Development and validation of bioimpedance prediction equations for fat-free mass in unilateral male amputees

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Background. Metabolic disease due to increased fat mass is observed in amputees (APTs), thereby restricting their activity. Systemic health management with periodic body composition (BC) testing is essential for healthy living. Bioelectrical impedance analysis (BIA) is a non-invasive and low-cost method to test BC; however, the APTs are classified as being exempted in the BIA.

Objective. We therefore developed five segmental estimated regression equations (sEREs) for determining fat-free mass (FFM, kg) suitable for APTs and aimed to improve the accuracy and validity of sEREs.

Methods. Seventy-five male APTs participated in this cross-sectional study. Multiple regression analysis was performed to develop highly accurate sEREs of BIA based on independent variables derived from anthropometric measurements, dual-energy X-ray absorptiometry (DXA), and BIA parameters. The difference in validity between the predicted DXA and sum of the segmentally-predicted FFM values by sEREs (Sum_sEREs) values was evaluated using bivariate linear regression analysis and the Bland–Altman plot.

Results. The coefficient of determination (R^2) and total error (TE) between DXA and Sum_sEREs were 71% and 5.4 (kg) in the cross-validation analysis.

Conclusions. We confirmed the possibility of evaluating the FFM of APTs through the sEREs developed in this study. We also identified several independent variables that should be considered while developing such sEREs. Further studies are required to determine the validity of our sEREs and the most appropriate BIA frequencies for measuring FFM in APTs.

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18 Abstract

19 Background. Metabolic disease due to increased fat mass is observed in amputees (APTs), thereby

20 restricting their activity. Systemic health management with periodic body composition (BC) testing is

21 essential for healthy living. Bioelectrical impedance analysis (BIA) is a non-invasive and low-cost

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28 anthropometric measurements, dual-energy X-ray absorptiometry (DXA), and BIA parameters. The

29 difference in validity between the predicted DXA and sum of the segmentally-predicted FFM values by

30 sEREs (Sum_sEREs) values was evaluated using bivariate linear regression analysis and the Bland–

31 Altman plot.

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34   Conclusions. We confirmed the possibility of evaluating the FFM of APTs through the sEREs developed

35   in this study. We also identified several independent variables that should be considered while developing

36   such sEREs. Further studies are required to determine the validity of our sEREs and the most appropriate

37   BIA frequencies for measuring FFM in APTs.

38

39

40   Introduction

41   Amputation refers to the removal of the upper or lower extremities to resolve the cause of a disability due

42   to disease or trauma (Hecht et al. 2003). Physical and functional constraints are more severe in amputees

43   (APTs) than in the general population (Coffey et al. 2014). Previously, most amputations were performed

44   following war-related or occupational injuries. Indeed, an increase in the number of APTs was observed

45   following World War II and the Iraq War (Kulkarni et al. 1998; Robbins et al. 2009).

46   In addition, cardiovascular diseases such as high blood pressure/stroke and metabolic diseases including

47   diabetes mellitus may necessitate amputation (Kopelman 2000; Rahman & Berenson 2010). Currently,

48   reports suggest that the rate of amputation continues to increase worldwide. Notably, the number of APTs

49   in the United States is predicted to increase from approximately 1.6 million (2008 data) to 3.6 million by

50   2050 (Ziegler-Graham et al. 2008).

51   Diabetes is associated with impaired blood supply to the extremities, which may lead to necrosis or severe

52   ulcers that necessitate amputation (Mishra et al. 2017). Post-amputation complications, such as atrophy,

53   phantom limb pain, and contracture, may lead to further activity constraints beyond those imposed by the

54   amputation, thereby exerting deleterious effects on one's overall health (Gallagher et al. 2001; O'Sullivan

55   et al. 2013; Ustun et al. 2003). Additional post-amputation complications include obesity or excessive fat

56   accumulation (Kurdibaylo 1996), atrophy due to decreased muscle mobilization for ambulation and joint

57   stability, and decreased physical strength (Centomo et al. 2008) (Isakov et al. 1996) (Renstrom et al.

58   1983a) (Renstrom et al. 1983b) (Sadeghi et al. 2001) (Bukowski 2006) (Zachariah et al. 2004).

