Estimating fat-free mass in recreationally resistance-trained young men: Longitudinal andcross-sectional validation of different methods

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A B S T R A C T

Several techniques exist to measure fat-free mass (FFM). Accordingly, this study is based on data from our recent trial comparing the sensitivity of the main field methods avail- able with that of dual-energy X-ray absorptiometry (DXA) as reference and analyzing the cross-sectional accuracy of these field methods in recreationally resistance-trained males. We hypothesized that the use of these techniques would lead to varying estimates of FFM compared with DXA. Participants ($N = 23$; 21.4 ± 3.3 years) completed a 10-week resistance training plus diet intervention designed to optimize hypertrophy. FFM was determined by bioelectrical impedance analysis (BIA), 23 anthropometric equations, and DXA. After the intervention, FFM increased significantly according to BIA and most anthropometric esti- mates, but this increase was not detected by 2 anthropometric equations or by DXA. Only 1of these 2 equations showed significant correlation with DXA and no standardized or signif-icant differences to this reference method, although it did display significant heteroscedas ticity. In our cross-sectional analysis, only 1 anthropometric equation gave rise to good ac-curacy as confirmed by DXA. Our findings indicate that the use of different techniques to assess FFM gains in response to a hypertrophic intervention yields different results. BIA with general embedded equations should not be used to monitor a young male adult's body composition. To monitor FFM over time, we would recommend the Dunne et al. equation
(2) as the most sensitive field method, and to assess FFM cross-sectionally, equation (1) ofthese authors is the most accurate field method.

Keywords:
Lean mass, Strength training, Bioelectrical impedance,Anthropometry, Dual X-ray absorptiometry

Abbreviations: %BF, percent body fat; BIA, bioelectrical impedance analysis; BM, body mass; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; ISAK, International Society for Advancement of Kinanthropometry; SKF, skinfold.

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1. Introduction

Changes in body composition are one of the main objectives of sports nutrition because increased fat-free mass (FFM) and reduced fat mass positively influence performance in a variety of sporting disciplines [1–3]. To determine the variations in FFM produced in response to an intervention, nutritional or training, there are many techniques that offer different sensitivity and accuracy [1] such as bioelectrical impedance analysis (BIA), anthropometry, or dual-energy X-ray absorptiometry (DXA) [4]. Methods designed to estimate FFM in sports nutrition should be accurate (i.e., there should be good agreement between the observed value and the true value), reliable, and reproducible [5]. Currently, DXA is the accepted reference method to assess body composition [4,6]. Although quick, ac-curate, and noninvasive, delivering a low-radiation dose, DXAhas shortcomings such as a high cost and that its use requires a high level of experience and certification [7,8]. Thus, other techniques such as BIA or anthropometric equations have become popular [9]. BIA is a noninvasive, simple-to-use technique that requires a short measurement time and is econom ically acceptable. However, numerous precautions must be taken such as avoiding physical exercise or maintaining a cer tain level of hydration before assessment, which is, of course,a considerable limitation for its use in athletes [10,11]. Finally, anthropometry is a cost-effective, acceptable, and practical method to assess body composition and FFM changes when conducted by a trained anthropometrist following appropriate guidelines [12]. Accordingly, many equations have been developed to estimate body composition (including FFM and fatmass) in both nonathletic and athletic populations. However,these equations differ substantially from each other and offer different and uncertain levels of accuracy [4,9,13,14].

In a recent systematic review, quantitative changes in FFMproduced in response to a resistance training intervention combined with vitamin C and E supplementation were examined [15]. However, the authors emphasized that because of the many instruments of different or uncertain accuracy used to assess FFM, a meta-analysis could not be carried out. Other studies have shown that BIA and most anthropometric equations underestimate fat mass in male athletes [4,13]. Therefore, we hypothesized that the use of BIA and anthropometric equations to assess body composition could result in overestimation of FFM gains compared with a reference method. It is also true that most studies that have addressed the accuracy of field methods for estimating body composition have focused on athletic populations such as soccer players [4,9,13,14,16] and, to our knowledge, no such research hasinvolved resistance-trained subjects.

Given this great heterogeneity of FFM analysis methods [4,15], the aim of this study conducted in participants of our recent randomized controlled trial [17] was to assess the sensitivity of various anthropometric equations and BIA using DXA as the reference standard. These procedures were used to the measure the FFM changes produced after a 10-week resi tance training program and dietary intervention targeting hypertrophy accompanied by the daily intake of vitamin C and Esupplements, as described in our previous report [17]. Besides longitudinal sensitivity, we also examined the cross-sectional(i.e., before and after the intervention) accuracy of FFM measured by BIA and anthropometric equations in relation to thepractical criterion (DXA).

2. Materials

This study was conducted as part of a previous double-blind randomized controlled trial whose methodology is detailed elsewhere [17].

2.1. Participants and intervention

Twenty-three recreationally resistance-trained men aged 18 to 32 years (21.4 ± 3.3 years) completed a 10-week resistance training protocol accompanied by a dietary intervention designed to optimize hypertrophy. Participants were randomly assigned to a group supplemented with vitamin C and E ($n = 12$) or a placebo group ($n = 11$). FFM in each participant was assessed before and after the 10-week intervention (April- June 2021). The training and supplementation protocols, dietary intervention, eligibility criteria, and ethics information are described elsewhere [17].

