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1 **Bioelectrical Impedance Analysis versus Reference Methods in the Assessment of Body Composition in Athletes** 

2 **Systematic Review**

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32 **Abstract**

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2 33 The present systematic review aimed to compare the accuracy of Bioelectrical Impedance Analysis (BIA) and
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4 34 Bioelectrical Impedance Vector Analysis (BIVA) vs. reference methods for the assessment of body composition in
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6 35 athletes. Studies were identified based on a systematic search of internationally electronic databases (PubMed and Scopus)
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8 36 and hand searching of the reference lists of the included studies. In total, 42 studies published between 1988 and 2021
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10 37 were included. The methodological quality was assessed using the Quality Assessment Tool for Observational Cohort
11
12 38 and Cross-sectional Studies as recommended by the National Institute of Health. Twenty-three studies had an overall
13
14 39 good rating in terms of quality, while 13 were rated as fair and six as poor, resulting in a low to moderate risk of bias. Fat
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16 40 mass was inconsistently determined using BIA vs. the reference methods, regardless of the BIA-technology. When using
17
18 41 the foot to hand technology with predictive equations for athletes, a good agreement between BIA and the reference
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20 42 methods was observed for fat-free mass, total body, intra and extra cellular water. However, an underestimation in fat-
21
22 43 free mass and body fluids was found when using generalized predictive equations. Classic and *Specific* BIVA represented
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24 44 a valid approach for assessing body fluids (Classic BIVA) and percentage of fat mass (*Specific* BIVA). The present
25
26 45 systematic review suggests that BIA and BIVA can be used for assessing body composition in athletes, provided that
27
28 46 foot-to-hand technology, predictive equations, and BIVA references for athletes are used.

29
30 47
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32 48 **Keywords:** Bioimpedance vector analysis; Classic BIVA; *Specific* BIVA; Phase angle; Tolerance ellipses; Resistance
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34 49 training; Nutrition

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63 **Abbreviations**

1		
2	64	BIA Bioelectrical impedance analysis
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4	65	BIVA Bioelectrical impedance vector analysis
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6	66	BIS Bioelectrical spectroscopy analysis
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8	67	DXA Dual-energy X-ray absorptiometry
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10	68	ECW Extracellular water
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12	69	FM Fat mass
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14	70	FFM Fat free mass
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16	71	ICW Intracellular water
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18	72	LST Lean soft tissue
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20	73	R Resistance
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22	74	Xc Reactance
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24	75	TBW Total body water
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26	76	UWW Underwater weighting
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28	77	4C Four compartment model
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94 **Introduction**

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Body composition describes the amount of the various components of the human body, such as fat (FM) and muscle mass or body fluids (Heymsfield et al. 2005). However, the assessment of body composition through direct procedures is not possible in humans (Heymsfield et al. 2005). For this reason, a number of indirect methods have been developed and implemented over the years, allowing to assess a wide range of body composition parameters (Heymsfield et al. 2005). Among these methods, some are considered as the gold standard for certain parameters, such as the dilution techniques for the body fluids assessment (Heymsfield et al. 2005). Other methods including energy X-ray absorptiometry (DXA), underwater weighing (UWW), air displacement plethysmography, magnetic resonance, and computed tomography are also classified as indirect approaches and used as reference methods in the body composition evaluation (Heymsfield et al. 2005).

However, most of the aforementioned techniques are expensive and require long procedures and highly specialized personnel (Campa et al. 2021b). For this reason, double-indirect methods have been implemented for obtaining estimations derived from indirect methods such as DXA, UWW or dilution techniques through validated regression equations. Over the recent years, the bioelectrical impedance analysis (BIA) has been identified as a possible alternative for assessing body composition. Although BIA is classified as a double-indirect approach, being noninvasive, portable, relatively low-cost, and technologically friendly, its use has gained attention in clinical and practical, as well as in research contexts (Lukaski and Raymond-Pope 2021). BIA is based on the different impedance of fat and lean tissues when a weak electric current flows through the body and several technologies have been designed and commercialized to date. These technologies include hand to hand, leg to leg, foot to hand direct or segmental approach, implying profound differences in both testing procedures (e.g., body position and electrodes placement) and final outcomes (Dellinger et al. 2021; Stratton et al. 2021). The hand to hand technology measures the upper body impedance, the foot to foot measures the lower body impedance, and the foot to hand measures the right hemisoma impedance, all estimating the remaining body sections through dedicated algorithms; on the contrary, the direct segmental technology measures the whole-body impedance (Campa et al. 2021b). Based on these four technologies, many devices have been produced by the manufacturers, working at a wide range of sampling frequency (from 1 to 1000 kHz), albeit the 50 kHz frequency has been identified as the most appropriate for measuring bioimpedance in humans (Kyle et al. 2004a, b).

