was monitored individually and the percentual amount of time in each intensity zone was classified. HR was monitored by a Polar RS800CX HR sensor (Polar Electro Oy Inc., Kempele, Finland) with the Polar ProTrainer 5 software (measuring range for HR: 5–95%; accuracy: $\pm 3\%$ between 15% and 90%).

The individual session rate of perceived exertion (RPE) was quantified during training. The 15-graded RPE scale [34] was shown to the players immediately after the training was completed. As shown in Table 1, the mean RPE of the players was 14.7 ± 1.7 , which implies that the training session was hard.

The blood collections for CK measurements were carried out by a qualified practitioner at PRE, POST, and POST24H and the subsequent analyses were conducted according to the procedures established in previous studies [7].

2.3.1. Anthropometric Assessment

The anthropometric measurements were performed according to the standard criteria of the International Society for the Advancement of Kinanthropometry (ISAK) [35]. The following anthropometric material was used: (A) a telescopic measuring rod (Seca 220[®], Birmingham, UK, measuring range: 85–200 cm; accuracy: 1 mm) to measure the height; (B) a scale (Seca 710[®], Birmingham, UK previously calibrated, capacity: 200 kg; accuracy: 50 g) to measure weight; and (C) anthropometric tape (Lufkin Executive[®], Lufkin, TX, USA, accuracy 1 mm) to place the electrodes and to measure the perimeters. To measure the perimeter of the thigh, the anthropometric tape was positioned perpendicular to the center of the long axis of the thigh, running from the inguinal crease to the superior part of the patella, while subjects were seated on anthropometric boxes. Body mass index (BMI) was calculated as body mass/height² (kg/m²).

2.3.2. Bioimpedance Assessment

R and Xc were measured using a BIA 101 Anniversary Sport Edition analyzer (Akern Srl, Florence, Italy) that emitted a 400 μ A alternating sinusoidal current at 50 kHz [36]. This device was previously calibrated with a known impedance circuit provided by the manufacturer. The system measurement errors were determined by an accuracy resistor (0.1% tolerance) and capacitor (tolerance 1%), where $<1 \Omega$ for R and $<2\%$ for Xc. The repeatability and accuracy of the real-time measurements of R and Xc allowed the smallest changes to be recorded, with 1 Ω accuracy for R and Xc.

Table 1. Individual values of training session details.

Subjects	HR (b/min)			HR Intensity Zones (min and %)												Total Session Time (Hours)	RPE—15-Point Borg Scale	Water Intake (L)	
	HR _{rest}	HR _{min}	HR _{mean}	Very Light (Min–80 b/min)		Light (81–100 b/min)		Moderate (101–120 b/min)		High (121–140 b/min)		Very High (141–160 b/min)		Maximum (161–Max b/min)					
				HR _{max}	Hours	%	Hours	%	Hours	%	Hours	%	Hours	%	Hours				%
1	61	72	132	189	0:30	19:9	0:32	21:2	0:24	15:9	0:27	17:9	0:29	19:2	0:09	6:0	2:31	15:0	1:40
2	56	71	134	190	0:19	12:6	0:49	32:5	0:35	23:2	0:20	13:2	0:16	10:6	0:12	7:9	2:31	16:0	1:20
3	52	63	129	183	0:16	10:7	0:33	22:0	0:35	23:3	0:23	15:3	0:30	20:0	0:13	8:7	2:30	15:0	1:50
4	56	68	128	190	0:22	15:3	0:40	27:8	0:29	20:1	0:19	13:2	0:23	16:0	0:11	7:6	2:24	17:0	2:25
5	53	61	117	180	0:12	7:9	0:27	17:8	0:27	17:8	0:33	21:7	0:35	23:0	0:18	11:8	2:32	12:0	2:25
6	55	65	122	177	0:10	6:8	0:15	10:2	0:24	16:3	0:36	24:5	0:37	25:2	0:25	17:0	2:27	13:0	1:60
7	58	66	129	184	0:20	13:2	0:30	19:9	0:33	21:9	0:24	15:9	0:28	18:5	0:16	10:6	2:31	15:0	1:60
Average	55.9	66.6	127.3	184.7	0:18	12.3	0:32	21.6	0:29	19.8	0:26	17.4	0:28	18.9	0:14	10.0	2:29	14.7	1.7
SD	3.0	4.0	5.9	5.2	0:06	4.5	0:10	7.1	0:04	3.2	0:06	4.3	0:07	4.7	0:05	3.7	0:02	1.7	0.4

HR, heart rate; RPE, rate of perceived exertion.

All players were instructed to take a cold shower (as cold as tolerable) for 10–15 min post-training, in order to reduce cutaneous blood flow and temperature and remove accumulated electrolytes [37], reducing a possible bias in the analysis caused by a change in body temperature. Body temperature was measured just before the BIA measurements; this verified the return to temperatures close to the pre-training values, thus no significant differences were recorded between individual body temperatures of each player (PRE: 37.1 ± 0.2 °C; POST: 37.3 ± 0.5 °C; POST24H: 37.2 ± 0.3 °C). The measurements of body temperature before and after the training session were performed with a tympanic thermometer in the right ear (Genius 1000, Sherwood, Nottingham, UK, measuring range: $-25 + 55$ °C, accuracy: 0.1 °C) connected to a “data logger” Squirrel 2010 model (Grant Instruments Ltd., Cambridge, UK). Ambient temperature and relative humidity were recorded every 30 min during the total duration of the training by a portable weather station (Kestrel Weather K4500, TEquipment, USA, temperature measurement range: $-29 + 70$ °C; accuracy: ± 1 °C), and the average was 22.3 ± 0.4 °C for room temperature and $55.2 \pm 1.5\%$ for relative humidity.

The bioelectrical measurements were conducted under controlled conditions through the 50 kHz single frequency tetrapolar technique [32]. The subjects were measured lying on a stretcher in the supine position with their arms separated from their body by 30 degrees and their legs apart from each other at 45 degrees to avoid adduction or crossing of limbs, which would shorten the circuit and reduce the impedance values [38]. The electrodes were placed using the anatomical landmarks identified by palpation and reference distances with the anthropometric tape, to measure localized spots of the selected muscles. Pre-gelled electrodes (Red Dot™ 2660-5, 3M Corporate Headquarters, St. Paul, MN, USA) with low impedance were used to minimize the measurement error produced by the bioimpedance between the electrodes and skin. The area where the electrodes were placed was shaved and cleaned with alcohol. The points at which they were placed were labelled with permanent marker to ensure that the location was identical before and after training. The bioelectrical measurements were recorded after a stabilization period of 5 min, in which the players remained lying motionless. From the 5 min mark, three measurements were performed every 60 s to ensure proper distribution of body fluids. Subsequently, the average value for the three measurements was used for the final calculations.

- Whole-body assessment: The proximal electrode (sensor) of the arm was placed on the dorsal surface of the right wrist, between the ulna and radius. The proximal leg electrode was placed on the anterior surface of the right ankle, between the protruding portions of the bones. The distal electrodes (nozzles) were placed on the dorsal surface of the third proximal phalanx of the right hand and foot [16]. The proximal electrodes were spaced 5 cm from the distal to prevent interaction between electric fields, which could lead to an overestimation of the impedance values.
- Rectus femoris muscle-localized assessment: The electrode placement procedures followed the recommendations of the Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) guidelines for the rectus femoris [39] and the ISAK standards and measurement protocol [35], as follows: 1. Locate the anterosuperior iliac spine (sartorius insertion), assuming that a more inferior (anteroinferior iliac spine) rectus femoris origin, and mark with a point; 2. Locate the superior pole of the patella with the knee in unforced extension, and mark with a point; 3. Connect both mark points with an anthropometric tape (running in a straight line along the immediately upper anterior thigh). Note the distance, in cm, between the two points and mark at 50% of this distance with a dot; 4. Placement of the electrodes: divide the distance between the origin and insertion by three. At one third of the origin (proximal) and one third of the insertion (distal), place the BIA sensor electrodes at a fixed distance of 5 cm (from the center of the electrodes) between the BIA injector electrodes (Figure 1).

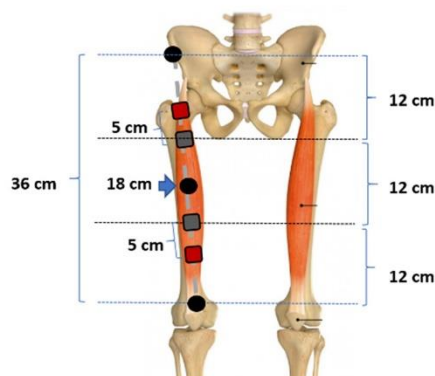


Figure 1. Rectus femoris muscle-localized BIA electrode placement example for 36 cm between anterosuperior spine and patella.

2.4. Statistical Analysis

The statistical values were expressed as mean $\pm$ standard deviation. After testing each variable for the normality of the distribution (Shapiro–Wilks test), the differences in anthropometric (BM and circumference of the thigh), bioelectrical (R, Xc, Z, R/h, Xc/h, Z/h, and PhA) and hematological data (CK) at PRE, POST, and POST24H were analyzed through the non-parametric Friedman test and, in the case of differences, the Wilcoxon test. The magnitude of ratio changes was computed as delta percent values ($\Delta\%$). Following this, the correlation was analyzed (Spearman coefficient) between increments or decrements (delta values) of BM, whole-body and localized R/h, Xc/h, and PhA, against CK. Whole-body individual bioimpedance vectors were analyzed by the standard reference RXc score graph, according to a healthy Italian reference population [40]. The RXc mean graph was performed to compare the whole-body mean vector of hockey players to the reference population. One and two-sample Hotelling's T^2 tests were used to determine the BIA vector differences between checkpoints and between hockey players vs. the reference population, respectively. An alpha value of 0.05 for statistical significance was set. IBM SPSS Statistics software v.20 was used for data management and statistical analysis.

3. Results

The BIVA mean graph (Figure 2) showed the mean hockey players' vector before training shifted to the left and upwards ($T^2 = 32.2$; $p = 0.0001$), and therefore had a greater PhA, in comparison with the reference population.

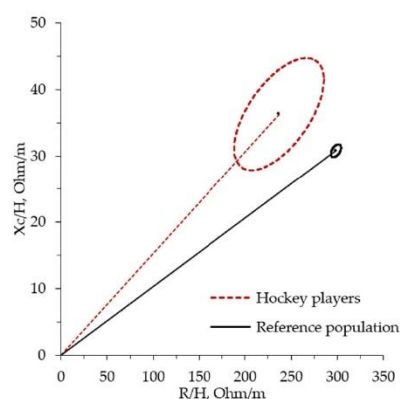


Figure 2. RXc mean graph. The 95% confidence ellipses for the mean impedance global vectors of hockey players (dotted line ellipse) and the healthy male reference population (solid line ellipse with vector) obtained from Piccoli et al. [40] are shown.

Anthropometric, bioelectrical, and CK data changes are reported at Table 2.

A significant decrease ($-1.1 \pm 0.6\%$) was recorded between the PRE weight (79.0 ± 9.8 kg) and POST weight (78.1 ± 9.7 kg), regardless of the 1.7 ± 0.4 l of water ingested during training. At POST24H, weight significantly increased to similar values to PRE (79 ± 9.9 kg). The perimeter of the thigh was significantly increased by $1.5 \pm 0.4\%$, and significantly decreased to 59.1 ± 4.5 at POST24H.

The whole-body bioelectrical analysis recorded a significant increase ($9.7 \pm 5.8\%$) between R/h PRE ($236.8 \pm 34.5 \Omega$) and R/h POST ($259.1 \pm 34.7 \Omega$). At POST24H ($246.3 \pm 30 \Omega$), the R/h values diminished significantly compared to POST R/h (Figure 3). No significant differences were registered in the kinetics of whole-body Xc/h. In the case of PhA, there was a significant reduction between PRE and POST24H ($-5.8 \pm 5.0\%$) but not between PRE and POST or POST and POST24H. The impedance module significantly increased by $9.6 \pm 5.7\%$ at POST and decreased by $4.8 \pm 3.2\%$ at POST24H.

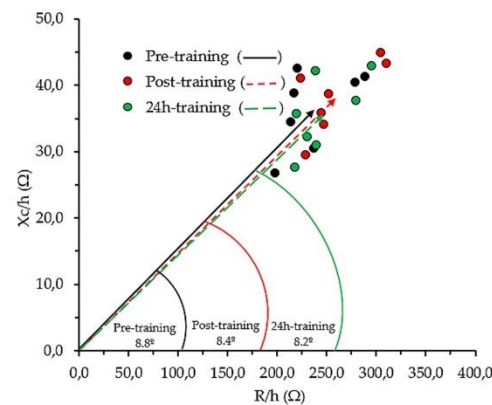


Figure 3. Displacement (PRE-POST-POST24H) bioimpedance global vector.

No significant changes were recorded in R/h in the localized analysis of the rectus femoris muscle. On the other hand, significant changes occurred in Xc/h in each of the three control points used in this study (PRE-to-POST: $38.47 \pm 44.7\%$; POST-to-POST24H: $-30.8 \pm 24.6\%$; PRE-to-POST24H: $-12.4 \pm 12.3\%$) (Figure 4). In the case of PhA, statistically significant differences were recorded between PRE and POST ($48.1 \pm 49.2\%$).

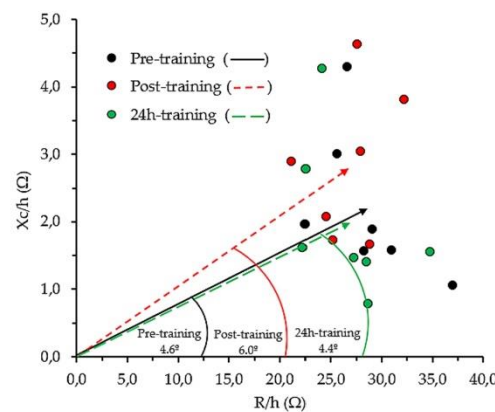


Figure 4. Displacement (PRE-POST-POST24H) rectus femoris localized bioimpedance vector.

Table 2. Anthropometric, bioelectrical (whole-body and localized), and CK values of the training session from the seven players analyzed PRE, POST and POST24H.

ANTHROPOMETRIC													
	PRE		POST		POST24H		Δ PRE-to-POST (%)		<i>p</i>	Δ POST-to-POST24H (%)		<i>p</i>	<i>p</i>
	WB	LOC	WB	LOC	WB	LOC	WB	LOC		WB	LOC		
BM (kg)	79 ± 9.8		78.1 ± 9.7		79 ± 9.9		−1.1 ± 0.6 *		0.02	1.2 ± 0.8 *		0.02	0.73
P thigh (cm)	59 ± 4.4		59.9 ± 4.4		59.1 ± 4.5		1.5 ± 0.4 *		0.02	−1.4 ± 0.6 *		0.02	0.39
BIOELECTRICAL													
	PRE		POST		POST24H		Δ PRE-to-POST (%)		<i>p</i>	Δ POST-to-POST24H (%)		<i>p</i>	<i>p</i>
	WB	LOC	WB	LOC	WB	LOC	WB	LOC		WB	LOC		
R (Ω)	417.5 ± 48	50.7 ± 8.2	457.2 ± 49.2	47.4 ± 6.3	434.8 ± 43	47.6 ± 7.6	9.7 ± 5.8 *	−5.3 ± 13	0.02	−4.8 ± 3.1 *	0.5 ± 10.5	0.02	1
R/h (Ω/m)	236.8 ± 34.5	28.6 ± 4.6	259.1 ± 34.7	26.8 ± 3.5	246.3 ± 30	26.9 ± 4.4	6.1 ± 12.3	38.7 ± 44.7 *	0.24	−6.2 ± 13.8	−30.8 ± 24.6 *	0.24	0.02
Xc (Ω)	64.1 ± 10.1	3.9 ± 2.1	67.4 ± 8.9	5 ± 2	62.8 ± 9.7	3.5 ± 2.2	6.1 ± 12.3	38.7 ± 44.7 *	0.24	−6.2 ± 13.8	−30.8 ± 24.6 *	0.24	0.02
Xc/h (Ω/m)	36.3 ± 6	2.2 ± 1.1	38.1 ± 5.4	2.8 ± 1.1	35.5 ± 5.7	2 ± 1.2	9.6 ± 5.7 *	−5.1 ± 13	0.02	−4.8 ± 3.2 *	0.3 ± 10.4	0.02	1
Z (m)	422.5 ± 48.1	50.9 ± 8.2	462.2 ± 49.5	47.7 ± 6.4	439.3 ± 43.4	47.8 ± 7.5	−2.9 ± 12.8	48.1 ± 49.2 *	0.55	−1.6 ± 13.5	−29.5 ± 29.1	0.24	0.06
Z /h (Ω/m)	239.6 ± 34.6	28.7 ± 4.5	261.9 ± 34.8	27 ± 3.5	248.9 ± 30.3	27 ± 4.3	−0.7	−		−	−		1
PhA (°)	8.8 ± 1.3	4.6 ± 2.6	8.4 ± 1	6 ± 2.2	8.2 ± 1	4.4 ± 3	−0.7	−		−	−		1
r(R/h vs. Xc/h)	0.61	−0.79	0.57	0.23	0.68	−0.7	−	−		−	−		1
HEMATOLOGICAL													
	PRE		POST		POST24H		Δ PRE-to-POST (%)		<i>p</i>	Δ POST-to-POST24H (%)		<i>p</i>	<i>p</i>
	WB	LOC	WB	LOC	WB	LOC	WB	LOC		WB	LOC		
CK (IU/L)	193.3 ± 75.5		283.6 ± 84.3		381.3 ± 147.7		53.4 ± 29.8 *		0.02	34.9 ± 29.5 *		0.02	0.02

Values are mean ± SD; BM, body mass; CK, creatine kinase; LOC, localized; P, perimeter; PhA, phase angle; POST, assessment after finishing the training session; POST24H, assessment 24 h after finishing the training session; PRE, measurements before starting the training session; R, resistance; R/h, resistance adjusted by height; r, Spearman correlation coefficient; WB, whole-body; Xc, reactance; Xc/h, reactance adjusted by height; |Z|, impedance module; |Z|/h, impedance module adjusted by height; * *p* statistical significance, *p* ≤ 0.05.

The bioimpedance vector analysis, analyzed together ($\frac{R/h}{X_c/h}$), showed a significant vector shift in the whole-body analysis in both PRE-to-POST ($T^2 = 25.7$; $p = 0.0001$) and PRE-to-POST24H ($T^2 = 14.8$; $p = 0.0001$), but not from POST-to-POST24H ($T^2 = 12.7$; $p = 0.1$) (Figure 3). Non-significant rectus femoris muscle-localized displacement vectors were recorded (PRE-to-POST: $T^2 = 6.7$, $p = 0.2$; PRE-to-POST24h: $T^2 = 7.3$, $p = 0.1$; POST-to-POST24H: $T^2 = 14.8$, $p = 0.1$) (Figure 4).

The graphical bioelectrical whole-body adaptations are represented by the BIVA score graph from PRE-to-POST (Figure 5a), from POST-to-POST24H (Figure 5b), and from PRE-to-POST24H (Figure 5c).

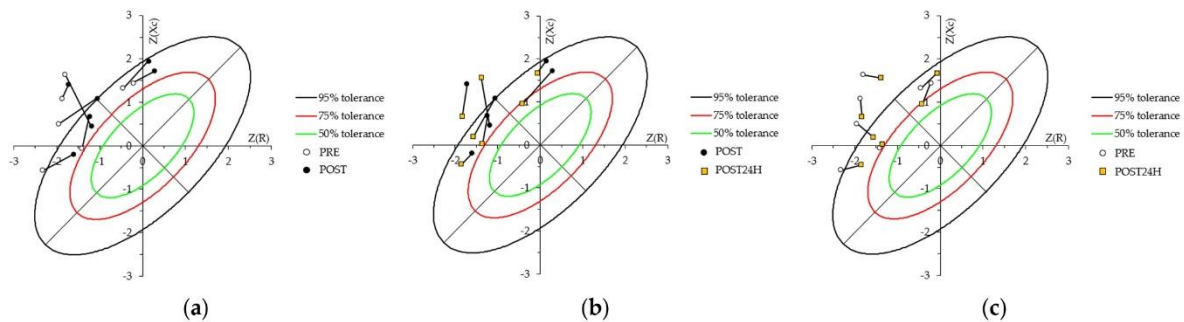


Figure 5. Individual global vector score values for the RXc score graph with 50%, 75%, and 95% tolerance ellipses of the male healthy reference population [40] plotted for: (a) PRE-to-POST, (b) POST-to-POST24H, and (c) PRE-to-POST24H. Z(R), resistance Z score; Z(Xc), reactance Z score.

CK significantly increased from PRE-to-POST ($53.4 \pm 29.8\%$), from POST-to-POST24H ($34.9 \pm 29.5\%$), and from PRE-to-POST24H ($109.2 \pm 76.4\%$). Furthermore, CK was correlated with R/h localized at the rectus femoris muscle in all the cases (Figure 6): PRE-to-POST: $r = 0.75$, $p = 0.05$; PRE-to-POST24H: $r = 0.81$, $p = 0.03$; POST-to-POST24H: $r = 0.82$, $p = 0.02$. No correlation was found between X_c/h , Z/h , or PhA with respect to CK.

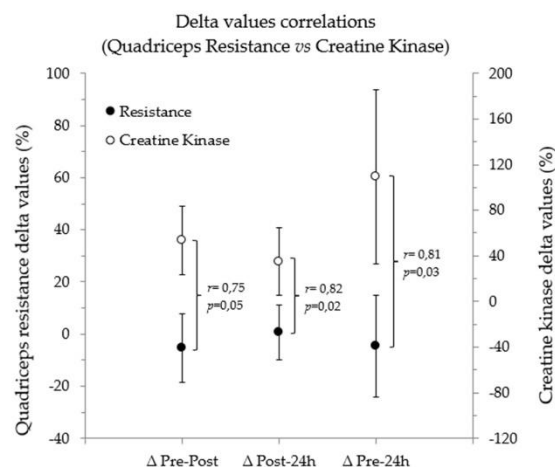


Figure 6. Delta value correlations of rectus femoris-localized BIVA (adjusted by height) and creatine kinase.

4. Discussion

The present study showed that professional rink hockey players experienced a significant vector displacement after an intense training session and during the recovery period due to physiological adaptations, that was detected by whole-body and localized BIVA.

The changes produced in the bioimpedance vector were different for the whole-body analysis than for the rectus femoris localized analysis. While the whole-body BIVA provides more focused information on hydration status, muscle-localized BIVA seems to be more appropriate for the analysis of cellular integrity.

In addition, we reported a correlation between the variation of the localized resistance in the rectus femoris muscle with the variation of CK, which is considered as a muscle damage marker. This novel correlation could help trainers to assess an optimal training load without the need to extract blood to obtain internal information.

4.1. Bioelectrical Patterns in the Rink Hockey Players

The BIVA score graph (Figure 5) showed that the hockey players' vector fell mostly outside the 75% tolerance ellipse in all three checkpoints and occupied a position more to the left of the major axis compared with the reference population. This location in the graph indicated a higher density of body cell mass than the reference population. Furthermore, the location of the subjects outside the 75% tolerance ellipse could mean an abnormal hydration state; however, no analysis was carried out before, during, or after the intervention to check the actual state of hydration. Castizo et al. [41] also plotted a group of triathletes outside the 50% of the tolerance ellipse in comparison with a healthy adult reference population [40]; however, in this case, urine testing was performed before training that showed the subjects were in a normal state of hydration. These results could indicate that the distribution of body fluids in athletes differs from that of the reference population, for example, through a higher concentration of intracellular water [42] due to the hypertrophy of muscle fibers [17].

The BIVA mean graph was also shifted to the left and upwards in comparison with the reference population (Figure 2), similar to other studies with athletes of different sports [17,41,43–45]; thus, this fact could provide information about the performance level of the athletes based on the position of the vector.

4.2. Anthropometrical Changes Evoked by Training

As was expected, a significant decrease in BM was recorded pre and post-training ($-1.1 \pm 0.6\%$) due to the overall dehydration induced by the training session, despite the fact that players were hydrating ($+1.7 \pm 0.4\text{ l}$) during the training session. The record of the BM loss was similar to that of other team sports [46].

The changes of hydration status may suppose variations in the RXc score graph, since the fluid loss leads the body to become less conductive; therefore, a greater resistance to the electrical current it is to be expected [47,48]. However, single bioelectrical assessments obtained by BIVA do not currently allow one to identify the type and magnitude of fluid loss, probably because the range of “normal hydration” comprised by the ellipses is wider than a hydration status/change considered as “dehydration” through other methodologies [41,48]. Therefore, the appropriate analysis of the hydration status in athletes with BIVA should be through the analysis of the vector length changes with a series of assessments. Twenty-four hours after the training session was completed, the players returned to their initial BM values.

There was a significant increase of $1.5 \pm 0.4\%$ in the perimeter of the thigh at the end of the training, due to the alteration of the intracellular and extracellular water balance induced by increased vascular permeability that produced a muscle swelling [49]. Similar to BM, the perimeter of the thigh returned to the initial values at POST24H.

4.3. Whole-Body Bioelectrical Changes Evoked by Training

Several studies analyzing whole-body BIVA show same impedance patterns as in the present study, reporting an increase in R/h and Xc/h parameters, as well as a vector migration along the major axis induced by short-term physical exercise [41,43,48,50]. The increase in R/h can be easily explained by the body fluid loss previously mentioned,

since R is the opposition of the conductor to the current flow [41]. The loss of body fluid triggers a decrease in BM and a decrease in the percentage of total amount of body water.

Contrary to R/h, the changes of Xc/h were not significant, although a similar tendency of the kinetic of this parameter to other studies was clearly seen [41,43], increasing at POST and decreasing at the recovery period (POST24H). However, the kinetic of Xc is not as easy to explain, and Castizo-Olier and Carrasco-Marginet in the book of Marini and Toselli [51] indicated that the variation of this parameter may be produced as a consequence of two different but related cellular adaptations. The first is fluid shifts between intracellular and extracellular compartments. Regarding this topic, Gatterer et al. [50] observed that changes in Xc/h were negatively related to plasma osmolarity changes; therefore, lower intracellular fluid losses mean greater plasma osmolality increases. The second is modifications in cell size affecting dielectric mass (cell membrane and tissue interfaces), as physical exercise generates acute processes that modify the characteristics of muscle cells, as the cell membrane becomes thinner, the cell swells, and capacitance increases, and the opposite happens as the cell shrinks, thus affecting Xc [41]. However, this kinetic needs to be studied further since multiple factors may affect Xc changes after performing exercise [13] and, as we previously mentioned, BIVA at 50 kHz does not penetrate inside the membrane cell and non-intracellular information is obtained [41]. Furthermore, as shown in Figure 5, six of the subjects reported the same pattern throughout the intervention; however, there was one subject for which the Xc/h kinetic was different from the rest at POST. There is not enough information to determine what happened to this specific subject, since he followed the same protocol and the same training session as the rest of the analyzed subjects.

A significant increase in the vector length ($9.6 \pm 5.7\%$) was observed, which, according to the body weight change, confirmed a loss of fluid since the length of the vector is inversely related to total body water (TBW) [32]. In fact, Campa et al. [52] reported that subjects who realized a cycling test to exhaustion on a cycle ergometer without dehydration through drinking during the training did not have a significantly altered vector length, as opposed to those who did not drink and became dehydrated. Furthermore, the cyclists in a state of dehydration had a worse performance, since hypohydration states attenuate strength by approximately 2% and power by approximately 3% [53].

At POST24, R/h significantly decreased by $4.8 \pm 3.1\%$, a fact that, in conjunction with the recovery of body weight and the shortening of the length vector ($-4.8 \pm 3.2\%$), indicated that the players were not dehydrated anymore and had recovered the fluid lost during training. Still, there were no significant changes regarding Xc/h at POST24H. However, regarding PRE, the direction of the vector changed since there was a significant difference between the PRE and POST24H PhA; hence, it was supposed that the ICW/ECW ratio had changed. In the sports field, a decrease in PhA can mean conditions of fatigue [54] or cell damage [20–24], thus this information could help physical trainers to assess the workload in their players.

4.4. Localized Bioelectrical Changes Evoked by Training

There were important pattern differences in the impedance vector changes analyzed in the rectus femoris muscle compared to those of the whole-body. To date, the studies analyzing muscle-localized BIVA short-term changes are completely different from the present study, and they analyze the impedance behavior in the biceps brachii during a fatiguing protocol exercise with resistances [55–58]; thus, there are no localized BIVA assessment studies focused on short-term adaptations in team sports training. This matter is important since the nature of the exercise is different and the muscle impedance adaptations can also be different; therefore, this should be considered when making comparisons.

According to muscle-localized BIVA studies, the kinetic of R is expected to decrease at the end of the training. Freeborn et al. [58] attributes three main reasons to explain this phenomenon: an increase in muscle blood flow, an increase in vasodilation, and an increase in muscular electrical conductivity due to an increase in metabolites. In this study, an increase in R/h was also reported; however, these differences were not significant

despite the fact that a clear tendency was observed. Another matter of interest was the possible correlation between R/h and muscle swelling, since muscle swelling is mainly due to fluid accumulation in the region and reduces the tissue resistance [57]. Freeborn et al. [58] reported significant impedance adaptations, both in R and Xc, at the maximum muscle swelling post-training. In the present study, such a correlation was not found, since non-significant adaptations were registered for localized R/h. A possible explanation for this lack of significance was that the fluid accumulation in the thigh was not as pronounced as that produced by resistance training. Additionally, a slight dehydration of the entire body renders R/h as a less appropriate parameter for evaluating the adaptations of this type of training exercise.

The Xc/h PRE-to-POST changes reported a significant increase ($38.7 \pm 44.7\%$), which was surprising since these results do not match with most previous localized-muscle studies [56–58]. The kinetic of Xc has yet to be clarified; however, it seems clear that is related to cell integrity, as it is not only altered as a consequence of muscle damage produced by strenuous exercise, but also when an injury occurs. In this regard, several studies show that the more severe the muscle injury is, the greater the reduction in the reactance component, which returns progressively to its pre-injury values while the subject recovers from the injury [20–24]. At POST24H, Xc/h significantly decreased beyond PRE-values, which could imply that muscles were not yet fully recovered. The characteristics of the sample, the type of training load, the assessment conditions, and the total workout time could have influenced these values. A possible hypothesis for this fact is related to cell adaptations, since after the dehydration produced by exercise, the main effect of losing extracellular fluid is to expel water from the interior of the cell such that the membrane thickens and the capacitance decreases, thus increasing Xc. In contrary to the aforementioned localized BIVA studies on biceps [55–58], there were no dehydration processes and the type of exercise promoted the accumulation of fluids in the exercised muscles, and the reverse mechanism could have occurred, hence the increasing Xc. This hypothesis cannot be demonstrated in the present paper as we lack the information on the adaptations occurring at intracellular level; thus, future studies could be targeted to this topic. Li et al. [59] analyzed the impedance impact of botulinum toxin injections on spastic muscles and reported a significant decrease in resistance, while no change was reported for reactance. This finding may suggest that localized muscle fluids do not necessarily modify reactance as occurs with resistance, and it is actually the biological tissue modifications (due to an injury or training) that cause this response.