2.2. Fat-free Mass Measurement

FFM (kg) was determined through standardized procedures of the methods DXA, BIA, and anthropometry [4]. Measurementswere made after a 3-hour fast including liquids in resting conditions. Subjects were asked to remove all metal objects, wearminimal clothing, and to urinate before measurements were taken in a well-ventilated examination room under conditionsof controlled temperature and humidity.

2.2.1. Anthropometry

Body mass (BM) and height were measured barefoot and in underwear to the nearest 0.1 kg and 0.1 cm using an analog scale (Asimed T2, Barcelona, Spain) and a stadiometer (An Sayol SL, Barcelona, Spain). Body mass index (BMI) was then calculated as weight (kg)/height (m^2). Six circumferences (arm relaxed, arm flexed, waist, hip, calf, and thigh), 8 skinfold (SKF) thicknesses (triceps, biceps, subscapular, iliac crest, supraspinal, abdominal, anterior thigh, and medial calf) and 2 bone biepicondylar widths (humerus and femur) were measured witha tape (Realmet), SKF caliper (Harpender), and caliper

(Real-met), respectively. In each subject, duplicate anthropometric measurements were obtained and the mean of the 2 measures entered in the analysis. When the technical error of measurement was more than 5% for SKF measurements and 1% for all other measurements, a third measurement was taken so that the median could be recorded. All anthropometric measurements were made by an accredited International Societyfor Advancement of Kinanthropometry (ISAK) level 1 anthropometrist following standard methods [18].

Twenty-three anthropometric equations were used to es- timate percentage body fat (%BF), and fat mass was subsequently subtracted from body weight to obtain FFM (kg). Four- teen of these equations were designed for populations of athletes [13,14,19-28]. In addition, FFM was also assessed using 9 anthropometric equations developed for healthy, normal- weight nonathletic subjects [29–36]. Both types of equationswere used as our participants were recreationally resistance-trained subjects. Because some of these equations only estimate body density, %BF was calculated from density using the Siri formula [37]. The different anthropometric equations used are provided in Table 1.

Table 1 – Anthropometric equations developed to estimate body fat percentage in athletes and the general population.

Author	Anthropometric equation
Athletes	
Carter [28]	%BF (males) = 0.1051 * (T + Sb + Sp + Ab + Th + Ca) + 2.58
Civar et al. [19]	%BF = (0.432 * T) + (0.193 * Ab) + (0.364 *B) + (0.077 * BM) - 0.891
Dunne et al. [20]	(1) %BF = 5.561 + (0.035 * Age) + (0.394 * T) + (-0.121 * Sb) + (0.184 * B) + (0.413 * IC) + (0.257 * Sp) + (0.285 * Ab) + (0.168 * Th) + (-0.265 * Ca) (2) %BF = 8.012 + (0.26 * Age) + (-0.497 * T) + (-0.103 * Sb) + (0.083 * B) + (0.376 * IC) + (-1.073 * Sp) + (0.647 * Ab) + (0.318 * Th) + (0.967 * Ca)
Evans et al. [21]	%BF = 8.997 + (0.24658 * [Ab + Th + T]) − (6.343 * Sex) − (1.998 * Race)
Faulkner [22]	%BF = 0.153 * (T + Sb + Sp + Ab) + 5.783
Forsyth and Sinning [23]	(1) BD = 1.103 − (0.00168 ∗ Sb) − (0.00127 ∗ Ab) (2) BD = 1.02415 - (0.00169 * Sb) + 0.00444* (Height/10) – 0.0013 * Ab
Munguia-Izquierdo et al. [14]	%BF = 3.867 + (0.516 * Sp) + (0.461 * T)
Reilly et al. [25]	%BF = 5.174 + (0.124 ∗ Th) + (0.147 ∗ Ab) + (0.196 ∗ T) + (0.13 ∗ Ca)
Suarez-Arrones et al. [13]	%BF = 8.047 + (0.616 * IC) + (0.189 * T)
White et al. [24]	BD = 1.0958 − (0.00088 ∗ IC) − (0.0006 ∗ Th)
Withers et al. [26]*	BD = 1.0988 − (0.0004 ∗ [T + Sb + B + Sp + Ab + Th + Ca])
Zemski et al. [27]	BF% = 5.896 + (0.265 * T) + (0.251 * Sp) + (0.394 * Ca)
General population	
Durnin and Rahaman [29]	BD = 1.161 − (0.0632 ∗ log [B + T + Sb + IC])
Durnin and Womersley [30]	18–19 years: BD = 1.162 − (0.063 ∗ log [B + T + Sb + IC]) 20–29 years: BD = 1.1631 − (0.0632 * log [B + T + Sb + IC]) 30–39 years: BD = 1.1422 − (0.0544 * log [B +T + Sb + IC])
Eston et al. [35]	%BF = (0.12 ∗ [B + T + Sb + IC]) + (0.36 ∗ [Ca + Th]) + 1.61
Lean et al. [34]	(1) %BF= 0.353 * WC + 0.756 * T + 0.235 * Age – 26.4 (2) %BF = 0.567 * WC + 0.101 * Age – 31.8
Lohman [33]	BD = 1.0982 − (0.000815 ∗ [T + Sb + Ab]) + (0.0000084 ∗ [T + Sb + Ab]²)
Peterson et al. [36]	%BF (males) = 20.94878 + (0.1166 * age) – (0.11666 * Height) + (0.42696 * [T + IC + Sb + Th] *) – (0.00159 * [T + IC + Sb + Th]²)
Sloan [32]	BD = 1.1043 – (0.001327 * Th) – (0.00131 * Sb)
Wilmore and Behnke [31]	BD = 1.08543 − (0.000886 * Ab) − (0.0004 * Th)