The traditional BIA approach allows the quantification of both absolute (kg or L) or relative amount (%) of a number of body composition parameters through predictive equations, thanks to the different conductance properties of each

125 biological tissue. In fact, FM shows poor conductive proprieties, while fat-free mass (FFM), including the lean soft tissue
126 (LST) and body fluids, is a good electrical conductor (Lukaski and Piccoli 2012). Following the procedures, the devices
127 may either provide the quantitative estimation of body composition parameters using predictive equations set by the
128 manufacturer or provide the raw resistance (R) and reactance (Xc) to be inserted into specific formulas up to the operator
129 (Campa et al. 2021b). In this regard, most of the formulas have been developed for non-athletic populations, and formulas
130 for athletes have been designed recently (Campa et al. 2021b). This may have an impact on the final outcomes, as shown
131 in athletes samples (Pichard et al. 1997; Houtkoop et al. 2001; Matias et al. 2016a,b; Deminice et al. 2016; Shiose et al.
132 2020; Coratella et al. 2021). However, these studies examined the difference in body composition outcomes comparing
133 BIA vs. reference methods considering only a single BIA technology or a given body composition parameter (e.g., FM
134 and FFM or body fluids) in athletes. Notwithstanding, many studies have compared the BIA vs. reference methods in
135 athletes using different BIA-technologies, predictive equations, and devices. All these studies have not been
136 systematically reviewed so far, in order to provide a comprehensive overview of the literature.

137
138 A further approach when using bioimpedance parameters is the bioelectrical impedance vector analysis (BIVA), proposed
139 by Piccoli et al. (Piccoli et al. 1994) in 1994 and modified by Buffa et al. (Buffa et al. 2013) in 2013. The initial approach
140 (Classic BIVA) consists of the simultaneous evaluation of R and Xc adjusted for the stature, plotting them as a vector
141 within a graph (Piccoli et al. 1994). The later approach (*Specific BIVA*), consists of the concurrent adjustment of R and
142 Xc for the stature and for the cross-sectional area of the arm, waist, and calf (Buffa et al. 2013). Classic and *Specific*
143 BIVA were developed with the aim to determine total body water (TBW) and %FM, respectively. The change in vector
144 length reflects the change in TBW (Classic BIVA) or %FM (*Specific BIVA*), while the lateral displacement of the vector
145 reflects the bioelectrical phase angle for both BIVA approaches, graphically represented as the angle between the vector
146 and the x-axis (Stahn et al. 2012). Particularly, the phase angle has been proposed as an indicator of cellular health, cell
147 membrane integrity (Stahn et al. 2012; Lukaski and Raymond-Pope 2021) and faithfully reflects the
148 intracellular/extracellular water (ICW/ECW) ratio (Marini et al. 2020; Campa et al. 2021b). As such, BIVA allows a
149 qualitative assessment of body composition, avoiding the use of prediction equations to estimate the different parameters.
150 Additionally, although BIVA does not quantify each component, the vector position can be evaluated within tolerance
151 ellipses drawn for each population, representing their percentile within that population distribution (Campa et al. 2019).
152 The use of BIVA in athletes has been implemented quite recently, and a few studies have assessed body composition in
153 athletes using both BIVA and reference methods. Some authors have systematically reviewed the use of BIVA in sports
154 practice (Castizo-Olier et al. 2018), albeit they did not focus on the comparison of BIVA vs. reference methods. In this
155 regard, this was not possible at that time, given that the first studies comparing BIVA vs. reference method in athletes

156 was only published in 2020 (Campa et al. 2020; Marini et al. 2020). Figure 1 depicts the key concepts of BIA and the
157 application of BIVA.

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159 ***Insert Figure 1 next here***

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161 Therefore, the main purposes of the present systematic review were to summarize the results of studies that compared i)
162 BIA vs. reference methods in the estimation of body composition parameters in athletes and ii) BIVA vs. reference
163 methods in the qualitative assessment of body composition parameters in athletes. Furthermore, we aimed to provide
164 appropriate strategies to assess body composition in athletes using BIA or BIVA, considering the different technologies
165 and predictive equations.