59   These complications may lead to an overall deterioration in physical health, which can be reflected by

60   changes in BC and muscle condition, ultimately resulting in a decreased quality of life (Chin et al. 2002)

61   (Rosenberg et al. 2013) (Suk et al. 2001). Periodic assessments of BC and weight management

62   interventions are critical for preventing secondary complications of amputation, such as excessive body

63   fat/obesity (Stone et al. 2006) (Yoo 2014). Such assessments and interventions may not only help prevent

64   obesity and muscle atrophy, but may also improve overall physical/mental health and quality of life.

65   Dual-energy X-ray absorptiometry (DXA) allows the measurement of bone mineral content (BMC), fat

66   mass (FM), and soft lean tissue mass (SLTM) by passing two X-ray beams through the body. Although

67   DXA is a highly accurate, validated method for the assessment of fat-free mass (FFM) (i.e.,

68   BMC+SLTM), these assessments are time-consuming and expensive. Furthermore, DXA requires

69   exposure to small amounts of radiation, and some participants may be uncomfortable given the limited

70   measurement space. Despite these limitations, DXA continues to be widely touted as the gold standard for

71   determining BC (Janssen et al. 2002) (Lee & Gallagher 2008) (Woodrow 2009).

72   Commercially available bioimpedance devices include single- and multi-frequency impedance analysis

73   (SFBIA and MFBIA) and bioimpedance spectroscopy (BIS) (Mulasi et al. 2015). The principle of

74   bioelectrical impedance analysis (BIA), including SFBIA, MFBIA, and BIS methods is to determine

75   electrical impedance as "resistance (R)" (Kyle et al. 2004a) for total body water (TBW) and "reactance

76   (Xc)" for body cell mass (Walter-Kroker et al. 2011). BIA is advantageous in that it is faster, more useful,

77   less invasive, less physically restrictive, and less expensive than DXA. In addition, BIA equipment

78   occupies far less space and requires less effort to operate than DXA equipment with a non-portable

79   nature, which limits its use (Buckinx et al. 2015) (Janssen et al. 2002). As a routine clinical BIA tool, BC

80   analysis was now readily available in a wider range of clinics and in the community (Ward 2019). Given

81   these advantages, BIA is performed in various settings. To estimate BC, BIA devices measure TBW

82   content by sending a micro-current of less than 800 µA throughout the body. An estimated regression

83   equation (ERE) is then used to predict FM and FFM (kg) (Kyle et al. 2004a). Recently, BIA has been

84   used for the quantitation of BC through a specific mathematical model without empirically derived

85   variables for athletic players (Sardinha et al. 2020) (Campa & Toselli 2018; Toselli et al. 2020), children

86   (Colica et al. 2018), older adults (Silveira et al. 2020), and young patients with cystic fibrosis (Charatsi et

87   al. 2016).

88   However, APTs are typically excluded from such BIA studies owing to differences in limb length

89   between the amputated and sound sides and irregular limb shapes (Dogan et al. 2012) because the height

90   is different and it is against the impedance index (ZI) formula (height^2/impedance), which is a more

91   significant single predictor of FFM than other anthropometric variables (Nguyen et al. 2007). Thus, the

92   use of BIA equations based on individuals (Beaudart et al. 2020) without amputation may not be accurate

93   and not be a valid measurement when applied in APTs.

94   In general, BC is predicted through variables considering the whole-body height on the wrist-ankle

95   method for reducing typical BIA errors.

96   However, we intend to proceed with a study on the ERE of APT through the variable of the ZI

97   considering the segmental length based on Tanaka's research (Tanaka et al. 2007). Appropriate segmental

98   estimated regression equation (sERE) for BC in APTs should thus consider residual limb properties

99   length and various other factors as independent variables including age, height, weight, gender, ZI, R, Xc,

100   and phage angle (PA) without segmental amputated limb weight that is not measurable.

101 In this study, the InBody S10 BIA instrument (InBody S10) was used. This instrument allows for the

102 analysis of various cellular properties through frequencies segmentally. It is actually placed at eight

103 precise tactile points with an adhere-type electrode to perform BIA in the comfortable and safe supine

104 position for ATPs.

