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Ramos IE, Coelho GM, Lanzillotti HS, Marini E, Koury JC. Fat-Free Mass Using Bioelectrical Impedance Analysis as an Alternative to Dual-Energy X-Ray Absorptiometry in Calculating Energy Availability in Female Adolescent Athletes. *Int J Sport Nutr Exerc Metab*, 32(5), 2022, 350-358.

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## 30 ABSTRACT

31 Energy availability (EA) is calculated by subtracting exercise energy expenditure from  
32 energy intake, adjusted for fat free mass (FFM) obtained using accurate methods, such as  
33 dual-energy X-ray absorptiometry (DXA). Unlike DXA, the bioelectrical impedance analysis  
34 (BIA) is low in cost, simple and easy to carry out. This study aimed to test the concordance  
35 between the calculation of EA using FFM values from four BIA predictive equations and  
36 FFM obtained using DXA in female adolescent athletes (n=94), recruited via social media.  
37 Paired Student's t-test, Wilcoxon test, Lin's concordance correlation coefficient (CCC), root  
38 means square error (RSME), limits of agreement (LOA) and mean absolute percentage error  
39 (MAPE) were used to evaluate agreement between the FFM values obtained by the four SF-  
40 BIA predictive equations and DXA. Regression linear analysis was used to determine the  
41 relation between FFM values obtained using DXA and the BIA predictive equations.  
42 Standardized residuals of the FFM and EA were calculated considering DXA values as  
43 reference. The most appropriate model for the FFM (LOA= 4.0/-2.6 kg, RMSE=1.9 kg,  
44 MAPE= 4.34%, CCC=0.926) and EA (LOA= 2.51/4.4 kcal/kg.FFM/d, RMSE=1.8 kcal/kg.  
45 FFM/d, MAPE 4.24%, CCC=0.992) was the equation with sexual maturity as a variable,  
46 while the equation with the greatest age variability was the one with the lowest agreement.  
47 FFM-BIA predictive equations can be used to calculate EA of female adolescent athletes.  
48 However, the equation should be chosen considering sex, age, and maturation status. In the  
49 case of athletes, researchers should use equations developed for this group.

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51

## 52 INTRODUCTION

53 Athletes' energy requirements depend on the volume, intensity, periodized training, and  
54 competition cycle. Some factors, such as exposure to cold, heat, high altitude, stress, physical  
55 injuries, and increases in fat-free mass (FFM), increase energy requirements above normal  
56 baseline levels (Thomas et al, 2016). Puberty is a biological phase of great growth and  
57 development, causing changes in body composition (Malina et al., 2011). In female  
58 adolescents, the age of menarche is a marker of sexual maturity leading to changes in body  
59 composition, such as accumulation of fat mass (FM) and FFM, the former mostly in certain  
60 body regions, such as the gluteal-femoral area, and the latter in a lower proportion than in  
61 male adolescents (Malina et al., 2011).

62 Energy balance is a primary factor in body composition, since energy intake higher than  
63 energy expenditure can lead to body weight gain, and energy expenditure higher than intake  
64 can reduce FFM and bone mineral density, cause changes in reproductive function, and

65 impact the growth of adolescents (Loucks, 2004). Differently from energy balance, the  
66 concept of energy availability (EA) is broader, as it considers the FFM as a variable for the  
67 calculation. EA is defined as the residual energy available to support an athlete's body  
68 functions. Low EA (L-EA) is a primary mechanism of the female athlete's triad that  
69 predisposes athletes to menstrual irregularities and low bone mineral density (Nattiv et al.,  
70 2007), and is considered a major factor in several health problems in athletes of both sexes,  
71 and it is described as a relative energy deficiency in sports (Mountjoy et al., 2018).

72 EA is obtained by subtracting exercise energy expenditure from energy intake, adjusted by  
73 FFM (Loucks et al, 2004, 2011). Each component of the equation relies on accurate  
74 measurement tools and a clear definition of what should be measured (Loucks et al., 2004).  
75 FFM can be obtained by gold standard methods, such as four compartments model, or high-  
76 accuracy, such as DXA (Shepherd et al., 2017). However, these methods are expensive and  
77 makes it difficult to study large groups of athletes (Nana et al., 2015). Besides that, 4C model  
78 is time-consuming and difficult to perform in children and adolescents (Sopher et al., 2004).  
79 Conversely, bioelectrical impedance analysis (BIA) is low in cost, simple and easy to carry  
80 out, and does not expose the subject to radiation (Kyle et al., 2004). Although the BIA  
81 method is widely disseminated, few studies have used FFM from BIA predictive equations to  
82 calculate EA (Koehler et al, 2013, Lagowska 2014, Lagowska et al, 2016, Brown et al., 2017,  
83 Braun et al, 2018). However, these studies did not test the concordance between the results of  
84 the predictive equation and those obtained by a more accurate method.

85 Considering the importance of predicting FFM in adolescent athletes by low-cost methods  
86 such as BIA, and of calculating EA in this vulnerable group, this study aimed to test the  
87 concordance between the calculation of EA using FFM values from four BIA predictive  
88 equations and values obtained using DXA in female adolescent athletes.