166 167 **Methods**

168 169 Search strategy and eligibility criteria

170 The present study was carried out following the preferred reporting items for systematic reviews and meta-analyses
171 (PRISMA) guidelines (Page et al. 2021). The bibliographic search was performed on August 15th, 2021, using Scopus
172 and PubMed online databases. The search query was applied to the source title, abstract, and keywords, and included
173 combinations of at least one of the terms identifying body composition, with at least one of the terms identifying the
174 bioimpedance techniques applied, a term on the reference technique, and a term on the field of application. The resulting
175 search query was:

176 (“body composition” OR “fat mass” OR “fat free mass” OR “muscle mass” OR “lean mass” OR “total body water” OR
177 “extracellular water” OR “intracellular water” OR FM OR FFM OR TBW OR ECW OR ICW) AND (BIA OR
178 bioimpedance OR “bioelectrical impedance” OR BIVA OR “bioelectrical impedance vector analysis” OR “vector
179 analysis” OR biavector) AND (DXA OR DEXA OR densitometry OR imaging OR CT OR tomography OR MRI OR
180 MRT OR magnetic OR RMT OR RM OR “dilution techniques” OR “deuterium dilution” OR “isotope dilution” OR
181 UWW OR hydrodensitometry OR “underwater weight” OR "BOD POD" OR ADP OR “air-displacement
182 plethysmography” OR “criterion method” OR “standard technique” OR “direct technique” OR “reference technique”)
183 AND (sport OR athletes). To identify additional relevant papers, hand searching of the reference lists of the included
184 papers was performed.

185
186 The inclusion criteria were:

- 187
- Peer-reviewed articles that assessed body composition in athletes involved in individual or team sports using BIA or BIVA and reference techniques.
- 188
- Accessible in English full text.
- 189
- Individuals aged above 16 years, and with no chronic diseases or health problems.
- 190

191 The exclusion criteria were:

- Reviews and case studies.
 - Articles aimed to develop predictive equations without a cross-validation group.
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195 Study selection and data processing

196 Based on the initial titles retrieved, duplicates were removed. Abstracts identified from the literature searches were
197 screened for potential inclusion by two authors (F.C. and G.C.) and a third author (L.G.) when there was a disagreement
198 between the first two. Data extraction included information about each article, such as: authors, year, study design,
199 participants' information (sex, age), ~~type of~~ sports code, bioimpedance methodology and devices, reference technique,
200 outcome measures and main results.

202 Quality assessment

203 Methodological quality was assessed using the Quality Assessment Tool for Observational Cohort and Cross-sectional
204 Studies in observational studies (NIH 2014) recommended by the National Institute of Health, U.S. Department of Health
205 and Human Services. The tool consists of 14 criteria that are used to assess quality, including whether the population
206 studied was clearly specified and defined, whether the outcome assessors were blinded, and an assessment of the
207 participation rate. The criteria were classified as “yes”, “no”, “unclear”, or “not applicable”. Quality rates were good, fair,
208 or poor as judged by two independent observers (F.C. and L.G.) following the instructions given by the National Institute
209 of and Human Services.

211 **Results**

213 Search outcomes

214 The literature search resulted in 554 articles. After removal of duplicates (n= 242) and abstract screening, 43 studies were
215 considered relevant. After the full text screening, 11 were further excluded, so that a total of 32 studies fully met the
216 eligibility criteria. Ten additional studies were included after a hand searching of the reference lists of the included articles.

217 The PRISMA flow chart is shown in Figure 1. Finally, 42 studies published between 1988 and 2021 were considered. Out
218 of these 42 articles, 37 presented a cross-sectional and five a longitudinal study design.

219
220 ***Insert Figure 2 next here***

221 222 Participants

223 A total of 2978 subjects (1962 men and 1016 women) participated in the selected studies. Regarding sports code, N=534
224 participants were involved in team sports, N=339 in individual sports, while the exact number of subjects for each sport
225 modality was not reported for N=2105 participants.

226 227 Risk of bias

228 The risk of bias resulted as low to moderate, as summarized in Supplementary Table 1. Measurement procedures (e.g.,
229 electrodes placement, hydration status, food and fluid intake before the test or time from the last exercise) of BIA were
230 sometimes not completely described. Furthermore, the predictive equations used to estimate body composition parameters
231 were not always reported. Twenty-three studies had an overall good rating in terms of quality, while 13 were rated as fair
232 and 6 as poor (Table S1).

