

External validation of bioelectrical impedance analysis equations for body composition against dual-energy X-ray absorptiometry in Mexican recreational runners

Validación externa de ecuaciones de composición corporal por análisis de impedancia bioeléctrica y la absorciometría dual de rayos X entre corredores recreativos mexicanos

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ABSTRACT

Sports professionals prioritize athlete body composition (BC) due to its relationship to performance. Bioelectrical impedance analysis (BIA) estimates BC using predictive equations, and validating these equations is essential to determine their utility. The aim of this study was to externally validate four bioelectrical impedance equations for predicting body composition in Mexican recreational runners using dual-energy X-ray absorptiometry (DXA) as a reference method. This external validation pilot study followed a comparative, cross-sectional design and included 30 Mexican male recreational runners (aged 38.0 ± 10.7 years). BC was measured using DXA and four BIA equations. A paired *t*-test was performed to evaluate differences between methods. Equivalent testing, simple linear regression, and Bland-Altman analysis were carried out to evaluate agreement between methods. Statistical significance was set at $p < 0.05$. Non-significant differences were found between DXA and predicted values with Macías *et al.* (2007) and Lukaski and Bolonchuk (1987) equations ($p < 0.05$). These equations provided equivalence at 5% regions and non-significant bias. In conclusion, Lukaski and Bolonchuk (1987) demonstrate the most accurate equations for the current sample. These equations showed promising results for measuring BC in a cohort of runners; however, caution is advised when applying them to individual tracking.

Keywords: Fat-free mass; athletes; body fat, sport.

RESUMEN

Los profesionales del deporte priorizan la composición corporal (CC) de los atletas por su vínculo con el rendimiento. El análisis de impedancia bioeléctrica (BIA) estima la CC mediante ecuaciones predictivas, cuya validación es necesaria para determinar su utilidad. El objetivo fue validar externamente cuatro ecuaciones de BIA para estimar la CC en corredores recreativos mexicanos, utilizando la absorciometría dual de rayos X (DXA) como método de referencia. En este estudio de validación externa con diseño transversal, 30 corredores recreativos mexicanos (edad 38.0 ± 10.7 años) fueron evaluados mediante DXA y cuatro ecuaciones de BIA. Se aplicó una prueba *t* pareada para comparar diferencias entre métodos.

Se emplearon pruebas de equivalencia, regresión lineal simple y análisis de Bland-Altman para evaluar la concordancia, con un nivel de significancia de $p < 0.05$. No se encontraron diferencias significativas entre DXA y las ecuaciones de Macías *et al.* (2007) y Lukaski y Bolonchuk (1987), ambas mostraron sesgo no significativo y equivalencia dentro del 5%. Las ecuaciones de Lukaski y Bolonchuk (1987) demostraron ser las más precisas para esta muestra. Estas ecuaciones podrían ser útiles para estimar la CC en corredores recreativos; sin embargo, se recomienda cautela en su uso para seguimiento individual.

Palabras clave: Masa libre de grasa; atleta; grasa corporal; deporte.

INTRODUCTION

Professionals in sports science are interested in assessing body composition (BC) of athletes due to the close relationship with sports performance (Suchomel *et al.*, 2016; Campa *et al.*, 2019; Cholewa *et al.*, 2019; Ferland *et al.*, 2020). In addition, understanding BC serves as the foundation for designing nutritional and training programs to improve their physical skills. Lastly, BC can also be used to predict sports success and provide valuable health information.

Currently, there are several highly accurate assessment methods available, known as reference methods; however these are primarily intended for research purposes and are often inaccessible to sports professionals. Traditionally, anthropometry has been used to assess athletes. However, this method may be time-consuming, and the reliability of anthropometric measurements often depends on the experience of the anthropometrist (Gavan, 1950; Ulijaszek and Kerr, 1999). Another accessible and commonly used method is bioelectrical impedance analysis (BIA). BIA applies a low electrical current and uses the electrical properties of the body to measure resistance (R) and reactance (Xc). Resistance refers to the body's resistance to the passage of an electric current, while reactance relates to the body's dielectric capacity. These properties vary according to the body component distribution and proportion, mainly fat mass (FM) and total body water (TBW) (Kyle *et al.*, 2004; Ward and Brantlov, 2023).