## 89 **METHODS**

## 90 **Participants**

91 The sample size was determined *a priori* using a statistical software (G\*Power 3.1.9.7  
92 Stuttgart, Germany) assuming: effect size= 0.2;  $\alpha$ = 0.5; Power (1- $\beta$  error probability) = 0.95.

93 The estimated number of participants was 70 individuals per group.

94 The participants were recruited via social media (Instagram advertisement  
95 [[https://www.instagram.com/saude\\_de\\_atleta/](https://www.instagram.com/saude_de_atleta/)]). After scheduling consultations, data were  
96 collected at the Interdisciplinary Laboratory of Nutritional Assessment, of the Institute of  
97 Nutrition of the University of the State of Rio de Janeiro, during the second semester of 2017.  
98 All participants in the study were adolescents (10 - 19 years; WHO, 1995). Only adolescents  
99 that had been playing sports in a sports club for at least 6 months and agreed to participate  
100 after being informed of the research objectives were included in the study.

101 This study was approved by the Ethics Committee of the Hospital Universitário Pedro  
102 Ernesto (CEP/HUPE649.202) in accordance with the Declaration of Helsinki. All guardians  
103 and participants provided prior written informed consent.

## 104 **Study design**

105 This was a cross-sectional study with female adolescent athletes. Individual data were  
106 collected on the same day, including anthropometric measurements and determination of  
107 body composition, using both single frequency SF-BIA and DXA methods. The athletes were  
108 divided into two groups according to EA cutoffs: adequate ( $\geq 45$  kcal/kg/FFM-1/d-1) and  
109 inadequate ( $< 45$  kcal/kg/FFM-1/d-1) energy availability (Nattiv et al., 2007).

110 The FFM values obtained by DXA were used as a reference for the tests of concordance of  
111 the results obtained by SF-BIA predictive equations. The FFM values obtained by the  
112 predictive equations were used to calculate EA. Besides that, the concordance between EA  
113 values obtained using DXA and SF-BIA was tested.

## 114 **Sexual maturity**

115 Sexual maturity was determined by the menarche occurrence, which was reported by  
116 the individuals. The participants whose menstrual cycle had already begun were classified as  
117 sexually mature, and those whose menstrual cycle had not begun were classified as sexually  
118 immature.

### 119 **Anthropometric measurements and body composition**

120 Body mass and height were obtained using a digital scale and a stadiometer (Filizola, Rio de  
121 Janeiro, Brazil). Fat-free mass and bone mineral density were estimated with the DXA  
122 method. The Lunar iDXA device with enCore 2008 software version 12.20 (GE Healthcare,  
123 Madison, Wisconsin, USA) was used in the automatic full-body scan mode. The participants  
124 were placed in a dorsal position and asked to remain immobile until the end of the procedure.  
125 The exams were performed by a single trained and qualified professional, following the  
126 quality control procedures recommended by the manufacturer, and the official  
127 recommendations of the International Society for Clinical Densitometry (Crabtree et al,  
128 2014). BMD z-scores  $< -1.0$  SD were classified as “low bone mineral density” (Nattiv et al,  
129 2004).

130 Body composition using the BIA method was measured using a single frequency  
131 bioimpedance analyser (Biodynamics-450 Corporation, Seattle, USA), [applying foot-to-hand](#)  
132 [technology](#). To avoid clinical disturbances in fluid distribution, participants were instructed to  
133 abstain from food and liquids for 4 hours and to abstain from caffeine intake and intense  
134 physical activity for 24 hours before [SF-BIA](#). [The adolescents were instructed not to undergo](#)  
135 [the exam during their menstrual period. Before each test, the analyser was checked with the](#)  
136 [impedance calibration \(resistance R= 500 ohms\); and the components inside the](#)  
137 [bioimpedance analyzer, such as the signal generator, the sensing apparatus, the scales of](#)  
138 [weight and height, and the electrical interference were tested as suggested by Kyle et al.](#)

139 (2004). The average of the two repeated measurements of R and Xc was used in the  
140 subsequent analyses.

141 The predictive equations were selected based on the following criteria: 1) validated by DXA;  
142 2) pertained to healthy participants and regular sports players; 3) BIA analyser used to  
143 develop the single frequency equation (50 kHz); 4) sex as a variation of the predictive  
144 equation; and 5) age range according to puberty. Four proposed predictive equations met the  
145 inclusion criteria: Deurenberg et al. (1991), Koury et al. (2019), Morrison et al. (2001), and  
146 Sun et al. (2003). The variables included in the calculation of FFM using SF-BIA predictive  
147 equations were: age, sex, reactance, body mass (except Koury et al. 2019), height<sup>2</sup>/resistance  
148 ratio, menarche occurrence (Koury et al. 2019, only). Table 1 shows the details of the  
149 selected equations.