233 234 Bioelectrical devices and technologies

235 The selected articles included different devices and technologies as shown in Figure 2. Considering the four different
236 technologies, 4 articles used the hand to hand (Esco et al. 2011; Loenneke et al. 2013; Graybeal et al. 2020; Syed-Abdul
237 et al. 2021), 6 the leg to leg (Civar et al. 2003, 2006; Dixon et al. 2005; Loenneke et al. 2013; Domingos et al. 2019;
238 Graybeal et al. 2020), 29 the foot to hand (Birzniece et al. 2015; Colville et al. 1989; Lukaski et al. 1990; Kirkendall et
239 al. 1991; Hortobágyi et al. 1992; Pichard et al. 1997; Williams and Bale 1998; Fornetti et al. 1999; De Lorenzo et al.
240 2000; Houtkoop et al. 2001; Andreoli et al. 2004; Svantesson et al. 2008; Company and Ball 2010; Matias et al. 2016a,
241 b, 2021; Deminice et al. 2016; Krzykała et al. 2016; Arias Téllez et al. 2019; Campa et al. 2020, 2021; Marini et al. 2020;
242 Graybeal et al. 2020; Silva et al. 2020; Sardinha et al. 2020; Shiose et al. 2020; Stagi et al. 2021; Francisco et al. 2021;
243 Coratella et al. 2021), and 9 the direct segmental technology (Loenneke et al. 2012, 2013; Esco et al. 2015; Krzykała et
244 al. 2016; Raymond et al. 2018; Brewer et al. 2019; Hartmann Nunes et al. 2020; Graybeal et al. 2020; Lee et al. 2021).
245 Particularly, more than one technology was used in some studies and for each technology, different devices were used.
246 Considering the dependent variables, 30 articles (Birzniece et al. 2015; Colville et al. 1989; Lukaski et al. 1990; Kirkendall
247 et al. 1991; Hortobágyi et al. 1992; Pichard et al. 1997; Williams and Bale 1998; Fornetti et al. 1999; De Lorenzo et al.

248 2000; Houtkoopr et al. 2001; Civar et al. 2003, 2006; Andreoli et al. 2004; Dixon et al. 2005; Svantesson et al. 2008;
249 Company and Ball 2010; Esco et al. 2011, 2015; Loenneke et al. 2012, 2013; Krzykała et al. 2016; Raymond et al. 2018;
250 Arias Téllez et al. 2019; Domingos et al. 2019; Brewer et al. 2019; Hartmann Nunes et al. 2020; Graybeal et al. 2020;
251 Sardinha et al. 2020; Lee et al. 2021; Matias et al. 2021; Syed-Abdul et al. 2021) assessed FM and FFM comparing BIA
252 with reference methods, although seven additional studies used more than one technology, resulting in 37 comparisons.
253 A total of 7 studies (Birzniece et al. 2015; Matias et al. 2016b, a; Deminice et al. 2016; Shiose et al. 2020; Coratella et al.
254 2021; Francisco et al. 2021) assessed body fluids comparing BIA with reference methods, using the foot to hand
255 technology. A total of 5 articles (Campa et al. 2020, 2021a; Marini et al. 2020; Silva et al. 2020; Stagi et al. 2021) assessed
256 body composition comparing BIVA with reference methods, using the foot to hand technology.

257
258 ***Insert Figure 3 next here***
259

260 BIA vs. reference methods: FM and FFM

261
262 Table 1 shows the study design, demographic information, bioimpedance methodology, reference methods, and the main
263 results for each study. Considering the 16 studies that used a foot to hand technology for assessing FM, eight (Colville
264 et al. 1989; Hortobágyi et al. 1992; Pichard et al. 1997; Williams and Bale 1998; Boileau and Horswill 2000; Houtkoopr
265 et al. 2001; Andreoli et al. 2004; Company and Ball 2010) showed an overestimation of the %FM obtained by BIA, five
266 studies an underestimation of %FM (Lukaski et al. 1990; De Lorenzo et al. 2000; Arias Téllez et al. 2019) and FM
267 (Svantesson et al. 2008; Birzniece et al. 2015), while three studies showed an agreement in the estimated %FM (Pichard
268 et al. 1997; Krzykała et al. 2016) and FM (Graybeal et al. 2020). Birzniece et al. (Birzniece et al. 2015) observed both a
269 cross-sectional and longitudinal underestimation of %FM obtained by BIA. Of these studies, only seven (Colville et al.
270 1989; Lukaski et al. 1990; Pichard et al. 1997; De Lorenzo et al. 2000; Houtkoopr et al. 2001; Andreoli et al. 2004;
271 Company and Ball 2010) reported the predictive equations used. Considering the 10 studies that used a foot to hand
272 technology for assessing FFM, four studies showed a good agreement between BIA and reference methods (Lukaski et
273 al. 1990; Fornetti et al. 1999; Graybeal et al. 2020; Matias et al. 2021), three studies showed an underestimation (Colville
274 et al. 1989; Hortobágyi et al. 1992; Pichard et al. 1997) and three studies showed an overestimation of FFM (De Lorenzo
275 et al. 2000; Svantesson et al. 2008; Birzniece et al. 2015). Birzniece et al. (Birzniece et al. 2015) observed both a cross-
276 sectional and longitudinal overestimation of FFM obtained by BIA. Of these studies, only seven (Colville et al. 1989;
277 Lukaski et al. 1990; Pichard et al. 1997; Fornetti et al. 1999; De Lorenzo et al. 2000; Houtkoopr et al. 2001; Matias et al.
278 2021) reported the predictive equations used. LST of the arm and legs was estimated by only one study (Sardinha et al.