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Due to the relationship between fat-free mass (FFM) and TBW, predictive equations have been developed using BIA variables and characteristics such as age, gender, weight, or height. Nevertheless, one of the main challenges of BIA equations is that most have been developed based on data from general populations, which do not consider athletes' characteristics. Therefore, biased results can be obtained when applying these equations to athletes, as the FFM and fluid compartments often differ significantly from the general population (Campa *et al.*, 2021; Coratella *et al.*, 2021). Therefore, it is essential to validate the currently available BIA equations with athlete populations, particularly those from the same country, to ensure their accuracy and applicability.

Recent reports highlighted that some equations developed for general populations could be applied to athletes (Campa *et al.*, 2022). Sports professionals could use this information to choose an equation that aligns with the variables measured regularly in their group of athletes. However, body profiles may vary considerably across sports and competitive levels, requiring further validation of available BIA equations. Therefore, this study aimed to externally validate four BIA equations in Mexican recreational runners using dual-energy X-ray absorptiometry (DXA) as a reference method.

MATERIAL AND METHODS

Participants and study design

This was an external validation pilot study following a comparative, cross-sectional design, including a non-representative sample of 30 healthy Mexican recreational runners (12 men and 18 women) aged between 18 and 50 years who were selected from a major research sample. Recruitment was performed through convenience sampling during 2018, with meetings held to explain the procedures to sports teams gathered in sports centers in northwest Mexico.

All participants had competition experience and a minimum of three years of exercise training and underwent rigorous physical activity screening using the short version of the International Physical Activity Questionnaire (IPAQ) before the study. Participants were nonsmokers, with no history of chronic cardiovascular disease or food allergies. Informed

consent was obtained from all participants. This research was conducted following the principles of the Declaration of Helsinki and received approval from the Research Centre for Food and Development Ethical Committee (Approval No. CE/010/2018).

Anthropometric and body composition assessment

Measurements were conducted at the Laboratory of Immunology, located within the Research Centre for Food and Development in Hermosillo, Mexico. Participants were instructed to arrive with a 12-hours fasting and abstain from vigorous exercise and alcohol consumption for 24 hours preceding their visit. Additionally, they were requested to empty their bladder before undergoing assessments.

Participants wore minimal clothing during the measurements. Body weight (BW) was measured using Seca flat scale model 874 (Seca, Hamburg, GER) and height (Ht) was measured using Seca stadiometer model 984 (Seca, Hamburg, GER). These measures were recorded to the closest 0.05 kg and 1 mm, respectively.

Hologic ASY-05119 was used for DXA scans (Hologic, Massachusetts, USA) to measure FFM as the main variable for equation validations. DXA equipment was calibrated according to the instructions provided by the manufacturer every assessment day. Scans were performed by a trained technician. A pregnancy test was conducted on female participants before DXA scans.

BIA was performed with subjects lying supine with their limbs slightly away from their bodies. Skin adhesive electrodes were placed following a hand and foot protocol. BIA device Quantum II (RJL System, Santeramo in Colle, ITALY) with a single frequency of 50 kHz was used. Resistance (R) and reactance (Xc) values were recorded, and the impedance index was calculated (Ht^2/R).

Additionally, FFM was predicted by applying the equations proposed by Macias *et al.* (2007) (EQ1), Matias *et al.* (2020) (EQ2), Lukaski and Bolonchuk (1986) (EQ3) and Matias *et al.* (2016) (EQ4), which are based on BIA and basic anthropometric variables and enable the prediction of FFM or TBW (Table 1). FFM from EQ4 was calculated as follows as $FFM = TBW/0.732$.

Table 1. Body composition predictive equations based on BIA and anthropometry characteristics.

Tabla 1. Ecuaciones predictivas de composición corporal basados en BIA y antropometría.

Authors	Equation	Sample	Reference method
EQ1	$FFM (kg) = (0.7374 \times [Ht^2 / R]) + (0.1763 \times BW) - (0.1773 \times Age) + (0.1198 \times Xc) - 2.4658$	155 Mexican healthy males and females, 20-50 years	ADP
EQ2	$FFM (kg) = -2.261 + (0.327 \times [Ht^2 / R]) + (0.525 \times BW) + (5.462 \times Sex)$	142 Portuguese trained male and female athletes, 22.9 ± 4.9 years	4C model by Wang <i>et al.</i> (2002)
EQ3	$FM (kg) = (0.734 \times [Ht^2 / R]) + (0.116 \times BW) + (0.096 \times Xc) + (0.876 \times Sex) - 4.03$	312 American healthy males and females, 19-50 years	HW
EQ4	$TBW = 0.286 + (0.195 \times [Ht^2 / R]) + (0.385 \times BW) + (5.086 \times Sex)$	212 Portuguese healthy active and elite athletes, 16-38 years	D ₂ O dilution

Sex was defined as 0 for females and 1 for males; Ht: Height; R: Resistance; BW: Body weight; Xc: reactance; ADP: Air displacement plethysmography; HW: Hydrostatic weighting; 4C: four compartments; D₂O: deuterium oxide.