#### 150 **Energy availability**

151 Two nutritionists applied a questionnaire, and each participant was interviewed once time by  
152 only one nutritionist. Energy intake was obtained by a 24-hour recall reported by the  
153 participants individually, and the target period of the interview was the previous typical day.  
154 Both nutritionists were trained to use the US Department of Agriculture Automated  
155 Multiple-Pass Method (Conway et al., 2003), which consists of five steps: 1) the participants  
156 are invited to verbally provide a quick list of foods and drinks consumed, without interruption  
157 as they speak; 2) the participants are asked about foods they might have forgotten to mention;  
158 3) the participants describe in detail the time at which each food was consumed; 4) the  
159 participants are encouraged to describe in detail the food and the respective quantities,  
160 reviewing the information about the time and occasion of consumption; and 5) the  
161 information is reviewed, and foods that were consumed and any food that has not been  
162 reported are identified. The participants received the Brazilian Photographic Manual of Food  
163 Portion Quantification (Monteiro, 2007) containing main household items and their

164 conversion into grams and millilitres (Pinheiro et al., 2004) to facilitate their description of  
165 the portions consumed. The Brazilian Table of Food Composition (Universidade Estadual de  
166 Campinas - UNICAMP, 2011) was used for the calculation of nutrient intake.

167 Metabolic equivalent task (MET) values were used for each sport as scored for moderate  
168 effort, using the youth compendium of physical activities (Ridley et al., 2008). Exercise  
169 energy expenditure was calculated individually using MET values and the period of time  
170 stated for exercise sessions. Energy availability was calculated as: energy intake minus  
171 energy expenditure divided by FFM (kg) values, obtained using DXA and the four SF-BIA  
172 predictive equations (Table 1).

173 To the best of our knowledge, there are no studies with specific cutoff points for energy  
174 availability in adolescents. Thus, the values for adult females have been used in studies on  
175 adolescents (Logue et al, 2020).

176 For young females who were physically active (Nattiv et al., 2007), energy availability was  
177 considered inadequate when the values were lower than 45 kcal/kg.FFM/d when assessing  
178 bone health, and when the values were lower than 30 kcal/kg.FFM/d when assessing  
179 reproductive function.

## 180 **Statistical analyses**

181 Statistical analyses were performed using SPSS software version 19 (IBM Corporation,  
182 Armonk, NY, USA) and MedCalc Statistical Software version 14.8.1 (MedCalc Software,  
183 Ostend, Belgium; <http://www.medcalc.org>; 2014). The Shapiro Wilk test was performed to  
184 determine whether data was normally distributed.

185 Anthropometric measurements and body composition compartments were expressed as  
186 mean±standard deviation. The independent Student's t-test was used to determine differences  
187 between groups, according to the EA classification (inadequate <45 kcal/kg.FFM/day, or <30

188 kcal/kg.FFM/day). Energy and nutrient intake were expressed as medians, and interquartile  
189 ranges and were compared using the Mann-Whitney-U test.

190 The paired t-test was used to determine the difference between the FFM values obtained by  
191 the four SF-BIA predictive equations and values obtained using DXA, and Wilcoxon test was  
192 used to compare EA values using FFM obtained using SF-BIA predictive equations. The  
193 mean absolute percent error (MAPE) and the root means square error (RSME) between FFM  
194 and EA observed (DXA) and predicted (SF-BIA) values were calculated.

195 Bland-Altman plots were also used to test individual-level agreement between each SF-BIA  
196 predictive equations and DXA values. Lin's concordance correlation coefficient (Lin, 1989)  
197 was used considering the strength-of-agreement criteria described by McBride (2005) (almost  
198 perfect: > 0.99; substantial: 0.95 - 0.99; moderate: 0.90-0.95; and poor: <0.90).

## 199 RESULTS

200 One hundred female athletes aged between 11 and 16 years agreed to participate in the study.  
201 Body composition data was obtained from 94 (40 [42%], sexually immature) of the  
202 participants, and all the necessary data for the calculation of EA (n= 16 [23%], sexually  
203 immature) was obtained from 70 (74%) of the participants. All participants trained regularly  
204 (6 times a week for at least 90 minutes/day).

205 The sports mentioned by the participants were soccer (n= 22), volleyball (n= 23), swimming  
206 (n= 14), table tennis (n= 15), and handball (n= 20). Regardless of the sport practiced, energy  
207 expenditure was similar between groups according to sexual maturity (immature - median =  
208 352.3 kcal/d [IQR = 283.1 - 413.6]; mature - median = 398.7 kcal/d [IQR 289.1 - 523.5]; p =  
209 0.155). The sport did not influence the other results.

210 Female adolescent athletes' energy and nutrient intake according to the availability of energy  
211 classification is shown in Table 2. L-EA (<30 kcal/kg.FFM/day (n=41,6%) or



212 <45kcal/kg.FFM/day (n=46,7%) female adolescent athletes presented lower carbohydrates,  
213 lipids, and proteins intake ( $p < 0.01$ ) than adolescents with adequate EA.

214 The raw SF-BIA parameters (R and Xc) and phase angle values were: reactance (n=94),  
215 mean $\pm$ SD= 70.4 $\pm$ 9.4  $\Omega$  (95%CI 68.4 – 72.1); resistance (n=94) mean $\pm$ SD= 666.4 $\pm$ 73.5  $\Omega$   
216 (95%CI 651.4 – 681.5), and phase angle (n=94), mean $\pm$ SD= 6.1 $^{\circ}$  $\pm$ 0.8 (95% CI 6.0 – 6.3).