279 2020), which showed an excellent agreement between BIA and the reference method and reported the predictive
280 equations.

281
282 Nine studies used a direct segmental technology for assessing FM, albeit Lee et al. (Lee et al. 2021) utilized three different
283 devices for a total of 11 comparisons. Six studies reported an overestimation of FM (Brewer et al. 2019; Hartmann Nunes
284 et al. 2020; Graybeal et al. 2020; Lee et al. 2021) and %FM (Loenneke et al. 2013; Lee et al. 2021). Of these six studies,
285 one used a regional approach for investigating the legs FM (Brewer et al. 2019), and one assessed the visceral FM
286 (Hartmann Nunes et al. 2020). Three studies (Esco et al. 2015; Krzykała et al. 2016; Raymond et al. 2018) showed an
287 underestimation of %FM, and one of them used a regional approach measuring the arms and legs FM (Raymond et al.
288 2018), three studies (Raymond et al. 2018; Brewer et al. 2019; Graybeal et al. 2020) showed no difference between BIA
289 and the reference methods. The study by Graybeal et al. (Graybeal et al. 2020) found higher %FM only in men, while
290 they reported a good agreement in women. None of these nine studies using the direct segmental technique reported the
291 equations. Five studies used a direct segmental technology for assessing FFM. Four studies showed an underestimation
292 compared with the reference method. Of these four studies, Graybeal et al. (Graybeal et al. 2020) found this result only
293 in men, while Raymond et al. (Raymond et al. 2018) referred to arms and Brewer et al. (Brewer et al. 2019) to arms and
294 legs FFM. Two studies reported an overestimation in FFM (Loenneke et al. 2012; Esco et al. 2015), and two studies
295 reported no difference in the FFM, but only when measuring women (Graybeal et al. 2020) or the trunk FFM (Raymond
296 et al. 2018). None of these five studies using the direct segmental technique reported the equations. Esco et al. (Esco et
297 al. 2015) was the only study that using a regional approach for assessing arm, leg, and trunk LST, reported a good
298 agreement between the methods.

299
300 Six studies used a leg to leg technology for assessing FM, albeit Loenneke et al. (Loenneke et al. 2013) utilized two
301 different predictive equations, as reported in Table 1, for a total of seven comparisons. Three of them reported an
302 underestimation in %FM (Civar et al. 2003; Loenneke et al. 2013) and FM (Dixon et al. 2005), two studies an
303 overestimation in FM (Domingos et al. 2019; Graybeal et al. 2020), while three studied showed no difference (Civar et
304 al. 2006; Loenneke et al. 2013; Graybeal et al. 2020). Considering the Graybeal et al. (Graybeal et al. 2020) study, higher
305 FM was found only in men, while no difference was reported for women. In the Loenneke et al. (Loenneke et al. 2013)
306 study, no difference was found when the device was set on the “non-athlete” mode, while FM was underestimated using
307 the “athletes” modality. Only Graybeal et al. (Graybeal et al. 2020) assessed FFM and reported a good agreement with
308 the reference method for women and an underestimation for men. No study involving leg to leg BIA-technologies reported
309 the predictive equations used.

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311 Four studies used a hand to hand technology and reported an underestimation in %FM (Esco et al. 2011; Loenneke et al.
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312 2013; Graybeal et al. 2020; Syed-Abdul et al. 2021), albeit Loenneke et al. (Loenneke et al. 2013) and Syed-Abdul et al.
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5
313 (Syed-Abdul et al. 2021) utilized different predictive equations, as reported in Table 1, for a total of eight comparisons.
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7
314 Of these four studies, Loenneke et al. (Loenneke et al. 2013) and Syed-Abdul et al. (Syed-Abdul et al. 2021) reported
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315 lower %FM when the devices were set on the “athlete” modality, and a good agreement when the devices were set on the
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11
316 “non-athlete” modality. Graybeal et al. (Graybeal et al. 2020) overestimated %FM in men, while no difference for women
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317 was found; additionally, FFM was underestimated in men and a good agreement for women was found. No studies
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318 involving hand to hand BIA technologies reported the predictive equations used.
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Insert Table 1 next here