The length of the vector decreased non-significantly, which means that the hydration status at thigh did not experience any great modification—at the extracellular level at least—as was expected since R/h also did not significantly change. However, the direction of the vector did change since PhA significantly increased by $48.1 \pm 49.2\%$ at POST. Regarding POST24H, there was a clear tendency of decrease; however, it did not become significant. The possible explanations for the behavior at POST were not consistent with the current knowledge and further study should be carried out. PhA in the sport field has become a promising tool to assess muscle quality and quantity, since an increase in PhA is associated with more active or younger subjects [51]; however, none of these studies analyze PhA through ML-BIVA after a sport team training, and the only study that analyze PhA through muscle-localized bioimpedance reported a significant reduction in PhA after a fatiguing exercise of different sets of bicep curls with repetition until task failure [57], thus neither the type of training nor the results are similar. These authors declared that monitoring R and Xc independently could provide more insights in relation to exercise.

4.5. Relationship between BIA Vector Changes and CK

As shown in Figure 6, there was a high correlation between the kinetic of the CK and the kinetic of muscle-localized R/h, which opens a new line of pioneering research in sports science.

Regarding CK, not only was there a significant increment at POST ($53.4 \pm 29\%$), but also at POST24H compared to POST ($34.9 \pm 29.5\%$) and to PRE ($109.2 \pm 76.4\%$), which would indicate that the muscles were not fully recovered and that the levels of CK were still increasing during the 24 h after the training ended, as seen in other studies [7].

A possible explanation for this correlation may be that the intracellular water is expelled from the inside of the cell due to the muscle damage caused by training, which would cause an increase in extracellular water and a reduction in the localized resistance, while the levels of CK in the blood increase during the high-intensity exercise.

However, it is important to be cautious with this correlation since, as was previously mentioned, the muscle-localized R/h changes were not significant. Therefore, we must ask ourselves to what extent the correlation between a significant change and a non-significant change is reliable. It could be possible in the case of athletes who were more sensitive to CK than R/h adaptations.

No correlation with localized reactance was obtained, which is surprising considering it is a parameter related to cell integrity that is affected by muscle damage and it would have made more sense to have a relation to CK rather than localized resistance.

The lack of studies published in the scientific literature that apply BIVA to assess changes in muscle hydration and CK induced by a training session prevent the present findings from being currently reliable in practice.

5. Limitations of the Study

At the time of assessing BIVA, some factors should be controlled in order to prevent measurement errors and provide accurate and reliable results [13]. This study attempted to control all these factors respecting the ecological design, which also implies certain limitations. For example, the players followed individual uncontrolled POST-to-POST24H recovery strategies (i.e., nutrition, hydration, physical activity, environmental conditions, etc.).

As BIVA at 50 kHz cannot penetrate inside the cell, we did not obtain any intracellular information; thus, some of the hypotheses presented in this study cannot be confirmed by this technique. However, this limitation may open up new lines of research in the future.

A 15-graded RPE scale was used instead of a category-ratio 10-scale (CR10), which could be a limitation as CR10 is commonly adopted for intermittent sports, such as rink hockey, and it might have been more suitable.

The last assessment was made 24 h after finishing the training, and it would have been more appropriate to have made a further assessment at 48 or 72 h, since CK may have still been rising when the last assessment was made.

Another limitation was the fact that the sample size was scarce, which implies the possibility of some bias presentation in the reported results.

Finally, there was also a lack of studies with which to compare the sample regarding rectus femoris muscle-localized assessments. Therefore, our results could not be compared with a reference population since there are no existing data, and the studies cited here to compare our results to used other types of BIVA protocols.

6. Practical Applications and Future Perspectives

The present study opened up some hypotheses that could not be answered using this method. For example, what causes the kinetic of X_c/h after a team sports training session, or why it seems that CK is correlated with R/h and not with X_c/h .

Despite these hypotheses, and more focused on the real applicability of BIVA in the sports field, we observed how the patterns of change in the whole-body analysis and in the muscle-localized analysis were completely different. On the one hand, whole-body BIVA is especially sensitive to changes in body hydration, which is important in high performance since a significant loss of body water means lower performance. On the other hand, the strength of ML-BIVA lies in the information that it provides on muscle cellular health and integrity, although the kinetics of some parameters are unclear and need to be further studied. The correlation between R/h and CK could help physical trainers to

control training load, for example, by establishing thresholds that could determine the intra-session intensity.

Regarding whole-body BIVA protocols, the foot-to-hand tetrapolar technique at 50 kHz is commonly used. However, with muscle-localized BIVA it is not clear where the electrodes should be placed on each muscle, and it is imperative to standardize a protocol. For this reason, we propose to follow the SENIAM project guidelines [39], that were designed initially for surface electromyography but can also be used in ML-BIVA.

In the present study, we only analyzed the bioelectrical values of the right thigh; however, it may also be interesting for future studies to address the possibility of analyzing both thighs and checking the possible differences between them, such that ML-BIVA could also become a method to assess asymmetries, as was shown by Stagi et al. [60] with segmental specific BIVA.

Finally, the muscle-localized data were normalized by the subject's height, because this is the predominant method in the current ML-BIVA studies; however, we believe that normalizing by muscle length would be more appropriate, as was executed by Mascherini et al. [61].

7. Conclusions

Despite the need for further research, the bioimpedance vector analysis based on variables of direct measurement, such as the resistance, reactance, or phase angle, emerges as a sensitive method to discriminate the adaptations in body water and cellular integrity induced by a high-intensity training session in professional rink hockey players.

Whole-body BIVA is more sensitive to body composition changes, while ML-BIVA may be a good method to control training load due to the correlation reported between CK and the significant X_c/h changes at all checkpoints.

The presented results suggest that BIVA may be a powerful tool in the exercise and sports field and could help trainers to optimize training and obtain information about body status and the body adaptations that are produced by exercise.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/app12020751/s1>, Details of the high-intensity training session.

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Article

Bioelectrical, Anthropometric, and Hematological Analysis to Assess Body Fluids and Muscle Changes in Elite Cyclists during the Giro d'Italia

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Simple Summary: Giro d'Italia is one of the most demanding races in cycling tour, in which, during the 3 weeks of competition, several physiological adaptations occur. This study aimed to analyze these changes with different methods (bioelectrical, anthropometrical, and hematological analysis) in order to see how body fluids vary in the whole body as the competition progresses, and also in three specific muscle groups (quadriceps, hamstrings, and calves). There were three checkpoint assessments: at the beginning, middle, and end of the race. Results indicated that bioelectrical impedance vector analysis is sensitive to the body fluid changes. A proper homeostatic adaptation was detected during the first half of competition, but the long-term, high intensity exercise would result in profound fluid loss and distribution in the soft tissues. Furthermore, most of these changes happened in the extracellular water compartment, indicating that the state of the cell membranes was maintained in good condition. The calves are the muscle group most sensitive to this analysis. In conclusion, bioelectrical impedance vector analysis is a reliable, non-invasive, and practical method to assess physiological adaptations in athletes.

Abstract: This study aimed to characterize and monitor the body fluid and muscle changes during the Giro d'Italia in nine elite cyclists via bioelectrical (whole-body and muscle-localized) anthropometric and hematological analysis. There were three checkpoint assessments: at the beginning, middle, and end of the race. The Wilcoxon signed-rank test was used to compare the data at baseline and follow up. The Spearman correlation was used to explore relationships between variables. Hotelling's T^2 test was used to determine bioelectrical differences in the complex vector. Bodyweight did not change during the competition, despite bioelectrical and hematological data indicating that at the first half of the race, there was a fluid gain, and in the second half a fluid loss occurred, reaching baseline values. These changes were especially prevalent in the extracellular water compartment. Significant correlations between whole-body bioelectrical vector changes and red blood cell parameter changes were reported. The muscle group most sensitive to changes were the calves. Quadriceps, hamstrings, and calves reported a PhA decrease trend during the first half of the race, and an increase during the second half. Bioelectrical impedance vector analysis appears to be sensitive enough to detect hydration and cellular integrity adaptations induced by competitions as demanding as the Giro d'Italia.

Keywords: BIVA; cycling; phase angle; sport; plasma volume

1. Introduction

In professional cycling, “Tour de France”, “Giro d’Italia”, and “Vuelta a España” are the most prestigious and demanding multistage races included in the International Cycling Union (UCI) World Tour. In these events, the cyclists ride a long distance of ~3500–4500 km over 3 weeks in 21–23 daily stages of different types.

In the current literature, there are different reports available showing the physiological changes produced by the high demands of these races [1–4]. However, knowledge is still sparse about the extent of potential dehydration due to prolonged strenuous cycling and its hematological acute effects [5]. The difficulty of systematically intervening with invasive tests in a professional group of cyclists during the 3 weeks of hard and strenuous competition could be one of the main reasons for the lack of data availability.

In this context, bioelectrical impedance vector analysis (BIVA) emerges as a method to assess body hydration and cellular status [6,7]. The classic BIVA approach works with the same basis of bioelectrical impedance analysis (BIA), but instead of using predictive equations to obtain quantitative data, it relies on the analysis of raw bioelectrical values (resistance, R ; reactance, X_c) standardized by conductor length. From these parameters, the derived impedance (Z) and the phase angle (PhA) can also be obtained, representing the vector length and vector direction, respectively [8]. According to classic BIVA, Z is inversely related to total body water (TBW) [6], whereas PhA is considered an indicator of cellular health and cell membrane integrity and is inversely related to the extracellular/intracellular water (ECW/ICW) ratio [9]. All interpretations should be based on the interpretation of Z and PhA jointly, along with the vector position on the Resistance-Reactance (RX_c) graphs [10]. BIVA can be performed through different protocols, depending on the device and the distribution of the electrodes [6]: whole-body BIVA (WB-BIVA) may be used to assess the composition of the entire body, whereas muscle-localized BIVA (ML-BIVA) may be used to assess the composition or status of one specific muscle or portion of muscle. In the sports field, some research has performed using both WB-BIVA and ML-BIVA to assess hydration changes and the muscle impact after a training session or competition of different sports [6,8,11].

In some studies made with elite cyclists during the Grand Tour competitions, a vector length shortening without changes in the direction has been reported, indicating a body fluid gain with the extracellular/intracellular water ratio remaining constant at the end of the race [2,3]. Another study made with segmental BIVA, which is an alternative method analyzing body limbs separately, reported that the body compartment most stressed by cycling races was the legs, showing a significant decrease in the PhA [12] and, therefore, an increase in the ECW/ICW ratio at the thighs.

Despite the advantages that BIVA presents as a method to assess hydration, there are other methods available, such as the haematological and serum parameter analysis, which has greater precision and accuracy than BIVA, but which is also invasive, costly, and demanding in terms of time. Regarding this method, Corsetti et al. [13] analysed some haematological parameters during the Giro d’Italia 2011 in order to define the variations across the stage race. They found that the hemoglobin (Hb) and the hematocrit (Hct) decreased during the first half of the race (indicating body fluid gain) with a stabilization in the second half due to an hemodilution typical of endurance sports. Therefore, some similarities can be observed between the interpretations of the hematological changes in relation to the bioelectrical changes reported in cycling grand events. This relationship could better identify changes in body fluid distribution and composition associated with adaptations after exercise and sport. Monitoring hydration status is thereby important for better sport performance, especially in endurance sports [14].

This study aimed to analyze body fluid and muscle changes evoked by the Giro d’Italia stage race by using bioelectrical (whole-body and muscle-localized) anthropometrical and hematological assessments. We hypothesized that both WB-BIVA and ML-BIVA are sensitive enough to detect subtle chronic fluid variations that occur in the body due to this

extremely long and demanding race. We also compared the BIVA characteristics of these elite professional cyclists with a healthy adult male Italian reference population [15].

2. Materials and Methods

2.1. Subjects

This prospective, quasi-experimental study involved 9 professional cyclists (age: 27.9 ± 2.4 years; height (H): 181.4 ± 6.1 cm; body mass (BM): 70.5 ± 6.1 kg; body mass index (BMI): 21.4 ± 0.4 kg/m²) from the Cannondale Pro-Cycling Team who participated in the 2013 Giro d'Italia, featured in Table 1. They were all in good health, as assessed by their team physician. No drugs or supplements influencing fluid balance were taken by athletes; only non-steroidal anti-inflammatory drugs and antibiotics were administered when needed, according to the “no needle” policy; and, lastly, therapies and drugs were allowed only for evident illnesses. Official urine and blood controls were performed by anti-doping agencies before the start of the race (day −3) and during the final phase (day 20), and one subject had an additional check on day 5. The study was approved by the Ethics Committee for Clinical Sport Research of Catalonia (Ethical Approval Code: 006/CE-ICGC/2023) and written informed consent was obtained from each cyclist prior to their participation. All the procedures were in accordance with the Declaration of Helsinki [16].

Table 1. Main features of the Giro d'Italia 2013 route.

Day	Stage (#)	Length (km)	Accumulated Slope (m)	Profile
−1 *	−	−	−	−
1	1	130.0	1274	F
2	2	17.4	329	Team TT
3	3	222.0	3308	MM
4	4	246.0	2528	MM
5	5	203.0	981	F
6	6	169.0	399	F
7	7	177.0	3664	MM
8	8	54.8	889	Individual TT
9	9	170.0	4786	MM
10 *	Rest	−	−	−
11	10	167.0	4183	HM
12	11	182.0	4235	MM
13	12	134.0	1411	F
14	13	254.0	2390	F
15	14	180.0	2790	HM
16	Rest	−	−	−
17	15	145.0	7095	HM
18	16	238.0	5798	MM
19	17	214.0	1136	F
20	18	20.6	1045	Individual TT
21	19	−	−	−
	(Cancelled)			
22	20	210.0	3354	HM
23 *	21	206.0	575	F
Total	21	3455.0	52,170	
Mean		164.5	2609	

F, flat; MM, medium mountain; HM, high mountain; TT, time trial; *, measurement days.

2.2. Experimental Design

The multistage race took place from 4 to 26 May 2013. The route’s main features are reported in Table 1. The mean completion time for the 9 participants was 86:47:29 h (range, 84:12:10–88:15:51 h) with a mean speed of 35.7 km/h.

As shown in Figure 1, bioelectrical, anthropometric, and hematological measurements were taken a day before the start (PRE), on the first resting day (MID), and on the final day

(POST). All measurements were performed between 7am and 9am in a fasted condition. There were 2 rest days (days 10 and 16) plus the cancelled stage of day 21.

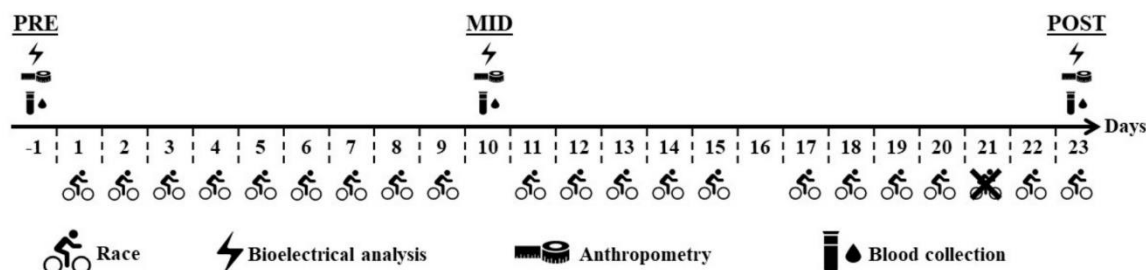


Figure 1. Graphical design of the study. PRE, first assessment one day before the start of the race; MID, second assessment at the middle of the race; POST, last assessment at the end of the race.

2.3. Bioelectrical Assessment

R and X_c were measured using a phase-sensitive multifrequency impedance plethysmograph (DS Medica, Human-Im PLUS Impedance meter unit 80C32, Milano, Italy) that emits an alternating sinusoidal electric current of 1 mA at 5 operating frequencies within the range of 5.5, 10, 50, 100, and 300 kHz, and PhA at 10, 50, and 100 kHz, continuously autocalibrated every 5 min with a known impedance provided by the manufacturer. The 50 kHz frequency was selected because of its best signal to noise ratio [7]. Bioelectrical variables were obtained by the same trained examiners in a thermo-neutral room (25 °C; ± 40 relative humidity), as recorded by a portable weather station (Kestrel Weather K4500, Nielsen-Kellerman, Boothwyn, Pennsylvania, PA, USA).

Subjects were tested with their arms and legs kept from touching the body by non-conductor foam objects to prevent adduction or the crossing of the limbs. Bioelectrical measurements were recorded after a stabilization period of 5 min, in which the cyclists remained lying motionless to ensure the proper distribution of body fluids. Three measurements were performed every 60 s and the average value was used for the final calculations. Before placing the electrodes, the skin was prepared by shaving the area to remove hair, and rubbing the area with gel and cleaning it with alcohol.

To analyze WB-BIVA, injector electrodes were placed on the dorsal surface of the right hand (proximal to the third metacarpal-phalangeal joint) and foot (proximal to the third metatarsal-phalangeal joint). The detector electrodes were placed proximally 5 cm from the injector ones in order to prevent interaction between electric fields, which could lead to an overestimation of the impedance values.

ML-BIVA was performed in both limbs. Once the symmetry between limbs was verified, the mean value of all parameters was calculated for each muscle analyzed. In the quadriceps, injector electrodes were placed at 5 cm from the anterior superior iliac spine and from the superior pole of the patella, and the detector electrodes were spaced 5 cm from the injector ones, proximal to the center of the muscle. In the hamstrings, injector electrodes were placed 5 cm from the ischial tuberosity and from the popliteal line, and the detector electrodes were spaced 5 cm from the injector ones, proximal to the center of the muscle. In calves, injector electrodes were placed 5 cm from the popliteal line and from the intermalleolar line, and the detector electrodes were spaced 5 cm from the injector ones, proximal to the center of the muscle.

Z was calculated as $\sqrt{(R^2 + X_c^2)}$, and PhA as $\tan^{-1} (X_c/R \cdot 180^\circ/\pi)$. R, X_c and Z were adjusted by height (R/H, X_c/H , Z/H). TBW, ICW and ECW were estimated by the athlete-specific prediction models proposed by Matias et al. [17] on the basis of height, BM, R, X_c and sex. RXc Z-score graph was used to plot the cyclists on the three checkpoints (PRE, MID, POST) regarding the 50%, 75%, and 95% tolerance ellipses for the general Italian healthy adult male population [15]. In accordance with the classic BIVA, the reference population selected may be divided into 4 quadrants: upper left quadrant representing athletes,

lower left quadrant representing obese individuals, upper right quadrant representing lean individuals, and lower right quadrant representing cachectic individuals. Furthermore, RXc paired graphs were used to compare bioelectrical differences over time and inter-limb symmetry. In these graphs, ellipses overlapping the origin indicate no differences, whereas non-centered ellipses indicate significant changes.

2.4. Anthropometric Assessment

The anthropometric measurements were taken according to the standard criteria of the International Society for the Advancement of Kinanthropometry (ISAK) [18]. The following anthropometric material was used: a telescopic measuring rod (Seca 220[®], Birmingham, UK, measuring range: 85–200 cm; accuracy: 1 mm) to measure the height; a scale (Seca 710[®]; Birmingham, UK; previously calibrated capacity: 200 kg; accuracy: 50 g) to measure BM; and an anthropometric tape (Lufkin Executive[®], Lufkin, TX, USA, accuracy 1 mm) to determine the position where electrodes (Red DotTM, 3M Corporate Headquarters, St. Paul, MN, USA) were placed. A waterproof pen was used to mark the anatomical sites for electrodes. BMI was calculated as BM/H^2 (kg/m²).

2.5. Hematological Measurements

The UCI and World Anti-Doping Agency (WADA) rules for sample collection and transportation of specimens were followed. Evacuated tubes (BD Vacutainer Systems, Becton-Dickinson, Franklin Lakes, NJ, USA) were used for hematological tests (BD K₂ EDTA 3.5 mL tubes), while 7 mL plain tubes (BD SSTII Advance) were used for clinical chemistry tests. Immediately after drawing, tubes were inverted 10 times and stored in a sealed box at 4 °C. Controlled temperature was assured during transportation using a specific tag (Libero Ti1, Elpro, Buchs, Switzerland) for temperature measurement and recording. The K₂ EDTA-anticoagulated blood was homogenized for 15 min prior to being analyzed. Plain tubes were centrifuged for 10 min, 1300× g, at 4 °C and the sera was stored at −80 °C until analysis. Clinical chemistry analyses were performed in a single batch and by the same technician. Hematological tests, which included Hb concentration and Hct, were performed on a Sysmex XE 2100 (Sysmex, Kobe, Japan). During the study, the analyzers were regularly calibrated and controlled by both internal and external quality control schemes. The imprecision of hematological tests was <2%. A day-by-day control of imprecision was performed on the Sysmex instrument by using fresh blood during the study, giving an imprecision of <1.6%. Estimated plasma volume (EPV) was calculated on the basis of the BM and Hct, using the Kaplan formula [19]. Biochemical analyses of sodium (Na⁺) and potassium (K⁺) (indirect potentiometry) were performed in an Advia 2400 automatic device (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA), following the International Federation of Clinical Chemistry's Committee on Reference Systems for Enzymes [20]. The osmolality was measured by an osmometer performed in an Osmo Station OM-6050 Arkray from Menarini (based on the decrease in cryoscopic point).

2.6. Statistical Analysis

Descriptive data are presented as mean ± standard deviation. After testing each variable for the normality of the distribution (Shapiro–Wilks test), differences in all variables over time (PRE, MID, and POST) were tested using the non-parametric Friedman test and, in the case of differences, the Wilcoxon signed-rank test. The magnitude of changes was computed as delta percent values (Δ%). Spearman's rank coefficient of correlation was applied to determine possible associations between hematological and bioelectrical delta percent changes. An RXc Z-score graph was used to characterize the sample. RXc paired graphs and paired one-sample Hotelling's T² test were used to identify bioelectrical changes over time and to check the bioelectrical inter-limb symmetry. The significance level was set at $p < 0.05$. SPSS (Chicago, IL, USA, ver. 21) and BIVA software [21] was used for data analysis.

3. Results

The BIVA point graph (Figure 2) indicates that almost all cyclists were between the 50 and 75 percentiles of the tolerance ellipses of the male adult Italian reference population during the three checkpoints. Its vector locations were also shifted to the left and upwards, and, therefore, with a greater PhA and body cell mass, corresponding with the athletic quadrant.

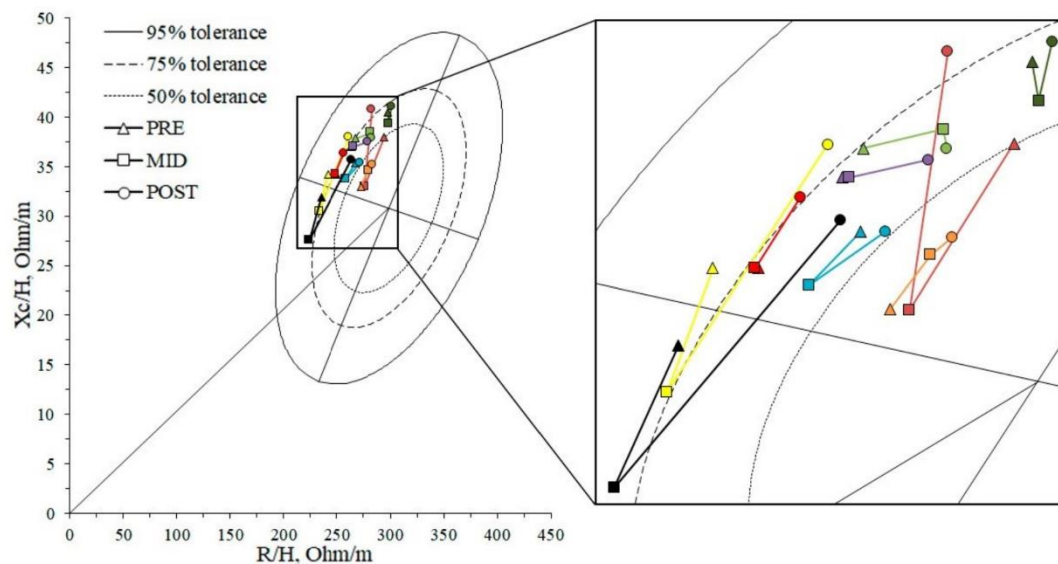


Figure 2. WB-BIVA Z-score point graph. Vector migration subjects over time were plotted with respect to the tolerance ellipse of the male adult Italian reference population [15]. R/H, height-adjusted resistance; Xc/H, height-adjusted reactance.

Several bioelectrical, anthropometric, and hematological changes were reported during and after the race (Table 2). Body fluid changes were not detected by BM changes ($p > 0.4$) nor by the water prediction equations ($p > 0.064$). Whole-body BIVA indicated a non-significant fluid gain during the first half of the race (PRE-MID) by means of a Z/H increase ($-1.1 \pm 3.8\%$, $p = 0.515$), and a significant body fluid loss in the second half of the race (MID-POST) as Z/H considerably increased ($5.5 \pm 6.0\%$, $p = 0.008$). In the entire race (PRE-POST), cyclists experienced a significant Z/H increase ($4.2 \pm 4.6\%$, $p = 0.038$). The predictive ECW/ICW ratio remained constant throughout the race ($p > 0.083$), even though some PhA changes were observed. Regarding the hematological analysis, fluid gain in PRE-MID is significantly indicated by the decrease of Hb and Hct ($p = 0.008$) and the increase of the EPV ($p = 0.011$) and Na^+ ($p = 0.027$). In MID-POST, the increase of Hb ($p = 0.007$) and Hct ($p = 0.015$), as well as the decrease of EPV ($p = 0.05$), indicate a fluid loss. Osmolality progressively increased over the race ($9.3 \pm 10.8\%$; $p = 0.011$), indicating dehydration at the end of the race.

Table 2. Hematological, anthropometric, and bioelectrical changes of elite cyclists during the Giro d'Italia 2013.

				PRE-MID		MID-POST		PRE-POST	
	PRE	MID	POST	Δ (%)	<i>p</i>	Δ (%)	<i>p</i>	Δ (%)	<i>p</i>
Body composition									
BM (kg)	70.5 ± 6.1	70.4 ± 6.2	70.7 ± 5.9	−0.1 ± 1.2	0.574	0.4 ± 1.3	0.400	0.3 ± 1.6	0.499
BMI (kg/m ²)	21.4 ± 0.8	21.4 ± 0.8	21.4 ± 0.7	−0.1 ± 1.2	0.589	0.4 ± 1.3	0.443	0.3 ± 1.6	0.673
TBW (L)	45.9 ± 3.7	46.1 ± 4.0	45.5 ± 3.3	0.3 ± 1.6	0.398	−1.2 ± 2.1	0.075	−1.0 ± 1.5	0.086
ECW (L)	15.3 ± 1.2	15.3 ± 1.2	15.1 ± 1.0	0.3 ± 1.7	0.683	−1.3 ± 2.2	0.105	−1.1 ± 1.6	0.064
ICW (L)	30.7 ± 2.5	30.8 ± 2.8	30.3 ± 2.2	0.3 ± 1.4	0.458	−1.0 ± 1.9	0.090	−0.8 ± 1.4	0.079
ECW/ICW ratio	0.50 ± 0.01	0.50 ± 0.01	0.50 ± 0.00	−0.1 ± 0.3	1.000	0.3 ± 0.3	0.083	0.2 ± 0.3	0.083
Whole-body BIVA									
R/H (Ω/m)	265.5 ± 21.5	262.8 ± 24.4	275.9 ± 13.9	−1.0 ± 3.7	0.515	5.4 ± 5.9	0.008 *	4.2 ± 4.6	0.038
Xc/H (Ω/m)	35.8 ± 2.8	34.3 ± 3.7	37.5 ± 2.2	−4.3 ± 6.8	0.108	10.5 ± 11.8	0.013 *	5.1 ± 4.6	0.018
Z/H (Ω/m)	267.9 ± 21.6	265.0 ± 24.6	278.4 ± 13.9	−1.1 ± 3.8	0.515	5.5 ± 6.0	0.008 *	4.2 ± 4.6	0.038
PhA (°)	7.7 ± 0.4	7.4 ± 0.4	7.8 ± 0.4	−3.4 ± 4.0	0.048 *	4.7 ± 7.7	0.089	1.0 ± 4.9	0.623
Mean quadriceps BIVA									
R/H (Ω/m)	14.7 ± 1.3	15.3 ± 1.3	15.1 ± 2.4	4.6 ± 10.6	0.236	−1.4 ± 13.6	1.000	2.8 ± 15.9	0.624
Xc/H (Ω/m)	5.1 ± 0.5	5.2 ± 0.5	5.4 ± 0.5	1.3 ± 12.2	0.671	5.8 ± 12.5	0.256	6.4 ± 11.2	0.122
Z/H (Ω/m)	15.6 ± 1.4	16.1 ± 1.3	16.0 ± 2.4	4.2 ± 10.4	0.236	−0.6 ± 13.1	0.722	3.2 ± 14.9	0.553
PhA (°)	19.4 ± 1.6	18.7 ± 1.3	20.1 ± 2.0	−3.1 ± 8.4	0.314	7.7 ± 10.3	0.085	4.2 ± 11.9	0.477
Mean hamstrings BIVA									
R/H (Ω/m)	14.5 ± 2.0	14.9 ± 1.1	14.6 ± 1.6	3.9 ± 13.7	0.463	−1.4 ± 9.4	0.766	2.0 ± 13.1	0.675
Xc/H (Ω/m)	4.9 ± 0.4	4.9 ± 0.5	5.0 ± 0.9	−1.1 ± 9.3	0.608	3.3 ± 13.0	0.440	2.3 ± 17.0	0.866
Z/H (Ω/m)	15.3 ± 2.0	15.6 ± 1.1	15.5 ± 1.7	3.3 ± 12.6	0.574	−0.9 ± 9.6	0.953	2.0 ± 13.2	0.722
PhA (°)	18.9 ± 1.9	18.1 ± 2.0	18.9 ± 2.1	−3.9 ± 9.3	0.262	4.2 ± 6.1	0.050 *	0.0 ± 8.8	0.953
Mean calves BIVA									
R/H (Ω/m)	36.2 ± 3.3	33.1 ± 3.3	34.7 ± 3.4	−8.7 ± 3.2	0.008 *	5.0 ± 4.5	0.017 *	−4.2 ± 3.7	0.018 *
Xc/H (Ω/m)	8.6 ± 1.2	7.6 ± 1.2	8.5 ± 1.2	−10.9 ± 7.3	0.007 *	11.5 ± 12.7	0.028 *	−1.1 ± 9.8	0.357
Z/H (Ω/m)	37.2 ± 3.4	33.9 ± 3.4	35.7 ± 3.6	−8.8 ± 3.3	0.008 *	5.3 ± 4.7	0.013 *	−4.1 ± 3.7	0.017 *
PhA (°)	13.3 ± 1.1	13.0 ± 1.2	13.7 ± 1.0	−2.5 ± 5.8	0.286	6.0 ± 9.2	0.097	3.1 ± 8.7	0.498
Hematological									
Hb (g/dL)	14.1 ± 0.7	13.1 ± 0.9	13.6 ± 0.8	−7.3 ± 3.5	0.008 *	4.1 ± 3.0	0.007 *	−3.6 ± 2.6	0.012 *
Hct (%)	42.0 ± 2.3	39.3 ± 2.3	40.6 ± 2.2	−6.4 ± 2.7	0.008 *	3.6 ± 3.5	0.015 *	−3.1 ± 3.3	0.028 *
EPV (mL)	2663.3 ± 280.2	2785.4 ± 309.4	2730.2 ± 257.9	13.1 ± 6.2	0.011 *	−5.9 ± 4.5	0.050 *	6.2 ± 5.4	0.028 *
POsm (mOsm/L)	285.4 ± 6.2	292.2 ± 16.9	312.2 ± 33.1	2.4 ± 5.5	0.362	7.2 ± 13.0	0.155	9.3 ± 10.8	0.011 *
Na ⁺ (mEq/L)	138.7 ± 1.9	141.0 ± 1.4	143.4 ± 6.0	1.7 ± 1.6	0.027 *	1.7 ± 4.0	0.233	3.5 ± 4.7	0.049 *
K ⁺ (mEq/L)	5.1 ± 0.2	4.7 ± 0.3	4.8 ± 0.3	−9.3 ± 5.9	0.012 *	3.2 ± 7.3	0.396	−6.7 ± 5.8	0.017 *

BIVA, bioelectrical impedance vector analysis; BM, body mass; BMI, body mass index; ECW, extracellular water; EPV, estimated plasma volume; Hb, hemoglobin concentration; Hct, hematocrit; ICW, intracellular water; K⁺, plasma potassium concentration; MID, second assessment at the middle of the race; Na⁺, plasma sodium concentration; PhA, phase angle; PRE, first assessment one day before the start of the race; POsm, plasma osmolality; POST, last assessment at the end of the race; R/H, height-adjusted resistance; TBW, total body water; Xc/H, height-adjusted reactance; Z/H, height-adjusted impedance; Δ , delta value (%); * *p* statistical significance, *p* < 0.05.