217 The paired t-test showed that the four equations tested provide different values than those  
218 obtained by DXA for FFM and for EA (Table 3). FFM data obtained by the SF-BIA  
219 predictive equations showed good symmetry (values between -0.5 and 0.5), and kurtosis  
220 showed flat curves (platykurtic). P-values for the Shapiro Wilk normality test indicated  
221 normal distribution results for all FFM predictive equations ( $P > 0.05$ ). The results of the EA  
222 calculated applying the FFM values using SF-BIA predictive equations showed symmetry in  
223 the maximum limit (0.5), kurtosis showed an elongated curve (leptokurtic). The P-values for  
224 the Shapiro Wilk test showed no normal distribution ( $P < 0.05$ ) for all predictive equations  
225 (Table 3).

226 The FFM values obtained by Deurenberg et al. (1991), Morison et al. (2001), and Sun et al.  
227 (2003) showed the lowest values of the concordance correlation coefficients (CCCs)  
228 classified as poor concordance by McBride (2005). The CCC values were corroborated by the  
229 different RMSE values obtained. The predictive equation proposed by Koury et al. (2019)  
230 showed a better result, less difference between the means, CCC classified as moderate  
231 (McBride, 2007), and lower RMSE. The Koury et al. (2019) ( $R^2 = 0.927$ ) and Sun et al. ( $R^2 =$   
232 0.913) predictive equations showed higher  $R^2$  values of the linear regression models for the  
233 sexually immature group. However, the RMSE and MAPE values showed a better  
234 performance for the Koury et al. (2019) equation (RMSE= 2.31 kg, MAPE= 4.34%) than Sun  
235 et al. (RMSE= 5.64 kg, MAPE= 12.58%) predictive equation (Figure 1).

236 All SF-BIA-predictive equations tested to calculate EA showed a high CCC value, being  
237 classified as excellent for the prediction of EA (McBride, 2005). Although the equation  
238 proposed by Sun et al. (2003) had a limit value to be considered as concordant (CCC= 0.95),  
239 the other equations had similar values (CCC= 0.99). Despite the high CCC values, the RMSE  
240 and MAPE values indicated that the predictive equation proposed by Sun et al. (2003) was  
241 the one with the greatest error (RMSE= 5.6 kcal/kg.FFM/day, MAPE= 12.05%), followed by  
242 those proposed by Morrison et al. (2001) (RMSE= 2.8 kcal/kg.FFM/day, MAPE= 5.31%),  
243 Deurenberg et al. (1991) (RMSE= 2.4 kcal/kg.FFM/day, MAPE= 4.34%), and Koury et al.  
244 (2019) (RMSE= 1.8 kcal/kg.FFM/day, MAPE= 4.23%) when compared with the EA values  
245 generated by DXA (Figure 2).

246 Considering all predictive equations, the standardized residuals for FFM showed that only  
247 one (1.8%) sexually mature participant was below the first quartile, and one was above the  
248 third quartile (1.8%), while 10 (25%) immature participants were below the first quartile. For  
249 EA, considering all predictive equations, standardized residuals showed that only one (2.5%)  
250 sexually immature participant was below the first quartile, and four (10%) were above the  
251 third quartile, while 8 (16%) sexually mature participants were below the first quartile and  
252 four (10%) above third quartile (Figure 3).

## 253 DISCUSSION

254 The present study is the first to assess the concordance between EA calculated with the FFM  
255 values, obtained from four SF-BIA predictive equations, and the FFM values obtained by  
256 DXA, in female adolescent athletes.

257 Although it is generally accepted that predictive equations of FFM using SF-BIA should be  
258 sex-specific for individuals over 13 years of age (Malina et al., 2011), only one study has  
259 proposed to estimate FFM-BIA considering maturity indicators in adolescent athletes, such as  
260 skeletal maturity for boys and sexual maturity for girls (Koury et al., 2019).

261 Among the four equations tested in the present study, two showed great variation in the age  
262 range: 82 years for Sun et al. (2003); 76 years for Deurenberg et al. (1991); one included an  
263 age range corresponding to childhood and adolescence (a variation of 11 years, Morrison et  
264 al., 2001); and the other one included an age range corresponding exclusively to adolescence,  
265 with a variation of 4 years, and a variable related to sexual maturity (Koury et al., 2019). The  
266 growth of adolescent athletes leads to specific adaptations in body composition (Toselli et al.,  
267 2020) that can be accurately detected by BIA method, in within- and between-athlete  
268 comparisons (Campa et al, 2022), when using adequate predictive equations.

269 The Koury et al. (2019) predictive equation presented the highest CCC, lowest bias, and  
270 lowest RMSE and MAPE values, all of which indicate a better fit, when compared to  
271 Deurenberg et al (1991) and Morrison et al (2003) predictive equations. These results are in  
272 line with the concept of Chumlea and Sun (2005), who stated that the equation with a high  
273 value of  $R^2$  and a low value of RMSE is likely to have lower bias. Considering the FFM  
274 obtained using predictive equations developed by Koury et al. (2019) and Sun et al. (2003),  
275 both presented similar values of  $R^2$  for the sexually immature participants. Despite the  
276 similarity, Koury et al. (2019) equation showed better performance in terms of RMSE and  
277 MAPE values for all participants and for the sexually immature group, confirming the  
278 importance of including the variable 'sexual maturity' in the equation. The results of the  
279 analysis of standardized residuals support the idea that the absence of sexual maturity as a  
280 variable in the predictive equation could overestimate the values of FFM of sexually  
281 immature participants.