BIA vs. reference methods: Body fluids estimations

Table 2 shows the study design, demographic information, bioimpedance methodology, reference methods, and the main results for each study. All seven studies assessing TBW were performed using the foot to hand technology, albeit different devices (Matias et al. 2016a) and procedures (Matias et al. 2016b; Deminice et al. 2016; Shiose et al. 2020; Coratella et al. 2021) for a total of 31 comparisons. Five studies reported no difference in TBW assessed by BIA vs. reference methods (Matias et al. 2016b, a; Shiose et al. 2020; Coratella et al. 2021; Francisco et al. 2021), four studies an underestimation (Matias et al. 2016a, b; Deminice et al. 2016; Coratella et al. 2021), and one study an overestimation (Matias et al. 2016a). Five studies used the foot to hand technology to assess ECW, albeit different devices (Matias et al. 2016a) and procedures (Matias et al. 2016a,b; Coratella et al. 2021) for a total of 12 comparisons. Five studies reported no difference in ECW assessed by BIA vs. reference methods (Birzniece et al. 2015; Matias et al. 2016a, b; Coratella et al. 2021; Francisco et al. 2021), four studies an underestimation (Birzniece et al. 2015; Matias et al. 2016a, b; Coratella et al. 2021), and no study reported an overestimation. Three studies (Matias et al. 2016a, b; Francisco et al. 2021) used the foot to hand technology to assess ICW, albeit different devices (Matias et al. 2016a) for a total of four comparisons. All comparisons showed no difference in ICW assessed by BIA vs. reference techniques. Only five out of these seven studies (Matias et al. 2016a, b; Deminice et al. 2016; Shiose et al. 2020; Coratella et al. 2021) reported the predictive equations used.

Insert Table 2 next here

341 BIVA vs. reference methods

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Five studies compared the BIVA's outcomes with body composition measurements obtained from reference methods, as shown in Table 3. All the analyzed studies were conducted using a foot to hand technology. Two studies were conducted with a cross-sectional design (Marini et al. 2020; Stagi et al. 2021), and showed that the *specific* vector length was correlated positively with %FM and the classic vector negatively with TBW, both at the whole body (Marini et al. 2020) and the segmental level (Stagi et al. 2021). These findings were also confirmed in three longitudinal studies (Campa et al. 2020, 2021a; Silva et al. 2020), which highlighted the effectiveness of BIVA in assessing changes in body composition over time. In addition to the vector length evaluation, its position along the minor axis of the BIVA ellipses, that is mainly due to phase angle variations, has been associated with the ICW/ECW ratio (Campa et al. 2020, 2021a; Marini et al. 2020; Silva et al. 2020).

Insert Table 3 next here

Discussion

The present systematic review aimed to compare i) BIA vs. reference methods in the estimation of body composition parameters in athletes and ii) BIVA vs. reference methods in the qualitative assessment of body composition parameters in athletes. Forty-two studies were included in the review, for a total of 2978 athletes involved in team or individual sports. Overall, most of the studies included used the foot to hand technology, allowing to draw a detailed picture of the BIA or BIVA vs. reference methods for FM FFM and body fluids, quantitative and qualitative assessment, respectively. The remaining hand to hand, leg to leg, and direct segmental technologies were used in few studies, and none of these assessed body fluids, so that the comparison is incomplete.

BIA vs. reference methods: FM and FFM

The studies that assessed FM and FFM using BIA vs. reference methods resulted in inconsistent findings. Concerning the FM, BIA showed poor accuracy vs. the reference methods, regardless of the technology used. However, while in some studies the authors used a direct formula to determine %FM (Colville et al. 1989; Pichard et al. 1997), in other studies FM was indirectly derived as the difference between the body mass and FFM (De Lorenzo et al. 2000; Houtkoop et al. 2001; Andreoli et al. 2004; Company and Ball 2010). In addition, although the same predictive equations were used (Oppliger et al. 1991), different reference methods were chosen to determine %FM, such as 4C (Andreoli et al. 2004) or

372 DXA (De Lorenzo et al. 2000). However, a lack of agreement between 4C and DXA was later observed when assessing
373 %FM in athletes (Santos et al. 2010), making the comparison between BIA and the reference methods challenging. To
374 further entangle this picture, several studies that used the foot to hand (Kirkendall et al. 1991; Hortobágyi et al. 1992;
375 Williams and Bale 1998; Houtkoop et al. 2001; Svantesson et al. 2008; Birzniece et al. 2015; Krzykała et al. 2016; Arias
376 Téllez et al. 2019; Graybeal et al. 2020), and all the studies that used the hand to hand, the leg to leg and the direct
377 segmental technology, did not report the predictive equations used for determining %FM. Interestingly, two studies
378 (Loenneke et al. 2013; Syed-Abdul et al. 2021) that used hand to hand devices found no difference between BIA and the
379 reference methods when the device was set on the "non-athlete" mode, while an underestimation in %FM was observed
380 when the "athlete" mode was set. According to the manufacturer of the device used by Loenneke et al. (Loenneke et al.
381 2013), the "athletic" mode is utilized for individuals who have exercised at least 10 hours a week consistently for at least
382 6 months, or who have a resting heart rate of 60 bpm or less. These factors may not exclude that an athlete may have
383 different characteristics. In this regard, an actual definition of athlete is advocated, so to define clearly when a specific or
384 generalized equation or modality should be used.