Whole-body BIVA paired graph (Figure 3A) detected a non-significant trend of fluid gain by a shortening of the vector in PRE-MID ($T^2 = 8.3$, *p* = 0.1), and a fluid loss in MID-POST by a vector lengthening ($T^2 = 10.9$, *p* < 0.001). The resultant PRE-POST vector change was a significant lengthening ($T^2 = 15.6$, *p* < 0.001). As seen in Figure 3B–D, the most sensitive muscle group to vector changes were the calves, with a significant shortening in PRE-MID ($T^2 = 60.4$, *p* < 0.001) and a lengthening in MID-POST ($T^2 = 11$, *p* < 0.001). The resultant vector change was a significant shortening ($T^2 = 12.1$, *p* < 0.001). Quadriceps and hamstrings did not report significant vector migrations. All three muscle groups reported a similar PhA trend, decreasing in PRE-MID (2.5–3.1%) and increasing in MID-POST (4.3–7.7%).

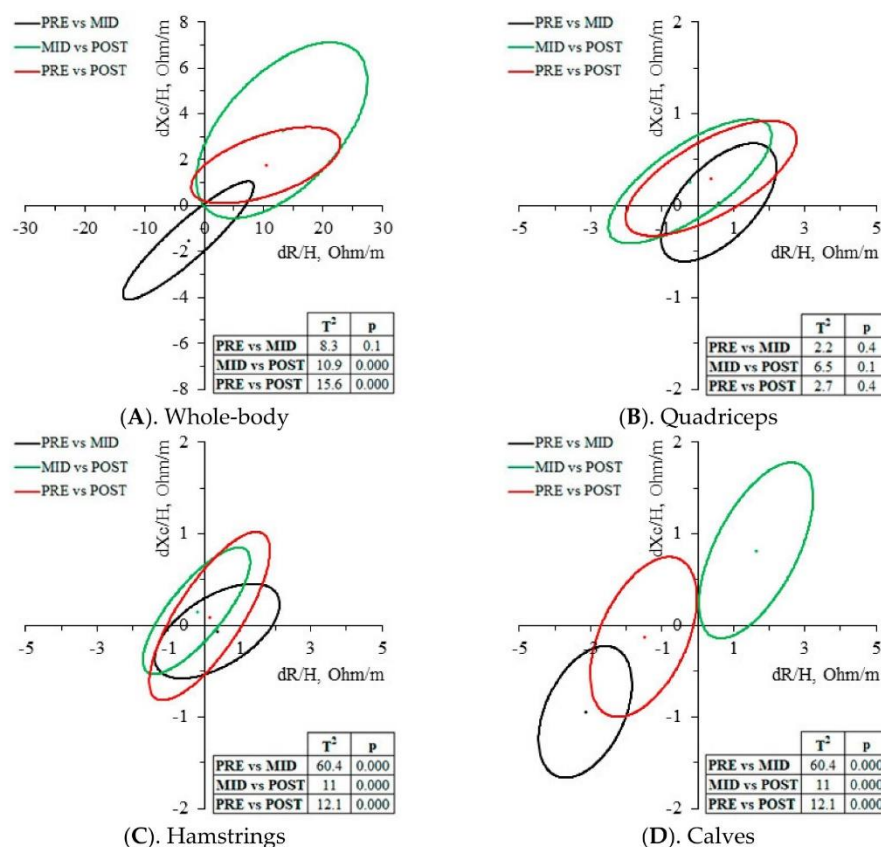


Figure 3. BIVA paired graph. Vector migration across the race. PRE, first assessment at the beginning of the race; MID, second assessment at the middle of the race; POST, last assessment at the end of the race; dR/H , height-adjusted resistance differences; dXc/H , height-adjusted reactance differences. T^2 , Hotelling's T-squared distribution; p statistical significance, $p < 0.05$.

Correlation analysis showed associations between some hydration markers changes with some WB-BIVA parameters changes during the race. Hb changes were correlated with Xc/H changes at PRE-MID ($r = 0.782$, $p = 0.013$) and with R/H , Xc/h , Z/h ($r = 0.711$, $p = 0.032$; $r = 0.833$, $p = 0.005$; $r = 0.711$, $p = 0.032$; respectively) at MID-POST. Hct ($r = 0.733$, $p = 0.025$) and EPV ($r = 0.733$, $p = 0.025$) changes were also correlated with Xc/H changes only at MID-POST.

4. Discussion

The present study highlighted several body changes evoked by the demands of the race, indicating a body fluid gain during the first half and a bigger posterior fluid loss during the second half. These changes were especially seen in the whole-body bioelectrical and hematological analysis that correlate between them, especially with the red blood cell parameters (Hb and Hct) and EPV. BM assessment and predictive equations could not detect hydration changes in the competition.

To our best knowledge, this is the first study to apply ML-BIVA in a group of elite cyclists. Results indicated that the muscle group most sensitive to the hydration changes produced by the race were the calves, with the same bioelectrical trend as the rest of the body.

Results confirmed the hypothesis that both WB-BIVA and ML-BIVA are sensitive to detecting fluid variations, encouraging BIVA as a method to monitor physiological adaptations in sport.

4.1. Athlete's Characterization

A greater PhA of our subjects in comparison with the reference group is reported in Figure 2, reflecting a better cell function [22] and differing fluid distribution [23], as has already been amply reported in athletes from different sports [24–27]. These data agree with the specific body composition of elite athletes (greater ICW/ECW ratio) characterized by a better soft tissue hydration, better cellular membrane exchange capacity, and better physical working capacity than the healthy adult population [28]. In fact, TBW, ICW, and PhA could be good indicators for muscle performance in all age groups [29,30]. Changes in these parameters, but especially in the intracellular water compartment, may alter the power generation capacity [31].

4.2. Whole-Body Changes

Our results did not show a significant decrease in BM both during and at the end of the race, confirming that the energetic balance between energy intake and energy expenditure was achieved. Current evidence establishes that, in the context of athletic performance, it is normal to lose >2% of BM, and this does not cause a clinically relevant reduction in TBW [5]. In fact, it is reported via urine specific gravity analysis that most cyclists of the highest competition level are not well hydrated prior to and also during an important competition [32].

For a better monitoring of hydration, then, it is convenient to use other methods beyond the BM analysis. Only one study analyzed quantitatively TBW in cycling grand tours (by means of BIVA), and reported a non-significant decrease ($0.4 \pm 1.1\%$, $p > 0.05$), but also a possible ICW reduction due to the reduction of body cell mass and PhA [33]. In neither the just mentioned study nor the present one (PRE-MID: $1.2 \pm 2.1\%$, $p = 0.075$; PRE-POST: $1.0 \pm 1.5\%$, $p = 0.086$), there seem to be magnitudes of change in TBW at a level sufficient to be considered relevant to the detriment of performance [5]. Likewise, it is possible to experience no BM or TBW changes, and yet to observe significant changes in the different water compartments [5]. Therefore, it is necessary to apply other methods to assess hydration [34]. Analyzing body water compartments (Table 2) through the athlete-specific prediction models [17], cyclists maintained hydration balance, and the homeostatic stability remained constant until the end of the race, despite the fact that there are non-significant trends.

However, both raw bioelectrical values and whole-body vector migration did report changes over the race: in the first half, there was a shortening trend of the vector, and in the second half there was significant lengthening, above baseline value. These results are similar to other cycling studies [2,3], and they indicate that although proper homeostatic adaptation was detected during the first week of competition, the long-term, high-intensity exercise would have resulted in TBW changes and its distribution in soft tissues, affecting R and Z. Moreover, Xc may be compromised by the oxidative stress associated with the cell damage and the antioxidant depletion generated by this type of physical exercise [35]. With respect to PhA, it describes a similar pattern to the one shown in Pollastri et al. [2], decreasing in the early stages of the race and subsequently increasing until reaching baseline values. PhA changes could reflect water compartment changes not detected by the predictive equations, indicating that major water changes were experienced in the extracellular compartment. The slight changes at the intracellular level indicate that the cell membrane remained intact in spite of the demands of the race.

4.3. Muscle-Localized Changes

The muscle group most sensitive to bioelectrical changes induced by the race were the calves, since R/H, Xc/H and Z/H significantly decreased at the middle of the race,

indicating a fluid gain in the surrounding area, and reversing the bioelectrical trend during the second half, indicating a fluid loss. Regarding the quadriceps, none of the bioelectrical parameters significantly changed across the race, while the hamstrings significantly increased in PhA in the second half of the race (MID-POST) only. Despite PhA changes only being significant in the hamstrings at MID-POST ($4.3 \pm 6.1\%$, $p = 0.05$), a similar non-significant trend was also seen both in the quadriceps ($7.7 \pm 10.3\%$, $p = 0.085$) and the calves ($6 \pm 9.2\%$, $p = 0.097$), which could imply that the greatest fluid shifts were provoked during the second half of the race, with water loss coming mainly from the extracellular compartment. In Figure 3, the null migration vector of both quadriceps and hamstrings can be observed (Figure 3B,C), while the mean calves' vector significantly shortened in the first half of the race, and lengthened in the second half, below the baseline values (Figure 3D).

Mascherini et al. [36] used ML-BIVA (analyzing also quadriceps, hamstrings and calves) as a method to assess long-term adaptations in 59 athletes of an elite Italian soccer team after 50 days of training. These players experienced a significant decrease in R (indicating an extracellular local fluid gain) in all three muscles groups studied (3–4.7%). However, only in the calves was there a significant decrease of the Xc (4.1%, $p = 0.007$). PhA remained constant, though. Results suggested that the calves were the most affected muscle group after 50 days of training. As in the present study, it should be analyzed whether the significance of these changes was due to the fact that the properties of the calves are more sensitive than the other muscle groups in the bioelectrical assessments, or because the calf muscles have been the most affected at the cellular level due to the high demands.

However, it is clear is that ML-BIVA is sensitive to some muscle adaptations evoked by the intensity of the race, since the gastrocnemius is one of the most severely affected muscles by fatigue during cycling [37].

4.4. Hematological Changes

As shown in Table 2, all the hematological variables were in agreement, reporting an initial gain fluid during the first half of the race, and a posterior dehydration due to the demands of the competition, as reported in the bioelectrical analysis.

The decrease in Hb, and, consequently, in Hct was the expected kinetic due to the hemodilution typical of such heavy efforts, as reported in other Giro d'Italia races [13,38]. According to the aforementioned studies, after the initial decrease in Hb, this parameter stabilizes for a period of time until the race becomes so demanding that reverses the kinetic, increasing both Hb and Hct. High levels of Hb and Hct are an indicator of the dehydration [39] that our cyclist has experienced, especially during the second half of the race, as it can also be confirmed with the vector migration (Figure 3A). Regarding the bioelectrical results, and knowing that R is dependent on lean tissue mass and tissue hydration, Xc is associated with cell size and integrity of the cell membranes, and an increase in EPV in the first phase of the race should be expected as well as a significant decrease at the end [40], according to the results. The osmolality also presents a behavior that indicates the dehydration of the athletes, although this increase occurs more progressively and without correlation with respect to the rest of the bioelectrical parameters, since the significant change was only seen at PRE-POST (PRE-POST: $9.3 \pm 10.8\%$, $p = 0.011$).

The most interesting fact regarding the hematological analysis is that some of these changes are correlated with some WB-BIVA parameter changes. More specifically, the highest correlations are given with the red blood cell parameters and with the EPV. Similarly, Giorgi et al. [41] found some large negative correlation of Z/H and Xc/H changes with EPV changes in some stages of the race, indicating possible fluid shifts. On the other hand, no correlations were found between the changes produced at the muscular level and the hematological changes, so ML-BIVA was shown not to be a good indicator for assessing hydration at a global level, and at a muscle level more research is also needed.

In accordance with this, it is well established that WB-BIVA has shown to be an adequate alternative to detect and rank changes in tissue hydration status, and to be more

sensitive than other conventional methods of body composition analysis based on the BM or classical BIA approximation, which cannot detect slight hydration changes.

The study has its five limitations. (1) Sample size was scarce and composed by men only, but that is difficult to amend given that the subjects are world class, and the limited sample also means that the results of this study are considered just preliminary. (2) There is a lack of information of the physical condition of the cyclists prior to race. (3) The lack of information regarding the hydration and nutrition strategies information both prior to and during the race. (4) The lack of length segment measurements to normalize ML-BIVA. (5) The assessments we performed were only in three different moments, but the ideal would have been to monitor the entire competition, as it was composed of different profile stages that could have produced different adaptations.

4.5. Practical Applications and Future Perspectives

The 3-week multi-stage cycling races such as the “Giro d’Italia” require constant care and supervision from medical and technical staff to monitor a cyclist’s health and performance in real time. This can only be achieved using a mobile laboratory to perform the pertinent tests with quick results. For these reasons, simple and reliable tests to assess hydration status, such as bioimpedance monitoring, can be useful to give some orientation and, consequently, to decide the best strategies regarding the health and performance of cyclists. Currently, we are still far from considering BIVA as the unique biomarker of hydration status, but, undoubtedly, its progressive use as a complementary measure to fully validated hematological hydration indicators allows us to parametrize its values and reflect its real possibilities in the near future. The present study could help physical staff to assess the status of their athletes in a precise, accurate, reliable, non-invasive, portable, cheap, and safe way, which could easily be used in real time.

5. Conclusions

BIVA appears to be sensitive enough to detect hydration changes induced by a 3-week multi-stage cycling race such as the Giro d’Italia. The bioelectrical kinetics of the whole-body bioimpedance vector coincides and is related with some hematological hydration biomarkers. The preliminary findings encourage BIVA applicability, previously validated in clinical settings, to detect hydration changes induced by multi-stage cycling races. ML-BIVA also appears to be sensitive to local muscle changes by exercise, despite more research being needed in order to better understand the reason for the bioelectrical changes. The calves were the most sensitive to ML-BIVA changes.

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Data Availability Statement: All data generated analyzed during the current study are available from the corresponding author on reasonable request.

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Estudi IV

Article publicat en *PeerJ* (2023), amb un factor d'impacte de 2.7 en la categoria de “ciències multidisciplinàries” (quartil 2 segons el *Journal of Citation Reports*):

JIF del JCR SCIE/SSCI 2.700 (2022)	Índex SJR 0.695 (2022)
Quartil JCR SCIE/SSCI Q2	Quartil SJR Q1
Categoria JCR SCIE/SSCI Multidisciplinary sciences	Categoria SJR Agricultural and biological sciences

Body fluids and muscle changes in trail runners of various distances

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Body fluids and muscle changes in trail runners of various distances

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ABSTRACT

Background: This study aims to investigate body fluids and muscle changes evoked by different trail races using anthropometric, bioelectrical, and creatine kinase (CK) measurements.

Methods: A total of 92 subjects (55 men, 37 women) participating in three different races of 14, 35, and 52 km were evaluated before (PRE) and after (POST) the races. Classic bioelectrical impedance vector analysis was applied at the whole-body level (WB-BIVA). Additionally, muscle-localized bioelectrical assessments (ML-BIVA) were performed in a subgroup of 11 men (in the quadriceps, hamstrings, and calves). PRE-POST differences and correlations between bioelectrical values and CK, running time and race distance were tested.

Results: Changes in whole-body vectors and phase angles disclosed an inclination towards dehydration among men in the 14, 35, and 52 km groups ($p < 0.001$), as well as among women in the 35 and 52 km groups ($p < 0.001$). PRE Z/H was negatively correlated with running time in the 35 km men group and 14 km women group ($r = -0.377$, $p = 0.048$; $r = -0.751$, $p = 0.001$; respectively). POST Z/H was negatively correlated with running time in the 14 km women group ($r = -0.593$, $p = 0.02$). CK was positively correlated with distance in men and women ($p < 0.001$) and negatively correlated with reactance and vector length in the 14 km men group ($p < 0.05$). ML-BIVA echoed the same tendency as the WB-BIVA in the 35 and 52 km runners, with the most notable changes occurring in the calves ($p < 0.001$).

Conclusions: WB-BIVA and CK measurements underscored a conspicuous trend towards post-race dehydration and muscle damage, displaying a weak association with performance. Notably, ML-BIVA detected substantial alterations primarily in the calves. The study underscores the utility of BIVA as a technique to assess athlete's body composition changes.

Subjects Anthropology, Anatomy and Physiology, Nutrition, Sports Injury

Keywords Body cell mass, Hydration, Phase angle, BIVA, Sport, Running

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INTRODUCTION

In recent years there has been a growing interest in natural environment running, and trail running (TR) has become an increasingly popular sport (*Carmona et al., 2019*).

The International Trail Running Association defines TR as “races which take place in a natural environment, with the minimum possible of paved roads (20% maximum)” (*ITRA, 2021*).

Many physiological changes occur during and at the end of the races (*Baiget et al., 2018; Carmona et al., 2019; Koral et al., 2022; Pradas et al., 2021; Roca, 2019*). Dehydration processes and neuromuscular fatigue, especially occurring in the lower limb (*Roca, 2019*), have been observed (*Baiget et al., 2018; Carmona et al., 2019; Koral et al., 2022; Pradas et al., 2021; Roca, 2019*). The hydration status prior to competition and strategies for maintaining euhydration during it are important, given their potential to influence athletic performance (*Gatterer, 2021*). The lack of body water increases the physiological strains and perceived effort in aerobic exercises (*Sawka et al., 2007*). In fact, there is general agreement that a $\geq 2\%$ body mass (BM) loss affects aerobic and cognitive performance (*Casa, Clarkson & Roberts, 2005*). Conversely, excessive fluid intake can lead to increased BM, causing a weight penalty and probably affecting the weight-bearing activities (*Gatterer et al., 2011; Noakes, 2007; Sawka et al., 2007*). Therefore, for achieving optimal endurance performance, attention should be paid to proper hydration status (*Gatterer, 2021*), that together with good neuromuscular conditions, represent useful predictors of running performance (*Baiget et al., 2018; Pastor et al., 2022*).

Neuromuscular fatigue is often accompanied by increases in plasma biomarkers of muscle damage, such as creatine kinase (CK) (*Brancaccio, Lippi & Maffulli, 2010*). When the muscle tissue cannot stand the intensity of the exercise overreaching its limit, CK is released into the interstitial space and transported into the bloodstream through the lymphatic system (*Brancaccio, Lippi & Maffulli, 2010*). CK has been widely studied in TR (*Carmona et al., 2019; Pradas et al., 2021*), revealing large increases at the end of the races. CK elevation is usually lower in trained individuals (*Carmona et al., 2019*), because their basal level and their exercise tolerance are usually higher (*Kim & Lee, 2015; Koutedakis et al., 1993*). Moreover, a positive correlation between race distance and CK levels has been observed (*Temesi et al., 2021*).

Various methods for analyzing hydration status exist within the sports field, like the plasma analysis or dilution techniques (*Roca, 2019*). While these methods offer valuable insights, some are impractical, costly, or invasive. Monitoring changes in BM, as well as food and fluid intake, constitute additional informative methods of the hydration status (*Lukaski, 2017*). A noteworthy addition to this array of methods is the bioelectrical impedance vector analysis (BIVA), which offers a comprehensive approach to assessing body hydration and cellular status (*Campa et al., 2022*). The classic BIVA approach works with the same basis of bioelectrical impedance analysis (BIA), but instead of using regression equations to obtain quantitative data, it relies on the analysis of raw bioelectrical values (resistance, R; reactance, Xc) standardized by conductor length. The derived impedance (Z) and phase angle (PhA) represent vector length and vector direction,

respectively (Piccoli *et al.*, 1994). According to classic BIVA, vector length is inversely related to total body water (TBW) (Campa *et al.*, 2022), whereas vector direction serves as an indicator of cellular health and cell membrane integrity and is inversely related to extracellular/intracellular water (ECW/ICW) ratio (Marini *et al.*, 2020). BIVA can be performed through different protocols depending on the distribution of the electrodes (Campa *et al.*, 2022; Stagi *et al.*, 2021a), including whole-body BIVA (WB-BIVA) for assessing the composition of the entire body, and muscle-localized BIVA (ML-BIVA), for assessing the composition of individual muscles or muscle segments. BIVA has been studied in different applications, such as in the clinical field (Norman *et al.*, 2012), or in body image and body composition (Stagi *et al.*, 2021b). In the sports field, some studies have employed both WB-BIVA and ML-BIVA to assess fluid fluctuations following training sessions or competitions (Campa *et al.*, 2022; Castizo-Olier *et al.*, 2018b; Cebrián-Ponce *et al.*, 2021, 2023; Nescolarde *et al.*, 2023). Notably, Castizo-Olier *et al.* (2018a) reported that classic whole-body vector migration remained consistent with fluid loss induced in an ultra-endurance triathlon event, even 48 h after the race finished. Nescolarde *et al.* (2020) established a connection between classic whole-body vector migration and certain serum and urine biomarkers post-marathon, providing insights into hydration status and renal function. Furthermore, a recent study revealed a positive correlation between resistance (BIVA) and muscle damage following a high-intensity hockey training (Cebrián-Ponce *et al.*, 2022). To the best of our knowledge, this study represents the first attempt to employ BIVA in the context of TR, offering valuable insights into the physiological responses elicited by these distinctive races.

This study aimed to analyze body fluids and muscle changes evoked by different trail running race profiles (14, 35 and 52 km) by using anthropometric, bioelectrical (whole-body and muscle-localized) and CK assessments.

MATERIALS AND METHODS

Subjects

As shown in Table 1, this observational and descriptive study involved 92 trail runners of both sexes (55 men and 37 women). Each participant engaged in one of the three races constituting the Volta a la Cerdanya Ultrafons® 2013 event (Fig. 1): (a) a 14 km race with 489 m of elevation gain; (b) a 35 km race with 1,600 m of elevation gain; (c) an 85 km race with 3,800 m of elevation gain. However, due to the adverse weather conditions during the competition, the 85 km race had to be shortened to 52 km, with 2,300 m of positive elevation gain. Participants were surveyed about their weekly training volume, revealing the typical heterogeneity among trail runners. Runners selected their race based on their preparedness and physical fitness. The inclusion criteria consisted of: (a) participants aged 18 and older; (b) absence of injuries or clinical conditions at the time of the study.

The study was performed following the Helsinki Declaration Statement. All runners voluntarily participated and delivered written informed consent prior to their participation. The research was previously approved by the Ethics Committee of the Catalan Sports Council (0099 S/690/2013).

Table 1 Characteristics of the study participants and performance.

		Men	Women
Age (years)	14 km	35.8 ± 8.4	35.1 ± 11.4
	35 km	37.2 ± 7.8	35.5 ± 6.7
	52 km	36.7 ± 4.8	34.3 ± 3.8
Height (cm)	14 km	176.8 ± 7.5	165.5 ± 6.4
	35 km	176.1 ± 6.4	164.2 ± 6.6
	52 km	175.9 ± 8.0	163.9 ± 3.7
Race time (min)	14 km	103.4 ± 20.5	123.2 ± 13.4
	35 km	226.3 ± 39.7	282.2 ± 52.4
	52 km	408.6 ± 75.8	464.8 ± 90.2

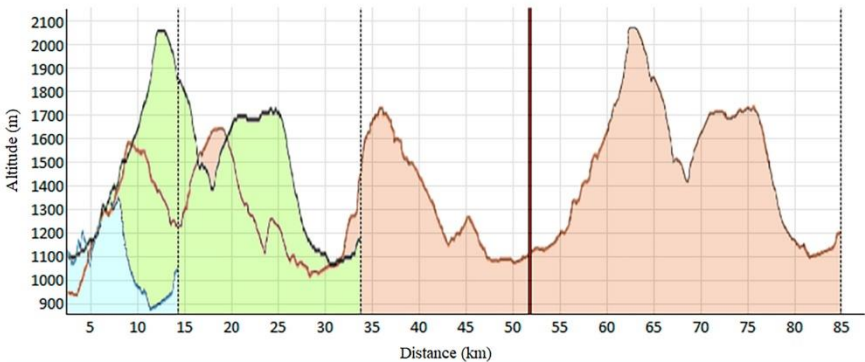


Figure 1 Elevation profile of the races. Blue: 14 km; Green: 32 km; Red: 85 km, shortened at 52 km (vertical red line). Full-size [DOI: 10.7717/peerj.16563/fig-1](https://doi.org/10.7717/peerj.16563/fig-1)

Procedures

Anthropometric, bioelectrical and hematological measurements were conducted in the morning under fasting conditions before (PRE) and at the end of the race (POST). Blood samples were collected from an antecubital vein by a physician, following the same protocol as previous studies (Carmona et al., 2019). CK analysis were performed in an Advia 2400 automatic device (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). Out of the 92 subjects, blood samples were obtained from all of them at PRE, but could not be obtained from seven women at POST.

Anthropometry

The anthropometric measurements adhered to the standard criteria established by the International Society for the Advancement of Kinanthropometry (ISAK) (Stewart et al., 2011), using the same equipment as in Cebrián-Ponce et al. (2023). The anthropometric

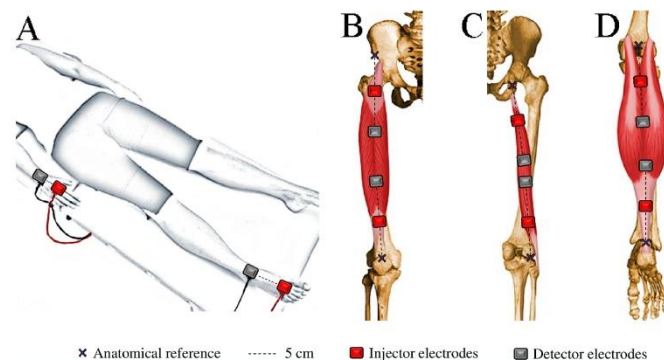


Figure 2 Electrode placement for bioelectrical impedance measurements in the whole-body (A), quadriceps (B), hamstrings (C), and calves (D). [Full-size](#) DOI: 10.7717/peerj.16563/fig-2

tape was also utilized to assess perimeters (mid-thigh and calf maximum) and length segments (quadriceps, hamstrings, and calves).

Bioelectrical impedance analysis

The impedance measurements were collected using the BIA device (BIA 101 Anniversary; Akern, Florence, Italy), applying a constant alternating sinusoidal electric current at a frequency of 50 kHz.

WB-BIVA and ML-BIVA (for quadriceps, hamstrings, and calves) data were obtained following the detailed procedures outlined in [Cebrián-Ponce et al. \(2023\)](#) and visually depicted in [Fig. 2](#). WB-BIVA is a conventional method widely used in various scientific and clinical settings to assess overall body composition. On the other hand, ML-BIVA is a more recent approach that allows us to examine specific muscle segments, providing valuable insights into localized muscle changes. This combined approach enhances our ability to comprehensively understand body composition alterations.

Z was computed as $\sqrt{(R^2 + Xc^2)}$, and PhA was determined using the formula $\tan^{-1} (Xc/R \cdot 180^\circ/\pi)$. The parameters R, Xc, and Z were adjusted with respect to height (R/H, Xc/H, Z/H) in the case of WB-BIVA, and with respect to segment length (R/L, Xc/L, Z/L) for ML-BIVA. The parameters Z/H and PhA were jointly analyzed to assess changes in TBW and ECW/ICW ratio ([Campa et al., 2022](#); [Marini et al., 2020](#)), using athlete-specific prediction models proposed by [Matias et al. \(2016\)](#) based on H, BM, R, Xc, and gender.