282 Predicting FFM and calculating EA can protect athletes from serious health problems. EA  
283 was initially proposed for adult females who consumed less energy than needed to support  
284 physiological functions, daily activities, and physical exercise. An EA frequently below the  
285 value considered adequate leads to endocrine adaptations that can cause amenorrhea (<30

286 kcal/kg. FFM/day), excessive weight loss, damage to bone mineral density, and eating  
287 disorders (Nattiv et al., 2007). The consequences of inadequate EA for adolescent athletes are  
288 more severe because it can occur concurrently with the period of growth and development,  
289 and may lead to irreparable impairment (Loucks et al., 2004; 2011).

290 In the present study, the FFM-BIA tested for EA calculation showed excellent CCC (95% to  
291 99%), despite having different degrees of concordance for FFM-DXA. The equation  
292 proposed by Sun et al. (2003) had the lowest CCC value. The smallest mean difference in the  
293 RMSE and the lowest percentage of the MAPE were obtained using the FFM predictive  
294 equation proposed by Koury et al. (2019). The observed CCC indicated that the insertion of  
295 FFM in the denominator of the EA equation reduces the margin of error when these equations  
296 are used to calculate EA, suggesting that the variables with the greatest impact are those  
297 found in the numerator (energy intake and exercise energy expenditure).

298 In the present study, it was observed that 58% and 66% of the participants presented EA  
299 values below the reference values for adult females of 30 or 45 kcal/kgFFM/day,  
300 respectively, considering the calculation based on FFM-DXA. Other authors have found a  
301 similar percentage in studies with female adolescent athletes, using different methods to  
302 predict FFM. Cherian et al. (2018) and Silva et al. (2018) used skinfolds and EA values  
303 higher than 30 kcal/kgFFM/day, and 45 kcal/kgFFM/day, respectively, as cutoff points for  
304 adequacy. Both studies found that 40% of the participants were below the cutoff points  
305 defined by the authors. Brown et al. (2018) used the BIA method to predict FFM, and  
306 observed that 53% of the participants were below the cutoff point of 30 kcal/kgFFM/day.

307 The main limitation of the present study is the absence of robust methods for predicting  
308 ingested and expended energy. However, to our knowledge, this is the first study to test FFM  
309 obtained using SF-BIA for EA calculation in female adolescent athletes. Furthermore, the  
310 present study included DXA analyses, which is considered an accurate method for FFM

311 measurement (Burke et al, 2018); and only athletes participated, thus reducing the margin of  
312 error.

313 In conclusion, our results suggest that FFM-BIA predictive equations can be used to calculate  
314 EA of female adolescent athletes. The model proposed by Koury et al. (2019) presented the  
315 best performance in the statistical tests used. Furthermore, the lack of statistical concordance  
316 and high MAPE percentage suggest that Sun et al. predictive equation should be interpreted  
317 with caution when estimating FFM in female adolescent athletes. In general, this suggests  
318 that the equation must be chosen considering sex, age group, and maturation. In the case of  
319 athletes, researchers should use equations developed for this group. The calculation of EA  
320 using more economical and less complex methods is of great use in the field of sports  
321 nutrition. Calculating the EA is of great importance because it makes it possible to treat  
322 eating disorders, and to avoid problems related to reproductive function and later to bone  
323 health.

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328 concept and design; IER acquisition of data; JCK, GMC, HLS, EM analysis and  
329 interpretation of data; JCK, GMC, EM, IER drafting the manuscript; JCK, EM, GMC, HLS  
330 statistical analysis; JCK, GCM study supervision; JCK, EM, GMC, HLS, IER original draft,  
331 writing- review and editing.

332 All authors have read and approved the final version of the manuscript and agree with the  
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**Table 1** – Characteristics of the selected equations for prediction of fat-free mass using BIA

<b>Authors</b>	<b>Age (years)</b>	<b>Sex</b>	<b>Fat-free mass predictive equation</b>	<b>SEE (kg)</b>	<b>R<sup>2</sup></b>
Deurenberg et al, 1991	≤15	F – M (n=166)	= 0.406 x H <sup>2</sup> /R + 0.36 x W + 0.0558 x H+ 0.56 x Sex - 6.48	1.7	0.97
Koury et al, 2019	11-15	F (n=151)	= -2.65+0.603 x Age+0.954 x (menarche occurrence)+ 0.713 x H <sup>2</sup> /R	2.2	0.84
Morrison et al, 2001	6-17	F (n=126)	= - 6.41 + 0.56 x H <sup>2</sup> /R + 0.34 x W+0.06 x Xc	3.3	0.99
(Sun et al, 2003	12-94	F (n=944)	= - 9.53 + 0.69 x H <sup>2</sup> /R + 0.17 x W+ 0.02x R	2.9	0.83

F = Female, M = Male; H= height (cm); W= weight (kg); R= resistance( $\Omega$ ), Xc = reactance ( $\Omega$ ); H<sup>2</sup>/R = Height<sup>2</sup>/Resistance; Sex: male = 1, female = 0; menarche occurrence: no = 0, yes = 1. SEE = standard error of estimate. R<sup>2</sup> = Coefficient of determination.