Abbreviations: %BF, body fat percentage; BD, body density; BM, body mass; DXA, dual-energy X-ray absorptiometry; WC, waist circumference. Skinfolds (millimeters): Ab, abdominal; B, biceps; Ca, calf; IC, iliac crest; Sb, subscapular; Sp, supraspinale; T, triceps; Th, thigh. Sex: male = 1, female = 0. Race: Black = 1, White = 0.
Age: years. Height: centimeters. Waist circumference: centimeters.
* Equation not fully published in the original but can be found in [25].

2.2.2. *Bioelectric impedance analysis*

BIA was performed using an InBody 770 device (Biospace, Seoul, South Korea). Before measurements, participants' palms and soles were wiped with an electrolyte tissue and participants were then asked to stand on the platform, making sure their soles made contact with the foot electrodes. Be-fore measurements, data including sex, age, and height were entered into the device and participants were asked to place their thumb and fingers at the set location and grasp firmly. During FFM measurement, the subject remains motionless toavoid errors in the results while maintaining the elbows fullyextended and shoulder joint abducted to a 30-degree angle.

2.2.3. Dual energy x-ray absorptiometry

Whole-body FFM (kg) was measured using a DXA instrument (Hologic QDR Discovery Wi 2013, Bedford, MA, USA) [38] equipped with Hologic APEX version 4.0.2. software. The instrument was calibrated using a lumbar spine phantom as recommended by the manufacturer. The test was the "whole- body" test in which subjects were asked to maintain a supine position with slight abduction and external rotation of the hip on a stretcher for 8 minutes. A single trained DXA technician positioned the participants and performed the scans with the National Health and Nutrition Examination Study body com- position correction function disabled [39].

2.3. Statistical Analyses

A sample size estimation was performed taking into account the correlation coefficient described by Núñez et al. [9] in a similar study comparing DXA reference values of FFM with those determined by different anthropometric equations and BIA. An alpha risk of 0.05 and beta risk of 0.2 were accepted in a bilateral contrast, assuming a minimum intersample correlation coefficient of 0.7 because this was the lowest value obtained in the study by Núñez et al. [9] when analyzing correlations among FFM values obtained through DXA, BIA, and anthropometric equations. Based on these data, the sample estimated was 18 subjects, considering a dropout rate of 25%.

Descriptive statistics are provided for each variable, and relative and absolute technical errors of measurements were calculated [40]. The normality of the distribution of data was verified by the Shapiro-Wilk test. To compare sensitivities to a change in FFM shown by DXA, BIA, and anthropometric equations and the cross-sectional accuracy of FFM values determined before and after the intervention, paired t tests, Pearson correlation (±90% confidence interval), bias, limits of agreement, and standardized differences (±90% confidence interval) of the differences within subjects were calculated. Correlation coefficients were qualitatively ranked by magnitude as trivial, $r < 0.1$; small, $0.1 < r < 0.3$; moderate, $0.3 < r < 0.5$; large, $0.5 < r < 0.7$; very large, $0.7 < r < 0.9$; almost perfect, $0.9 < r < 1.0$; and perfect $r = 1.0$ [41]. The effect size of standardized differences in FFM was determined by Cohen d statistic, and Hopkins' scale was used to determine the magnitude of the effect size where 0 to 0.2 = trivial, 0.2 to 0.6 = small, 0.6 to 1.2 = moderate, 1.2 to 2.0 = large, and > 2.0 = very large [41]. Agreement between methods was assessed using Bland- Altman plots, including 95% levels of agreement [42]. The as- sociation between the difference and the magnitude of the change measurement (i.e., heteroscedasticity) was examined by regression analysis.

All statistical tests were carried out using Statistical Pack- age for the Social Sciences version 23 (IBM, Chicago, IL, USA) and Microsoft Excel software (Microsoft, Redmond, WA, USA). Significance was set at $P < .05$.

3. Results

The study sample comprised 23 participants, aged 21.4 ± 3.3 years, with a mean BM of 76.3 ± 10.9 kg and body height of 177.1 ± 4.0 cm (BMI, 24.3 ± 3.1 kg/m2). Technical errors of anthropometric measurements were all lower than the standards indicated by ISAK guidelines [18].

3.1. Longitudinal analysis

BM measured with an analog scale and BMI increased significantly from pre- (BM, 73.7 ± 1.4 kg; BMI, 23.7 ± 0.5 kg/m2) to postintervention (BM, 74.8 ± 1.4 kg; BMI, 24.0 ± 0.5 kg/m2) (both P = .009).

The magnitude of the FFM changes produced from baseline to after the hypertrophic intervention as determined by DXA, BIA and the anthropometric equations are shown in Table 2. A significant increase in FFM was observed according to BIA and most anthropometric estimates ($P < .05$) but not with DXA or the equations by Lohman [33] and Dunne et al. [20] (Eq. 2).