105 Therefore, in the present study, we aimed to develop an ERE for BIA in APTs using DXA measurements

106 as the reference standard. The development of a valid ERE for use in APTs may improve health

107 management by providing more accurate and convenient assessments of FFM through BIA. We

108 confirmed the possibility of evaluating FFM of APTs through the sERE developed in this study. For an

109 overview of abbreviations and parameters, see Table 1.

110

111

112 Materials & Methods

113 *Participants*: This study was approved by the Institutional Review Board (No. 1040875-201707-SB-030).

114 After receiving a complete description of the study, all participants provided written informed consent.

115 A total of 75 male APTs were recruited and the sERE development was applied to them. A total of 75

116 individuals who were unilateral APTs were included in the study. The mean participant age was 43.6±12

117 years. Seventeen participants had previously undergone upper limb amputation (trans-humeral

118 amputation: n=5; trans-radial amputation: n=12), and 58 had previously undergone lower limb amputation

119 (trans-femoral amputation: n=32; trans-tibial amputation: n=26). APTs with disarticulation and

120 multilateral (bi-, tri-) amputation were excluded. Additional participant characteristics including residual

121 limb length (cm) and onset (postoperative period, years) are presented in Table 2.

122

123 *Experimental device (DXA)*: DXA (Lunar Corp., Madison, WI, USA) measurements of FFM were used as

124 the reference standard in the development of the ERE. This instrument was calibrated through the spine

125 phantom provided by the manufacture daily. To standardize the scan, files from the original DXA system

126 were transferred to iDXA software, version 4.0.2. The scan process was blinded and fulfilled by one

127 radiologist who wore protective clothing. The segmentation method based on Heymsfield et al. (1990)

128 was applied to uniform measurement (Heymsfield et al. 1990).

129 Participants were instructed to wear comfortable clothing for the assessment. Under the guidance of a

130 professional examiner, each participant lay comfortably in the supine position on the assessment table,

131 following which they were asked to spread both the upper and lower limbs. Whole-body DXA was

132 performed for approximately 15 min.

133

134 *Experimental device (BIA)*: BIA of the tetrapolar 8-point electrode type (InBody S10 for the supine

135 measure, InBody Co. South Korea) was performed in this study. This BIA model uses eight electrodes

136   positioned at each hand and foot and enables multifrequency impedance measurement of the arms, trunk,

137   and legs. Impedance parameters were measured with alternating current of 80 and 100 mA at frequencies

138   of 1, 5, 50, 250, 500, and 1000 kHz for InBody S10. After checking the precision error of FFM about

139   repeatability through biplicate measurements by same APT based on previous study (Buckinx et al.

140   2015), the InBody S10 was used, which was designed for single measurements in the supine position and

141   operated using eight electrodes on the right and left upper and lower sides while being in the supine

142   position on a non-conductive surface through an SFBIA of only 50 kHz. In the sound limb, adhesive gel

143   electrodes were placed at defined anatomical sites cleaned with alcohol before attaching the electrode on

144   the dorsal surfaces of the hand, wrist, ankle, and foot as follows: the proximal edge of the wrist electrode

145   was attached from an imaginary line bisecting the styloid process of the ulna and the proximal edge of the

146   finger electrode on an imaginary line from the imaginary line bisecting the metatarsophalangeal joint of

147   the middle finger. The proximal edge of the ankle electrode was attached from an imaginary line bisecting

148   the medial malleolus and the distal edge of the toe electrode was placed from an imaginary line through

149   the metatarsophalangeal joints of the second toe as shown in Figure 1b. In the residual limb, the distal and

150   proximal electrodes were attached from the end of the stump (=distal part) to the region (=proximal part)

151   by keeping the distance according to the instructions of InBody S10. Additionally, fixed-distance of

152   electrodes was used with a 5-cm standard distance as shown in Figure 1c (Kaysen et al. 2005; Kriemler et

153   al. 2009). The device was calibrated every morning using the standard control circuit supplied by the

154   manufacturer. We confirmed that the precision error was less than 2% (Figure 1).