**Table 2-** Intake of energy and nutrients by female adolescent athletes according to energy availability.

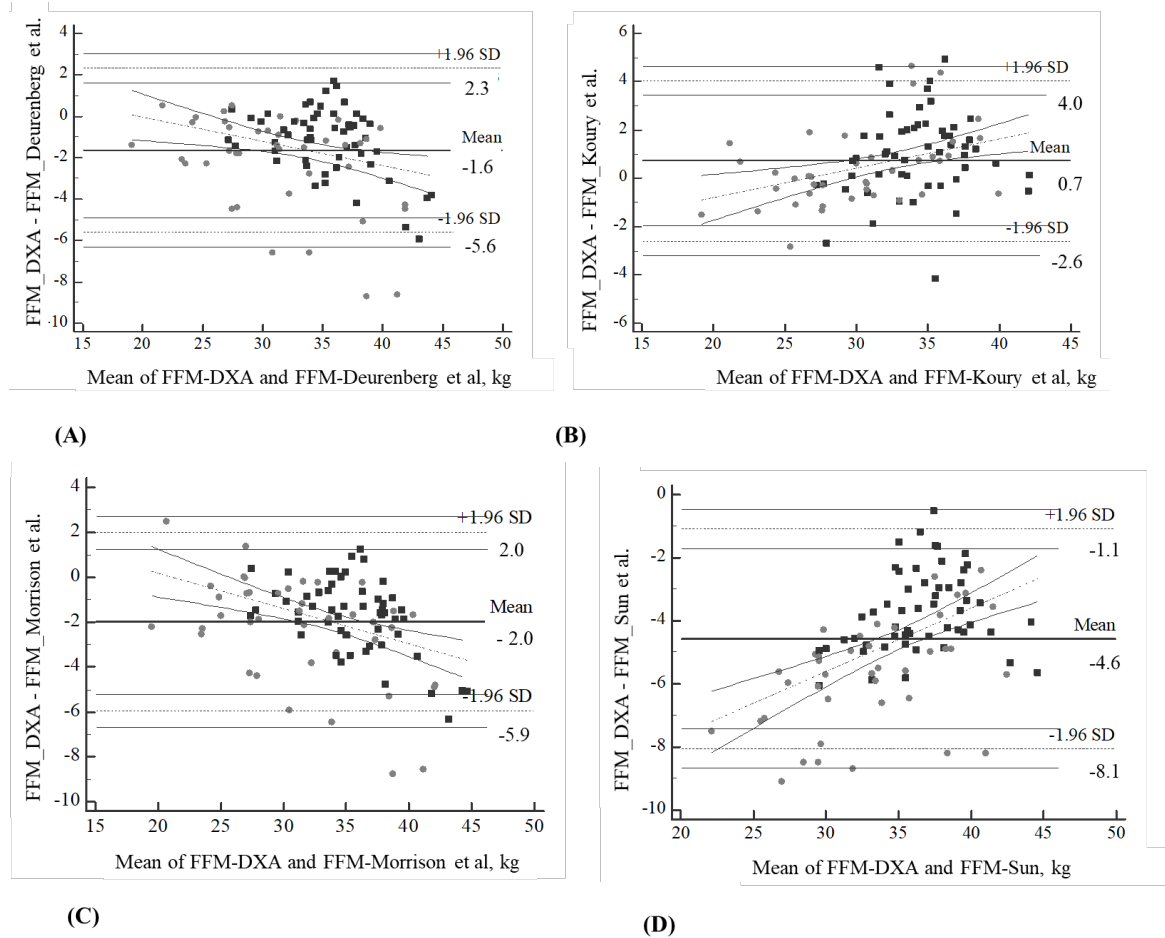
	All (n=70)	≥ 30kcal/kg.FFM/d (n=29)	< 30kcal/kg.FFM/d (n=41)	<i>p</i>	≥ 45kcal/kg.FFM/d (n=24)	< 45kcal/kg.FFM/d (n=46)	<i>p</i>
Energy, kcal	1,654.8 (1,246.4 – 1,987.3)	1,941.1 (1,682.9-2,505.1)	1,231.6 (1,003.4-1,431.4)	0.007	2,251.1 (1,787.7 - 2,679.9)	1,395.9 (1,101.1 - 1,654.8)	0.005
Carbohydrates, g	230.3 (174.5 – 287.5)	275.9 (230.8-370.2)	171.6 (142.9-200.3)	0.004	308.6 (242.5 - 408.1)	198.8 (159.9 - 236.8)	0.003
Lipids, g	45.1 (29.0 – 62.8)	55.3 (40.4-82.3)	34.3 (24.7-45.1)	0.003	75.6 (50.1 – 92.4)	34.7 (26.8 – 49.4)	0.005
Proteins, g	71.8 (53.2 – 84.7)	77.5 (70.0-95.4)	53.9 (41.8-69.6)	0.009	82.2 (71.9 – 101.7)	65.5 (46.5 – 76.8)	0.008

Median (interquartile range). FFM = fat-free mass. Mann-Whitney-U test.