Table 3 shows the correlations, bias, limits of agreement, significant and standardized differences between DXA-determined FFM changes and the other practical estimates of FFM changes produced after the training period. Of the 2 equations [21,34] that did not detect the increase in FFM observed using most field procedures, equation (2) of Dunne et al [20] showed a large significant correlation with DXA (r = 0.51), whereas the Lohman equation [33] did not. Further, Dunne equation (2) did not give rise to standardized differences versus DXA and also showed the least bias.

Table 2 – Pre-post training changes produced in FFM determined by DXA and other practical methods in recreationallyresistance-trained young men (n = 23).

Method/equation used	Pretraining FFM(mean ± SD)	Posttraining FFM(mean ± SD)	Mean difference (SD)	% difference (mean ± SD)	*P* value	Standardized difference (90% CL)
DXA	56.68 ± 5.05	57.21 ± 5.04	0.53 ± 1.26	0.96 ± 2.25	.058	0.10 (0.09)
BIA	62.34 ± 5.59	63.63 ± 6.03	1.29 ± 1.76	2.06 ± 2.86	.002	0.22 (0.11)
Durnin and					.012	0.13 (0.08)

Womersley [30]	61.71 ± 5.02	62.38 ± 5.15	0.66 ± 1.16	1.08 ± 1.88		
Faulkner [22]					.002	0.16 (0.08)
Carter [28]	63.93 ± 5.24	64.82 ± 5.42	0.89 ± 1.21	1.40 ± 1.88	.002	0.17 (0.08)
Civar et al. [19]	66.00 ± 5.46	66.95 ± 5.68	0.95 ± 1.29	1.45 ± 1.92	.008	0.15 (0.09)
Evans et al. [21]	62.87 ± 4.78	63.60 ± 5.21	0.73 ± 1.19	1.14 ± 1.84	.001	0.18 (0.08)
Munguia-Izquierdo et al. [14]	63.06 ± 5.31	64.06 ± 5.67	1.01 ± 1.25	1.58 ± 1.87	<.001	0.18 (0.08)
Reilly et al. [25]	63.81 ± 5.11	64.74 ± 5.45	0.93 ± 1.17	1.45 ± 1.76		
Suarez-Arrones et al. [13]					.002	0.17 (0.08)
Peterson et al. [36]	63.85 ± 5.33	64.80 ± 5.63	0.95 ± 1.28	1.49 ± 1.94	.015	0.16 (0.10)
Eston et al. [35]	59.42 ± 4.77	60.21 ± 4.90	0.79 ± 1.43	1.34 ± 2.36		
Forsyth and Sinning [23] (1)					.001	0.16 (0.07)
Forsyth and Sinning [23] (2)	58.25 ± 4.89	59.09 ± 5.04	0.83 ± 1.02	1.44 ± 1.70	.004	0.16 (0.08)
White et al. [24]	62.08 ± 5.34	62.96 ± 5.63	0.88 ± 1.29	1.41 ± 1.97	.017	0.14 (0.09)
Withers et al. [26]	61.77 ± 5.10	62.48 ± 5.12	0.72 ± 1.32	1.19 ± 2.12		
Durnin and Rahaman [29]					.019	0.13 (0.09)
Lohman [33]	61.40 ± 5.25	62.11 ± 5.24	0.71± 1.34	1.19 ± 2.16	.002	0.18 (0.09)
Wilmore and Behnke [31]					.003	0.16 (0.08)
Lean et al. [34] (1)	64.94 ± 5.37	65.92 ± 5.55	0.98 ± 1.36	1.52 ± 2.03	.010	0.13 (0.08)
Lean et al. [34] (2)	63.50 ± 5.18	64.34 ± 5.48	0.84 ±1.22	1.32 ± 1.83		
Sloan [32]	61.23 ± 4.84	61.90 ± 4.97	0.67 ± 1.14	1.11 ± 1.87	.074	0.14 (0.12)
Zemski et al. [27]					.003	0.18 (0.09)
Dunne et al. [20] (1)	58.45 ± 5.27	59.19 ± 5.40	0.74 ± 1.90	1.32 ± 3.30		
Dunne et al. [20] (2)	61.60 ± 5.18	62.54 ± 5.47	0.94 ± 1.34	1.53 ± 2.08	.003	0.17 (0.09)
					.003	0.17 (0.09)
	62.79 ± 4.45	63.59 ± 4.85	0.80 ± 1.13	1.25 ± 1.74	.001	0.16 (0.08)
	62.27 ± 3.93	62.95 ± 4.08	0.68 ± 0.99	1.09 ± 1.58	.004	0.16 (0.09)
	63.56 ± 5.62	64.52 ± 5.77	0.96 ± 1.22	1.52 ± 1.91	.019	0.16 (0.11)
	63.28 ± 5.19	64.13 ± 5.49	0.85 ± 1.28	1.34 ± 1.96	.202	0.11 (0.14)
	55.32 ± 4.78	56.13 ± 5.10	0.80 ± 1.52	1.45 ± 2.68		
	52.07 ± 5.31	52.66 ± 5.74	0.59 ± 2.15	1.16 ± 3.96		

Abbreviations: BIA, bioelectric impedance analysis; CL, confidence level; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass.
Differences between pre- and posttraining were determined by paired *t* test (1 tailed).