Statistical analysis

Descriptive data are presented as mean $\pm$ standard deviation. After evaluating the normality of the distribution for each variable using the Shapiro-Wilks test, differences between PRE and POST values were assessed using the paired Student's t-test for parametric distribution and the Wilcoxon signed-rank test for non-parametric distribution. The magnitude of changes in all values was expressed as delta percent values ($\Delta\%$). Pearson's correlation coefficient was employed to identify potential associations between changes in bioelectrical parameters and CK delta percent values, as well as

between PRE and POST bioelectrical parameters and running time. Spearman rank-order correlation coefficient was applied to examine the relationships between race distance and CK changes. Resistance-reactance paired graphs and paired one-sample Hotelling's T^2 test were used to compare bioelectrical PRE-POST differences. In these graphs, ellipses overlapping the origin indicate no differences between PRE and POST bioelectrical values, whereas non-centered ellipses indicate significant changes. The significance level was set at $p < 0.05$. Data analysis was conducted using SPSS (Version 21; Chicago, IL, USA) and BIVA software (Piccoli & Pastori, 2002) were used for data analysis.

RESULTS

Several anthropometric, bioelectrical and CK changes were reported after the race in all groups (Table 2).

Whole-body BIVA

All runners showed a decrease of BM (0.8–2.6%). WB-BIVA highlighted an increase of Z/H, especially in the 14 and 35 km groups, and PhA, especially in the 35 and 52 km groups. Paired graphs (Fig. 3) showed a migration of the vector in almost all the groups, except for women who ran 14 km. These bioelectrical changes suggest a body fluid loss, more likely at the extracellular level, as indicated by vector length and PhA increase, respectively. The trend was confirmed by the water prediction equations, showing a tendency to TBW, ECW, ICW, and ECW/ICW reduction in the majority of the cases (Table 2). PRE Z/H was negatively correlated with running time in the 35 km men group and 14 km women group ($r = -0.377$, $p = 0.048$; $r = -0.751$, $p = 0.001$; respectively). POST Z/H was negatively correlated with running time in the 14 km women group ($r = -0.593$, $p = 0.02$).

CK levels increased in all groups (81.6–433.5%), and it was positively correlated with distance ($r = 0.521$, $p < 0.001$ for men, and $r = 0.628$, $p < 0.001$ for women) and negatively correlated with R/H, Xc/H and Z/H in the group of men who ran 14 km only ($r = -0.645$, $p = 0.017$; $r = -0.592$, $p = 0.033$; $r = -0.647$, $p = 0.017$; respectively).

Muscle-localized BIVA

In the 11 subjects who performed ML-BIVA, the muscles with major changes were the calves (Fig. 4), with a significant increase in vector length and PhA in 35 km and, especially, 52 km runners (Table 3). Hamstrings vector length' change showed the same pattern than calves, particularly in the 52 km group. Quadriceps changes were not significant. The changes of PhA followed the same trend as the whole-body in all cases and muscles, increasing its value (4.1–6.6%).

DISCUSSION

The present study, which is the first one analyzing physiological changes by means of WB-BIVA and ML-BIVA in trail runners, highlighted body changes evoked by the demands of the races.

Table 2 Anthropometric, whole-body bioelectrical and CK changes evoked by the races.

	Men				Women			
	PRE	POST	% Δ	<i>p</i>	PRE	POST	% Δ	<i>p</i>
BM (kg)								
14 km	77.4 ± 8.1	76.7 ± 7.8	−0.8 ± 0.8	0.003	63.0 ± 9.5	62.5 ± 9.2	−0.9 ± 0.7	0.000
35 km	72.1 ± 8.6	70.2 ± 8.4	−2.6 ± 1.6	0.000	56.5 ± 6.0	55.7 ± 5.7	−1.3 ± 1.9	0.006
52 km	71.7 ± 8.4	70.1 ± 8.4	−2.2 ± 0.8	0.000	55.1 ± 3.3	54.1 ± 3.1	−1.8 ± 1.3	0.039
R/H (Ωm)								
14 km	266.9 ± 26.5	285.7 ± 38.0	6.8 ± 6.3	0.003	358.3 ± 38.5	364.0 ± 43.4	1.6 ± 5.1	0.214
35 km	268.3 ± 22.9	282.0 ± 25.0	5.1 ± 3.0	0.000	353.5 ± 41.0	365.2 ± 40.4	3.4 ± 3.1	0.000
52 km	275.1 ± 31.3	278.5 ± 32.7	1.2 ± 3.6	0.217	356.8 ± 37.0	359.7 ± 35.5	1.0 ± 4.6	0.702
Xc/H (Ω/m)								
14 km	35.3 ± 3.8	37.6 ± 3.9	6.9 ± 6.5	0.002	38.9 ± 5.3	39.1 ± 5.9	0.6 ± 7.1	0.726
35 km	34.4 ± 3.8	37.8 ± 4.1	9.9 ± 5.9	0.000	39.6 ± 4.9	42.2 ± 6.2	6.5 ± 6.9	0.002
52 km	34.2 ± 4.0	36.0 ± 4.3	5.5 ± 5.8	0.003	36.3 ± 1.9	38.7 ± 1.8	6.8 ± 2.3	0.002
Z/H (Ω/m)								
14 km	269.3 ± 26.7	288.2 ± 38.1	6.9 ± 6.3	0.003	360.5 ± 38.7	366.1 ± 43.7	1.5 ± 5.1	0.218
35 km	270.5 ± 23.1	284.5 ± 25.3	5.2 ± 3.0	0.000	355.7 ± 41.3	367.6 ± 40.7	3.4 ± 3.1	0.000
52 km	277.2 ± 31.4	280.8 ± 32.8	1.3 ± 3.6	0.196	358.7 ± 36.9	361.8 ± 35.4	1.0 ± 4.6	0.678
PhA (°)								
14 km	7.5 ± 0.5	7.6 ± 0.6	0.1 ± 4.1	0.894	6.2 ± 0.5	6.1 ± 0.6	−1.0 ± 2.8	0.179
35 km	7.3 ± 0.5	7.6 ± 0.5	4.5 ± 5.0	0.000	6.4 ± 0.4	6.6 ± 0.5	3.0 ± 6.4	0.098
52 km	7.1 ± 0.7	7.4 ± 0.6	4.1 ± 3.9	0.002	5.8 ± 0.4	6.2 ± 0.6	5.8 ± 3.8	0.033
TBW (l)								
14 km	48.2 ± 4.3	47.2 ± 4.5	−2.2 ± 1.6	0.000	33.7 ± 4.8	33.3 ± 4.8	−1.0 ± 1.3	0.017
35 km	46.0 ± 4.3	44.7 ± 4.2	−2.9 ± 1.4	0.000	31.2 ± 3.4	30.6 ± 3.2	−1.8 ± 1.5	0.000
52 km	45.6 ± 4.7	44.9 ± 4.7	−1.6 ± 1.3	0.001	30.5 ± 1.4	30.1 ± 1.4	−1.5 ± 2.2	0.197
ECW (l)								
14 km	19.1 ± 1.7	18.6 ± 1.7	−2.6 ± 2.0	0.001	14.8 ± 1.8	14.7 ± 1.9	−0.7 ± 2.0	0.221
35 km	18.4 ± 1.7	17.7 ± 1.6	−3.7 ± 1.7	0.000	13.9 ± 1.4	13.6 ± 1.3	−2.3 ± 1.5	0.000
52 km	18.3 ± 1.8	17.9 ± 1.8	−2.2 ± 1.7	0.000	13.8 ± 0.5	13.5 ± 0.4	−2.3 ± 1.7	0.042
ICW (l)								
14 km	29.2 ± 2.7	28.6 ± 2.8	−1.9 ± 1.4	0.000	18.9 ± 3.0	18.7 ± 3.0	−1.2 ± 1.0	0.000
35 km	27.6 ± 2.7	27.0 ± 2.6	−2.4 ± 1.5	0.000	17.3 ± 2.0	17.1 ± 1.9	−1.5 ± 2.2	0.009
52 km	27.3 ± 3.0	26.9 ± 2.9	−1.3 ± 1.2	0.001	16.7 ± 0.9	16.6 ± 1.0	−0.9 ± 2.6	0.498
ECW/ICW ratio								
14 km	0.66 ± 0.01	0.65 ± 0.01	−0.8 ± 1.0	0.082	0.78 ± 0.04	0.79 ± 0.04	0.4 ± 1.5	0.089
35 km	0.67 ± 0.02	0.66 ± 0.02	−1.4 ± 1.4	0.000	0.80 ± 0.03	0.79 ± 0.03	−0.8 ± 2.4	0.154
52 km	0.67 ± 0.02	0.67 ± 0.02	−0.9 ± 1.1	0.013	0.83 ± 0.02	0.82 ± 0.02	−1.4 ± 1.1	0.034
CK (IU/l)								
14 km	204 ± 90	378 ± 144	108.5 ± 91.9	0.002	102 ± 36 †	174 ± 72	81.6 ± 63.4	0.002
35 km	234 ± 246	414 ± 186	153.1 ± 123.2	0.001	138 ± 54 ‡	366 ± 318	156.3 ± 115.3	0.001
52 km	210 ± 102	1,020 ± 600	425.2 ± 266.9	0.001	138 ± 48 §	696 ± 390	433.5 ± 244.5	0.068

Note:

BM, body mass; R/H, height-adjusted resistance; Xc/H, height-adjusted reactance; Z/H, height-adjusted vector length; PhA, phase angle; TBW, total body water; ICW, intracellular water; ECW, extracellular water; CK, creatine kinase; PRE, assessment before the race; POST, assessment after the race. Men: 14 km (*n* = 13), 35 km (*n* = 28), 52 km (*n* = 14); Women: 14 km (*n* = 15), 35 km (*n* = 17), 52 km (*n* = 5). †, *n* = 12; ‡, *n* = 14; §, *n* = 4.

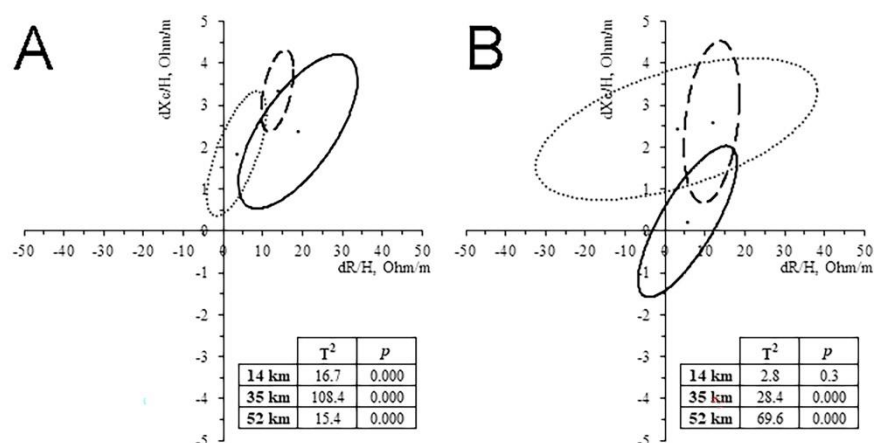


Figure 3 Paired graph. Intra-group classic whole-body BIVA differences evoked by the race in men (A) and women (B). Solid line: 14 km; Dashed line: 32 km; Dash-dotted line: 52 km.

Full-size [DOI: 10.7717/peerj.16563/fig-3](https://doi.org/10.7717/peerj.16563/fig-3)

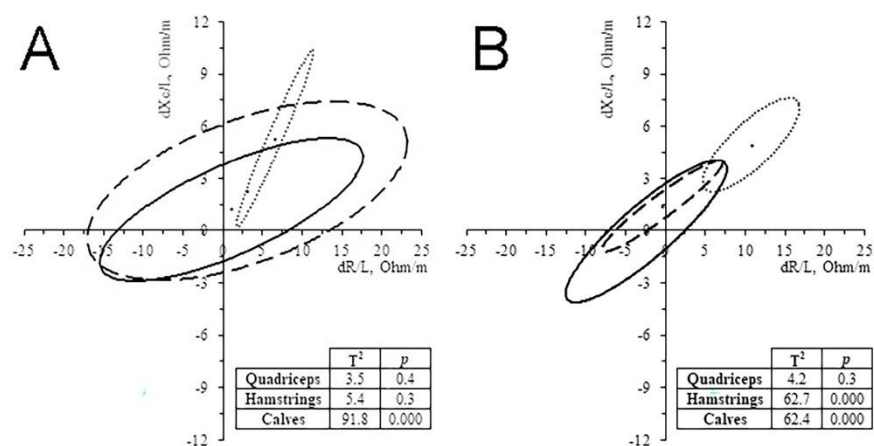


Figure 4 Paired graph. Muscle-localized BIVA differences evoked by the race in 35 km group (A) and 52 km group (B). Solid line: quadriceps; Dashed line: hamstrings; Dash-dotted line, calves.

Full-size [DOI: 10.7717/peerj.16563/fig-4](https://doi.org/10.7717/peerj.16563/fig-4)

Whole-body changes

All groups experienced a decrease in BM and in TBW (more likely at the extracellular level), as indicated by the prediction equations, and by bioimpedance vector lengthening and PhA increase (Campa et al., 2022; Marini et al., 2020). The vector migration was similar in the three groups of runners, although some differences can be perceived. The group that lost more water was the 35 km for both men and women, and the group showing the less accentuated trend was the 14 km women group. Such differences among groups of runners could be attributed to several factors, such as the initial hydration status,

Table 3 Anthropometric and muscle-localized BIVA changes evoked by the races.

	PRE	POST	% Δ	p
Thighs perimeter (cm)				
35 km	52.3 ± 3.5	51.5 ± 2.6	-1.4 ± 1.8	0.148
52 km	53.1 ± 3.0	53.0 ± 2.7	-0.2 ± 1.3	0.753
Calves perimeter (cm)				
35 km	38.3 ± 1.9	37.2 ± 1.3	-2.7 ± 2.2	0.057
52 km	37.5 ± 0.9	37.0 ± 1.0	-1.3 ± 1.9	0.143
Quadriceps				
R/L (Ω/m)				
35 km	74.5 ± 6.4	75.5 ± 10.4	1.3 ± 9.5	0.760
52 km	78.1 ± 9.4	75.7 ± 12.7	-3.5 ± 8.5	0.363
Xc/L (Ω/m)				
35 km	21.3 ± 2.2	22.5 ± 1.3	6.4 ± 9.2	0.209
52 km	23.0 ± 2.8	23.0 ± 1.8	0.7 ± 10.8	0.974
Z/L (Ω/m)				
35 km	77.5 ± 5.9	78.9 ± 9.7	1.7 ± 9.3	0.697
52 km	81.5 ± 9.3	79.1 ± 12.6	-3.2 ± 8.4	0.402
PhA (°)				
35 km	16.1 ± 2.6	16.9 ± 2.9	4.8 ± 5.5	0.105
52 km	16.6 ± 2.3	17.2 ± 2.3	4.1 ± 5.2	0.123
Hamstrings				
R/L (Ω/m)				
35 km	86.6 ± 7.2	89.7 ± 6.0	4 ± 10.6	0.487
52 km	92.4 ± 6.8	92.0 ± 9.3	-0.5 ± 4.9	0.838
Xc/L (Ω/m)				
35 km	26.4 ± 2.6	28.7 ± 2.3	9.1 ± 8.7	0.087
52 km	29.5 ± 5.1	31.0 ± 4.5	5.4 ± 6.5	0.074
Z/L (Ω/m)				
35 km	90.6 ± 7.1	94.3 ± 5.2	4.5 ± 10.2	0.415
52 km	97.1 ± 6.8	97.1 ± 9.2	0.0 ± 4.9	0.975
PhA (°)				
35 km	17.0 ± 1.9	18.0 ± 2.2	5.4 ± 7.1	0.154
52 km	17.7 ± 3.1	18.7 ± 2.9	5.6 ± 3.0	0.001
Calves				
R/L (Ω/m)				
35 km	143.2 ± 12.9	149.7 ± 12.7	4.6 ± 1.7	0.002
52 km	165.9 ± 13.8	176.7 ± 12.2	6.6 ± 2.5	0.001
Xc/L (Ω/m)				
35 km	37.0 ± 4.9	42.3 ± 5.4	14.5 ± 6.6	0.006
52 km	39.9 ± 7.1	44.8 ± 7.3	12.7 ± 4.7	0.001

(Continued)

Table 3 (continued)

	PRE	POST	% Δ	<i>p</i>
Z/L (Ω/m)				
35 km	147.9 $\pm$ 13.7	155.6 $\pm$ 13.7	5.3 $\pm$ 2.0	0.003
52 km	170.7 $\pm$ 14.5	182.4 $\pm$ 12.7	6.9 $\pm$ 2.6	0.001
PhA ($^{\circ}$)				
35 km	14.5 $\pm$ 0.7	15.7 $\pm$ 0.8	4.6 $\pm$ 1.7	0.009
52 km	13.5 $\pm$ 1.9	14.2 $\pm$ 2.0	6.6 $\pm$ 2.5	0.001

Note:

R/L, length segment-adjusted resistance; Xc/L, length segment-adjusted reactance; Z/L, length segment-adjusted vector length; PhA, phase angle; PRE, assessment before the race; POST, assessment after the race. 32 km ($n = 5$), 52 km ($n = 6$).

the amount of water they drank during the race, the profile of the race (concentric and eccentric component) but could also be due to their physical fitness and tolerance to the race demands. It's worth noting that similar vector migrations have been observed in two previous studies on endurance (not trail) runners (*Castizo-Olier et al., 2018a; Nescolarde et al., 2020*). In particular, *Castizo-Olier et al. (2018a)*, examining experienced ultra-endurance triathletes, reported an increase of the vector length and PhA (3.8 $\pm$ 2.3%, 3.7 $\pm$ 4.9%, respectively). *Nescolarde et al. (2020)* showed the same trend in non-elite marathon runners (4.0% and 6.2% increase in mean for R/H and PhA, respectively). These studies performed a third assessment 48 h after the end of the race, showing that runners returned to their initial bioelectrical values after following an adequate hydration and nutritional recovery strategies. Other methods rather than BIVA, as the plasma analysis (*Kim & Lee, 2015; Pradas et al., 2021*), urine tests (*Baiget et al., 2018*), or dilution techniques (*Tam, Nolte & Noakes, 2011*) confirmed the tendency to dehydration in TR races.

The significant loss of TBW and BM exceeding 2% on average in some runner groups is consistent with findings in a study on 56 km ultra-distance runners using dilution techniques (*Tam, Nolte & Noakes, 2011*). Although it is proposed in the literature that BM losses greater than 2% impairs endurance performance (*Casa, Clarkson & Roberts, 2005*), it is actually unclear what are the real effects of water and BM loss on running performance (*Goulet, 2012*). Our study suggests that hydration status at the end of the race does not necessarily correlate with performance, with the exception of the 14 km women's group. In this specific group, we found a positive correlation between POST Z/H and running time, indicating that those runners who finished the race in a more dehydrated state tended to perform worse. This correlation might be linked to variations in their athletic potential and their experience with effective hydration strategies, especially when compared to runners participating in longer races.

The negative correlation between PRE Z/H and running time in the 35 km men's group was low, making it poorly relevant for our analysis. In contrast, this correlation held significance in the 14 km women's group. Interestingly, a counterintuitive pattern emerged in these groups: higher hydration levels at the start of the race were associated with worse performance outcomes. This finding contradicts (*Baiget's et al., 2018*) observation that the

most dehydrated trail runners tended to be slower. It's important to note that this relationship wasn't consistently supported by other studies, such as [Casa et al. \(2010\)](#) and [Noakes \(2007\)](#). [Noakes \(2007\)](#) proposed that human physiology is naturally inclined to maintain plasma osmolality rather than focusing solely on body mass, suggesting that excessive water intake might lead to a "weight-penalty," potentially impairing performance, as discussed previously ([Gatterer et al., 2011](#); [Noakes, 2007](#)). Despite the fact that ECW/ICW ratio is considered an important parameter, since it is well-reported that ICW is associated with power and strength ([Silva et al., 2014](#)), it did not demonstrate a consistent relationship with performance in our study.

Muscle damage, reflected by significant CK increases, was particularly noticeable in long-distance runners. Similar results have been reported in several studies ([Carmona et al., 2019](#); [Pradas et al., 2021](#)) and could be attributed to the continuous eccentric muscle contractions during running, leading to the release of muscle proteins like CK into the bloodstream ([Brancaccio, Lippi & Maffulli, 2010](#)). Notably, longer-distance runners might be expected to exhibit higher CK levels post-race, as observed in previous research ([Temesi et al., 2021](#)). However, it is essential to consider the superior physical fitness of longer-distance runners in comparison to other groups, which may result in their increased tolerance for elevated CK levels ([Kim & Lee, 2015](#); [Koutedakis et al., 1993](#)). Furthermore, it is worth mentioning that the 52 km runners were preparing for an 85 km race, but the race had to be halted at the 52nd km due to a storm. Therefore, it's plausible that they did not reach their physical limits, which could explain the lower-than-expected CK levels observed in this group.

In the present sample, significant negative correlations were detected between CK and R/H, Xc/H and Z/H in the group of men who ran 14 km only. The correlation with Xc/H was due to a decrease in cellular integrity after the race. However, a positive correlation was expected regarding R/H and Z/H, due to the crenation phenomenon that occurs in the skeletal muscle cells as a process of restoring the ECW loss ([Lozano et al., 2005](#)). In fact, a previous study with a small sample of seven subjects reported a positive correlation between R/H and CK changes ([Cebrián-Ponce et al., 2022](#)). Thus, caution is required in the interpretation of these correlations and continue researching.

Muscle-localized changes

ML-BIVA showed greater changes in the calf muscles, as indicated by large lengthening's of the vectors and PhA increases. Hamstrings reported similar but less accentuated trends as calves, while quadriceps displayed comparatively minor changes. These bioelectrical shifts suggest a propensity towards dehydration. Based on the interpretation of whole-body PhA, it is assumed that even at the muscular level, water loss comes mainly from the extracellular compartment.

No previous studies used ML-BIVA in TR, although the technique has been applied in other sports. A study examining quadriceps before and after a high-intensity training session in rink hockey players ([Cebrián-Ponce et al., 2022](#)) detected an increase in PhA, similar to the findings in our runner sample, with subsequent normalization after 24 h. However, it is worth noting that, in contrast to our results, the hockey players experienced

a decrease in Z/H along with an increase in thigh perimeter, indicative of fluid retention in the lower limb. This disparity could be attributed to the differing physiological demands of high-intensity interval efforts in hockey compared to the sustained, aerobic exertion in TR. ML-BIVA has also been employed to assess chronic adaptations to exercise in elite soccer players following 50 days of training (*Mascherini, Petri & Galanti, 2015*) and during the Giro d'Italia (*Cebrián-Ponce et al., 2023*). In alignment with our findings, the most significant changes were observed in the calf muscles.

Despite the limited existing studies on ML-BIVA in the realm of sports, particularly in TR, muscle changes have been evaluated using various methods. Multiple studies have reported reductions in isometric maximum voluntary contractions (IMVC) of knee extensors and plantar flexors following TR races (*Fourchet et al., 2012; Koral et al., 2022; Pastor et al., 2022; Temesi et al., 2021*), with more pronounced effects observed in longer-distance events (*Temesi et al., 2021*). For instance, *Koral et al. (2022)* observed reductions of 16% in knee extensor IMVC and 13% in plantar flexor IMVC in TR races shorter than 60 km, which further increased to 29% and 26%, respectively, in races longer than 100 km. Moreover, *Baiget et al. (2018)* and *Pradas et al. (2021)* documented significant decrements in various vertical jump tests (rebound jump; squat jump and countermovement jump; respectively). These tests serve as indicators of lower-body musculature functionality and force-generating capacity.

As evident from the literature, TR races induce peripheral fatigue, particularly in the lower limbs, resulting in reduced muscle function and performance. The calf muscles, integral to the plantar flexors, undergo significant strain during mountain running, leading to the expected erosion observed at the end of these races (*Fourchet et al., 2012*). The irregular terrain and high eccentric demands likely contribute to heightened activation and subsequent fatigue in posterior muscles such as the hamstrings and calves (*Roca, 2019*), accompanied by local fluid loss. This body of knowledge (*Baiget et al., 2018; Fourchet et al., 2012; Koral et al., 2022; Pastor et al., 2022; Pradas et al., 2021; Roca, 2019; Temesi et al., 2021*) suggests that BIVA is sensitive to the neuromuscular fatigue induced by TR races, particularly in the calf muscles.

Limitations and strengths

The present study has its limitations. Firstly, food and water intake during the races was not controlled. Secondly, the sample size for ML-BIVA was relatively small, comprising only male participants. Thirdly, data on POST CK levels were missing for seven participants. Fourthly, the wide variation in participant's exercise experience, ranging from non-trained individuals to international-level athletes, may introduce potential confounding factors. Finally, the dilution technique, which typically serves as the reference method for assessing body hydration, could not be applied.

With regard to the strengths of the study, this is the first study analyzing BIVA in TR and ML-BIVA in endurance sports. Also, the study design allowed to compare the physiological responses among athletes practicing different profiles of races.

Future perspectives

In various endurance sports, continuous health and performance monitoring is essential and often necessitates mobile laboratories for swift, real-time assessments. In this context, the utilization of simple and reliable methods, such as bioimpedance monitoring, can offer valuable insights into an athlete's hydration status, which is pivotal for their well-being and performance.

The results presented encourage further investigation in different lines of research and applications: (a) testing BIVA as a method to assess body fluid changes associated with different exercise modalities; (b) investigating the relationship between vector migration and muscle fatigue and damage, both on a whole-body and localized level; (c) extending the application of ML-BIVA to other groups and female participants. The outcomes of these investigations could equip coaches with non-invasive and straightforward tools to gain essential information about their athletes.

CONCLUSIONS

This study underscored the notable impact of trail races on both whole-body and localized parameters, with medium and long-distance runners experiencing more pronounced alterations. Anthropometrical and bioelectrical changes indicated a loss of body mass and body fluids, especially at the extracellular level. Muscle-localized BIVA showed that the major changes were at the calf level. Interestingly, our findings did not establish a straightforward link between hydration status and endurance performance, highlighting the complexity of these interactions. Muscle damage, indicated by CK changes, increased with distance.

Our study reinforces the utility of BIVA as a cost-effective, non-invasive, and efficient tool for monitoring dynamic shifts in body fluids and cellular properties. While ML-BIVA in sports remains an emerging field, our results suggest that this method may serve as a sensitive indicator of peripheral fatigue induced by high-intensity training or competitive endeavours. Further exploration in these areas could provide valuable insights into optimizing athlete health and performance.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Álex Cebrián-Ponce analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Elisabetta Marini analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Silvia Stagi analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jorge Castizo-Olier conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Marta Carrasco-Marginet conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Manuel Vicente Garnacho-Castaño performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Zeasseska Noriega performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Javier Espasa-Labrador performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Alfredo Irurtia conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

The research was approved by the Ethics Committee of the Catalan Sports Council (0099 S/690/2013).

Data Availability

The following information was supplied regarding data availability:

The data is available at Zenodo: Álex Cebrián Ponce, Elisabetta Marini, Silvia Stagi, Jorge Castizo Olier, Marta Carrasco Marginet, Manuel V Garnacho-Castaño, Zeasseska Noriega, Javier Espasa Labrador, & Alfredo Irurtia. (2023). Body fluids and muscle changes in trail runners of various distances [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.8342016>.

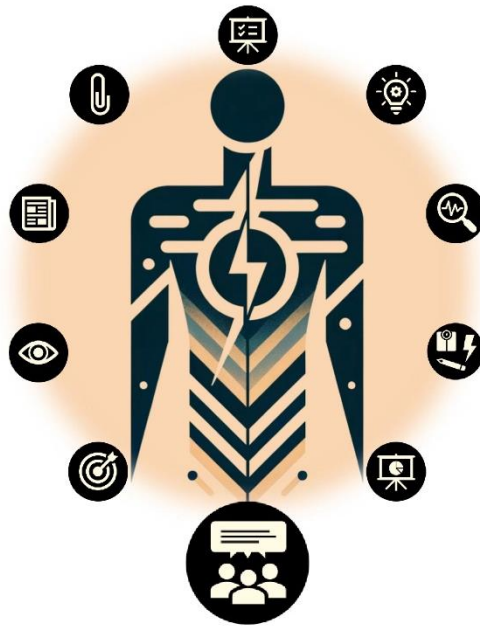
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DISCUSSIÓ

5. Discussió

A continuació es realitza la discussió d'aquesta tesi per compendi de publicacions en base a l'anàlisi estructural de tres grans apartats.

Inicialment, es realitza una caracterització prèvia del vector, establint un marc conceptual i metodològic que permet entendre les bases sobre les quals es construeix la investigació. Aquesta secció posa les bases per a les discussions posteriors i ajuda a contextualitzar els resultats dins d'un marc teòric adequat. A continuació, es revisen els canvis del vector de WB-BIVA induïts per l'exercici físic, analitzant aquest fenomen en diferents períodes de temps: curt, mig i llarg termini. Aquest enfocament temporal ofereix una comprensió més detallada dels canvis que es produeixen en els paràmetres bioelèctrics segons el tipus d'exercici. Finalment, es tracta l'àmbit de la BIVA muscular, considerant els canvis induïts per factors com l'edat, les lesions musculars i l'exercici físic (curt, mig i llarg termini), oferint una panoràmica integral de l'impacte d'aquests factors sobre la composició i funció musculars. A més, es discuteix sobre l'estandardització del protocol d'estudi, degut a les necessitats observades durant la realització d'aquesta tesi. Aquest punt és clau si l'objectiu es garantir la fiabilitat i la reproductibilitat dels resultats.

A través d'aquesta estructura, es busca analitzar en profunditat cada un dels aspectes mencionats i establir connexions entre ells, ressaltant la seva interdependència i importància dins del context més ampli de la investigació en ciències del esport.

5.1. Caracterització prèvia del vector

Considerant les limitacions inherents de BIA amb l'ús d'equacions predictives específiques per a esportistes, juntament amb la necessitat de monitorar els canvis fisiològics induïts per l'exercici físic, destaca la importància de l'anàlisi del vector com a mètode d'avaluació. Això subratlla la necessitat d'identificar el punt de partida de les propietats electrofisiològiques del cos, orientant així la formulació d'objectius específics.