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386 Using the foot to hand technology coupled with predictive equations developed for athletes, BIA showed no difference
387 with reference methods for estimating FFM (Lukaski et al. 1990; Fornetti et al. 1999; Graybeal et al. 2020; Matias et al.
388 2021) and its LST component (Sardinha et al. 2020). On the contrary, when generalized equations were used, inconclusive
389 findings were observed. Regarding the direct segmental technology, BIA showed an underestimation of FFM compared
390 to the reference methods. Only one study assessed FFM using the leg to leg and hand to hand technology, reporting
391 underestimation in men and good agreement in women for both technologies (Graybeal et al. 2020). In conclusion, the
392 present state of the art needs to be implemented with procedures including the gold standard procedure for determining
393 %FM and FFM (4C) and predictive equations for athletes, reported in the protocol. Considering that the Matias et al.
394 (Matias et al. 2021) equation is the only one developed with 4C, its use should be preferred when assessing FFM in
395 athletes.

396 397 BIA vs. reference methods: body fluids estimations

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399 All the studies comparing BIA vs. reference methods to assess body fluids were conducted using the foot to hand
400 technology and the dilution techniques as the reference method. Such a consistency allows more robust outcomes when
401 summarizing the results. Considering all the studies, the use of predictive equations for athletes (Matias et al. 2016b)
402 resulted in good agreement with the dilution techniques. Notably, the Matias' equations (Matias et al. 2016b) were

403 developed for the foot to hand technology at a 50 kHz frequency, and are to date the only available ones. In contrast, the
404 use of generalized predictive equations (Kushner and Schoeller 1986; Van Loan and Mayclin 1987; Lukaski and
405 Bolonchuk 1988; Kushner et al. 1992; Sergi et al. 1994; Schoeller and Luke 2000; Morgenstern et al. 2002; Sun et al.
406 2003) led to an overall underestimation of the body fluids. Lastly, BIA was also shown to be a valid method for assessing
407 body fluids in person with varying hydration status (Francisco et al. 2021).

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409 BIVA vs. reference methods.

410 BIVA is an alternative method to qualitatively assess body composition in athletes. It allows the analysis of the
411 ICW/ECW ratio and the amount of TBW (Classic BIVA) or %FM (Specific BIVA) (Campa et al. 2021b). Since these
412 techniques are based on raw data, BIVA does not require the use of predictive equations, avoiding possible errors due to
413 their improper application. On the other hand, BIVA does not provide estimates of volume or mass, but a classification
414 (e.g., more or less body fluids or %FM) and ranking (e.g., better or worse after treatment or intervention) tool (Lukaski
415 and Raymond-Pope 2021). In this regard, a rightward or leftward displacement of the BIVA vector is interpreted as a
416 decrease or increase in the ICW/ECW ratio, respectively; moreover, longer vectors corresponds to lower TBW (Classic
417 BIVA) or higher %FM (*Specific BIVA*) and vice versa (Campa et al. 2021b). All the selected studies agree in suggesting
418 BIVA as a valid method for assessing body composition in athletes compared to the reference methods. Specifically, the
419 standard reference methods were 4C (Marini et al. 2020), dilution techniques (Marini et al. 2020; Campa et al. 2020; Silva
420 et al. 2020) and DXA (Marini et al. 2020; Stagi et al. 2021; Campa et al. 2021a). Notably, BIVA was derived from the
421 foot to hand technology only. This leads to some considerations. In first instance, the reference tolerance ellipses for
422 athletes have been designed for the foot to hand technology, so that different technology should not be used due to the
423 lack of agreement between the technologies (Silva et al. 2019; Dellinger et al. 2021; Stratton et al. 2021). Secondly, when
424 the aim of the research is to compare an athlete with his peers, the tolerance ellipses have been designed for some athletic
425 populations, such as soccer (Micheli et al. 2014; Bongiovanni et al. 2020), volleyball (Campa and Toselli 2018), cycling
426 (Giorgi et al. 2018), or endurance, sports team or power/velocity (Campa et al. 2019) athletes. All other sports should be
427 redirected to the tolerance ellipses for generic athletic population (Campa et al. 2019).