In Fig. 1, the Bland-Atman plot provided differences be- tween DXA-determined FFM changes and those detected by the equation yielding the most similar magnitude of change, positive significant correlation, and no standardized differences versus DXA. This plot reveals a significant relationship between the difference and magnitude of FFM changes estimated with Dunne equation (2) (beta = 0.545; $P < .05$).

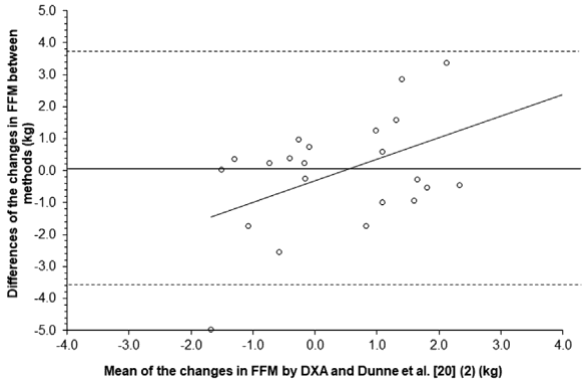

Fig. 1 – Bland-Altman plot of the difference between DXA-determined FFM changes and FFM changes estimated with anthropometric equation (2) of Dunne et al. [20]. Each point represents individual differences between methods. The upper and lower lines represent the 95% limits of agreement, and the central line represents the standard error. Abbreviations: DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass.

3.1. Cross-sectional analysis

Preintervention and postintervention correlations, biases, limits of agreement, and standardized and significant differences between FFM estimated by DXA and the field methods are shown in Tables 4 and 5, respectively.

In our cross-sectional analysis of FFM accuracy before the intervention, all the equations and BIA gave rise to very large to almost perfect correlation (r = 0.77–0.98) with DXA mesurements. Although all the practical estimates of FFM revealed significant and standardized differences versus DXA, equation (1) of Dunne et al. [20] showed the smallest standardized differences and least bias.

Similarly, in the postintervention analysis, correlations be- tween FFM measures determined by the field methods

versus DXA ranged from large to almost perfect (r = 0.84–0.98). All the anthropometric equations and BIA showed significant and standardized differences versus DXA, except for Dunne equation (1), which did not give rise to significant differences and also showed the lowest bias.

Table 3 – Correlation, bias, limits of agreement, standardized and significant differences between FFM changes produced after the intervention detected by DXA and other practical methods in recreationally resistance-trained young men (n = 23).

Method/equation used	Correlation (90% CI)	*P* value correlation	Bias (± LoA)	Standardized difference (90% CL)
BIA	0.57 (0.27–0.77)	.005	0.76 (± 2.89)	0.58 (0.40)[a]
Durnin and Womersley [30]	0.84 (0.70–0.92)	<.001	0.14 (± 1.35)	0.10 (0.19)
Faulkner [22]	0.86 (0.73–0.93)	<.001	0.37 (± 1.29)	0.28 (0.18)[a]
Carter [28]	0.86 (0.73–0.93)	<.001	0.42 (± 1.32)	0.32 (0.18)[a]
Civar et al. [19]	0.76 (0.55–0.87)	<.001	0.20 (± 1.69)	0.16 (0.24)
Evans et al. [21]	0.70 (0.46–0.84)	<.001	0.48 (± 1.92)	0.37 (0.27)[a]
Munguia-Izquierdo et al. [14]	0.67 (0.41–0.83)	<.001	0.41 (± 1.95)	0.31 (0.27)
Reilly et al. [25]	0.81 (0.65–0.91)	<.001	0.43 (± 1.52)	0.33 (0.21)[a]
Suarez-Arrones et al. [13]	0.84 (0.69–0.92)	.002	0.39 (± 1.38)	0.30 (0.19)[a]
Peterson et al. [36]	0.71 (0.48–0.85)	<.001	0.31 (± 1.75)	0.23 (0.24)
Eston et al. [35]	0.69 (0.44–0.84)	<.001	0.35 (± 1.97)	0.27 (0.28)
Forsyth and Sinning [23] (1)	0.52 (0.20–0.74)	.011	0.19 (± 2.49)	0.14 (0.35)
Forsyth and Sinning [23] (2)	0.49 (0.17–0.72)	.017	0.18 (± 2.57)	0.14 (0.36)
White et al. [24]	0.80 (0.63–0.90)	<.001	0.45 (± 1.62)	0.35 (0.23)[a]
Withers et al. [26]	0.72 (0.49–0.85)	<.001	0.32 (± 1.83)	0.24 (0.26)
Durnin and Rahaman [29]	0.83 (0.68–0.92)	<.001	0.15 (± 1.38)	0.11 (0.19)
Lohman [33]	0.04 (–0.32 to 0.38)	.867	0.22 (± 4.40)	0.17 (0.61)
Wilmore and Behnke [31]	0.73 (0.51–0.86)	<.001	0.41 (± 1.88)	0.32 (0.26)[a]
Lean et al. [34] (1)	0.42 (0.08–0.67)	.047	0.27 (± 2.54)	0.21 (0.35)
Lean et al. [34] (2)	0.84 (0.64–0.92)	<.001	0.15 (± 1.35)	0.12 (0.19)
Sloan [32]	0.67 (0.41–0.82)	<.001	0.43 (± 1.99)	0.33 (0.28)
Zemski et al. [27]	0.75 (0.54–0.87)	<.001	0.33 (± 1.77)	0.25 (0.25)
Dunne et al. [20] (1)	0.31 (–0.05 to 0.59)	.157	0.28 (± 3.25)	0.21 (0.45)
Dunne et al. [20] (2)	0.51 (0.19–0.73)	.013	0.06 (± 3.65)	0.05 (0.51)

Abbreviations: BIA, bioelectric impedance analysis; CI, confidence interval; CL, confidence level; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; LoA, limits of agreement.