Sexo fue definido como 0 para mujeres y 1 para hombres; Ht: Estatura; R: Resistencia; BW: Peso corporal; Xc: Reactancia; ADP: Pletismografía por desplazamiento de aire; HW: Pesaje hidrostático; 4C: Cuatro compartimentos; D₂O: Óxido de deuterio.

Statistical analysis

Results are presented as means and standard deviations (SD). The normality of the data distribution was confirmed by the Shapiro–Wilk test. FFM was considered as the main variable, with DXA as the reference method. Differences between sexes were analysed using independent sample *t*-tests. To establish an accurate prediction of FFM at the group level, paired sample *t*-test and equivalence testing with a 5% equivalence region (Dixon *et al.*, 2018) around mean DXA FFM were conducted. The strength of association between equations and DXA was analyzed through Pearson's correlation coefficient (*r*); association was classified according to *r* values of 0.00 to 0.10 (Negligible), 0.10 to 0.39 (Weak), 0.40 to 0.69 (Moderate), 0.70 to 0.89 (Strong) and 0.90 to 1.00 (very strong) (Schober and Schwarte, 2018). Simple linear regression analysis was performed to evaluate the variability of DXA FFM, explained through equations and R^2 and standard estimation error (SEE). To evaluate the agreement between methods, Bland-Altman analysis was performed with a one sample *t*-test comparing the difference between methods against a null hypothesis equals to zero to identify systematic bias (Bland and Altman, 1986; 1999). Additionally, a simple linear regression analysis between method differences and method means was used to evaluate proportional bias; bias and 95% limits of agreement were reported (Doğan, 2018). Statistical significance was set at $p < 0.05$. Statistical analyses were carried out using IBM SPSS Statistics 26.0 (IBM corp, NY, USA). Graphics were generated using GraphPad Prism 8.0 (GraphPad, Boston, USA).

RESULTS AND DISCUSSION

Independent sample *t*-tests revealed significantly greater values in BW, Ht, Ht^2/R , and DXA FFM in the male group, while R and DXA percentage FM (%FM) were greater in the female group ($p < 0.05$). All participants characteristics are presented in Table 2.

Equations were subjected to validation based on sex and the whole sample (Table 3). The paired sample *t*-test showed significant differences in predicted FFM and DXA FFM ($p < 0.05$). DXA FFM consistently differed significantly

from the EQ2 and EQ4 values in both sexes and the whole sample, which predictions resulted in higher FFM values. Non-significant differences were observed between EQ1 and EQ3 FFM compared with DXA FFM ($p > 0.05$).

Regarding the equivalence testing, EQ3 demonstrated equivalence with DXA in the male group and the whole sample meaning that differences between these methods may be within ± 2.66 kg for males and ± 2.25 kg for the whole sample. Meanwhile, EQ1 demonstrated equivalence with DXA only in the whole sample. No equivalence with DXA was observed in the other equations.

Table 3 presents the results of validating each equation against DXA. A statistically significant and moderate-to-strong relationship was observed between DXA FFM and all the equations across all groups (*r* ranging from 0.70 to 0.95; $p < 0.01$), indicating a good to excellent linear association. In the male group, EQ2 and EQ4 showed the highest R^2 with DXA FFM ($R^2 = 0.91$ and 0.90 , respectively); both regression models were statistically significant ($p < 0.01$). However, the bias observed by the Bland–Altman plot for these equations was -4.33 kg for EQ2 and -5.11 kg for EQ4, both of which resulted statistically different from zero, indicating a systematic bias ($p < 0.01$). A similar behavior was observed in the female group, where EQ2 and EQ4 showed the highest R^2 with DXA FFM, resulting in statistically significant ($p < 0.01$). Systematic bias for these equations also resulted statistically significant ($p < 0.05$). On the other hand, although EQ1 and EQ3 exhibited lower R^2 than EQ2 and EQ4, no significant systematic bias with DXA was observed in either males or females ($p > 0.05$).