En dos dels estudis de la recerca doctoral de Castizo-Olier (2018) sobre BIVA i esport, es van identificar patrons bioelèctrics específics en subgrups de dones de natació sincronitzada (Carrasco-Marginet et al., 2017) i en homes triatletes (Castizo-Olier, Carrasco-Marginet, et al., 2018), evidenciant la capacitat de BIVA de diferenciar entre esportistes de diferents modalitats, sexe, edats i nivells de rendiment (Figura 20).

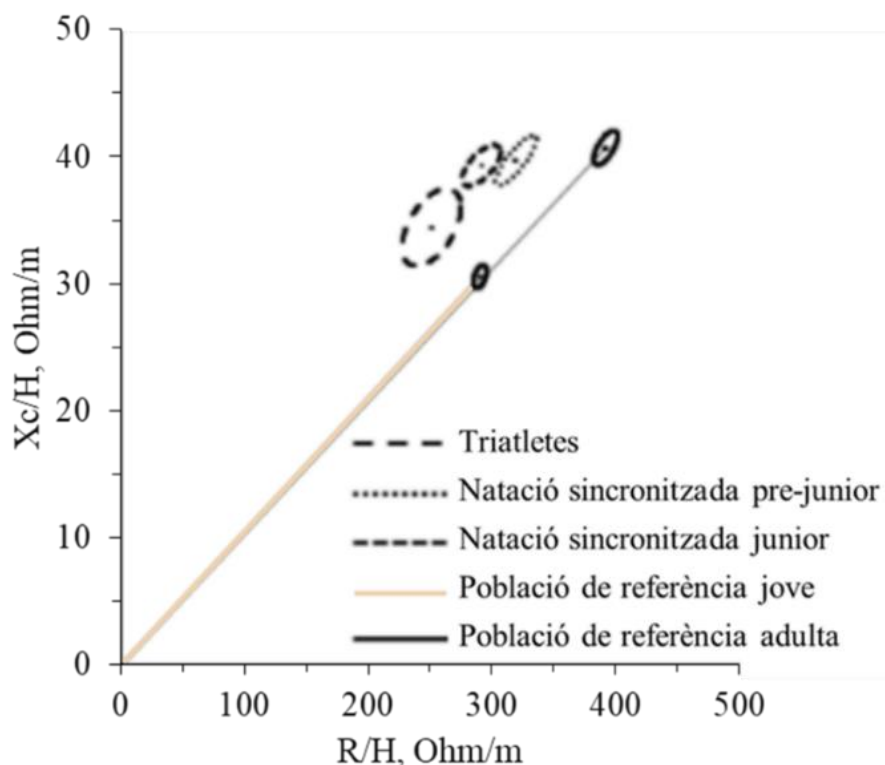


Figura 20. Gràfic de valor mitjà de triatló i de natació sincronitzada en relació a la seva població de referència. Adaptat de Castizo-Olier (2018). R/H , resistència ajustada per l'estatura; X_c/H , reactància ajustada per l'estatura.

En la revisió narrativa de Campa et al. (2021) es van proporcionar altres el·lipses de tolerància per a poblacions concretes d'esportistes: jugadors d'elit masculins adults de futbol (Bongiovanni et al., 2020; Micheli et al., 2014), de vòlei (Campa & Toselli, 2018) i de ciclisme (Giorgi, Vicini, et al., 2018); esportistes masculins adults d'esports de resistència, d'equip i de velocitat/potència (Campa, Matias, Gatterer, et al., 2019); esportistes femenines d'esports de resistència, d'equip i de velocitat/potència (Campa, Matias, Gatterer, et al., 2019); esportistes masculins adolescents i adults d'esports generals (Koury et al., 2014); i jugadors joves d'elit de futbol (Toselli et al., 2020). A més d'altres més recents com d'esportistes masculins i femenins (Marini et al., 2020), d'àrbitres de futbol (Izzicupo et al., 2023) o de culturistes (Petri et al., 2023). Aquestes el·lipses ofereixen una valoració més detallada i adaptada tant de la composició corporal com del nivell de condició física dels esportistes, facilitant el coneixement de les necessitats en termes d'entrenament i recuperació.

Dins d'aquesta tesi, els estudis publicats no han derivat en la creació de noves el·lipses de tolerància per a col·lectius específics, degut a la limitada mida de la mostra. No obstant això, els esportistes que formen la mostra total, presenten un desplaçament del vector cap

a l'esquerra envers a la població de referència d'adults sans de Piccoli et al. (1994) (Figura 21A). Cal destacar que el subjectes de l'Estudi III no es troben representats en aquest gràfic a causa de l'ús d'un dispositiu de bioimpedància diferent (DS Medica) al de la resta d'estudis (Akern), en el qual no hi ha dades de referència pre representar. Aquesta variació dels esportistes envers a la població de referència s'associa amb un millor funcionament cel·lular, manifestat per un increment en el PhA, fet que està en línia amb els altres grups d'esportistes mencionats. Aquestes diferències són el resultat d'adaptacions específiques relacionades amb la pràctica esportiva, evidenciant una major massa de teixits tous i una distribució diferent dels líquids corporals (major quantitat de TBW i de proporció ICW/ECW) (Andreoli et al., 2001). En canvi, en comparació amb una població de referència d'esportistes de 21.5 ± 5.0 i 20.7 ± 5.1 anys per homes i dones respectivament, el vector situa als subjectes majoritàriament en la meitat dreta de l'el·lipse, evidenciant un menor PhA (Figura 21B). Aquest desplaçament del vector cap a la dreta s'observa especialment en els corredors de trail, estudi on es va mostrar una major variabilitat respecte a la condició física dels esportistes.

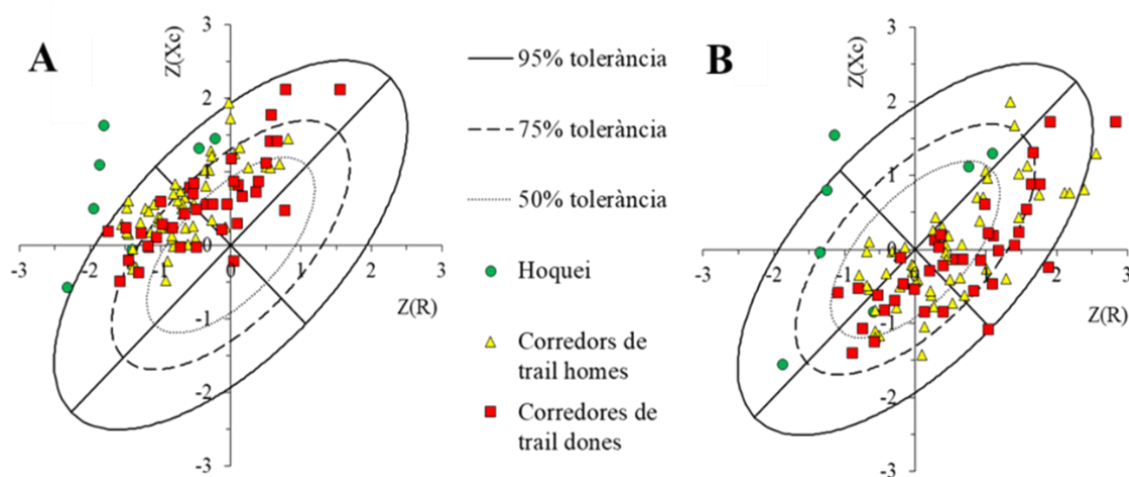


Figura 21. Gràfic de punts Z dels subjectes masculins que integren els estudis d'aquesta tesi doctoral en relació a les poblacions de referència. A: adults sans; B: esportistes. Z(R), z-score de resistència; Z(Xc), z-score de reactància.

5.2. BIVA global

La interacció entre BIVA i els canvis induïts per l'exercici físic és complexa i es veu influenciada per múltiples factors. Aquests canvis derivats de l'exercici poden tenir un impacte significatiu tant en l'àmbit del rendiment com de la salut. No obstant això, és crucial considerar certes limitacions. A través dels Estudis II, III i IV, aquesta investigació

pretén desxifrar aquesta complexitat, oferint una visió detallada de com l'exercici físic modifica els paràmetres bioelèctrics.

5.2.1. Canvis a curt termini

L'impacte de l'exercici físic en els paràmetres de bioimpedància a curt termini queda clarament evidenciat en els diversos estudis inclosos en aquesta tesi (Figura 22).

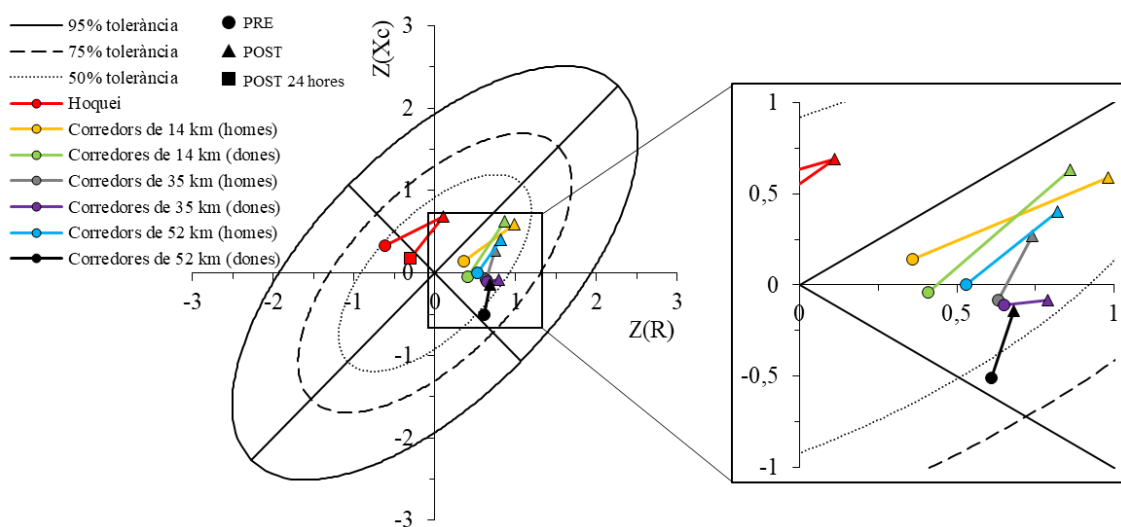


Figura 22. Canvis globals bioelèctrics en l'Estudi II i IV. POST, després de l'exercici; PRE, abans de l'exercici; $Z(R)$, z-score de resistència; $Z(Xc)$, z-score de reactància.

Els estudis II i IV examinen les variacions bioelèctriques en competicions de curta durada (inferiors a 24 hores): el primer en relació amb un entrenament intensiu d'hoquei i el segon, dins del marc de curses de trail running. Aquests estudis de curta durada tendeixen a ser més sensibles a elements externs com la ingesta de líquids i aliments o la temperatura corporal, ja que són factors que poden alterar les mesures bioelèctriques. Tot i així, tant en aquests estudis com en altres investigacions en contextos similars de diverses disciplines esportives (Carrasco-Marginet et al., 2017; Castizo-Olier, Carrasco-Marginet, et al., 2018; Gatterer et al., 2014; Heavens et al., 2016), s'han observat modificacions en els valors de R i Xc , indicatiu de canvis en la distribució dels fluids corporals i en la funcionalitat cel·lular. La R segueix el mateix patró, amb un increment que suggereix una reducció del volum de líquids corporals, tot i que en ambdós estudis els subjectes tenien la llibertat de consumir tants líquids com desitgessin. Tots els grups van experimentar un descens de la BM ($\Delta = 1.1\%$ per als jugadors d'hoquei i entre $0.8\text{-}2.2\%$ pels corredors/es de trail) però no es va detectar cap correlació significativa entre els canvis en la BM i migracions del vector. Situació que ja s'ha observat en altres estudis (Carrasco-Marginet

et al., 2017; Castizo-Olier, Carrasco-Marginet, et al., 2018; Gatterer et al., 2014) i que podria explicar-se degut a la ingesta consistent de líquids i aliments. Encara que també contrasta amb altres estudis que sí han identificat una correlació entre les modificacions bioelèctriques i les de la BM (Gatterer et al., 2013; Piccoli et al., 1996).

També s'ha detectat un augment post-exercici en la Xc en tots dos estudis, en línia amb els estudis de Carrasco-Marginet et al. (2017), Castizo-Olier, Carrasco-Marginet, et al. (2018) i Gatterer et al. (2014), el que reflecteix moviments de líquids entre compartiments intra i extracel·lulars (Gatterer et al., 2014). Paral·lelament als canvis en la Xc, el PhA també experimenta modificacions, sent aquest inversament proporcional al rati ECW/ICW (Marini et al., 2020). Tenint en compte que en ambdós estudis es va registrar una pèrdua d'aigua (disminució en la R), indicaria que la major pèrdua d'aquesta prové predominantment del compartiment extracel·lular. No obstant, cal una investigació més profunda sobre la dinàmica de la Xc i el PhA després de l'exercici, ja que són diversos els factors que poden influir en aquests paràmetres (Kyle et al., 2004b). Per exemple, Campa, Gatterer, et al. (2019) van observar que just després d'haver finalitzat un protocol d'exercici, els valors de Xc (i PhA) van disminuir, i després d'una dutxa freda de 10 minuts, aquests van incrementar-se de nou.

Per tant, cal destacar que les mesures de bioimpedància es poden veure afectades per aspectes com la temperatura corporal i cutània o el flux sanguini (Buono et al., 2004). Així, els resultats suggereixen que és necessari anar amb cura en els anàlisis a curt termini, tenint en compte que factors com la ingesta de líquids i aliments, o adaptacions corporals a l'exercici, com l'increment de la temperatura de la pell o la vasodilatació, poden modificar significativament els resultats, i per tant, les interpretacions.

5.2.2. Canvis a mig termini

Aquesta dinàmica adquireix una rellevància particular en el marc de les adaptacions corporals a través de diferents períodes de temps. Es sap que un increment en la R senyala la presència d'elements menys conductors en l'organisme (com la FM) o la pròpia absència d'elements conductors. D'altra banda, un augment en la Xc podria indicar canvis en aspectes com la mida, l'espessor, la composició i la proximitat de les membranes cel·lulars (Sperelakis, 2011). Així doncs, una anàlisi comparativa entre diversos intervals temporals més amplis, podria revelar diferències significatives en aquestes adaptacions, com en el cas de l'Estudi II i l'Estudi IV.

En l'Estudi II, els jugadors d'hoquei dels que s'ha parlat en la secció anterior van ser reavaluats 24 hores després de l'entrenament, observant que, tot i la reducció en la R, aquesta no va revertir als nivells basals. Aquest aspecte indica una reposició de líquids no complerta, malgrat que la BM sí que es va restablir. Això reforça la hipòtesi plantejada anteriorment sobre la manca de correlació entre BIA i la BM. La dinàmica observada en la Xc es correspon amb el mateix patró identificat en l'estudi de Castizo-Olier, Carrasco-Marginet, et al. (2018). Inicialment, es produeix un augment de la Xc post-exercici, el qual pot estar relacionat amb canvis en es fluids intracel·lulars o amb alteracions en les propietats elèctriques dels teixits, tal i com s'ha esmentat. A continuació, durant la fase de recuperació, s'observa una reducció de la Xc que arriba a ser inferior als nivells pre-exercici. Aquesta disminució podria reflectir una reorganització dels líquids corporals entre els compartiments intra i extracel·lular, probablement com a conseqüència dels processos de rehidratació i recuperació posterior a l'exercici intens. El fet que la Xc disminueixi fins a nivells inferiors als basals pot suggerir l'existència de canvis persistents en les propietats físiques de les membranes cel·lulars o en la composició dels teixits, els quals no es recuperen completament després del període d'exercici. Aquesta situació podria indicar un possible dany muscular, el qual no es resol plenament durant la recuperació (Carmona et al., 2015). Aquesta interpretació subratlla la importància d'analitzar en detall les variacions de la Xc per entendre les adaptacions i les possibles lesions a nivell muscular després de l'activitat física.

Pel que fa a l'Estudi III (Figura 23), els resultats van mostrar una baixada de la R durant la primera meitat del Giro d'Itàlia i un augment en la segona meitat, superant els valors basals. Aquests resultats, coherents amb altres estudis en el ciclisme (Corsetti et al., 2012; Pollastri, Lanfranconi, Tredici, Burtscher, et al., 2016; Pollastri, Lanfranconi, Tredici, Schenk, et al., 2016), apunten a una adaptació homeostàtica inicialment adequada, però que l'exercici de llarga durada i alta intensitat podria haver alterat la TBW i la seva distribució en els teixits tous, influenciant la R. A més, l'estrès oxidatiu derivat de l'exercici físic podria haver compromès la Xc a causa del dany cel·lular i l'esgotament dels antioxidants (Powers & Jackson, 2008).

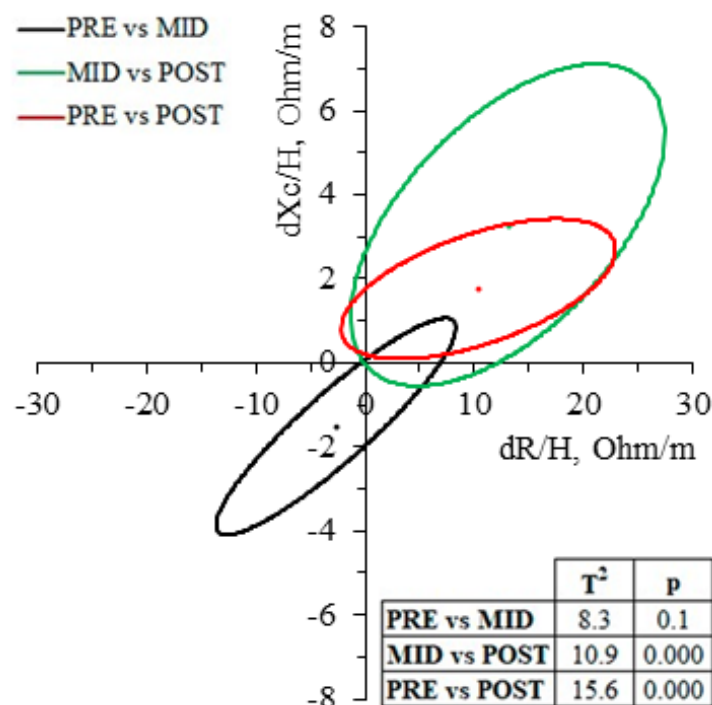


Figura 23. Canvis globals bioelèctrics en l'Estudi III. MID, a la meitat de la competició; p, p-valor; POST, al final de la competició; PRE, abans de la competició; R/H, resistència ajustada per l'estatura; T^2 , prova T-quadrada d'Hotelling; Xc/H, reactància ajustada per l'estatura.

L'Estudi III també revela una interacció dinàmica entre els canvis bioelèctrics i les variables hematològiques durant exercicis intensos. Aquesta interacció es manifesta a través de correlacions significatives entre els canvis bioelèctrics i els paràmetres relacionats amb els glòbuls vermells, com l'hemoglobina (Hb) i l'hematòcrit (Hct), així com el volum plasmàtic estimat, aspecte que també van identificar Giorgi, Sanders, et al. (2018). Aquesta relació proporciona una perspectiva detallada de la resposta fisiològica del cos a l'exercici. Durant exercicis extenuants, es detecta un fenomen d'hemodilució que es caracteritza per un increment del volum plasmàtic, seguit d'una disminució en els nivells d'Hb i Hct, una seqüència que reflecteix els canvis en la hidratació corporal (Lombardi et al., 2012). Aquesta seqüència de canvis es troba en línia amb altres estudis (Corsetti et al., 2012; Giorgi, Sanders, et al., 2018), que documenten una hemodilució inicial seguida d'una etapa de deshidratació, evidenciada per una disminució en els valors d'Hb i Hct.

5.2.3. Canvis a llarg termini

Tot i que cap dels tres estudis quasi-experimentals d'aquesta tesi es centra específicament en les adaptacions a llarg termini, aquesta aproximació és molt important, ja que permet un major control dels factors que podrien alterar els resultats, així com proporcionar un

període de temps més ampli per observar els efectes. Aquestes adaptacions bioelèctriques es poden evidenciar en investigacions realitzades durant temporades esportives complertes (Bongiovanni et al., 2020; Campa, Matias, Marini, et al., 2019; Cebrián-Ponce, Petri, et al., 2023; Mascherini, Gatterer, et al., 2015; Reis et al., 2020). Aquestes fluctuacions en els valors bioelèctrics són atribuïbles a canvis tant en la BMC com en la massa extracel·lular (ECM), elements que reflecteixen respectivament les variacions de la ICW i de la ECW (Gatterer et al., 2011).

En una investigació addicional sobre jugadors de rugbi durant una temporada competitiva (Cebrián-Ponce, Petri, et al., 2023) (Annex 1), i en línia amb les recerques prèviament esmentades, s'observa que tant la composició corporal com els valors bioelèctrics experimenten variacions significatives en funció de les diferents fases de la temporada competitiva (Mascherini, Gatterer, et al., 2015; Reis et al., 2020). Al llarg de gairebé nou mesos d'estudi, es van identificar dues tendències clares en les diverses avaluacions: des de l'inici fins a mitjans de temporada es va registrar una reducció del vector bioelèctric i un augment del PhA, mentre que des de la meitat fins al final de la temporada hi va haver un allargament progressiu del vector amb un PhA que va disminuint, indicant una pèrdua de líquids corporals i un augment de la relació ECW/ICW. Aquests canvis reflecteixen no només les fluctuacions en la salut cel·lular, on un PhA més alt indica una millor salut, sinó també la importància de la dinàmica del contingut hídric corporal i la relació ECW/ICW en el rendiment esportiu. A més, aquestes modificacions en la ICW mostren una relació directa amb millores en la força i la potència (Silva et al., 2010, 2011, 2014). Pel que fa a Hetherington-Rauth et al. (2021), van establir una correlació positiva entre el PhA i la potència relativa, així com la força relativa i absoluta. De manera similar, Hartmann-Nunes et al. (2020) van identificar correlacions inverses entre BIA i diversos indicadors de rendiment, com la velocitat màxima i la capacitat de sprint, així com en proves de rendiment neuromuscular i algunes variables de rendiment en partits de jugadors de rugbi. Matias et al. (2022) van trobar una associació positiva entre el PhA i el VO₂màx en jugadors de futbol sala. Així mateix, Mascherini, Gatterer, et al. (2015) van descobrir una correlació inversa entre la longitud del vector i el rendiment, mitjançant la prova de resistència yo-yo. Tots aquests resultats subratllen que l'avaluació mitjançant BIVA pot oferir un suport valuós per a la valoració de la condició física en esportistes, actuant com una eina de monitoratge per augmentar el rendiment.

5.3. BIVA muscular

5.3.1. Revisió sistemàtica (Estudi I)

Aquesta tesi neix d'una revisió sistemàtica centrada exclusivament en BIVA aplicada a nivell muscular en contextos d'exercici físic i salut (Cebrián-Ponce et al., 2021). 19 estudis es van classificar en tres categories diferents: 6 estudis caracteritzant els valors bioelèctrics musculars, 5 estudis informant sobre els canvis en els paràmetres bioelèctrics durant lesions muscular en esportistes i 8 estudis centrats en els canvis bioelèctrics durant o després d'un protocol d'exercici. S'ha de tenir en compte que el tipus de dispositiu o la col·locació dels elèctrodes entre estudis varien de manera considerable.

Canvis amb l'edat

Els estudis van constatar que com més edat tenien els subjectes, el PhA era menor. Això era especialment notori quan tenien més de 60 anys (Aaron et al., 2006), mostrant una disminució més acusada en els músculs de les extremitats inferiors (Janssen et al., 2000). Aquest patró coincideix amb la pèrdua d'atròfia muscular i força associada a l'envelliment, coneguda com a sarcòpenia (Cruz-Jentoft et al., 2019). Davant de la necessitat de mètodes d'avaluació en aquest context, ML-BIVA sorgeix com un mètode per poder realitzar avaluacions ràpides de la qualitat i quantitat muscular en l'envelliment o la sarcopènia, optimitzant tant la cura personalitzada dels pacients com el desenvolupament de la recerca clínica. Encara que els estudis amb animals són prometedors (Rutkove, Callegari, et al., 2023; Rutkove, Chen, et al., 2023; Verga et al., 2022), la recerca en humans és escassa (Aaron et al., 2006; Cheng et al., 2022).

Canvis davant de lesions musculars

Les lesions musculars esquelètiques, com contusions, elongacions o distensions, són freqüents en l'esport i representen un desafiament significatiu en la medicina esportiva, incloent la prevenció, determinació del moment òptim per tornar a jugar i la reducció de la taxa de recaiguda (Baoge et al., 2012). En aquest context, ML-BIVA permet analitzar la hidratació del teixit tou i la integritat de la membrana cel·lular, factors que es veuen afectats al moment d'una lesió i durant el procés de curació (Järvinen et al., 2005; Lukaski & Moore, 2012).

Lukaski & Moore (2012) van iniciar aquesta aproximació a nivell muscular avaluant ferides en les cames, i investigacions posteriors en futbolistes d'elit amb la mateixa

aproximació han demostrat que els valors de R , X_c i PhA disminueixen d'acord amb la gravetat de la lesió muscular, millorant a mesura que es produeix la curació (Nescolarde et al., 2013, 2015, 2017, 2020). Aquests descobriments han estat posteriorment resumits en la revisió de Nescolarde et al. (2023), proporcionant una base teòrica i pràctica per a la identificació de les lesions musculars i el seguiment fins a la recuperació.

Per tant, ML-BIVA s'estableix com una eina valuosa per a l'avaluació de lesions musculars. Utilitzant la cama contralateral com a referència, aquesta metodologia permet fer comparacions directes entre l'àrea lesionada i la sana. Es recomana iniciar les medicions 24 hores després de la lesió i continuar fent revisions periòdiques durant la recuperació. Aquest procediment permet quantificar la severitat de la lesió i fer un seguiment de la recuperació mitjançant el $\% \Delta$ de les dades bioelèctriques entre les zones afectades i les no afectades.

Els resultats dels estudis demostren que la disminució dels valors de R són indicatius de l'acumulació de líquids en l'àrea lesionada, mentre que les disminucions en X_c i PhA indiquen alteracions en la integritat de les membranes cel·lulars, traduint-se en un dany als teixits i cèl·lules musculars (Nescolarde et al., 2013, 2015, 2017, 2020). A mesura que les lesions es curen (confirmades amb MRI), s'observa un augment de la R , X_c i PhA progressiva, proporcionant així una correlació positiva entre els paràmetres de ML-BIVA i la recuperació física dels teixits (Figura 24).

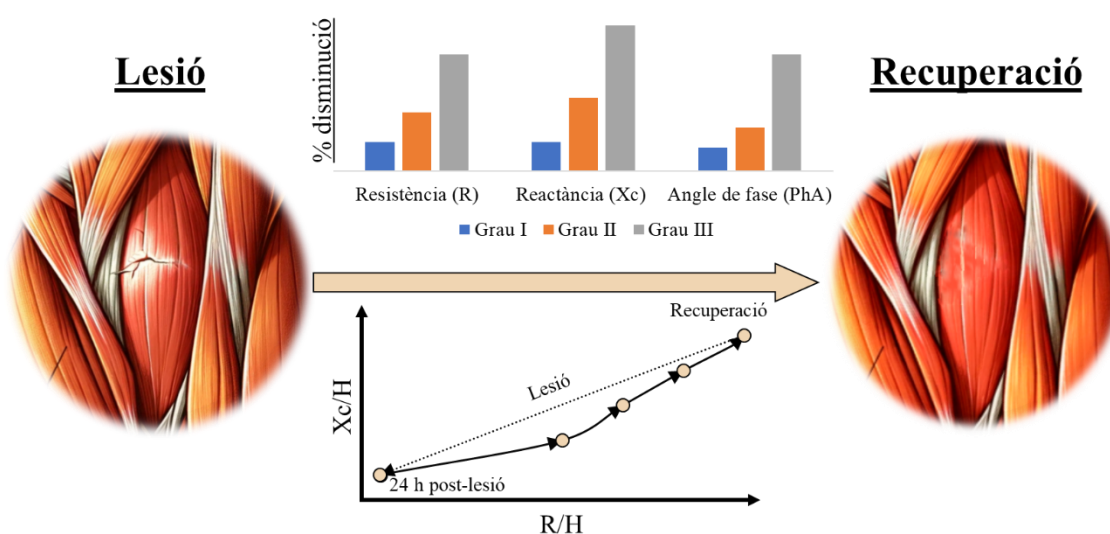


Figura 24. Esquema de la utilitat de ML-BIVA en diagnòstic i seguiment de lesions. R/H , resistència ajustada per l'estatura; X_c/H , reactància ajustada per l'estatura.

ML-BIA destaca per la seva elevada sensibilitat, especialment evident en l'anàlisi del $\% \Delta$ de X_c 24 hores després del moment de la lesió. Això subratlla la funcionalitat de ML-BIVA no únicament en la identificació de la gravetat de les lesions, sinó també en la valoració del procés de recuperació i enfortiment muscular per tornar a la competició esportiva.

Canvis davant de l'exercici físic

En l'àmbit de la investigació en l'exercici físic i l'esport, ML-BIVA sorgeix com una eina valuosa per a l'avaluació dels canvis morfològics i fisiològics (aguts i crònics), encara que aquesta aproximació es troba en una etapa més inicial.

De la mateixa forma que WB-BIVA, en la revisió sistemàtica es van identificar adaptacions a curt, mig i llarg termini. Dins de les adaptacions a curt termini, s'inclou una altra aproximació mitjançant la miografia d'impedància elèctrica dinàmica (D-EIM o ML-BIVA dinàmica), és a dir, analitzant els valors bioelèctrics durant l'execució d'un exercici.