**Table 3** - Comparison between DXA and predictive equations from BIA for fat-free mass and energy availability of female adolescent athletes (n=94)

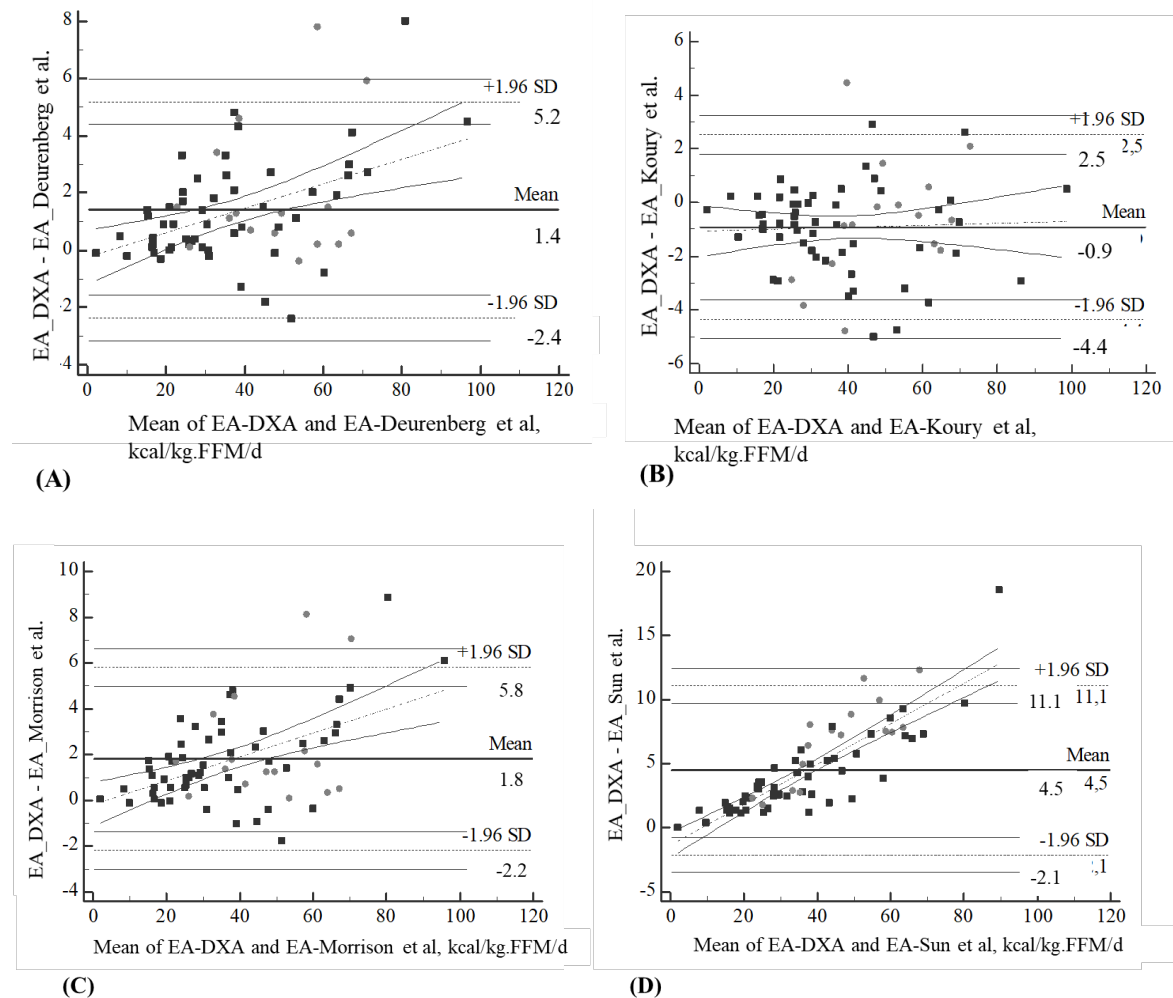
	<b>Fat-free mass (n=94)</b>				
	DXA	Deurenberg et al, 1991	Koury et al, 2019	Morison et al, 2001	Sun et al, 2003
Mean (kg)	32.8±4.9	34.3±5.6	32.1±4.4	34.8±5.8	37.4±4.1
IC 95%	33.0 - 33.8	32.3 – 35.6	31.5 – 33.4	33.6 – 36.0	36.5 – 38.2
(P-value <sup>1</sup> )		0.01	0.01	<0.01	<0.01
Skewness		-0.1	-0.4	-0.2	-0.09
Kurtosis		-0.04	0.3	-0.09	0.04
(P-value <sup>2</sup> )		0.54	0.12	0.69	0.95
	<b>Energy availability (n=70)</b>				
Median (kcal/kg.FFM /d)	36.8	34.5	37.6	34.2	32.5
IQR	25.3 – 50.6	23.3 – 52.5	25.8 – 53.7	22.9 – 52.2	22.2 – 44.8
(P-value <sup>3</sup> )		< 0.01	<0.01	< 0.01	< 0.01
Skewness		0.5	0.5	0.5	0.5
Kurtosis		1.7	0.9	1.2	1.9
(P-value <sup>2</sup> )		0.03	0.04	0.03	0.05