[a]Significant differences between reference (DXA) and other practical methods of estimating FFM changes using paired t test*

Table 4 – Cross-sectional analysis of the accuracy of pre-intervention FFM values determined by BIA or anthropometric equations, using DXA as reference, in recreationally resistance-trained young men (n = 23).

Method/equation used	Correlation (90% CI)	Bias (± LoA)	Standardized difference (90% CL)
BIA	0.97 (0.94–0.99)	5.66 (± 2.93)	1.08 (0.10)[a]
Durnin and Womersley [30]	0.96 (0.92–0.98)	5.03 (± 2.81)	0.96 (0.10)[a]
Faulkner [22]	0.97 (0.93–0.98)	7.24 (± 2.64)	1.38 (0.09)[a]
Carter [28]	0.97 (0.94–0.99)	9.32 (± 2.52)	1.78 (0.09)[a]
Civar et al. [19]	0.97 (0.94–0.99)	6.18 (± 2.43)	1.18 (0.08)[a]
Evans et al. [21]	0.96 (0.92–0.98)	6.43 (± 3.17)	1.22 (0.10)[a]
Munguia-Izquierdo et al. [14]	0.98 (0.95–0.99)	7.12 (± 2.22)	1.36 (0.08)[a]
Reilly et al. [25]	0.98 (0.95–0.99)	7.14 (± 2.58)	1.37 (0.08)[a]
Suarez-Arrones et al. [13]	0.94 (0.89–0.97)	2.74 (± 3.25)	0.52 (0.11)[a]
Peterson et al. [36]	0.95 (0.90–0.98)	1.42 (± 2.86)	0.30 (0.11)[a]
Eston et al. [35]	0.94 (0.87–0.97)	5.40 (± 3.65)	1.03 (0.13)[a]
Forsyth and Sinning [23] (1)	0.88 (0.77–0.94)	5.08 (± 4.83)	0.97 (0.17)[a]
Forsyth and Sinning [23] (2)	0.86 (0.73–0.93)	4.71 (± 5.31)	0.90 (0.19)[a]
White et al. [24]	0.96 (0.92–0.98)	8.26 (± 2.87)	1.58 (0.10)[a]
Withers et al. [26]	0.96 (0.92–0.98)	6.81 (± 2.85)	1.30 (0.10)[a]
Durnin and Rahaman [29]	0.96 (0.92–0.98)	4.54 (± 3.07)	0.87 (0.09)[a]
Lohman [33]	0.77 (0.57–0.88)	1.77 (± 6.93)	0.34 (0.24)[b]
Wilmore and Behnke [31]	0.95 (0.90–0.98)	4.92 (± 3.09)	0.94 (0.11)[a]
Lean et al. [34] (1)	0.95 (0.89–0.97)	6.11 (± 3.29)	1.17 (0.11)[a]
Lean et al. [34] (2)	0.92 (0.84–0.96)	5.58 (± 4.11)	1.07 (0.14)[a]
Sloan [32]	0.90 (0.80–0.95)	6.87 (± 4.84)	1.31 (0.17)[a]
Zemski et al. [27]	0.98 (0.96–0.99)	6.60 (±2.04)	1.26 (0.07)[a]
Dunne et al. [20] (1)	0.85 (0.71–0.93)		–0.26 (0.18)[b]

Dunne et al. [20] (2)	0.84 (0.70–0.92)	–1.36 (± 5.28) –4.62 (± 5.70)	–0.88 (0.20)[a]

Abbreviations: BIA, bioelectric impedance analysis; CI, confidence interval; CL, confidence level; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; LoA, limits of agreement.
Significant differences between reference (DXA) and other practical methods of estimating FFM using the paired t test: [b]$P < .05$, [a]$P < .001$.

Table 5 – Cross-sectional analysis of the accuracy of post-intervention FFM values determined by BIA or anthropometric equations, using DXA as reference, in recreationally resistance-trained young men ($n = 23$).