ML-BIVA dinàmica i adaptacions a curt termini

En els treballs realitzats per Shiffman et al. (2003) i Li, Shin, et al. (2016), es van investigar els canvis en la impedància muscular en contraccions isomètriques màximes voluntàries. Shiffman et al. (2003) es van enfocar en la variació de la R i X_c en els músculs de l'avantbraç durant l'exercici. Els resultats van mostrar un augment exponencial d'ambdós paràmetres durant la contracció i un descens ràpid al deixar d'aplicar força (R es mantenia per sota dels valors basals i X_c per sobre). A més, la tendència era acumulativa entre sèries. D'altra banda, l'estudi realitzat per Li, Shin, et al. (2016) en el bíceps braquial van mostrar un patró similar en la R , la qual s'incrementava notablement durant la contracció (especialment a majors intensitats), i retornant ràpidament als valors basals una vegada finalitzada la força aplicada. Es va revelar una correlació moderada entre el temps fins a la fallada muscular i la disminució de la R , probablement deguda a l'acumulació de metabòlits i fluids intracel·lulars durant la contracció, la qual podria millorar la conductivitat del múscul. D'altra banda, els canvis en la X_c no van ser significatius. La discrepància entre els resultats relatius a la X_c en els diferents estudis podria atribuir-se a variacions en la col·locació dels elèctrodes (Rutkove, 2009), subratllant la importància de desenvolupar protocols estandarditzats i garantint la coherència i la comparabilitat entre els resultats obtinguts.

Fu & Freeborn (2018) van realitzar mesures d'impedància en el bíceps braquial durant l'execució d'exercicis isotònics de curl de bíceps fins a la fallada muscular, aplicant intensitats del 60 i 75% de la 1 repetició màxima (1RM), amb un límit de fins a 10 sèries. Van registrar una disminució significativa en els valors de R i Xc post-exercici en ambdós grups, però no entre intensitats.

Freeborn & Fu, (2019), amb un protocol anàleg, van constatar que els valors de R, Xc i PhA tendien a reduir-se progressivament, acumulant aquestes disminucions al llarg de les sèries. Aquesta tendència reforça la hipòtesi que la R és el primer indicador de canvis substancials, relacionant aquest fenomen amb l'increment del volum de fluid local degut a l'edema muscular. Aquesta hipòtesi queda reforçada amb l'estudi de Yasuda et al. (2015), els quals van demostrar un increment en el volum del bíceps braquial posterior a diverses sèries consecutives de curl, notant un augment progressiu de l'edema muscular després de cada sèrie addicional. Els canvis en els valors de Xc podrien no respondre tan directament a l'edema muscular i a l'acumulació de fluids com els canvis en R.

Huang et al. (2020) van analitzar el bíceps braquial durant contraccions isotòniques d'un curl a intensitats del 20, 40 i 60% de la contracció màxima voluntària, observant una reducció significativa de la R en totes tres intensitats. Segons els autors, els subjectes van assolir un estat de semi-fatiga quan la disminució de la R arribava aproximadament a 4 Ω . Quan aquesta disminució s'aproximava als 8 Ω , la fatiga muscular era total. Així mateix, van constatar que la disminució en la R varia segons els diferents nivells de càrrega, amb una disminució més accelerada a mesura que la càrrega incrementa.

Adaptacions musculars bioelèctriques a mig i llarg termini

Freeborn et al. (2020) van analitzar els canvis bioelèctrics derivats de l'exercici excèntric en un curl de bíceps al 90% de la 1RM, avaluant juntament el diàmetre del bíceps i el dolor autopercebut, en intervals temporals preestablerts (pre, immediatament posterior, i 24, 48, 72, i 96 hores post-exercici). Els resultats van demostrar una disminució pronunciada en la R i Xc del bíceps, així com un increment del diàmetre del braç i el dolor, destacant principalment a les 72 i 96 hores després de l'activitat.

Mascherini, Petri, et al. (2015), van utilitzar ML-BIVA per analitzar les adaptacions musculars en futbolistes d'elit (en els quàdriceps, isquiotibials i tríceps sural), abans d'iniciar la pretemporada i després de 50 dies d'entrenament. Els resultats van revelar una

disminució en la resistència normalitzada per longitud (R/L) en tots els grups musculars analitzats, el que reflecteix canvis significatius en la composició corporal segmentària. No obstant això, en els quàdriceps va haver-hi una disminució més pronunciada en R/L, acompanyada d'un augment significatiu en el PhA. En el tríceps sural es va observar també una reducció destacada en la reactància normalitzada per longitud (Xc/L).

5.3.2. Estudi quasi-experimentals (Estudis II, III i IV)

A diferència de la major part dels estudis que s'inclouen en la revisió sistemàtica, els canvis bioelèctrics musculars han estat analitzats en contextos més globals (involucrant múltiples articulacions i grups musculars simultàniament) i no tan analítics, amb un component més esportiu. Malgrat la diversitat en la modalitat i naturalesa dels exercicis, s'han identificat diversos patrons clau que subratllen la importància i la consistència dels canvis bioelèctrics a nivell muscular en resposta a l'exercici físic (Figura 25).

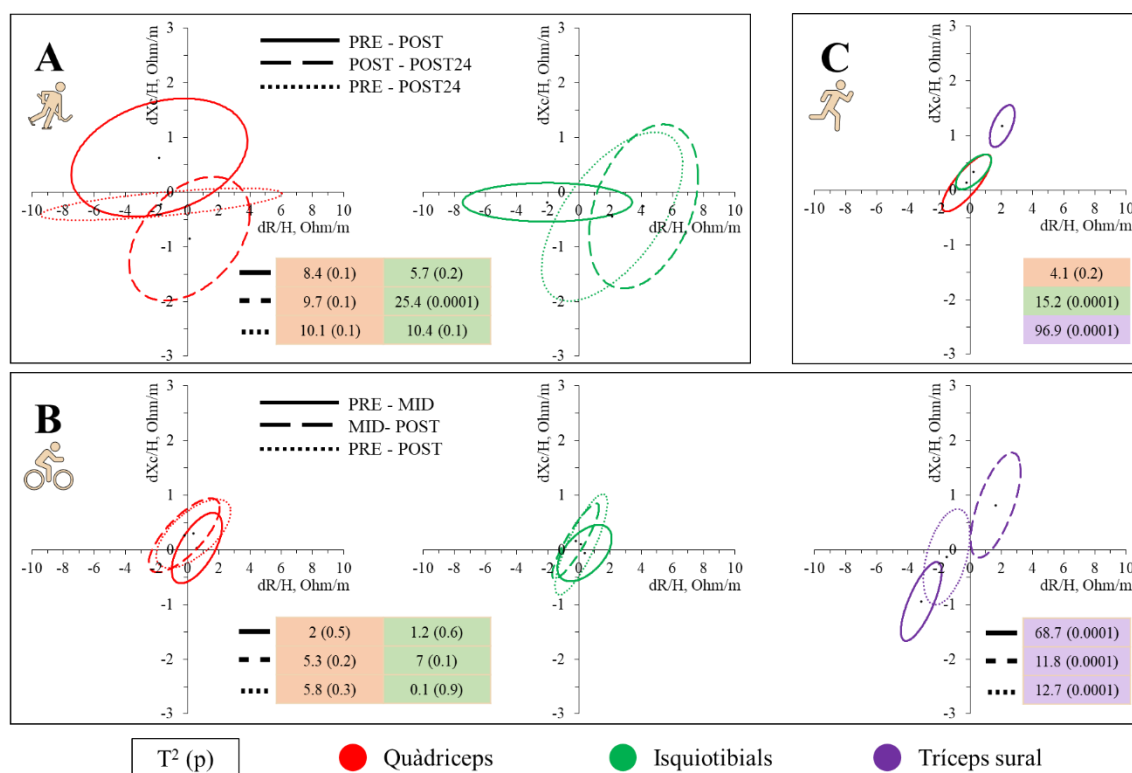


Figura 25. Gràfica aparellats de ML-BIVA dels estudis quasi-experimentals. A: estudi II (inclou dades no publicades); B: estudi III; C: estudi IV. MID, a la meitat de l'exercici; p, p-valor; POST, al finalitzar l'exercici; POST24, a les 24 hores de finalitzar l'exercici; PRE, abans de l'exercici; R/H, resistència ajustada per l'estatura; T², prova T-quadrada d'Hotelling; Xc/H, reactància ajustada per l'estatura.

Es pot apreciar com en general, ML-BIVA és sensible en la detecció dels canvis fisiològics musculars induïts per l'exercici físic, encara que aquestes adaptacions poden variar significativament segons l'esport, la temporalitat o el múscul analitzat.

En l'Estudi II (Cebrián-Ponce et al., 2022), es van explorar les variacions bioelèctriques del quàdriceps, seguint un entrenament d'alta intensitat en hoquei sobre patins. Es van registrar aquestes dades abans, immediatament després de l'entrenament i també 24 hores més tard. Tot i que es va observar una tendència cap a una reducció en la R i un increment en Xc, els canvis no van resultar estadísticament significatius. De manera similar, la recuperació dels valors de R i Xc fins a les 24 hores posteriors, tampoc van mostrar diferències significatives.

En dades complementàries no publicades de l'estudi, s'observa que els músculs isquiotibials presentaven un patró semblant (Figura 25A). No obstant, en el període de recuperació fins a les 24 hores posteriors a l'exercici, es va observar un desplaçament significatiu del vector, atribuïble principalment a un augment de la R. Les alteracions observades en les tendències post-exercici poden ser atribuïdes a l'augment del volum muscular (Hirono et al., 2020), el qual modifica el balanç d'aigua dins i fora de les cèl·lules, provocant inicialment un increment del perímetre de la cuixa de $1.5 \pm 0.4\%$ després de l'entrenament, que després disminueix durant les 24 hores següents. Aquesta dinàmica està vinculada a la resposta inflamatòria i a l'acumulació de fluids degut a microlesions musculars (Peake et al., 2017), el que provoca un augment del flux sanguini muscular, una major vasodilatació i un increment de la conductivitat elèctrica muscular a causa de l'augment de metabòlits (Freeborn et al., 2020). Aquest fenomen és el mateix que s'ha observat en els diversos estudis amb exercicis de resistència recollits en la revisió sistemàtica de l'Estudi I (Freeborn et al., 2020; Freeborn & Fu, 2019; Fu & Freeborn, 2018; Huang et al., 2020).

A més, l'estudi mostra que hi ha una relació inversa entre els canvis en la R i els nivells de CK, el que va ser inesperat ja que es preveia que la Xc (relacionada amb la integritat cel·lular), es veuria més afectada pel dany muscular. Aquest resultat suggereix que el dany muscular podria influir directament sobre la resistència bioelèctrica a través d'alteracions en la composició d'ECW i ICW. No obstant això, cal interpretar amb precaució aquests resultats, donat que els canvis en R no van ser estadísticament significatius.

En l'Estudi III (Cebrián-Ponce, Irurtia, et al., 2023), es va investigar el comportament bioelèctric dels quàdriceps, els isquiotibials i el tríceps sural en ciclistes professionals durant el Giro d'Itàlia (Figura 25B). Les avaluacions es van efectuar en tres moments diferents: al començament, a la meitat i al final de l'esdeveniment. Els resultats no van mostrar variacions significatives als quàdriceps ni als isquiotibials, encara que hi va haver tendència cap a un increment de la R, degut a una possible pèrdua de fluids. Per contra, el tríceps sural va presentar canvis bioelèctrics notables, oposats als observats en els altres dos grups musculars, evidenciant inicialment una disminució tant de la R com de la Xc. Això va indicar un augment del fluid en l'àrea circumdant i un deteriorament de la funció de la membrana cel·lular. Aquesta tendència va revertir-se en la segona meitat de la competició, indicant una notable pèrdua de fluid local, superant fins i tot els valors inicials. El tríceps sural, i en concret el gastrocnemi, és un del músculs més demandats en el ciclisme, per sobre d'altres com el quàdriceps o el isquiotibials (Dingwell et al., 2008).

La tendència del PhA va ser similar en els tres grups musculars, disminuint en el període inicial a mitjans de la competició i incrementant-se cap al final. Aquests resultats, juntament amb l'elongament del vector, van indicar que la major pèrdua de fluids va tenir lloc durant la segona meitat de la prova, predominantment des del compartiment extracel·lular.

En l'estudi IV (Cebrián-Ponce, Marini, et al., 2023), es van analitzar 11 corredors de trail running abans i després de participar en una cursa de 35 o 52 kilòmetres, analitzant la resposta bioelèctrica dels músculs dels quàdriceps, isquiotibials i tríceps sural (Figura 25C). En l'estudi original, els corredors van ser dividits en dos grups segons la distància de la cursa (5 subjectes per la de 35 km i 6 per la de 52 km). No obstant això, per aquesta anàlisi, es va optar per tractar els 11 corredors com un únic grup, donat que els patrons bioelèctrics observats son molt similars entre ells (per a una comparativa detallada entre els grups, consultar l'estudi original).

Un cop més, destaca que el tríceps sural va experimentar els canvis més significatius, evidenciant un increment notable en els paràmetres R, Xc i PhA. Els isquiotibials van mostrar una tendència similar, encara que en menor mesura. Addicionalment, es va observar una reducció en els perímetres de les cuixes i les cames, indicant una tendència diametralment oposada a la registrada en l'Estudi II. Aquesta disparitat pot ser atribuïda a les diferents demandes fisiològiques entre els esforços d'alta intensitat intermitent

típiques de l'hoquei i l'exercici aeròbic sostingut característic del trail running. Aquests canvis bioelèctrics indiquen una tendència cap a la pèrdua de fluids a nivell muscular, principalment del compartiment extracel·lular.

Les curses de trail running generen una fatiga perifèrica notable en les extremitats inferiors, que comporta una reducció significativa tant en la funcionalitat muscular com en el rendiment atlètic (Baiget et al., 2018; Koral et al., 2022; Pastor et al., 2022; Pradas et al., 2021; Temesi et al., 2021). Destaca particularment l'afectació del tríceps sural, que experimenta un estrès considerable durant les competicions de muntanya (Fourchet et al., 2012). A més, el terreny irregular i les exigències excèntriques elevades d'aquest tipus de curses poden provocar un augment de l'activació i, en conseqüència, una major fatiga en els músculs posteriors, com els isquiotibials i el tríceps sural, fenomen que sovint es veu acompanyat de pèrdues locals de fluids (Roca, 2019).

Tal i com s'ha vist en la revisió sistemàtica, Mascherini, Petri, et al. (2015) van aplicar ML-BIVA (en els quàdriceps, isquiotibials i tríceps sural) per analitzar les adaptacions a llarg termini en futbolistes d'elit després de 50 dies d'entrenament. Aquests jugadors van experimentar una disminució significativa en la R en tots tres grups musculars, encara que només en el tríceps sural es va registrar una disminució significativa de la Xc, suggerint que el tríceps sural es va veure afectat per l'entrenament.

A partir d'aquests resultats, es constata que el tríceps sural és especialment propens a la fatiga i a l'erosió en els esports analitzats. Resulta fonamental prosseguir amb la investigació per aïllar o controlar possibles factors que poden influenciar aquests resultats. Aquesta investigació hauria d'incloure l'anàlisi del tipus d'esport i l'activació muscular específica, així com les característiques pròpies d'aquests músculs, com ara la implicació dels tendons o la seva disposició biomecànica, que podrien ser elements rellevants per a l'anàlisi de les dades. En aquest context, Baumert et al. (2021) van suggerir que els músculs esquelètics amb una major concentració de fibroblasts poden exhibir una capacitat superior per a la reorganització de la matriu extracel·lular complexa, facilitant així una recuperació més àgil en cas de dany muscular, el que podria ser un indicador de les diferències bioelèctriques entre músculs. La realització d'aquests estudis addicionals és essencial per a una millor comprensió de les demandes fisiològiques i biomecàniques específiques en l'esport, i per desenvolupar estratègies més efectives per a la seva preparació i recuperació.

En definitiva, aquests estudis reafirmen la rellevància de ML-BIVA com a eina potent en la valoració de canvis bioelèctrics musculars, demostrant la seva aplicabilitat en diversos àmbits de l'exercici i la salut. La seva capacitat per detectar adaptacions musculars subratlla la seva rellevància tant en la medicina esportiva com en el rendiment esportiu. La continuïtat de la recerca i l'adopció d'aquesta metodologia en pràctiques clíniques i d'entrenaments poden millorar significativament l'atenció a l'esportista, promovent una recuperació més eficient i una preparació física més adaptada.

5.3.3. Estandardització del protocol

Tal com s'ha vist en els articles que integren la tesi, ML-BIA ha esdevingut una eina notable per a la identificació de canvis bioelèctrics que reflecteixen les variacions morfològiques i fisiològiques dins del teixit muscular. Aquesta sensibilitat demostrada obre nombroses possibilitats dins de l'àmbit de la recerca i l'aplicació clínica. No obstant això, per realitzar una implementació efectiva i garantir comparacions consistents entre estudis, és imperatiu estandarditzar el protocol de mesurament. Alguns dels factors crítics en l'estandardització són:

Influència de la capa de greix subcutani (SFL): l'espessor de la SFL és un factor determinant en la interpretació de les mesures d'impedància. Estudis com els realitzats per Jafarpour et al. (2013), Sung et al. (2013) i Li, Li, et al. (2016), han posat de manifest com aquesta variable pot alterar significativament els valors de R , X_c i PhA . Dins d'aquests paràmetres, la X_c presenta una menor susceptibilitat a les variacions provocades per increments en l'espessor de la SFL. A més, s'ha observat que l'efecte de la SFL sobre les mesures bioelèctriques pot ser atenuat al utilitzar configuracions d'elèctrodes amb amplituds majors. Malgrat això, és crucial assenyalar que aquest efecte de mitigació no elimina completament la influència de la SFL, sinó que només la redueix. Aquests descobriments ressalten la importància de considerar la SFL en el disseny del protocol, suggerint que una selecció metòdica de la distància entre els elèctrodes i la seva orientació pot mitigar l'impacte de la variabilitat de l'espessor de la SFL.

Col·locació i Configuració dels Elèctrodes: Sanchez et al. (2016, 2021) informen sobre com la variació en la posició dels elèctrodes pot influir en la forma i amplitud de la senyal registrada (Figura 26), especialment quan es modifica l'orientació respecte a l'eix principal de configuració dels elèctrodes. Aquestes variacions poden alterar profundament R i X_c , apuntant a la necessitat d'una col·locació precisa per minimitzar

errors. Una major distància entre els elèctrodes facilita la reproductibilitat i minimitza les interferències de la SFL, encara que una separació excessiva pot introduir distorsions per la inclusió de teixits adjacents en les mesures (Rutkove et al., 2017). A més, és important considerar les propietats anisotròpiques dels teixits, com la pell, la SFL i el múscul, per a una anàlisi d'impedància més precisa. Això ressalta la necessitat crítica d'elaborar i seguir un protocol estandarditzat per a la col·locació dels elèctrodes, assegurant així la consistència en la recollida de dades tant en estudis longitudinals com en la comparació entre diferents individus.

En aquest context, al llarg de la tesi hem utilitzat les denominacions de quàdriceps, isquiotibials i tríceps sural per referir-nos als grups musculars examinats mitjançant ML-BIVA. No obstant això, és pertinent assenyalar que la ubicació dels elèctrodes per a les mesures dels diferents estudis es va determinar basant-se en la localització anatòmica dels músculs recte anterior, bíceps femoral i gastrocnemi. Donat que la penetració del corrent elèctric pot arribar a diverses profunditats i pot ser influenciada pels músculs adjacents, es va optar per utilitzar aquestes nomenclatures més àmplies.

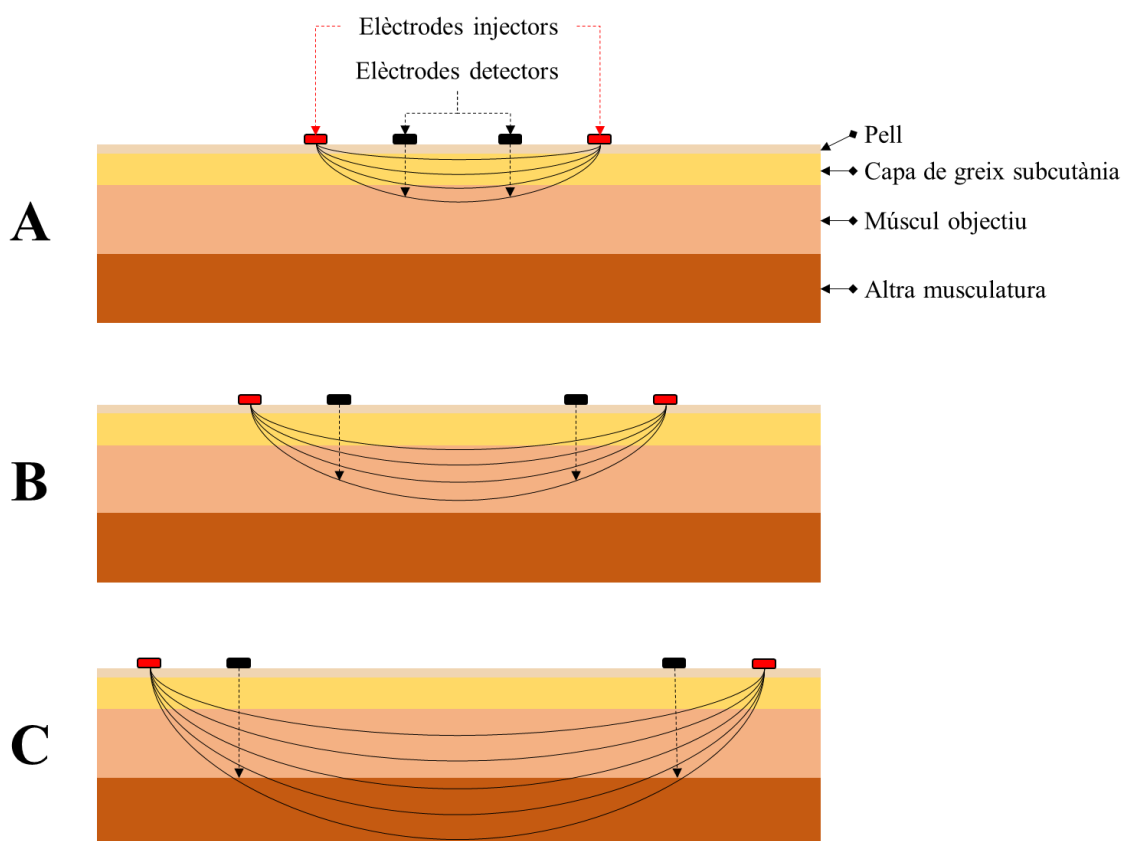


Figura 26. Profunditat del senyal segons la col·locació dels elèctrodes. A: distància entre els elèctrodes estreta; B: distància entre els elèctrodes mitjana; C: distància entre els elèctrodes àmplia.

Selecció de la Forma d'Elèctrodes: la comparativa entre formes d'elèctrodes, específicament circulars contra rectangulars, evidencia la influència significativa de la geometria en la distribució del corrent i, en conseqüència, en la fiabilitat de les mesures de bioimpedància. Segons l'estudi d'Ahad et al. (2023), els elèctrodes circulars, per la seva configuració, propicien una transmissió de corrent més directa i menys dispersa comparada amb els rectangulars, degut al seu perímetre reduït i la conseqüent injecció més perpendicular de corrent. Aquesta característica fa que estiguin menys afectats per les variacions en la SFL, contrastant amb els elèctrodes rectangulars, on el corrent tendeix a estendre's més horitzontalment, augmentant la seva dissipació. Així, la selecció d'elèctrodes circulars s'identifica com una estratègia més eficaç per a minimitzar l'impacte de la variabilitat de la SFL.

Adicionalment, el desenvolupament de noves configuracions d'elèctrodes, que combinen els rols d'injectors i detectors en una única peça adhesiva, permeten mitigar les problemàtiques associades amb la mala alineació i les distàncies variables entre elèctrodes, facilitant una col·locació més precisa i uniforme. Aquesta innovació podria representar un avanç significatiu en la reducció de les variabilitats en les mesures de bioimpedància, afavorint una major estandardització i fiabilitat en les avaluacions.

Normalització de les dades: sovint s'ha fet servir l'estatura dels subjectes per realitzar la normalització de les dades, de la mateixa forma que en WB-BIVA (R/H , X_c/H , Z/H). No obstant això, dins del context de ML-BIVA, es proposa una adaptació: la normalització segons la longitud del múscul (R/L , X_c/L , Z/L). Aquesta aproximació, que alinea els paràmetres bioelèctrics amb les dimensions específiques de la porció muscular analitzada, pot oferir una representació més precisa de les seves propietats bioelèctriques. Aquest pas permetria realitzar comparacions de millor forma entre diferents estudis.

Tot i els aspectes previs a considerar, l'estudi de Martinez-Gonzalez et al. (2020) van mostrar una alta fiabilitat tant intra- com inter-avaluador utilitzant ML-BIVA sobre els quàdriceps de subjectes sans, mostrant una notable consistència entre diferents sessions i dins de la mateixa sessió, especialment en el PhA, que es va revelar com el menys influenciat per l'avaluador o el moment de la medició. De manera similar, Ahad et al. (2023) van reportar una fiabilitat des de bona fins a excel·lent en l'avaluació dels isquiotibials, ressaltant, un altre cop, la robustesa del PhA com a indicador consistent. Aquestes investigacions reafirmen la validesa de ML-BIVA per avaluacions musculars

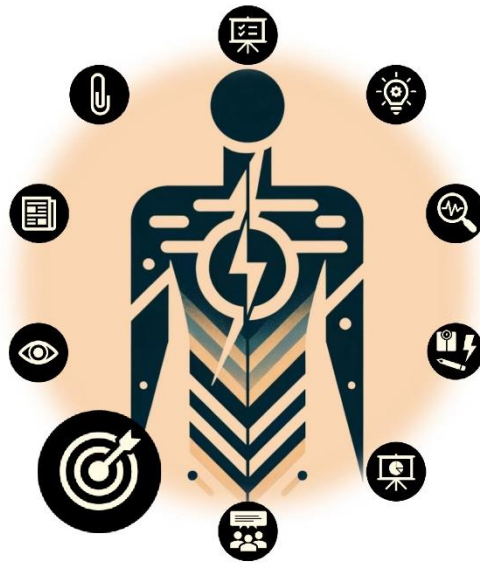
ràpides i exactes, demostrant la seva aplicabilitat tant en entorns clínics com en estudis longitudinals.

5.4. Limitacions

En el marc dels estudis realitzats per aquesta tesi doctoral, s'han observat diverses qüestions que mereixen ser mencionades, encara que no afecten de manera significativa la integritat global dels resultats:

1. **Diversitat en les tècniques d'avaluació:** s'ha identificat una certa variabilitat en els protocols i dispositius utilitzats en l'avaluació de la impedància elèctrica. Aquesta diversitat reflecteix l'adaptabilitat dels mètodes a diferents contextos d'investigació, encara que pot limitar la comparabilitat directa entre estudis.
2. **Control de variables externes:** malgrat els esforços per minimitzar les fonts d'error, com la gestió de la ingesta d'aliments i líquids, algunes variables externes van romandre fora del control complet dels investigadors, reflectint les condicions naturals en les quals es realitzen activitats físiques.
3. **Ampliació del marc teòric:** la limitada informació científica disponible en algunes àrees específiques (com ML-BIVA) ressalta la necessitat de futura investigació, tot i que també indica l'àrea de contribució potencial d'aquest treball.
4. **Característiques de les mostres:** encara que la mida de la mostra en alguns estudis fos reduïda i no sempre diversa en termes de gènere, aquestes mostres proporcionen una base important per a conclusions preliminars i específiques al context.
5. **Temporalitat i abast de l'avaluació:** la recopilació de dades es va realitzar en intervals específics, que, tot i ser adequats per als objectius dels estudis, podrien beneficiar-se d'un seguiment més extens en investigacions futures per comprendre millor les dinàmiques a llarg termini.
6. **Tècniques i mètodes específics:** la selecció de mètodes d'avaluació, com la manca d'ús de tècniques de referència, es va basar en el marc pràctic i disponible dels estudis, que podria ser ampliat en futures recerques per aprofundir en els resultats.

Aquests punts són mencionats per garantir la transparència i proporcionar un context complet als lectors, tot subratllant que, malgrat aquestes qüestions, els estudis aporten valor significatiu en el camp de la ciència de l'exercici físic i de l'esport.



CONCLUSIONS

6a. Conclusions (català)

BIVA ha demostrat ser una eina significativa en l'avaluació de les propietats electrofisiològiques del cos dins del context esportiu, superant algunes de les limitacions de BIA tradicional.

Aprofundint en l'estudi de WB-BIVA, observem que a curt termini, els canvis en la R i Xc reflecteixen alteracions en la distribució de líquids corporals i la funcionalitat cel·lular post-exercici, sent indicadors de les adaptacions immediates a l'activitat física. A mig termini, es detecten canvis significatius que podrien indicar una reorganització dels líquids corporals i canvis en les propietats de les membranes cel·lulars, destacant-se com a resultats dels processos de rehidratació i recuperació. A llarg termini, les fluctuacions en els valors bioelèctrics durant temporades esportives completes evidencien canvis en la composició corporal i la salut cel·lular. Aquests canvis es correlacionen amb millores en la força, la potència i altres indicadors de rendiment esportiu, destacant l'aplicabilitat de BIVA com a eina de monitoratge en l'entorn esportiu. És crucial destacar que la temporalitat és un factor de gran importància en les avaluacions, ja que a curt termini es presenten múltiples factors que poden ser difícils de controlar en contextos ecològics. A més, les adaptacions bioelèctriques, segons el marc temporal, poden variar en la seva interpretació, sent això clau per a una correcta valoració dels canvis físics i fisiològics.

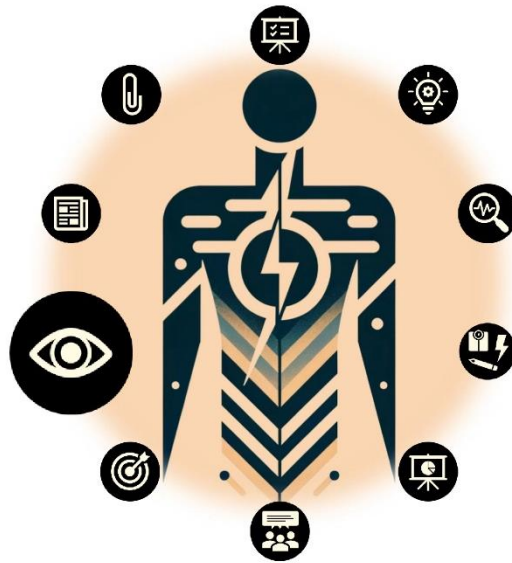
ML-BIVA permet fer una avaluació no invasiva de la salut muscular i facilita la identificació i seguiment de recuperació de lesions. Això és especialment rellevant en el monitoratge dels esportistes, destacant per la seva utilitat en la millora de l'atenció personalitzada i el desenvolupament de programes d'entrenament. A través de l'anàlisi de múltiples grups musculars i contextos d'exercici, s'observen patrons consistents que reforcen la utilitat de ML-BIVA en la detecció de canvis morfològics i fisiològics. Especialment notable és la resposta del tríceps sural, que es veuen particularment afectats en diferents modalitats esportives i després d'entrenaments intensius. La continuïtat en la recerca d'aquesta metodologia potenciarà la seva integració en la pràctica clínica i esportiva, millorant així l'atenció i el rendiment dels esportistes. Encara que per a una implementació efectiva de ML-BIVA, el primer pas ha de ser realitzar una estandardització rigorosa pel protocol d'avaluació. L'estandardització d'aquest mètode milloraria la fiabilitat i la validesa de les mesures, facilitant la comparabilitat entre estudis i la interpretació longitudinal dins del mateix subjecte.