Regarding the whole sample analysis (Fig. 1), EQ1 showed a significant linear association with DXA ($R^2 = 0.77$, $p < 0.001$) (Fig. 1a), with a non-significant intercept ($p = 0.718$), indicating consistent differences across the FFM range. This was supported by the Bland-Altman analysis, which resulted no systematic or proportional bias ($p > 0.05$) (Figure 1b).

For EQ2, the linear regression was also significant ($R^2 = 0.89$, $p < 0.001$), but a significant intercept ($p < 0.01$) suggests a systematic bias between methods (Fig. 1c). Bland-Altman, confirmed this with a significant systematic bias of -3.52 kg ($p < 0.05$) (Fig. 1d), which resulted in an overestimation of FFM.

Table 2. General participants characteristics grouped by sex.

Tabla 2. Características generales agrupadas por sexo.

	Male (n = 12)	Female (n = 18)	Whole sample (n = 30)
Age (years)	40.5 ± 11.8	36.3 ± 10.0	38.0 ± 10.7
BW (kg)	68.6 ± 11.5	60.8 ± 9.2*	63.9 ± 10.8
Ht (cm)	1.69 ± 0.1	1.62 ± 0.0**	1.7 ± 0.1
BMI (kg/cm ²)	23.9 ± 3.9	22.9 ± 2.8	23.3 ± 3.3
R (Ω)	512.9 ± 53.2	602.8 ± 77.7**	566.9 ± 81.4
Xc (Ω)	64.8 ± 7.7	68.3 ± 11.1	66.9 ± 9.9
Ht^2/R (Ω)	56.4 ± 6.5	44.6 ± 6.6***	49.3 ± 8.7
DXA FFM (kg)	53.3 ± 5.9	41.2 ± 4.3***	46.1 ± 7.8
DXA FM (%)	21.53 ± 5.9	30.2 ± 8.9**	26.7 ± 8.8

BW: Body weight; Ht: Height; R: Resistance; Xc: Reactance; FFM: Fat-free mass; FM: Fat mass; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

BW: Peso corporal; Ht: Estatura; R: Resistencia; Xc: Reactancia; FFM: Masa libre de grasa; FM: Masa grasa; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3. External validation of FFM predictions through BIA equations with DXA as a reference method.**Tabla 3.** Validación externa de las predicciones de FFM por ecuaciones de BIA con DXA como método de referencia.

		FFM	Bias (95% IC)	ET	<i>r</i>	<i>R</i> ²	SEE	95% LOA
Male (n=12)								
	DXA	53.3±5.8	-	-	-	-	-	-
	EQ1	51.8±6.8	1.52 (-0.65, 3.69)	No	0.86**	0.75**	3.07	-5.19, 8.23
	EQ2	57.6±7.6 [†]	-4.33 (-6.02, -2.63)*	No	0.95**	0.91**	1.85	-9.56, 0.91
	EQ3	52.4±5.3	0.90 (-0.58, 2.37)	Yes	0.92**	0.84**	2.45	-3.68, 5.47
	EQ4	58.4±7.3 [†]	-5.11 (-6.71, -3.50)*	No	0.95**	0.90**	1.92	-10.05, -0.17
Female (n=18)								
	DXA	41.2±4.3	-	-	-	-	-	-
	EQ1	42.8±4.7	-1.65 (-3.34, 0.04)	No	0.72**	0.52**	3.06	-8.32, 5.02
	EQ2	44.2±6.2 [†]	-2.99 (-4.76, -1.21)*	No	0.83**	0.69**	2.45	-9.98, 4.01
	EQ3	42.3±4.7	-1.08 (-2.81, 0.66)	No	0.70**	0.49**	3.15	-7.92, 5.77
	EQ4	44.2±5.9 [†]	-2.99 (-4.66, -1.32)*	No	0.83**	0.69**	2.45	-9.58, 3.60
Whole sample (n=30)								
	DXA	46.1±7.8	-	-	-	-	-	-
	EQ1	46.5±7.1	-0.38 (-1.76, 1.01)	Yes	0.88**	0.77**	3.57	-7.56, 6.88
	EQ2	49.6±9.5 [†]	-3.52 (-4.74, -2.30)*	No	0.94**	0.89**	2.53	-9.91, 2.86
	EQ3	46.4±7.0	-0.29 (-1.47, 0.90)	Yes	0.91**	0.83**	3.24	-6.54, 5.96
	EQ4	49.9±9.6 [†]	-3.84 (-5.02, -2.65)*	No	0.95**	0.91**	2.38	-10.06, 2.41