155

156   *Definition of segmental ZI (ZI) and regions of interest (ROI) in DXA*: Impedance indices (*ZI*) for each

157   body part were determined considering the residual limb length in each participant. *ZI* values were

158   calculated in accordance with methods described by Tanaka et al. (Tanaka et al. 2007). As shown in

159   Equation 1, *ZI* was calculated by dividing height^2 by *Z* (based on values for non-APTs). We then

160   calculated the body part length *ZI* (*ZI*$_{BPL}$) by dividing the body part length (BPL)^2 by Z. (Tanaka et al.

161   2007). BPL was measured for the following five areas: left arm (LA), right arm (RA), left leg (LL), right

162   leg (RL), and trunk (TR). The reference positions for the lengths of the upper/lower limbs and trunk were

163   defined as follows: the area between the humeral head (=acromion) and styloid processes of the wrist

164   (ulna) for the upper limbs; the area from the anterior superior iliac spine to the medial malleoli for the

165   lower limbs; and the posterior length between the seventh cervical vertebra prominens to the center of the

166   posterior superior iliac spine for the trunk (Beattie et al. 1990) (Hackenberg et al. 2003) (Jamaluddin et al.

167   2011) (Lee et al. 2016) (Neelly et al. 2013) (Ross 1972). The results of the experiment were analyzed by

168   matching the physical measurements to the BIA electrode locations and DXA ROI based on these

169   measurement standards.

170 According to the Heymsfield's protocol (1990), the boundaries of the ROI are defined as follows: 1) for

171 the upper limbs of the ROI (right and left), the arms are isolated by running a line through the humeral

172 head and 2) for the lower limbs, the pelvis cut is placed just above the pelvic brim and the computer

173 automatically draws the lower pelvic lines to bisect the hip joints (Heymsfield et al. 1990; Jeon et al.

174 2020).

175

$$ZI = \frac{H^2}{Z}$$ (Equation 1)

H: Height of the whole body, Z: Impedance

176

$$ZI_{BPL} = \frac{BPL^2}{Z}$$ (Equation 2)

BPL: Body part length, Z: Impedance

177

178 *Independent variables for segmental BIA*: Independent variables included in the EREs were determined

179 for the five body areas as follows: R was applied by differentiating among the LA (R_{LA}), RA (R_{RA}), LL

180 (R_{LL}), RL (R_{RL}), and trunk (R_{TR}). Using notations identical to those for R, impedance (Z), reactance (Xc),

181 and PA were also calculated for each body part and expressed in terms of R_{BPL}, Z_{BPL}, Xc_{BPL}, and $PA_{BPL.}$

182

183 *Experimental procedures*: Prior to the measurements of FFM, participants were instructed to abstain from

184 excessive dehydration-accompanied exercises and excessive alcohol use. In addition, they were instructed

185 to fast for at least 6 h and to abstain from drinking for at least 4 h.

186 We checked that the urinary bladder was voided in all participants within 30 minutes before

187 measurement, and we ensured that all participants wore non-conductive and comfortable sportswear. All

188 conductive materials, prosthetic limbs, and amputation covers (silicone, amputation protection, etc.) were

189 removed. First, DXA and then BIA test were measured consistently. For the measurements, after lying on

190 a DXA sheet, stability (stabilization of BC) measurement was taken for 5 min in the supine position

191 considering the potential for variable fluid shifts, and the DXA scan was performed, and BIA was

192 measured on the place immediately after the DXA scan without changing the position. All participants

193 maintained an abduction angle of approximately 15° for the shoulder and hip joints. The elbow and knee

194 joints were extended in a straight anatomical position. The physical contact of each electrode was ensured

195 in accordance with the criteria recommended by the manufacturer. Each measurement took approximately

196 5–15 min. Participants were instructed to maintain a comfortable position without any movement during

197 the examination (Brantlov et al. 2017; Kyle et al. 2004d).

198

199 *Sum of the segmentally-predicted FFM values by sERE (Sum_sEREs):* After the development of the

200 sEREs of the five body parts in the APTs, the sum of the segmentally-predicted FFM values by sERE was

201 calculated. We confirmed the validity and accuracy between DXA and the sum of sERE_BIA about FFM

202 through bivariate linear regression and the Bland_Altman plot.