6b. Conclusions (english)

BIVA has proven to be a significant tool in assessing the electrophysiological properties of the body within the sports context, surpassing some of the limitations of traditional BIA.

Deep diving into the study of WB-BIVA, we observe that in the short term, changes in R and Xc reflect alterations in the distribution of body fluids and post-exercise cellular functionality, being indicators of immediate adaptations to physical activity. In the medium term, significant changes are detected. This could indicate a reorganization of body fluids and changes in the properties of cellular membranes, standing out as results of the rehydration and recovery processes. In the long term, fluctuations in bioelectrical values during full sports seasons show changes in body composition and cellular health. These changes correlate with improvements in strength, power, and other indicators of sports performance, highlighting the applicability of BIVA as a monitoring tool in the sports environment. It is crucial to highlight that temporality is a factor of great importance in the evaluations, as in the short term there are multiple factors that can be difficult to control in ecological contexts. Furthermore, the bioelectrical adaptations, according to the time frame, may vary in their interpretation, being this key for a correct assessment of physical and physiological changes.

The ML-BIVA allows for a non-invasive evaluation of muscular health and facilitates the identification and tracking of injury recovery. This is especially relevant in monitoring athletes, standing out for its utility in improving personalized care and the development of training programs. Through the analysis of multiple muscle groups and exercise contexts, consistent patterns are observed that reinforce the utility of ML-BIVA in detecting morphological and physiological changes. Especially remarkable is the response of the triceps surae, which are particularly affected in different sports modalities and after intense training sessions. Continuity in the research of this methodology would enhance its integration into clinical and sports practice, thus improving the care and performance of athletes. However, for an effective implementation of ML-BIVA, the first step should be to carry out a rigorous standardization of the evaluation protocol. The standardization of this method would improve the reliability and validity of the measurements, facilitating comparability between studies and longitudinal interpretation within the same subject.



LÍNIES FUTURES D'INVESTIGACIÓ

7. Línies futures d'investigació

Algunes de les futures línies d'investigació derivades d'aquesta tesi són:

1. Ampliació i diversificació de la base de dades amb esportistes

Augmentar la robustesa i la capacitat de generalització dels estudis actuals, mitjançant l'ampliació de les bases de dades per incloure mostres més àmplies i diverses d'esportistes, per als diferents tipus d'aproximació de la bioimpedància. La inclusió d'individus de diverses disciplines, amb una àmplia gamma d'edats, gèneres i nivells de rendiment, contribuiria a millorar la fiabilitat i la generalització dels resultats obtinguts.

2. Realització d'estudis longitudinals en períodes competitiu

Implementar seguiments detallats de les variacions bioelèctriques durant temporades esportives complertes, permetent la correlació d'aquests canvis amb el calendari de competicions, els cicles d'entrenament, els patrons nutricionals, els períodes de descans i altres variables rellevants. La interpretació d'aquests canvis pot variar en funció de la temporalitat, ja sigui a curt, mig o llarg termini, reflectint diferents dinàmiques i adaptacions dels esportistes al llarg de la temporada.

3. Estudi de la interacció entre canvis bioelèctrics i altres paràmetres

Ampliar la investigació per aprofundir en la comprensió de l'impacte que l'exercici físic té en diversos paràmetres, no limitant-se únicament als canvis bioelèctrics, sinó abastant també altres indicadors significatius com els nivells d'Hb, Hct i CK. Aquests paràmetres són essencials com a marcadors de deshidratació i dany muscular, elements clau en l'estudi de les respostes fisiològiques a l'exercici. Addicionalment, es proposa una investigació detallada sobre la relació existent entre la bioimpedància i els nivells de glucogen muscular. Aquest enfocament es justifica pel fet que l'acumulació de glucogen en els músculs influeix significativament en el balanç hídric corporal. Així, els canvis en el contingut hídric corporal poden alterar les mesures de bioimpedància, oferint, per tant, una possible via indirecta per a l'estimació dels nivells de glucogen muscular.

4. Integració de noves tecnologies en els processos d'avaluació

Investigar la integració de la bioimpedància amb altres eines de diagnòstic i seguiment, incloent per exemple la MRI o l'electromiografia, desenvolupant un enfocament holístic

i multidimensional que faciliti una avaluació més exhaustiva i precisa de l'estat fisiològic, la condició de salut i el rendiment dels esportistes. Mitjançant la combinació de la bioimpedància, amb l'alta resolució estructural que ofereix la MRI, es podrien obtenir imatges detallades dels teixits corporals, permetent una comprensió més profunda de les variacions internes relacionades amb l'entrenament i la recuperació. A més, la incorporació de l'electromiografia, que registra l'activitat elèctrica produïda pels músculs, podria complementar aquesta informació amb dades valuoses sobre el funcionament i l'estat dels músculs en diferents estats de repòs i activitat. L'aplicació d'aquest enfocament multidisciplinari podria ser particularment beneficiosa en la prevenció de lesions, en l'optimització del rendiment i en la gestió eficient de la recuperació post-esforç.

5. Desenvolupament i estandardització de ML-BIVA

Degut a la manca d'un protocol estàndard per a la col·locació dels elèctrodes en l'anàlisi de ML-BIVA, es proposa desenvolupar un nou protocol inspirat en les directrius del SENIAM (Hermens et al., 2000), àmpliament reconegut per a la col·locació d'elèctrodes en l'electromiografia de superfície (sEMG). Aquesta proposta sorgeix de la necessitat d'adaptar les bones pràctiques ja existents de la sEMG a les particularitats de ML-BIVA, per millorar la precisió i la reproductibilitat de les mesures bioelèctriques a través dels diferents grups musculars. A més, no únicament és important la mesura aïllada de R i X_c , sinó la consistència en la col·locació dels elèctrodes a través de les diferents mesures, per assegurar que els canvis observats en els valors de bioimpedància reflecteixin fidelment les adaptacions fisiològiques reals del subjecte, més enllà de la idoneïtat de la posició inicial dels elèctrodes. Això inclouria la documentació detallada de la ubicació dels elèctrodes, l'ús de marcadors cutanis o plantilles i la capacitació dels avaluadors per garantir una metodologia homogènia, en base a referències anatòmiques. Així, s'establiria una metodologia uniforme basada en referències anatòmiques, millorant la fiabilitat de les dades i facilitant l'anàlisi comparativa dels canvis bioelèctrics, tant a nivell individual com entre diferents subjectes.

En conclusió, les línies d'investigació futures suggerides representen possibles avanços interessants per ampliar el nostre coneixement en la fisiologia de l'exercici envers a la bioimpedància, oferint perspectives prometedores per a la millora substancial de l'avaluació, el rendiment i la salut dels esportistes.



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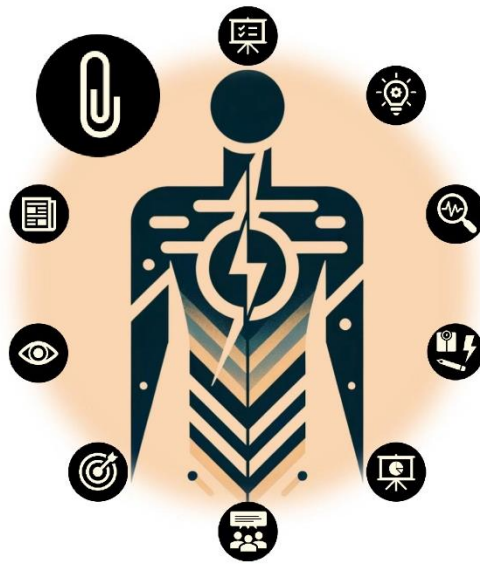
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ANNEXOS

9. Annexos

9.1. Annex 1

Article publicat en *Sports* (2023):

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Quartil JCR SCIE/SSCI -	Quartil SJR Q1
Categoria JCR SCIE/SSCI -	Categoria SJR Orthopedics and sports medicine

In-Season Longitudinal Hydration/Body Cell Mass Ratio Changes in Elite Rugby Players

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Article

In-Season Longitudinal Hydration/Body Cell Mass Ratio Changes in Elite Rugby Players

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Abstract: Background: Hydration status has a direct role in sports performance. Bioelectrical Impedance Vector Analysis (BIVA) and Urine Specific Gravity (USG) are commonly used to assess hydration. The study aims to identify the sensitivity and relationship between BIVA and USG in a field sports setting. Methods: BIVA and USG measurements were conducted five times throughout one rugby season. 34 elite male rugby players (25.1 ± 4.4 years; 184.0 ± 7.8 cm; 99.9 ± 13.4 kg) were enrolled. Differences over time were tested using one-way repeated measures ANOVA, and Bonferroni's post-hoc test was applied in pairwise comparisons. Resistance-reactance graphs and Hotelling's T2 test were used to characterize the sample and to identify bioelectrical changes. A repeated measures correlation test was conducted for BIVA-USG associations. Results: Two clear trends were seen: (1) from July to September, there was a vector shortening and an increase of the phase angle ($p < 0.001$); and (2) from December to April, there was a vector lengthening and a decrease of the phase angle ($p < 0.001$). USG reported neither changes nor correlation with BIVA longitudinally ($p > 0.05$). Vector variations indicated a body fluid gain (especially in the intracellular compartment) and a body cell mass increase during the preseason, suggesting a physical condition and performance improvement. During the last months of the season, the kinetic was the opposite (fluid loss and decreased body cell mass). Conclusions: Results suggested that BIVA is sensitive to physiological changes and a better option than USG for assessing hydration changes during a rugby sports season.

Keywords: urine; phase angle; bioelectrical; BIVA; team sport

1. Introduction

Rugby is considered an aerobic sport with an intermittent activity profile of frequent changes between high- and low-intensity periods, requiring physical qualities such as endurance, speed, agility, power and flexibility [1,2]. These athletic qualities are influenced by body composition characteristics [3,4]. The body of evidence suggests that as competitive standards rise, rugby players require higher functional body mass (BM) with a lower percentage of body fat [2]. These anthropometric characteristics and athletic aptitudes change across different season periods [5,6]. Therefore, strength and conditioning coaches should consider tracking these parameters to maximize sports performance.

Among the methods developed to assess body composition, bioelectrical impedance analysis (BIA) is an easy and viable technique for obtaining quantitative assessment [7].

However, this approach has some limitations due to the potential inaccuracy of the predictive equation when applied to populations with different characteristics, as well as a minimum standard error of estimation, even when the equation is accurate [8]. In recent years, bioelectrical impedance vector analysis (BIVA) has been introduced within the sports field to investigate body composition and its changes with sport-specific training [9–11], overcoming the aforementioned limitation, since BIVA allows the identification of changes in hydration status and mass of soft tissues by solely considering the raw impedance components (resistance, R ; reactance, X_c) independently of regression predictions and assumptions of the constant chemical composition of the fat-free body [7,12,13]. The derived impedance (Z) and the phase angle (PhA) can also be obtained from these parameters. According to the classic BIVA approach, Z is inversely related to total body water (TBW) [10]. PhA indicates cellular health and cell membrane integrity, and is inversely related to the extracellular/intracellular water (ECW/ICW) ratio [14]. Therefore, all interpretations should be based on the interpretation of Z and PhA jointly, along with the vector position on the Resistance-Reactance (RX_c) graphs [15]. In these graphs, vector displacements parallel to the major axis of tolerance ellipses indicate changes in tissue hydration (vector lengthening indicates fluid loss, whereas a vector shortening indicates fluid gain). Vector shifts parallel to the minor axis of the ellipses indicate changes in cell mass and ECW/ICW ratio: a leftward shift indicates increased cell mass and a decreased ECW/ICW ratio, whereas a rightward shift indicates a decreased cell mass and an increased ECW/ICW ratio [7,12]. Although BIVA has been studied in many sports, the scientific literature on BIVA applied in rugby is scarce, especially at the elite level.

The measurement of urine-specific gravity (USG), which measures the weight of the urine and the particles contained in it [16], is also a commonly used method to assess hydration status among athletes [16–18]. However, USC's day-to-day reliability has not been reported yet [19]. Nevertheless, some research has been made using USG in rugby, detecting that most players are, in principle, hypohydrated [19,20].

As BIVA and USG are standard methods to assess hydration, the present study aimed to examine the sensitivity and relationship between BIVA and USG at multiple time points throughout a competitive rugby season in elite players to measure hydration and body cell mass status. We hypothesized that BIVA and USG methods are sensitive enough to detect the physiological changes in the body in the different stages of the season, with a correlation between them. In addition, we also hypothesized that the bioelectrical change, according to the time of the competitive season, is due to the adaptations of the physical training.

2. Materials and Methods

2.1. Subjects

This retrospective, quasi-experimental study involved 34 Italian elite rugby players (age: 25.1 ± 4.4 years; height (H): 184.0 ± 7.8 cm; BM: 99.9 ± 13.4 kg; body mass index (BMI): 29.4 ± 3.2 kg/m²) from the Rugby Rovigo team who participate in the “Top 10 Championship”. They were all in good health, as assessed by their team physician. No drugs or supplements influencing fluid balance were taken. All athletes participated voluntarily, being informed of the entire process and methodology used, providing their consent in writing in accordance with the Declaration of Helsinki 2013 [21]. The Ethics Committee for Clinical Sport Research of Catalonia approved the study (Ethical Approval Code: 0022/CEICGC/2023). All physical performance data were anonymized before analyses to ensure player confidentiality.

2.2. Procedures

Five measurements were performed during an entire season (2014/2015). The first measurement was performed on Jul 22 (T1), the second on Jul 27 (T2), the third on Sep 6 (T3), the fourth on Dec 19 (T4), and the fifth on Apr 18 (T5). All measurements were performed in the same place and conditions (in the morning and after at least 12 h without exercise) and by the same operator (C.P.).

2.2.1. Anthropometric Measurements

BM was measured to the nearest 0.1 kg and H to 0.5 cm using a mechanical column scale model Seca 700[®] with stadiometer Seca 220[®] (Seca GmbH & Co., Hamburg, Germany). BMI was calculated as BM/H^2 (kg/m^2).

2.2.2. Bioelectrical Measurements

R and X_c were measured using a BIA 101 Anniversary Sport Edition analyzer (Akern Srl, Florence, Italy) that emitted a 400 mA alternating sinusoidal current at 50 kHz ($\pm 0.1\%$). This device was previously calibrated with a known impedance circuit provided by the manufacturer, whose impedance values were $R = 383 \pm 10 \Omega$ and $X_c = 45 \pm 5 \Omega$.

Subjects were tested according to the manufacturer guidelines, that is to say, with their arms and legs kept from touching the body by non-conductor foam objects to prevent adduction or the crossing of the limbs. Bioelectrical measurements were recorded in the morning before the training session and after at least 12 h without exercise and after a stabilization period of 5 min, in which the players remained laying motionless to ensure the proper distribution of body fluids. The ambient temperature was 22 to 24 °C. Injector electrodes (Biatrodes, Akern, Florence, Italy) were placed on the dorsal surface of the right hand (proximal to the third metacarpal-phalangeal joint) and foot (proximal to the third metatarsal-phalangeal joint). The detector electrodes were placed proximally 5 cm from the injector ones to prevent interaction between electric fields, which could lead to overestimating the impedance values.

Z was calculated as $(R^2 + X_c^2)^{0.5}$, and PhA as $\tan^{-1} (X_c/R \cdot 180^\circ / \pi)$. R, X_c and Z were adjusted by height (R/H , X_c/H , Z/H). R , X_c and Z were adjusted by height (R/H , X_c/H , Z/H). RXc graph was used to plot the five-time points of the rugby players (in confidence ellipses) regarding the 50%, 75% and 95% tolerance ellipses of the reference population, which consists of 139 athletes (age: 21.5 ± 5.0 years; H: 183.3 ± 9.1 cm; BM: 77.2 ± 11.4 kg; BMI: 22.9 ± 2.6 kg/m^2) of 11 different sports: athletics, basketball, handball, judo, karate, modern pentathlon, rugby, soccer, swimming, triathlon and volleyball [14]. Furthermore, RXc-paired graphs were used to compare bioelectrical differences over time. In these graphs, confidence ellipses overlapping the origin indicate no differences, whereas non-centered confidence ellipses indicate significant changes.

2.2.3. Urine-Specific Gravity Measurements

USG was determined using a refractometer model PEN-Urine S. G. (ATAGO PEN-Urine S.G) [22]. The cut-off criteria for urinary dehydration markers (USG, UOSM) were based on published guidelines [23] and defined as >1.02 ($g \cdot mL^{-1}$) and >1.03 ($g \cdot mL^{-1}$) for hypohydration and severe hypohydration, respectively.

2.3. Statistical Analysis

Descriptive analysis was calculated, and data are presented as mean $\pm$ standard deviation (SD). After testing each variable for the normality of the distribution (Shapiro–Wilks test), differences in all variables over time were tested using a one-way repeated measures ANOVA. The sphericity assumption was checked using the Mauchly test, and a post-hoc test with Bonferroni correction was applied in pairwise comparisons. An RXc graph was used to characterize the sample. RXc paired graphs and paired one-sample Hotelling's T2 test were used to identify bioelectrical changes over time. Pearson correlation's coefficient was used to examine the transversal sensitivity and relationship between BIVA parameters (R/H , X_c/H , Z/H , PhA) and USG in each time point. Repeated measures correlation analysis (rmcorr) [24] was conducted to evaluate intra-individual associations between the same BIVA parameters and USG longitudinally during the season. The relationship was assessed as: small (0–0.3), moderate (0.3–0.5), significant (0.5–0.8) and highly relevant (0.8–1.0). The significance level was set at $p < 0.05$ (two-sided). SPSS (Chicago, IL, USA, ver. 21), RStudio (RStudio Inc., Boston, MA, USA, ver. 2023.03.0) and BIVA software [25] were used for data analysis.

3. Results

A total of 34 elite rugby players were measured five times during the season. Each time point is plotted in Figure 1, compared to the athlete's reference population, including rugby players. Despite the visible differences between the 95% ellipses of time points, they all lie in the lower-left quadrant of the reference tolerance ellipse, mainly within 95% and 50%.

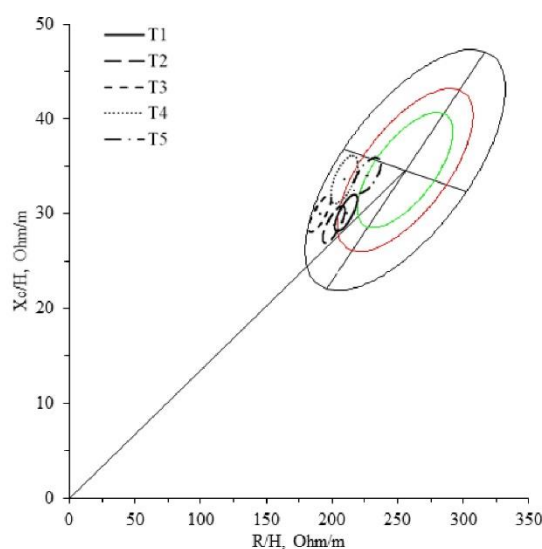


Figure 1. Confidence ellipses of each measurement plotted in the athlete's reference population [14]. Green line for 50%, red line for 75%, and black line for 95% tolerance.

Table 1 shows the BM, bioelectrical and USG changes during the season using five different timepoint measurements. BM progressively increased, reaching its highest point at the end of the season (T1 vs. T5: $p = 0.022$; T3 vs. T5: $p = 0.021$). R/H and Z/H reported the same kinetic, statistically decreasing in T2 (T1 vs. T2: $p < 0.002$) and T3 (T1 vs. T3 and T2 vs. T3: $p < 0.001$). After reaching the lowest values in T3, values increased in T4 (T3 vs. T4: $p < 0.001$), at the baseline (T1 vs. T4: $p = 1$), and also in T5 (T4 vs. T5: $p < 0.001$), well above the baseline (T1 vs. T5: $p < 0.001$). Xc/H follows a similar trend, but being the lowest point at T2, T3 is already increasing, with no differences to baseline (T1 vs. T3: $p = 1$). Both T4 and T5 are significantly higher than T1, T2 and T3 ($p < 0.001$), but there are no differences between T4 and T5 ($p = 1$). PhA remained equal in T2 ($p = 1$), and increased in T3 (T1 vs. T2 and T2 vs. T3: $p > 0.001$). From T3 to T4, there was no increase ($p = 1$). Finally, PhA decreased in T5 (T4 vs. T5, $p = 0.007$) remaining above baseline (T1 vs. T5, $p = 0.02$). USG remained constant throughout the season, with no statistical differences ($p = 0.754$).

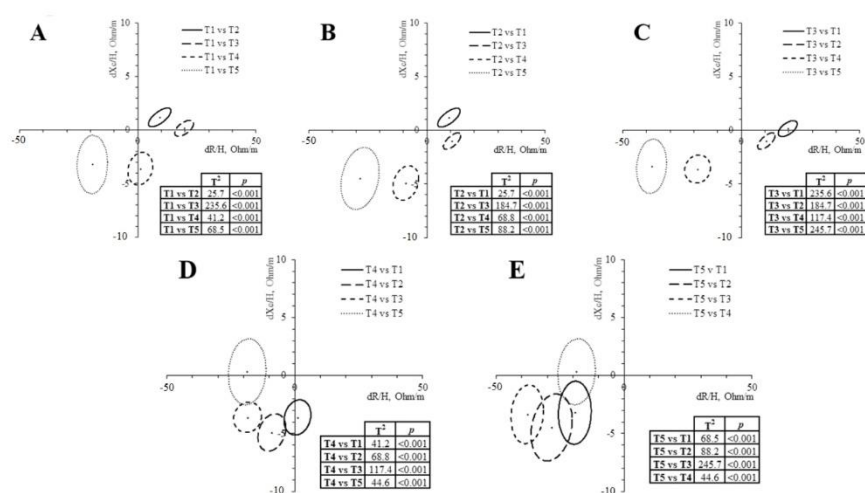
In addition to the raw bioelectrical changes, paired graphs (Figure 2) report statistical migration of the complex vector in all time points ($p > 0.001$). Vector shortening suggests a progressive body fluid gain until T3 due to increased ICW, as indicated by the PhA increase. From T3 to T5, a progressive vector lengthening suggests a body fluid loss, peaking at T5. PhA indicated that the ratio ECW/ICW remained constant in T4 but not in T5, due to an increase in ECW or a decrease in ICW.

Players were hypohydrated for almost the whole season, according to Sawka et al. [23], except for T4 when they were euhydrated.

Table 1. Longitudinal values through the competitive season.

	T1	T2	T3	T4	T5	ANOVA
BM (kg)	100.7 ± 13.0 ^e	100.9 ± 13.2	100.9 ± 12.9 ^e	102.1 ± 13.2	102.3 ± 13.1 ^{a,c}	<0.001
R/H (Ω/m)	204.6 ± 18.4 ^{b,c,e}	195.2 ± 18.3 ^{a,c,d,e}	185.0 ± 18.9 ^{a,b,d,e}	205.0 ± 23.0 ^{b,c,e}	224.3 ± 24.6 ^{a,b,c,d}	0.003
Xc/H (Ω/m)	29.6 ± 4.3 ^{b,d,e}	28.3 ± 4.0 ^{a,c,d,e}	29.3 ± 4.2 ^{b,d,e}	33.3 ± 5.8 ^{a,b,c}	34.0 ± 4.5 ^{a,b,c}	0.001
Z/H (Ω/m)	206.7 ± 18.7 ^{b,c,e}	197.2 ± 18.6 ^{a,c,d,e}	187.3 ± 19.2 ^{a,b,d,e}	207.8 ± 23.4 ^{b,c,e}	226.9 ± 24.8 ^{a,b,c,d}	0.004
PhA (°)	8.2 ± 0.8 ^{c,d,e}	8.2 ± 0.8 ^{c,d,e}	9.0 ± 0.8 ^{a,b,e}	9.2 ± 1.2 ^{a,b,e}	8.6 ± 0.9 ^{a,b,c,d}	<0.001
USG (g·mL ⁻¹)	1.021 ± 0.009	1.023 ± 0.008	1.023 ± 0.005	1.019 ± 0.009	1.021 ± 0.007	0.754

Legend: BM, body mass; PhA, phase angle; R/H, height-adjusted resistance; USG, urine-specific gravity; Xc/H, height-adjusted reactance; Z/H, height-adjusted impedance. T1, first assessment; T2, second measurement; T3, third measurement; T4, fourth measurement; T5, fifth measurements; ^a, statistically different from T1; ^b, statistically different from T2; ^c, statistically different from T3; ^d, statistically different from T4; ^e, statistically different from T5.

**Figure 2.** Paired graphs of each measurement. (A), differences regarding T1; (B), differences regarding T2; (C), differences regarding T3; (D), differences regarding T4; and (E), differences regarding T5.

BIVA (R/H and Z/H) and USG were statistically correlated at T5 only ($r = 0.43$, $p = 0.019$; $r = 0.42$, $p = 0.021$, respectively). No correlation was detected between any bioelectrical parameter changes for the non-significant USG changes longitudinally throughout the season (Table 2).

Table 2. Transversal and longitudinal BIVA and USG correlations.

USG												
Pearson's Correlation										Rmcorr		
T1		T2		T3		T4		T5		Whole Season		
	r	p	r	p	r	p	r	p	r	r	p	p
R/H	0.23	0.216	0.15	0.452	0.07	0.716	−0.08	0.687	0.43	0.019	−0.08	0.438
Xc/H	0.03	0.858	0.01	0.956	−0.05	0.801	−0.15	0.463	0.15	0.428	−0.17	0.082
Z/H	0.23	0.226	0.14	0.462	0.07	0.729	−0.09	0.678	0.42	0.021	−0.08	0.422
PhA	−0.16	0.394	−0.14	0.468	−0.18	0.359	−0.13	0.546	−0.27	0.144	0.15	0.134

4. Discussion

This study aimed at assessing elite rugby players' hydration and body cell mass changes throughout a whole season with two different methods (BIVA and USG) that measure similar aspects, and to check the correlation between these two methods. The main findings of the present investigation were threefold: (1) BIVA identifies hydration/body

cell mass changes, while USG is not able to do so; (2) no correlation between BIVA and USG was found; and (3) it is necessary to take into account the temporality of the bioelectrical assessments in the rugby players since the values change significantly.

The rugby players were plotted in the classic tolerance ellipse of an athlete's population (different sports, including rugby) [14] as the most similar population currently found in the literature (Figure 1). The players' confidence ellipses were plotted separately according to the assessment time. They all fit within the lower left quadrant, indicating an above-average amount of body water and body cell mass compared to other athletes. These data agree with Campa et al. [26], which indicates that team sport athletes have a greater PhA (and therefore cellular health) than other sports (such as endurance) due to a mesomorphic phenotype and higher muscle mass [27,28]. In addition, the players' PhA fit within the 50th and >95th percentile of a 147 rugby male sample [26], indicating that the physical condition of our players was above average to their homonyms.

Although all the confidence ellipses' assessments are within the same quadrant, they are all significantly different from one another (Figure 2). These results align with previous investigations conducted in different sports, reporting that body composition and bioelectrical values change across the competitive season phases [29,30]. During almost nine months of the study, two clear trends can be seen between the different evaluations, especially before and after T3 (the start of the season). From T1 (pre-season) to T3, there was a significant shortening of the vector length ($-9.2 \pm 4.3\%$, $p < 0.001$) jointly with an increase in the PhA ($9.8 \pm 4.8\%$, $p < 0.001$), indicating a body fluid gain and a decrease of the ECW/ICW ratio. In contrast, from T3 to T5 (end of the season), there was a progressive lengthening of the vector ($20.4 \pm 7.5\%$, $p < 0.001$), while PhA remained stable at T4 ($2.2 \pm 9.5\%$, $p = 1$) and decreased at T5 ($-5.3 \pm 7.1\%$, $p < 0.001$), indicating a body fluid loss and an increase in the ECW/ICW ratio. From T1 to T5, there was a significant lengthening of the vector ($9.5 \pm 6.9\%$, $p < 0.001$) jointly with an increase in the PhA ($5.4 \pm 7.7\%$, $p < 0.001$), indicating that the players experienced a body fluid loss. However, the ECW/ICW ratio also decreased.

The kinetic of body water content (inversely related to vector length [9]) and ECW/ICW ratio (inversely related to PhA [14]) are important, since it is well reported that these parameters (particularly ICW) play a fundamental role in sports performance, since increases in ICW are associated with power and strength improvements [31–33]. Due to the lack of performance data in this study, the knowledge of the changes in ICW, ICW/ECW ratio and PhA are important in order to understand the physical condition of the players throughout the season. In the non-rugby player population, PhA was positively associated with relative power and relative and absolute strength ($p < 0.05$) [34], and vector length shortening was associated with improvements in endurance performance ($p = 0.034$) [29]. Nunes et al. [4] found negative correlations between BIVA and peak running velocity ($r^2 \geq 0.49$), neuromuscular performance tests (vertical jumps, $r^2 \geq 0.30$; and sprinting ability $r^2 \geq 0.65$) and some match performance variables ($r^2 \geq 0.30$) in rugby players. However, these correlations were not based on the raw BIVA values but on the fat mass calculated based on the raw bioelectrical values. These data indicate that our players experienced changes in body composition and physical condition throughout the season that may influence their sports performance [35,36]. The time of the season when players were fittest, according to PhA, was at T3/T4. These data confirm the importance of assessing athletes according to the specific time of the season, as previously reported [29,30].