ET: Equivalence testing at 5% from DXA FFM corresponding to ± 2.25 kg for the whole sample, ± 2.66 kg for males, and ± 2.06 for females; *r*: Pearson's correlation coefficient; *R*²: Simple linear regression determination coefficient between the EQ and DXA; SEE: Standard estimation error; LOA: Limits of agreement. **p* < 0.05; ***p* < 0.001; [†]Significant difference to DXA FFM.

ET: Prueba de equivalencia al 5% de la FFM de DXA correspondiente a ± 2.25 kg para la muestra total, ± 2.66 kg para hombres y ± 2.06 para mujeres; *r*: Coeficiente de correlación de Pearson; *R*²: Coeficiente de determinación de regresión lineal simple entre ecuaciones y DXA; SEE: Error estándar de la estimación; LOA: Límites de concordancia. **p* < 0.05; ***p* < 0.001; [†]diferencias estadísticas con DXA FFM.

In addition, a significant proportional bias was observed for EQ2 (*R*² = 0.28, *p* < 0.05), indicating that disagreement increased at higher FFM values.

EQ3 presented a significant linear association with DXA (*R*² = 0.83, *p* < 0.001) (Figure 1e) and a non-significant intercept (*p* = 0.887), meaning a constant difference between methods across the range. This was consistent with Bland-Altman results, which showed no significant systematic or proportional bias. (Fig. 1f).

Lastly, EQ4 showed a significant association (*R*² = 0.91, *p* < 0.001) (Fig. 1g), but with a significant intercept (*p* < 0.05), suggesting non-constant discrepancies. Bland-Altman analysis confirmed a systematic bias of -3.84 kg (*p* < 0.05), and a significant proportional bias (*R*² = 0.32, *p* < 0.001), indicating overestimation increased with higher FFM values (Fig 1h).

The main finding of the current study is the potential suitability of EQ1 and EQ3 in Mexican recreational runners when analyzed collectively. The equations selection employed was based on their suitability for athletes, particularly EQ2 and EQ4 as these were developed involving athletes from different disciplines. Additionally, EQ3 has been previously validated in athletes (Lukaski *et al.*, 1990) and EQ1 was considered due to its development using data from the northwestern Mexican population.

Among the equations included, EQ3 provides the most accurate prediction of FFM compared with DXA on the male group and the whole sample group. Even though the paired sample *t*-test showed no differences in mean DXA FFM in the female group, equivalence testing was rejected. This means that differences in DXA FFM and EQ3 predictions may be

larger than ± 2.03 kg for this group, considered clinically relevant for longitudinal assessment. Additionally, the remaining validation metrics suggest caution in the use of EQ3 in this group. Interestingly, the whole sample improved EQ3 accuracy metrics, given that bias was slightly reduced.

To our knowledge, this is the first study externally validating EQ1 in Mexican recreational runners. Non-significant differences were observed in DXA FFM and EQ1 in both male and female groups. However, considering a more specific analysis, EQ1 rejected the equivalence testing for each sex group, although the whole sample improved this result. This suggests that using EQ1 in a sex-specific participant cohort may reduce the accuracy of FFM estimations, which could be clinically relevant, given that a 5% region of equivalence is set as a rigorous criterion for evaluating agreement between methods (Dixon *et al.*, 2018). Moreover, even though sex was not included as a predictive variable in EQ1, *R*² differed considerably between sexes. On the other hand, EQ1 showed improved *R*² and lower bias values in the whole sample compared to the subgroups. This improvement may be partially explained by the larger variability in FFM values when both sexes are combined, which increases the variance explained by the model. This highlighted the capability of EQ1 to predict FFM in a cohort of athletes, including both sexes. On an individual basis, even if reasonable *R*² and non-significant bias were observed, the use of EQ1 may not be appropriate due to the wider LOA obtained through Bland-Altman analysis.