Method/equation used	Correlation (90% CI)	Bias (± LoA)	Standardized difference (90% CL)
BIA	0.95 (0.91–0.98)	6.42 (± 3.82)	1.23 (0.13)[a]
Durnin and Womersley [30]	0.95 (0.91–0.98)	5.16 (± 3.05)	0.99 (0.11)[a]
Faulkner [22]	0.97 (0.93–0.98)	7.61 (± 2.79)	1.46 (0.10)[a]
Carter [28]	0.98 (0.95–0.99)	9.74 (± 2.64)	1.86 (0.09)[a]
Civar et al. [19]	0.97 (0.94–0.99)	6.39 (± 2.48)	1.22 (0.09)[a]
Evans et al. [21]	0.97 (0.94–0.99)	6.83 (± 3.41)	1.31 (0.10)[a]
Munguia-Izquierdo et al. [14]	0.97 (0.93–0.98)	7.53 (± 2.71)	1.44 (0.09)[a]
Reilly et al. [25]	0.98 (0.96–0.99)	7.50 (± 2.74)	1.45 (0.09)[a]
Suarez-Arrones et al. [13]	0.92 (0.84–0.96)	3.00 (± 3.90)	0.57 (0.14)[a]
Peterson et al. [36]	0.95 (0.89–0.97)	1.60 (± 3.36)	0.36 (0.11)[a]
Eston et al. [35]	0.95 (0.90–0.98)	5.75 (± 3.54)	1.10 (0.12)[a]
Forsyth and Sinning [23] (1)	0.90 (0.79–0.95)	5.27 (± 4.56)	1.01 (0.16)[a]
Forsyth and Sinning [23] (2)	0.87 (0.75–0.94)	4.89 (± 5.07)	0.94 (0.18)[a]
White et al. [24]	0.96 (0.91–0.98)	8.71 (± 3.17)	1.67 (0.11)[a]
Withers et al. [26]	0.97 (0.94–0.99)	7.13 (± 2.68)	1.36 (0.09)[a]
Durnin and Rahaman [29]	0.95 (0.90–0.98)	4.59 (± 3.55)	0.90 (0.11)[a]
Lohman [33]	0.82 (0.66–0.91)	1.98 (± 6.21)	0.38 (0.22)[b]
Wilmore and Behnke [31]	0.96 (0.93–0.98)	5.33 (± 2.87)	1.02 (0.10)[a]
Lean et al. [34] (1)	0.94 (0.88–0.97)	6.38 (± 3.35)	1.22 (0.12)[a]
Lean et al. [34] (2)	0.92 (0.84–0.96)	5.74 (± 3.99)	1.10 (0.14)[a]
Sloan [32]	0.92 (0.83–0.96)	7.30 (± 4.56)	1.40 (0.16)[a]
Zemski et al. [27]	0.97 (0.95–0.99)	6.92 (± 2.52)	1.32 (0.09)[a]
Dunne et al. [20] (1)	0.87 (0.75–0.94)	–1.09 (± 5.07)	–0.21 (0.18)
Dunne et al. [20] (2)	0.84 (0.69–0.92)	–4.55 (± 6.12)	–0.87 (0.21)[a]

Abbreviations: BIA, bioelectric impedance analysis; CI, confidence interval; CL, confidence level; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; LoA, limits of agreement.
Significant differences between reference (DXA) and other practical methods of estimating FFM using the paired t test [b]$P < .05$, [a]$P < .001$.

4. Discussion

This study based on data from our previous double-blind, randomized controlled trial [17] sought to compare, using DXA as reference, the sensitivity of BIA and anthropometry when used as field methods to measure FFM before and after a 10-week hypertrophy intervention in recreationally resistance- trained men. In a further analysis, the cross-sectional accuracy prior and postintervention of these field methods com- pared with DXA was also determined. Our main findings were that BIA and all the anthropometric equations except two [20,33] detected a significant increase in FFM in response to the training/nutrition intervention, whereas DXA analysis detected no such change. Therefore, our hypothesis that the use of different body composition assessment techniques could overestimate FFM gains was confirmed. Moreover, only the anthropometric equation (2) of Dunne et al. [20] was able to estimate the changes in FFM revealed by DXA. Finally, in a cross- sectional analysis, we noted that the equation (1) of Dunne et al. [20] was the most accurate to measure FFM.

In our longitudinal analysis, Dunne's anthropometric equation (2) [20] emerged as highly sensitive to estimate changes in FFM compared with DXA. When the magnitude of the FFM change increases, the difference between values derived from these equations versus DXA also increases. Dunneequation (2) was developed through regression analysis of data from professional jockeys using DXA (Lunar Prodigy)- derived measures as the dependent variable. Anthropometric data were obtained using Harpender calipers and following ISAK guidelines, and the equations developed included8 SKF measurements and age. Although the study population differed from our sample, similarities between the stud- ies are the use of DXA as reference and the same methodology and instruments used for the anthropometric measurements. Because this equation of Dunne et al. (2) is recent (2020), no study has assessed its accuracy, and more investigations are needed for its validation. Moreover, to our knowledge, no study has examined which field methods are more sensitive to quantify FFM gains in resistance-trained subjects.The only similar investigation found was the one by Núñezet al. [19], who examined which field method was the most effective to quantify FFM changes in elite young soccer players.These authors identified the equations of Durnin and Womer- sley [30], Carter [28], Slaughter et al. [43], Reilly et al. [25], and Munguia-Izquierdo et al. [14]

as the most sensitive. Because our study participants differed, the 2 studies are difficult to compare. Pending further investigation, the Dunne equation (2) could be used with caution to monitor body composition changes in recreationally resistance-trained young men in response to a hypertrophy intervention. It should, nevertheless,be considered that if the magnitude of FFM change is large, this will lead to greater differences with respect to the real change produced.