DXA = dual-emission X-ray absorptiometry. BIA = bioelectrical impedance. FFM = fat-free mass. CI = confidence interval. SD= standard deviation. (P-value<sup>1</sup>) obtained using Paired t-test, comparison between DXA values and each SF-BIA predictive equation (<0.05 significant difference); (P<sup>2</sup>-vlaue) obtained using Shapiro Wilk test to test normal distribution (>0.05 normal distribution), (P<sup>3</sup>-vlaue) obtained using Wilcoxon test, comparison between DXA values and each SF-BIA predictive equation for EA calculation (<0.05 significant difference)



Predictive equations	R <sup>2</sup>	RMSE, kg	MAPE%	CCC
A	0.871	2.6	5.0	0.885
B	0.888	1.9	4.3	0.926
C	0.886	2.8	6.0	0.871
D	0.887	4.8	12.6	0.613

Figure 1-Bland-Altman analysis and regression line between the FFM obtained using BIA predictive equations (A) Deurenberg et al; (B) Koury et al; (C) Morrison et al and (D) Sun et al and DXA measured for female adolescent athletes considering sexual maturity. R<sup>2</sup> considered all participants, RMSE=Root mean square error; MAPE= mean absolute percentage error; CCC=concordance correlation coefficient. Gray circle= sexual immature; dark square= sexual mature.



Predictive equations	R <sup>2</sup>	RMSE, kcal/kg.FFM/d	MAPE%	CCC
A	0.992	2.4	4.3	0.992
B	0.997	1.8	4.2	0.992
C	0.991	2.8	5.3	0.989
D	0.991	5.6	12.1	0.954

Figure 2- Bland-Altman analysis and regression line between the EA obtained using BIA predictive equations (A) Deurenberg et al; (B) Koury et al; (C) Morrison et al and (D) Sun et al and DXA measured for female adolescent athletes considering sexual maturity. R<sup>2</sup> considered all participants, RMSE=Root mean square error; MAPE= mean absolute percentage error; CCC=concordance correlation coefficient. Gray circle= sexual immature; dark square= sexual mature.

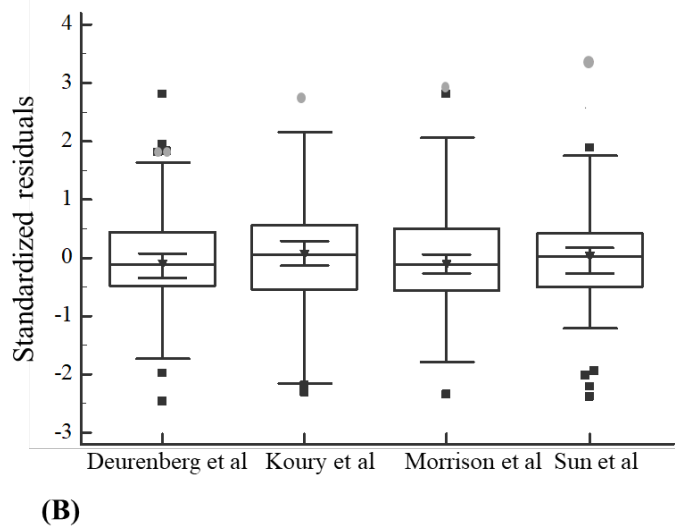
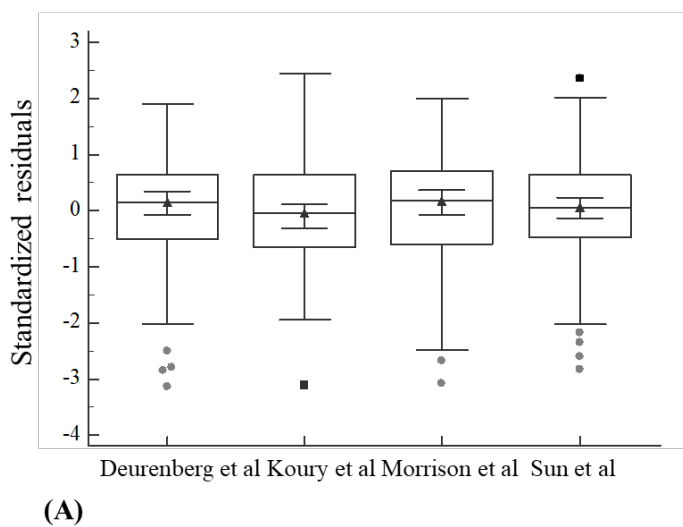


Figure 3- Box plot of the standardized residuals of FFM (a) and EA (b) obtained using four BIA predictive equations. Standardized by FFM and EA obtained using DXA. Gray circle = sexually immature, black square = sexually mature. 95%CI for median