For a better understanding of the current findings, a FFM prediction from EQ3 from individual data is presented as this equation resulted to be the most accurate compared

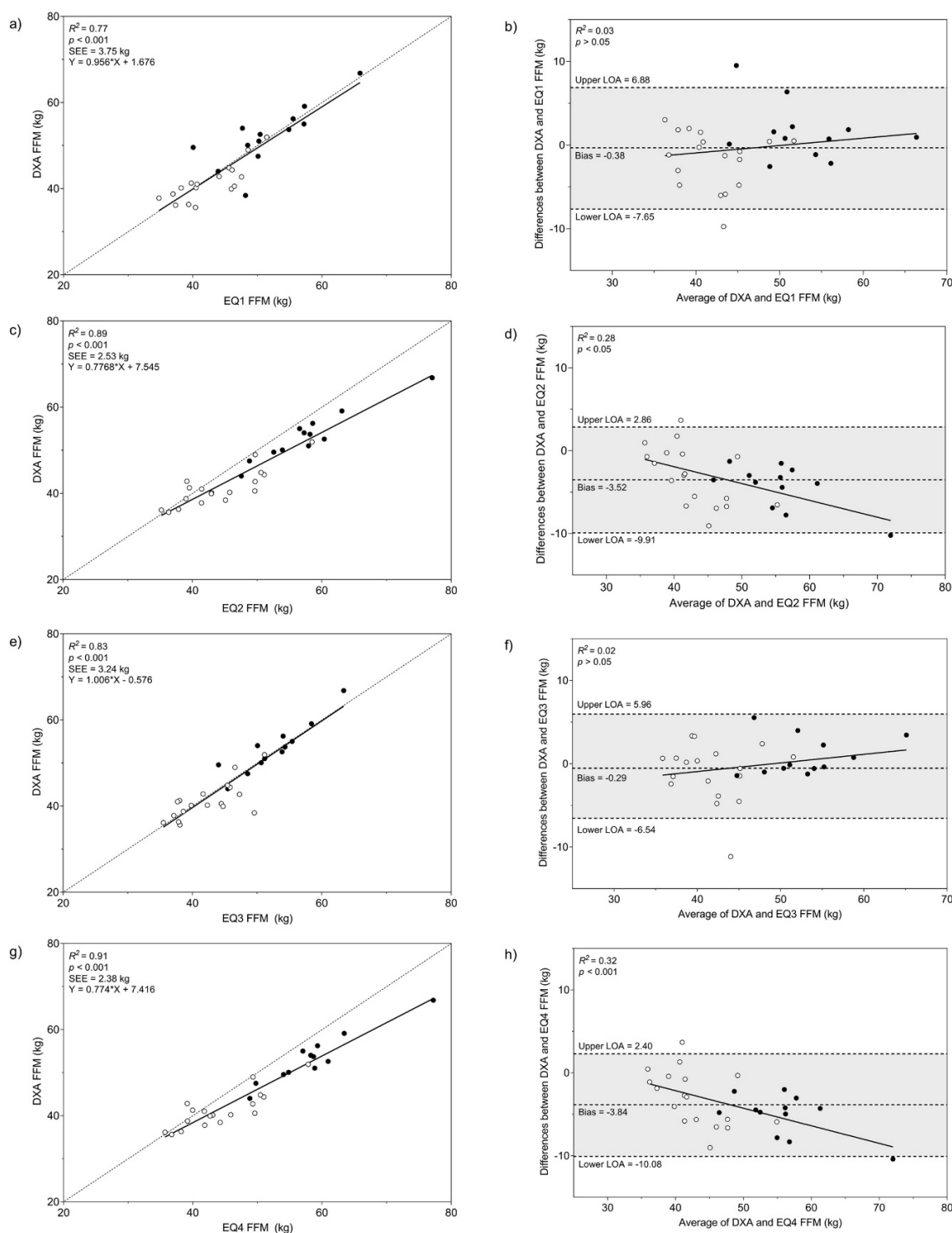


Figure 1. Accuracy analysis between DXA and FFM predictions from four BIA-based equations. Panels (a), (c), (e), and (g) display the linear regressions analysis, showing significant associations for all equations, with non-significant intercepts for EQ1 and EQ3, and significant intercepts for EQ2 and EQ4. Panels (b), (d), (f), and (h) show Bland-Altman plots, where EQ1 and EQ3 exhibited no significant systematic or proportional bias, while EQ2 and EQ4 showed both, indicating that these equations tend to overestimate FFM, particularly in individuals with higher values. White dots represent females; black dots represent males.