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429 Limitations of the review and future perspectives

430 A few limitations to this review should be acknowledged. Firstly, we classified the results according to the BIA
431 technology used in the selected studies. However, even within the same technology, there could be confounding factors.
432 For example, the positioning of the electrodes used in the foot to hand technologies and their typology could lead to
433 different outcomes, increasing the variability within the results. In this regard, recent guidelines have been proposed to

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434 avoid inconsistent procedures (Campa et al. 2021b). Secondly, several devices have been considered, which could have
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435 different characteristics that may represent a further confounding factor, such as the amperage and their reliability.
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436 Furthermore, regardless of the BIA-devices and technologies used, the athlete's evaluation must consider numerous
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437 factors such as the hours since last exercise and the nutrition prior to the test (Lukaski et al. 1990). A number of future
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438 perspectives also arise from these results. For example, future longitudinal studies are warranted, assessing the
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439 responsiveness of different BIA technologies in comparison with the reference methods. Moreover, further studies are
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440 needed to understand which factors (e.g., amperage, body segments measured, experimental conditions) other than
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441 technologies increase the between-device variability. **Lastly, authors are encouraged to provide raw bioelectrical data to
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442 a more transparent assessment of body composition through BIA and BIVA.**
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443 19 2044 **Conclusions**

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2245 Regardless of the BIA-technology, the assessment of FM% results in lack of agreement with the reference methods. When
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2446 estimating FFM using predictive equations developed for athletes and the foot to hand technology, a good agreement with
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2647 the reference methods has been observed. Generalized equations lead to an underestimation of FFM. Similarly, body
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2848 fluids are accurately estimated using predictive equations for athletes and the foot to hand technology, while overall
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3049 underestimated using generalized equations. Regarding BIVA, Classic and *Specific* approaches represented two valid
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3250 methods for assessing body fluids (Classic BIVA) and percentage of fat mass (*Specific* BIVA). The present systematic
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3451 review suggests that BIA and BIVA could be used for assessing body composition in athletes, provided that equations
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3652 and BIVA references developed for athletes are used. Figure 4 summarizes the main finding of the present systematic
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3853 review.
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Insert Figure 4 next here

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669 **Figure captions**

670 **Figure 1.** Key concepts of bioelectrical impedance analysis.

671 **Figure 2.** PRISMA Flow chart of the studies' selection.

672 **Figure 3.** Bioelectrical devices and technologies involved in the selected studies.

673 **Figure 4.** Bioelectrical impedance analysis (BIA) vs. reference methods in athletes.

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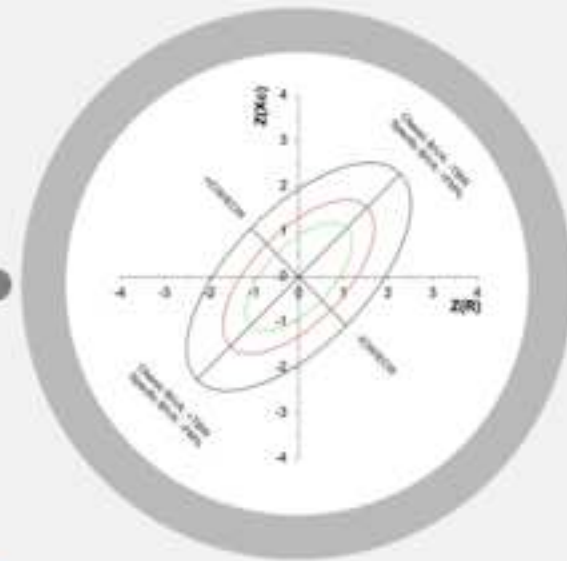
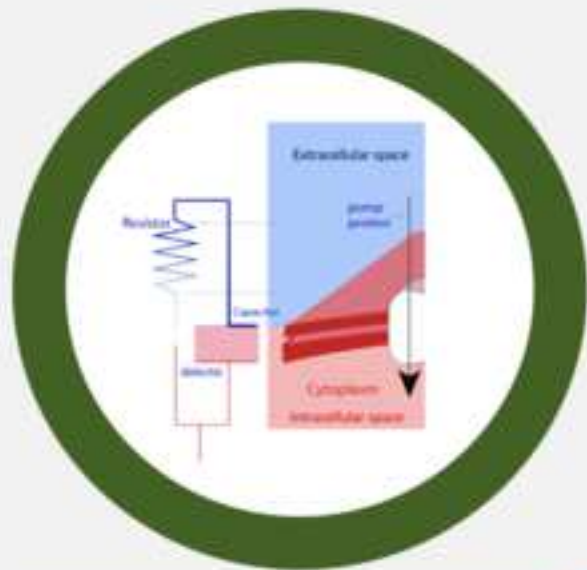
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What does BIA measure?

- Impedance
- Resistance (R)
- Reactance (Xc)

How can BIA values be used?

- Insert it into predictive equations to estimate body composition
- Directly evaluated by bioelectrical impedance vector analysis (BIVA)

What does BIA provide?

- Quantification of body composition parameters
- Bivariate interpretation of R and Xc against the reference population percentiles