We observed that, compared with 23 anthropometric equations, BIA showed the highest bias and greatest standardized differences versus DXA, as reported by Núñez et al. [9] for the same BIA device (InBody 770). These results could be perhaps explained by the lack of use of athlete-specific BIA equations [10]. Furthermore, the prediction equations used by the device used in these studies are not available to the public and the user cannot manipulate them [14]. In effect, a recent investigation compared athlete-specific and general BIA (IS 4200, Xitron Technologies) equations with DXA in resistance-trained athletes. Interestingly, only the athlete-specific equation showed good agreement with DXA, whereas general equations over- estimated FFM changes. However, an important limitation of these specific equations is that raw BIA parameters from a 50- kHz device, which have not been tested in a multifrequency device, are needed [44]. In conclusion, it seems that compared with DXA, FFM analysis based on the equations embedded in BIA devices is not a valid and accurate method to assess FFM changes in resistance-trained subjects.

To our knowledge, the present study is the first to ex- amine both the longitudinal and cross-sectional accuracy of FFM analysis. We observed that correlations between all the anthropometric equations or BIA and DXA ranged from very large to almost perfect, both before and after the intervention. However, most procedures gave rise to significant and standardized differences with respect to DXA. UsingDXA as reference, equation (1) of Dunne et al. [20] emerged as the most accurate to estimate pre- and postintervention FFM. However, as stated previously, this equation is recent, and no prior research has assessed the accuracy of this formula outside the study population in which it was developed.Further work is needed to develop specific equations vali- dated with a reference method such as the 4-compartment method for different populations because it has been established that sport-specific equations show greater accuracy [4,27,45].

To date, there is no universally recognized gold standard methodology or tool to assess body composition [11]. Accordingly, the main limitation of the present study is that DXA was taken as the standard criterion, and, indeed, it is considered an acceptable method for FFM [4,6,11]. In addition, the 2-compartment model was used, and, as a con- sequence, it was assumed that body hydration and density were stable [10]. Although the repeated use of DXA to detect composition changes produced over time could lead to measurement errors, this can be improved by implementing standardization protocols of subject positioning on the scanning bed and manipulation of the scan results [6].

When an intervention is designed to produce skeletal muscle hypertrophy, FFM should be monitored; numerous types of devices and brands exist for this purpose [46]. Consequently,as can be seen here, we could easily reach an erroneous conclusion concerning how effective an intervention is. This isan important limitation related to any hypertrophic intervention that targets modifications in body composition, as foundin a recent systematic review [15]. Hence, the method used toassess FFM needs to be carefully selected. Although DXA isa widely accepted reference method for this purpose in athletes, it can also have limitations such as the use of different devices, or different manufacturers or software versions, which may lead to disparate results [47]. Moreover, the equipment for DXA is costly and not usually accessible. For this reason, field methods are most frequently used for monitoring body composition in athletes [11]. When BIA is used in athletic populations, some authors recommend specific equations whereby raw bioelectrical parameters can be introduced, rather than using the body composition data provided by thesoftware. Additionally, there is a need for sport-specific equations [45,48,49]. To resolve the problems of conventional BIA,bioelectrical impedance vector analysis could be another option [48]. A further important limitation to consider is relatedto the prerequisites of BIA, which include refraining from exercise [11]. If we consider anthropometry, this method has the drawback that it needs to convert SKF measurements into body composition data. Although there is a need for more studies to develop specific equations for resistance-trained subjects, according to our results, equations (1) and (2) of Dunne et al. [20] are the best options, respectively, to analyzechanges in FFM and measure FFM cross-sectionally in recreationally resistance-trained young adults. Another interesting practical method to consider is the sum of SKFs to monitor body composition changes over time, as proposed by other authors [4,50].

5. Conclusions

The use of different field techniques to measure FFM gains produced after a hypertrophic intervention yields different results that could invalidate the hypothesized effectiveness of the intervention. BIA assessment using the general equation embedded in the device should not be used to measure FFM changes in young male athletes because it correlates poorly with DXA. Until specific anthropometric equations are developed for resistance-trained populations, when DXA is un- available, anthropometric methods used by a trained anthropometrist following appropriate guidelines such as Dunne equation (2) could be a good option to assess FFM changes inrecreationally resistance-trained young men. It should nevertheless be taken into account that for greater FFM changes, discrepancies with DXA also increase. To examine cross- sectional FFM data in this study population, the other equation of Dunne et al. (1) [20] emerged here as the most accurate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

María Martínez-Ferrán: Conceptualization, Methodol- ogy, Validation, Investigation, Data curation, Visualization, Supervision, Project administration, Writing – original draft. **Luis A. Berlanga:** Methodology, Investigation, Re- sources, Visualization, Supervision, Project administration. **Olga Barcelo-Guido:** Investigation, Resources, Data curation, Visualization. **Michelle Matos-Duarte:** Investigation, Resources, Project administration, Visualization. **Davinia Vicente-Campos:** Investigation, Resources, Data curation, Visualization. **Sandra Sánchez Jorge:** Investigation, Resources, Visualization. **Carlos Romero-Morales:** Resources, Visualization. **Soraya Casla-Barrio:** Formal analysis, Visualization. **Diego Munguía-Izquierdo:** Validation, Formal analysis, Data curation, Visualization, Methodology, Writing – review & editing. **Helios Pareja-Galeano:** Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Projectadministration.

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