Figura 1. Análisis de precisión entre DXA y las predicciones de masa libre de grasa (FFM) a partir de cuatro ecuaciones basadas en BIA. Los paneles (a), (c), (e) y (g) muestran los análisis de regresión lineal, evidenciando asociaciones significativas para todas las ecuaciones, con interceptos no significativos para EQ1 y EQ3, y significativos para EQ2 y EQ4. Los paneles (b), (d), (f) y (h) presentan los gráficos de Bland-Altman, donde EQ1 y EQ3 no mostraron sesgo sistemático ni proporcional significativo, mientras que EQ2 y EQ4 mostraron ambos, lo que indica que estas ecuaciones tienden a sobreestimar la FFM, particularmente en individuos con valores más altos. Los puntos blancos representan a las mujeres; los puntos negros representan a los hombres.

with DXA. A random male participant aged 25 years old with a BW of 63.4 kg and a DXA FFM of 55.0 kg corresponding to 13.20% FM, resulted in the EQ3 FFM predictions of 55.40 kg (Δ -0.37 kg). In this case, FFM differences fall into the equivalence region, and these are considered clinically irrelevant. However, these differences may not be that small in all participants as differences of up to 5.55 kg were observed, in other participants considered clinically relevant as the %FM increased from 25.10% to 33.48%, thereby resulting in his category shifting from overfat to obese category (Gallagher *et al.*, 2000).

In contrast, EQ2 and EQ4 values exhibited significant differences when compared with DXA FFM, thereby rejecting equivalence testing, meaning that group differences may be larger than ± 2.25 kg of FFM. Both equations also exhibited significant systematic bias, with an average overestimation of 3.52 kg (EQ2) and 3.84 kg (EQ4) across the whole sample. This bias was particularly evident in participants with FFM above 50 kg. Additionally, a significant proportional bias was observed in the Bland–Altman analysis for both equations, indicating that the magnitude of overestimation increased with higher FFM values. The regression analyses also revealed statistically significant intercepts, suggesting systematic disagreement across the measurement range. These findings suggest that neither EQ2 nor EQ4 are appropriate for accurately estimating FFM in samples similar to ours. Since gender is a variable included in EQ2 and EQ4, differences in BC in males and females may contribute to prediction error, as numerous equations have included this variable.

It could be expected to find certain differences in DXA measurements and predictions as these have been developed using other reference methods. Systematic bias could be found in BC across different methodologies and populations (Lohman and Chen, 2005). Furthermore, compared with other reference methods, DXA has been reported to overestimate FM (Santos *et al.*, 2010). Despite these factors, we believe that the main reason for the differences in equation predictions and DXA is the overall characteristics of the samples studied. Accuracy of predictive equations is influenced by the characteristics of the subjects evaluated, and it is considered important that these characteristics closely match those involved in the equation development. Our sample showed similarities to the sample included in the development of EQ1, particularly in age, Ht, R, and FFM. However, females in the EQ1 study were smaller than our sample was. Although Ht were not directly employed in the equations, these were indirectly used in the form of Ht^2/R . Disparities between the DXA and EQ1 values may be attributed to differences in BW and %FM as our sample was leaner than the sample from EQ1 study, mainly in the female group.

There is limited information available regarding EQ3 development sample. The subjects ranged in age from 19 to 50 years, and the mean FFM was 60.2 kg, which was notably higher than our sample (Lukaski *et al.*, 1986). Nevertheless, due to the fair agreement observed between the DXA and EQ3 values in the present study, we assumed that some of the characteristics in their sample were comparable to ours.

In a previous study (Lukaski *et al.*, 1990), EQ3 was tested on athletes from various sports and reported an $R^2 = 0.988$ using hydrostatic-weighting as the reference method. Considering these results and the findings of the current study, it is likely that EQ3 may be more suitable for subjects with higher FFM, as both the original sample and the athlete validation sample included individuals who were heavier in terms of FFM than our participants. In other studies, a weak relationship between DXA and EQ3 predictions has been reported, particularly among female athletes. Houtkooper *et al.* (2001) reported an $R^2 = 0.10$ for a %FM when comparing DXA using EQ3 in a group of nineteen heptathletes. The equation overestimated the FM in the Houtkooper *et al.* (2001) sample by 4.4%. It is worth mentioning that the authors did not provide a detailed discussion of these findings. Noteworthy, their sample was considerably leaner than the sample included in the EQ3 development.