Alternatively to BIVA, USG has also been widely used to assess hydration status [17]. However, our players not only showed no USG differences throughout the season ($p = 0.754$), but also showed no correlation for any of the bioelectrical parameter changes ($p > 0.08$). Only at T5 was there found to be a transversal moderate positive correlation between R/H and Z/H and USG, which is clearly not enough to state that the two methods are correlated. Marathon runners have also reported this lack of correlation [37]. In contrast, a weakly negative correlation between BIVA and USG has been found in divers, which is the opposite of what the authors expected, since such results indicated that the higher the

amount of water, the higher the urine density [38]. In a recent study, Francisco et al. [39] showed that athletes with a low water intake reported higher USG values. However, low and high water intake differences were found in no TBW, ICW, ECW and ECW/ICW ratio (all these parameters were determined using dilution techniques). Based on this discovery, the authors recommend considering USG not as an indicator of hydration status but as a biomarker resulting from those mechanisms to maintain homeostasis. Similarly, Zubac et al. [40] indicated that urinary dehydration markers such as USG seem inappropriate diagnostic tools to screen for fluid deficits in real-life scenarios. Furthermore, it should be noted that athletes with a large muscle mass may present a higher USG without being dehydrated [19] since, according to the cut-off proposed by Sawka et al. [23], our athletes would be hypohydrated during most of the season, contrary to what BIVA indicates, since plotting our subjects in the lower half of the reference tolerance ellipse (Figure 1). These data suggest that if the goal of research or sports teams is to identify changes in the amount of water or its compartments, BIVA is a much more sensible option than USG. It is already well known in the literature that BIVA is a valid, economical and practical method for assessing hydration status and changes in athletes [9–11]. However, it can also serve as a method to assess athletes' cellular quality and physical condition throughout the season. Furthermore, these findings may be engaging in the sports field, since they may help athletes and their physical and technical staff to track the workload and physical condition quickly, easily and economically to improve sports performance. Nonetheless, some limitations must be addressed. First, the most effective strategy for determining hydration is the combination of other methods (plasma osmolality with the dilution technique) and non-BIVA with USG [41]. However, this combination cannot be used due to the in-field nature of the assessments of the present study. Therefore, interpreting these results relies on methodologies that may not provide information directly. In the case of field settings, finding evaluation solutions that allow the selection of the best possible test battery becomes essential, and the proposal with two practical and transportable methods, such as BIVA and USG, could be a useful solution. Second, the position of the players on the field may influence the body composition (and therefore the bioelectrical values) due to the design training strategies or morphological characteristics. In this study, however, this fact was not considered due to the normality of the sample and the fact that the sample is not excessively large. Third, this study did not collect data on the daily fluid intake of rugby players. However, future studies could predict how the differences found in the present study could change based on the different athletes' hydration statuses experienced during an entire competitive season.

5. Conclusions

The present study suggests that BIVA is sensitive to rugby players' hydration and body cell mass status, which changes during the regular season. During the pre-season, the players' physical condition presumably increased and decreased at the end of the season, as vector changes detected it. USG reported no changes, and a moderate correlation with R/H and Z/H only was found in one-time points. This rugby sample presents higher body water content (especially in the intracellular compartment, which predicts strength and power) than other sports. BIVA could thus be a promising tool for monitoring fluid and body cell mass status for rugby coaches and nutritionists.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee for Clinical Sport Research of Catalonia (Ethical Approval Code: 0022/CEICGC/2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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9.2. Annex 2

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Article

Unsupervised Exercise Intervention vs. Adherence to a Mediterranean Diet Alone: The Role of Bioelectrical Impedance Vector Analysis and Cardiovascular Performance in Liver-Transplanted Recipients

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Abstract: (1) Background: Cardiovascular disease is one of the leading causes of mortality after liver transplantation. Body composition and cardiovascular performance assessment represent a potential approach for modulating lifestyle correction and proper follow-up in chronic disease patients. This study aimed to verify the additional role of an unsupervised physical activity program in a sample of male liver transplant recipients who follow the Mediterranean diet. (2) Methods: Thirty-three male liver transplant recipients were enrolled. Sixteen subjects followed a moderate-intensity home exercise program in addition to nutritional support, and seventeen received advice on the Mediterranean diet. After six months, bioelectrical vector impedance analysis (BIVA) and cardiopulmonary exercise testing (CPET) were performed. (3) Results: No differences in CPET (VO₂ peak: exercise 21.4 ± 4.1 vs. diet 23.5 ± 6.5 mL/kg/min; $p = 0.283$) and BIVA (Z/H: exercise 288.3 ± 33.9 vs. diet 310.5 ± 34.2 Ω/m; $p = 0.071$) were found. Furthermore, the BIVA values of resistance correlate with the submaximal performance of the Ve/VCO₂ slope ($R = 0.509$; $p < 0.05$) and phase angle with the maximal effort of the VO₂ peak ($R = 0.557$; $p < 0.05$). (4) Conclusions: Unsupervised physical exercise alone for six months does not substantially modify liver transplant recipients' cardiovascular performance and hydration status, despite their adherence to a Mediterranean diet. The body composition analysis is useful to stratify the risk profile, and it is potentially associated with better outcomes in transplanted subjects.

Keywords: exercise prescription; nutrition; body composition; BIVA; cardiopulmonary exercise testing; CPET; solid organ transplant

1. Introduction

Solid organ transplantation is a therapeutic strategy in the end-stage of the disease or in emergency cases. Surgery and care techniques have improved in recent years, ensuring increasingly greater survival after transplantation. In the last ten years in Italy, there has been a 26% increase in the number of solid organ transplants, among which liver transplants have had one of the largest increases, standing at 44% [1].

Lifestyle, combining regular physical activity and adherence to a healthy diet, represents a treatment for many chronic diseases [2]. Cardiovascular disease represents a major cause of mortality after liver transplantation [3], and it is long-established that

healthy habits reduce risk factors by improving physical fitness parameters related to health in solid organ transplant recipients [4].

Physical inactivity is a crucial driver of progression and adverse outcomes in liver diseases [5]. In particular, exercise's role in reducing mortality is currently debated [6] in liver transplant recipients. After a successful liver transplantation, the patients show impaired exercise capacity and fatigue due to a minor effort [7]. This fatigability is explained by prolonged bed rest after transplantation, immunosuppressive drugs, associated comorbidities (e.g., obesity, diabetes, hyperlipidemia, hypertension, and metabolic syndrome), and sarcopenia [8]. In this context, the evidence suggests that exercise programs improve two parameters related to cardiovascular adverse events after liver transplantation: maximal oxygen uptake [9,10] and body composition [9]. Therefore, physical activity is increasingly recommended as a therapeutic approach after solid liver transplantation [11].

Cardiovascular risk factors after a liver transplant are also modifiable by eating habits. These patients consume a high-energy, low-quality diet in the long term [12], and they do not adhere to the dietary guidelines for cardiovascular disease prevention [13]. Studies initially focused on controlling caloric, fat, and protein intake, also using supplements [9,14]. More recently, attention has begun, with promising results, to focus on promoting the Mediterranean diet to increase healthy eating habits after liver transplantation [15,16].

In the three years following liver transplantation, there is an average weight gain of about 10 kg, and most patients are overweight or obese [17,18]. Furthermore, sarcopenia is present in more than half of liver transplant recipients [19]; therefore, one might wonder whether, in some cases, weight gain could be considered sarcopenic obesity [20]. An early and inappropriate increase in fat mass characterizes changes in the body composition of transplant recipients. In contrast, the restoration of cell mass and fluid distribution appears to occur more slowly and is incomplete [21]. Recently, patients on the liver transplant waiting list have been evaluated using bioelectrical impedance vector analysis (BIVA). The placement of subjects in the RXc graph quadrants in vector impedance interpretation has been found to have a prognostic factor [22].

Body composition and cardiovascular performance assessment represent an approach for modulating lifestyle correction and carrying out a proper follow-up. This study aimed to verify the intervention's effectiveness on lifestyle by comparing the promotion of a Mediterranean diet alone vs. a Mediterranean diet plus an unsupervised physical activity program in a sample of male liver transplant recipients.

2. Materials and Methods

2.1. Participants

Thirty-three male liver transplant recipients, aged 61.4 ± 8.0 years and 1.2 ± 0.7 years post-transplant, were enrolled in this study from February 2021 to January 2023. Inclusion criteria were to be transplanted for at least one year and clinically stable (e.g., absence of liver-related complications in the previous six months, including acute rejection episodes and increased serum transaminases two times the upper limit). Exclusion criteria were combined transplantation, re-liver transplantation, physical limitations, cardiovascular contraindications to exercise, and psychiatric or severe debilitating neurological disorders. A total of 19 participants had mild or moderate hypertension and were under antihypertensive treatment (calcium channel blockers, ACE inhibitors, or ARBs); 14 participants had no hypertension. All participants assume immunosuppressive therapy, including drugs such as calcineurin inhibitors (Ciclosporin or Tacrolimus), in combination with Mycophenolate or Everolimus, and steroids (Methylprednisolone). Comorbidities, such as diabetes, hypertension, or other metabolic diseases, were not a reason for exclusion. None of them assumed beta-blockers. This study was conducted in accordance with the Declaration of Helsinki and subsequent modifications and approved by the

ISRCTN registry (study ID: ISRCTN66295470, 19/01/2017). All participants provided written, informed consent.

2.2. Physical Exercise Program and Dietary Intervention

Sixteen transplanted subjects followed a tailored home-based exercise program with moderate intensity with nutritional support. An unsupervised physical exercise program was chosen to prevent participants from traveling to a specific health club to carry out the training to achieve a lower probability of absence from exercise and greater adherence to the program in the long run.

The physical exercise program consisted of mixed physical activity (endurance and resistance exercise) for 60 min thrice weekly, following the American College of Sports Medicine guidelines [23]. Endurance exercises were prescribed for up to 30 min with an intensity of around 60% of the maximal heart rate. In particular, the heart rate range indicated was established individually using the Karvonen formula [24]. Resistance exercise involved training eight major muscle groups for three sets of ten repetitions for the remaining 30 min after the endurance exercise. The exercises were chosen based on the possibility of being performed safely at home (such as a bodyweight squat and glute bridge for the lower limbs, a lateral raise, and a biceps curl for the upper limbs). Furthermore, a qualified kinesiologist demonstrated resistance exercise, followed by repetition by the patient as a learning test. In order to verify adherence to the prescribed physical exercise, the International Physical Activity Questionnaire (IPAQ) was used [25]. Data collected with the IPAQ were reported as a continuous measure and expressed as METs/min/week (MET: metabolic equivalent of task; one MET is defined as the amount of oxygen consumed at rest, which corresponds to 3.5 mL of O₂ per kg of body weight × min). Achieving at least a value of 600 was considered moderately active, and 1500 was considered vigorously active.

Despite being physically active, the remaining seventeen subjects were outside of a structured exercise program and received indications for the Mediterranean diet. Specifically, the recommendation was about long-term nutritional support after liver transplantation [26]. In particular, it was recommended to limit fat intake and consume adequate amounts of lean protein to promote muscle development, in parallel with ensuring an adequate calorie intake to avoid protein utilization as an energy source. Finally, no added salt (a daily maximum of 3 g of sodium) was recommended to prevent water retention. In order to verify adherence to nutritional advice on the Mediterranean diet, the MEDI-LITE score was used [27]. Subjects reporting a score higher than 8.5 on the MEDI-LITE questionnaire should be considered adherents to the Mediterranean diet.

Six months after receiving lifestyle recommendations, exercise prescriptions, or Mediterranean diet indications, the evaluations were performed for both groups to establish the effectiveness of each program. Body composition assessment and cardiopulmonary exercise testing (CPET) were performed to measure the nutritional and hydration status as well as the cardiovascular and respiratory performances.

2.3. Cardiopulmonary Exercise Testing

The cardiopulmonary exercise testing (CPET) was conducted by an electromagnetic brake cycle ergometer (Ergoline) and a specific gas measure machine (COSMED Quark). Every participant was invited to avoid strenuous physical exertion the day before the evaluation and to refrain from eating solid foods or carbohydrate drinks at least three hours before the test. The CPET was performed in the morning in a room with controlled conditions (temperature 18–24 °C; humidity 30–60%). The protocol was established based on sex, age, height, and weight, and the training evaluation was declared. The ramp was also individualized on predicted weight values to achieve muscle exhaustion between 8 and 12 min [28]. Participants were equipped with an orofacial mask connected to a gas measuring device [29]. Exhaled CO₂ and O consumed were measured using the breath-by-breath method. The lowest possible increase in watts (1, 2, or 5) was set for each ramp

to obtain the most linear possible increase in load and, consequently, a more physiological response. After the first 3 min of warming up, by cycling without load at a cadence of 50 rpm (revolutions per minute), the test started. At the beginning of the actual effort, cycling was required at a cadence between 60 and 80 rpm until muscle exhaustion was reached. The test ended when the participants could no longer maintain their cycling cadence despite verbal encouragement [30]. The test was considered maximal with at least two of the following criteria:

- Respiratory exchange ratio (RER) > 1.10.
- Heart rate (HR) > 85% according to age.
- Exercise duration between 8 and 12 min.

CPET was stopped in cases of cardiovascular signs and symptoms (complex ventricular arrhythmias, a drop in systolic blood pressure, dizziness, etc.). The 12-lead ECG and oxygen saturation were monitored continuously.

During the test, oxygen consumption (VO_2), carbon dioxide production (VCO_2), tidal volume (TV), respiratory rate (RR), minute ventilation (VE), heart rate (HR), and workload (WL, in watt) were obtained. The two ventilatory thresholds (VT1 and VT2) were indirectly determined using the combination of V-slope and the ventilatory equivalents approach. In addition, other variables analyzed were the relationship between oxygen consumption and heart rate (oxygen pulse, VO_2/HR), minute ventilation/carbon dioxide production slope (VE/ VCO_2 slope), and the relationship between oxygen consumption and workload [31] (VO_2/WL slope, as a measure of circulatory efficiency).

2.4. Body Composition Analysis

Bioimpedance analysis was chosen to evaluate body composition. The bioelectrical parameters of resistance (R) and reactance (Xc) were measured with a BIA 101 Anniversary Sport Edition analyzer (Akern Srl, Florence, Italy) emitting an alternating sinusoidal current of 400 mA at 50 kHz ($\pm 0.1\%$). Before each evaluation, this device was calibrated with a known impedance circuit provided by the manufacturer.

The assessments were carried out according to the guidelines, with arms and legs abducted to prevent contact with the body. The measurements were recorded after a 5 min stabilization period, in which the participants remained still to ensure a homogeneous distribution of body fluids. Injector electrodes were placed on the dorsal surface of the right hand (proximal to the third metacarpophalangeal joint) and right foot (proximal to the third metatarsophalangeal joint). The sensing electrodes were placed approximately 5 cm from the injector to prevent interaction between the electric fields and to avoid overestimating the impedance values.

Impedance (Z) was calculated as $(R^2 + Xc^2)^{1/2}$, and phase angle (PhA) as $\tan^{-1}(Xc/R \cdot 180^\circ/\pi)$. R, Xc, and Z were adjusted by height (R/H, Xc/H, Z/H). According to classic BIVA, Z/H is inversely related to total body water (TBW) [32]. In contrast, vector direction indicates cellular health and cell membrane integrity and is inversely related to the extracellular/intracellular water (ECW/ICW) ratio [33]. All interpretations should be based on the interpretation of Z/H and PhA jointly, along with the vector position on the Resistance-Reactance (RXc) graphs [34]. In this graph, shifts in vectors parallel to the major ellipse axis indicate differences in tissue hydration (a longer vector indicates less fluid, while a shorter vector indicates more body fluids). Shifts in the vector parallel to the minor axis of the ellipses indicate differences in cell mass and ECW/ICW ratio (a shift to the left indicates an increase in cell mass and a reduction in the ECW/ICW ratio, while a shift to the right indicates a reduction of cell mass and an increase in the ECW/ICW ratio [35]).

2.5. Statistical Analysis

Descriptive analysis was calculated, and the data are presented as mean $\pm$ standard deviation (SD). After testing each variable for the normality of the distribution (Shapiro–Wilks test), differences in all the variables were tested using a student's *t*-test in cases of

normal distribution and a Mann–Whitney test in cases of the data not being distributed normally. The relative effect sizes (ES) were calculated using Cohen’s *d* [36] to estimate the relevance of the differences analyzed. According to Cohen, ES is defined as small (≤ 0.20), medium (≤ 0.50), and large (≤ 0.80). Pearson’s correlation test (Spearman’s test for not normally distributed values) was applied to examine the relationships between BIVA and CPET. RXc point graph was used to plot the points of the subjects regarding the 50%, 75%, and 95% tolerance ellipses of the healthy reference population [37]. RXc mean graph and two-sample Hotelling’s T^2 test were used to check the differences in the complex vector between groups. The significance level was set at $p < 0.05$. SPSS (Chicago, IL, USA, ver. 21) and BIVA software [38] were used for data analysis.

3. Results

Thirty-three males who had undergone liver transplantation 1.2 ± 0.7 years previously were enrolled. From this total sample, 16 subjects (age 61.7 ± 8.8 years, BMI 26.5 ± 3.3 kg/m²) performed the unsupervised exercise program, and 17 subjects (age 61.1 ± 7.5 years, BMI 26.9 ± 1.7 kg/m²) were adherent to nutritional advice relating to the Mediterranean diet with a score > 8.5 .

The physical exercise group reported higher but not statistically significant values of weekly METs from IPAQ (1269.3 ± 1272.4 vs. 951.9 ± 875.5 ; $p = 0.408$, ES = 0.290), and the diet group reported higher but not statistically significant values of the MED-LITE score (11.7 ± 2.1 vs. 10.4 ± 2.3 ; $p = 0.236$, ES = 0.590).

The results relating to cardiorespiratory performance, assessed with the CPET, show no differences between the two groups (Table 1).

Table 1. Data obtained from liver transplant recipient samples at CPET.

	Physical Exercise (<i>n</i> = 16)	Diet (<i>n</i> = 17)	<i>p</i> -Value	ES
VO ₂ peak (mL/kg/min)	21.4 ± 4.1	23.5 ± 6.5	0.283	0.386
VO ₂ /HR	12.5 ± 2.0	12.7 ± 2.8	0.790	0.082
Power (Watt)	128.8 ± 34.0	142.4 ± 52.4	0.385	0.308
Ve/VCO ₂ Slope	33.6 ± 6.0	33.6 ± 6.1	0.996	0.000
VO ₂ /WL	10.2 ± 0.9	10.6 ± 1.3	0.295	0.358
HR VT1 (bpm)	97.8 ± 15.3	100.8 ± 15.8	0.574	0.192
HR VT2 (bpm)	120.9 ± 21.4	124.2 ± 17.8	0.627	0.167
HR max (bpm)	153.6 ± 18.0	152.9 ± 15.8	0.909	0.041
VE peak (L/min)	70.8 ± 14.5	80.0 ± 22.8	0.183	0.481
SBP rest (mmhg)	121.9 ± 10.3	123.8 ± 16.0	0.682	0.141
DBP rest (mmhg)	73.4 ± 11.4	74.7 ± 7.2	0.702	0.136
SBP peak (mmhg)	167.2 ± 21.6	165.3 ± 20.1	0.796	0.091
DBP peak (mmhg)	71.9 ± 15.2	72.4 ± 11.3	0.919	0.037

Legend: Data are expressed as mean ± s.d. VO₂ peak = oxygen consumption achieved at peak performance; VO₂/HR = ratio between oxygen consumption and heart rate; Ve/VCO₂ slope = minute ventilation/carbon dioxide production slope; VO₂/WL = ratio between oxygen consumption and workload; HR VT1 = heart rate corresponding to the first ventilatory threshold; HR VT2 = heart rate corresponding to the second ventilatory threshold; HR max = heart rate at the peak of exertion; VE peak = peak ventilation; SBP rest = systolic blood pressure at rest; DBP rest = diastolic blood pressure at rest; SBP peak = systolic blood pressure at the peak of exertion; DBP peak = diastolic blood pressure at the peak of exertion.

Table 2 and Figure 1 compare body composition assessed with the impedance vector analysis. No significant differences between the two groups were found, even if the different positioning of the two groups on the RXc mean graph (Figure 1B) seems to be more attributable to the R component (physical exercise group = 286.6 ± 33.8 vs. diet group

= 308.8 ± 33.9 ; $p = 0.069$, ES = 0.656) rather than the Xc component (exercise group = 30.1 ± 5.7 vs. diet group = 32.3 ± 6.4 ; $p = 0.314$, ES = 0.363).

Table 2. Bioimpedance vector analysis (BIVA) data of the two groups of patients undergoing liver transplantation.

	Physical Exercise ($n = 16$)	Diet ($n = 17$)	p -Value	ES
R/H (Ω/m)	286.6 ± 33.8	308.8 ± 33.9	0.069	0.656
Xc/H (Ω/m)	30.1 ± 5.7	32.3 ± 6.4	0.314	0.363
Z/H (Ω/m)	288.3 ± 33.9	310.5 ± 34.2	0.071	0.652
PhA ($^{\circ}$)	6.0 ± 1.0	5.9 ± 0.7	0.847	0.116

Legend: Data are expressed as mean $\pm$ s.d. R/H = ratio between resistance and height; Xc/H = ratio between reactance and height; Z/H = ratio between impedance and height; PhA = phase angle.

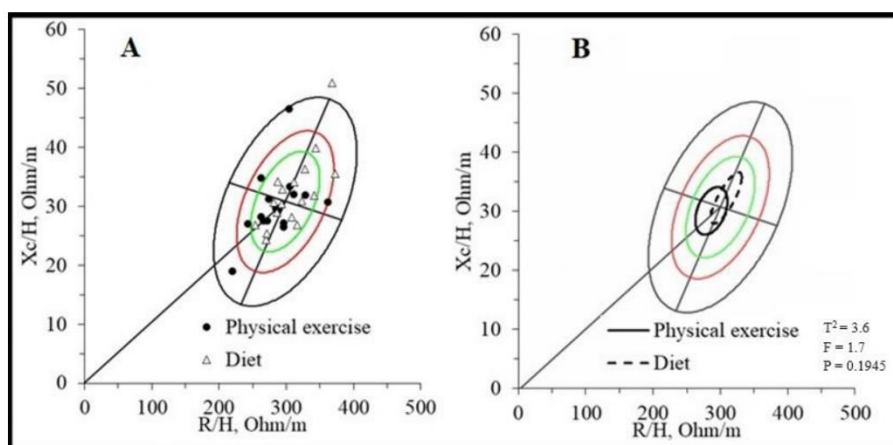


Figure 1. BIVA Resistance-Reactance (RXc) graphs of liver transplant recipients. Legend: (A). RXc point graph; (B). RXc mean graph. The green, red and black ellipses represent the 50%, 75% and 95% tolerance respectively.

Finally, the analysis of the correlations between the BIVA and CPET parameters shows how lower resistance values (R) are linked to better submaximal performance. At the same time, the PhA provides information on maximal performance with a direct relationship with oxygen consumption and ventilation at peak effort (Table 3).

In detail, analyzing these correlations divided based on the two study groups, the R parameter appears to have greater relevance for the performance of the exercise group about VO_2/HR ($R = -0.648$; $p < 0.05$), Ve/VCO_2 slope ($R = 0.573$; $p < 0.05$), and peak VE ($R = 0.516$; $p < 0.05$). While in the diet group, the phase angle shows a greater relationship with power ($R = 0.568$; $p < 0.05$) and with the VE peak ($R = 0.579$; $p < 0.05$).

Table 3. Matrix of correlations between bioimpedance vector analysis (BIVA) and CPET performance parameters.

	R/H	Xc/H	Z/H	PhA
VO_2 peak	−0.013	0.294	0.019	0.557 *
VO_2/HR	−0.392 *	−0.107	−0.391 *	0.230
Power	−0.237	0.101	−0.233	0.509 *
Ve/VCO_2 slope	0.509 *	0.112	0.506 *	−0.222
VO_2/WL	0.403 *	0.267	0.403 *	0.132
HR VT	0.183	0.369 *	0.316	0.377 *
HR max	−0.002	0.053	0.001	0.213

VE peak	0.087	0.392 *	0.092	0.506 *
SBP rest	−0.109	−0.253	−0.114	−0.214
DBP rest	0.198	−0.075	0.193	−0.135
SBP peak	0.072	−0.119	0.072	−0.116
DBP peak	0.278	0.296	0.275	0.177

Legend: R/H = ratio between resistance and height; Xc/H = ratio between reactance and height; Z/H = ratio between impedance and height; PhA = phase angle; VO₂ peak = oxygen consumption achieved at peak performance; VO₂/HR = ratio between oxygen consumption and heart rate; Ve/VCO₂ slope = minute ventilation/carbon dioxide production slope; VO₂/WL = ratio between oxygen consumption and workload; HR VT = heart rate corresponding to the ventilatory threshold; HR max = heart rate at the peak of exertion; VE peak = peak ventilation; SBP rest = systolic blood pressure at rest; DBP rest = diastolic blood pressure at rest; SBP peak = systolic blood pressure at the peak of exertion; DBP peak = diastolic blood pressure at the peak of exertion. * $p < 0.05$.

4. Discussion

This study investigates the potential effectiveness of the additional role of unsupervised exercise programs in a group of liver-transplanted males that follow nutritional advice. Under stable clinical conditions, the study sample was divided into two groups. One group was encouraged to lead healthy eating habits with dietary advice based on the Mediterranean diet, and the other group was encouraged to lead an active lifestyle in addition to nutritional advice. Both groups performed their program for six months, and then evaluations were done to establish the effectiveness of each program.

Based on the questionnaires, the two groups should be considered moderately physically active (around 1000 METs with IPAQ) and follow the Mediterranean diet (both groups have a MED-LITE score above 8.5). The IPAQ questionnaire was recently administered to liver transplant candidates to assess how physical activity levels are related to physical performance and frailty [39]. In this study, patients showed low-to-medium levels. However, in another two studies [40,41], patients who have already undergone a liver transplant reported higher values than those in the present study. In particular, Kotarska et al. [40] showed rather high values compatible with daily workouts of vigorous intensity. The results obtained from our study show that the declared levels are, although not significantly, slightly higher in the exercise group and compatible with moderate-intensity activity. Therefore, the exercise prescription proposed in this study may be reasonable because adult transplant recipients are among the most sedentary of all populations with chronic disease, with a daily step count of 3164 ± 2842 steps [5]. The values MED-LITE recorded in this study align with the data recently published on a larger sample of the same geographical area following indications on the Mediterranean diet [16]. The group that received only indications on the Mediterranean diet, albeit not significantly, showed higher values than the group that also followed the exercise program. The exercise group also obtained a mean MED-LITE value >8.5 and, therefore, can still be considered adhering to the principles of the Mediterranean diet; however, the greater effort required by physical exercise could compromise, at least in part, the adherence to the nutritional advice.

Interestingly, the two groups had no significant differences in oxygen consumption parameters and the other parameters of CPET. However, the VO₂ peak value reported in the present or can be considered above the mean of 20.5 mL O₂/kg/min reported at the end of three previous supervised exercise interventions performed in three randomized controlled trials [9,10,42]. In addition, Totti et al. [43] report that 12 months of supervised physical exercise with a training program comparable to that of the present study did not increase the VO₂ peak in a group of liver transplant recipients. Therefore, the cardiorespiratory performances recorded in the present study allow for establishing this sample as a liver-transplant recipient with a high level of cardiovascular fitness. Furthermore, the current ACSM guidelines [23] do not indicate the physical activity

regimen post-transplantation, and there is yet to be evidence of the stronger benefits of an intensive physical activity program in this particular population of patients.

The present study reports the bioelectrical impedance vector analysis assessment in liver transplant recipients for the first time. The data suggest a medium mortality risk profile due to incorrect water distribution. A study [22] shows that patients on the liver transplant waiting list have vector impedance placed mainly in the lower quadrants of the RXc graph, indicating increased body water content. In addition, vector placement in the lower right quadrant and ascites or edema were independent risk factors for the wait list and 1-year post-transplant mortality. The placement in the RXc graph of the sample of this study (Figure 1) shows how the subjects are, on average, in the center of the reference ellipse (Figure 1B), with only four subjects in the lower right quadrant (Figure 1A) within the 50th percentile and, therefore, with a more favorable prognostic perspective. Another study, conducted by the same research center as the present study, performed bioelectrical impedance vector analysis on thirteen kidney transplant patients who performed an unsupervised exercise program [44]. Their placement is reported to be, on average, within the 50th percentile of the lower right quadrant. However, it must be considered that the state of hydration and the intra/extracellular distribution may differ due to the different transplanted solid organs, where renal function in the hydro-salt balance plays a leading role compared to liver function.

The relationship between bioelectrical parameters and physical performance in liver transplant recipients is a new direction of study never investigated before, especially in this population. Lower resistance values are compatible with a greater state of hydration, which could allow better cellular function and more efficient thermoregulation and, therefore, better submaximal endurance performance. At the same time, the role of PhA in muscle performance is well established in the literature, even in subjects with chronic disease [45]. The present study confirms that higher PhA values that reflect greater cellularity support physical performance close to maximum individual effort.

Lifestyle correction plays a relevant role as a therapeutic intervention in many non-communicable chronic diseases, and it has also recently been promoted in post-transplanted subjects [46]. Lifestyle intervention is normally proposed as diet correction and/or physical activity combination. However, more data should be available regarding the efficacy of the additional component of the unsupervised exercise activity in the presence of sufficient adherence to the Mediterranean diet.

This study has, therefore, some strengths and points of interest. Firstly, the sample size aligns with and even exceeds existing similar studies. Secondly, the same healthcare professionals trained in CPET and BIVA evaluated all subjects with the same instrumentation. Thirdly, the evaluation methodologies were direct measurements and not an indirect estimation: cardiorespiratory performance was measured through a CPET, unlike other studies, which estimated VO_2 peak through the six-minute walking test, while body composition was evaluated with the BIVA method rather than an estimation through a regression equation derived from anthropometric parameters.

The authors are aware that the absence of an initial evaluation of the enrolled subjects does not allow a comparison with the values obtained after six months of joining the program. However, baseline assessments are not compatible with the care pathway. In addition, the values obtained from this study sample at CPET allow us to speculate that they are compatible with a healthy lifestyle.

5. Conclusions

In summary, unsupervised physical exercise appears feasible and sustainable; however, the long-term efficacy should be studied. The data are in any case in agreement to a frailty in post-transplanted subjects and to the importance of physical activity to improve quality of life and mitigate the CV risk. Despite the absence of specific guidelines or recommendations in this category, promoting more intensive and potentially supervised training is reasonable. Body composition analysis seems fundamental in the

initial phase to stratify the risk profile due to excessive water compartmentalization. In addition, the results obtained in this study suggest that an approach based on a single aspect of the lifestyle, like diet and moderate physical activity, could not be sufficient to influence cardiovascular parameters.

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