Notwithstanding the development of EQ1 and EQ3 from the general population, their wide applicability across various populations, including recreational athletes, is evident due to the broad range of values in the variables included in equation development. This is confirmed by the accurate prediction of FFM of EQ3 in athletes by Lukaski *et al.* (1990). We find EQ1 and EQ3 to be viable options for predicting FFM in a group of recreational runners, despite the wide 95% LOA that may be considered for individual predictions. Moreover, it is important to highlight the consistency between validation metrics as the capacity of the equation to accurately predict BC. Compared with DXA, EQ3 demonstrated to be the most consistent equation with a fair accuracy, given that most of the metrics were adequate in males and the whole sample analysis. However, it is important to note that the Bland–Altman analysis revealed considerably wide LOAs, ranging from -7.92 kg to 5.96 kg depending on the subgroup. Therefore, caution should be exercised when using these equations at the individual level, as various authors emphasize that agreement between methods is primarily determined by LOA (Atkinson and Nevill, 1998; Giavarina, 2015). Given these results, individual agreement between DXA FFM and the predicted values cannot be confirmed, and such discrepancies may be substantial in practice—particularly when BC estimates are used for personalized training or nutritional decision-making.

In the case of EQ2, the sample used in its development was different from ours. These subjects were considerably younger, larger, and leaner. Similarly, the development of EQ4 had a similar sample. Differences in sample characteristics could explain the lack of agreement between DXA and predictions of both equations, in addition to differences in methods employed for the use of a 4C model in EQ2. Moreover, the use of the hydration factor to estimate FFM from EQ4 could increase bias in different age ranges, as the hydration factor may vary slightly on the individual basis (Bossingham *et al.*, 2005; Sagayama *et al.*, 2020).

Athletes may develop body profiles according to their physical demands. Even though, recreational athletes may differ from a higher level athletes, participants in the current study presented similarities with findings from previous

reports on Ht, BW and BMI. However, in other reports, recreational runners were relatively leaner, even when considering the same age range. This was reported by Nikolaidis *et al.* (2020), with a mean %FM of $19.6 \pm 4.7\%$ for women and $17.7 \pm 4.0\%$ for male participants. Another study documented a FM of $16.3 \pm 5.6\%$ in male recreational runners with BMI values similar to our sample (Knechtle and Tanda, 2013). It is important to note, that the assessment method used by these authors was anthropometry which may add differences in the results. It is worth highlighting that elite athletes, particularly in endurance sports, are generally expected to be leaner than recreational athletes. Specifically in runners, the %FM has been observed to fall within the range of 14.9% to 21.0% in females (Piasecki *et al.*, 2018; Carbuhn *et al.*, 2022) and 7.29% to 11.4% in males (Mooses *et al.*, 2013; Carbuhn *et al.*, 2022). Notably, a particularly low FM of 10.31% was reported by Mooses *et al.* (2013) in the case of recreational athletes although these authors did not provide explicit criteria for athlete classification. Nonetheless, while we acknowledge that there may be differences in FM in the current study and prior reports, a portion of these variations may be attributed to the different methods employed and the populations studied.

Limitations of the study and further recommendations

The main limitation of the current study is the relatively small sample size, which may limit the generalizability of the findings and the precision of some estimates, particularly in subgroup analyses. Further studies are encouraged with a larger sample size to strengthen the results by including more subjects with a wider variety of body characteristics. Our sample differed from recreational athlete characteristics according to other studies. However, categorizing athletes could be difficult due to the lack of standardizing criteria. Nevertheless, these results show the importance of identifying pertinent predictive equations to track and evaluate accurately BC, and the potential of equations to be used in recreational athletes who may not suit the standard athletic body.

CONCLUSIONS

Equations one and three demonstrated a greater level of accuracy in predicting BC within the current sample, and Equation three was more consistent between groups of data. These equations exhibit the effectiveness to predict FFM with a notable degree of accuracy when applied to a group of athletes with similar characteristics as the current sample. However, agreement on an individual basis was not accomplished. Researchers are encouraged to evaluate the accuracy of the different equations available to provide information for their universal utility.

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CONFLICTS OF INTEREST

The author(s) have no conflicts of interest relevant to this article.

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