203

204 *Statistical analysis:* The physical characteristics of the APTs group are presented as means with SDs. For

205 confirming the normality test, if there are fewer than 30 people, the normality test is required, but the

206 number of participants was 75; therefore, we assumed normality and analyzed all data (Kwak & Kim 2017;

207 Sang Gyu et al. 2019). The forward stepwise multiple linear regression analysis was used to develop sEREs

208 in the APTs group. The significance level was set to $p \leq 0.05$. Variables included in the initial analyses

209 contained ZI_{BPL}, R_{BPL}, Xc_{BPL}, PA_{BPL}, age (yr), height (cm), and weight (kg). The developmental equations

210 were selected by measures of goodness-of-fit statistics, including coefficient of determination (R^2), the

211 standard error of estimate (SEE), acceptable subjective rating of SEE (i.e. good to excellent) according to

212 the minimally acceptable standard for prediction errors (Buckinx et al. 2018; Heyward 2004; Lohman

213 1992a), and the variance inflation factor (VIF). The SEE measures the variation in the actual values from

214 the predicted values. The SEE represents the degree of deviation of individual scores form the regression

215 line. It is computed using the following formula:

216 $SEE = \sqrt{\sum(Measured\ \text{FFM} - Estimated\ \text{FFM})^2 / (N - p - 1)}$ where p = number of predicter

217 variables. The VIF assesses how much the variance of an estimated regression coefficient increases when

218 predictors are correlated for estimating collinearity/multicollinearity. In case of higher values of more than

219 10, it can be assumed that the regression coefficients are poorly estimated due to multi-collinearity to

220 remove predictors from the model. In our study, with values less than 10 (Lee et al. 2018; Wickramasinghe

221 et al. 2008), we could proceed with our regression analysis. In the cross-validation, the group predictive

222 accuracy of the Sum_sEREs was tested by calculating R^2, total error (*TE*: The TE represents the degree of

223 deviation from the line of identity using the formula: Total Error =

224 $\sqrt{\sum(Measured\ FFM - Estimated\ \text{FFM})^2 / N}$), and acceptable subjective rating of TE (Heyward &

225 Wagner 2004; Lohman 1992b). The individual predictive accuracy of these equations was also tested by

226 Bland-Altman plots that includes the bias of the mean difference between measured values of DXA and

227 predicted values of Sum_sEREs. We used the 95% limits of agreement (LOA) between equations, and

228 concordance correlation efficient ($r_{\text{y-y',mean}}$). Data were analyzed using Microsoft Office Excel Ver. 2013

229 (Microsoft, Redmond, WA, USA) and SPSS version 18.0 (IBM, USA).

230

231 Results

232   Segmental Estimated Regression Equation

233   DXA measurements of FFM were used as the dependent variable in the development of the EREs for use

234   in APTs. Various independent variables were entered to ensure optimal model development. Our model

235   considered factors such as R^2, multicollinearity (tolerance and variance inflation factor [VIF]), and

236   standard error estimates (SEE). Using these factors, we developed sEREs for the left and right

237   upper/lower limbs as well as the trunk. ZI_{LA}, Xc_{LA}, height, and age were entered as independent variables

238   in the final sERE model for FFM in the left arm (LA_{FFM}). Values for the final sERE for LA_{FFM} were as

239   follows: $R=0.95$, $R^2=0.90$, and adjusted $R^2=0.89$. ZI_{RA} and Xc_{RA} were entered as independent variables in

240   the final sERE model for FFM in the right arm.

241   Values for the final sERE for RA_{FFM} were as follows: $R=0.86$, $R^2=0.74$, and adjusted $R^2=0.73$. R_{BPL}, Xc_{BPL},

242   ZI_{BPL}, and weight were entered as independent variables in the final models for both the left and right

243   lower limbs. The LL_{FFM} model included R_{LL}, Xc_{LL}, ZI_{LL}, and weight. The final sERE values for LL_{FFM} were

244   as follows: $R=0.95$, $R^2=0.91$, and adjusted $R^2=0.90$. The highest correlation coefficients were observed for

245   RL_{FFM}: $R=0.97$, $R^2=0.94$, and adjusted $R^2=0.93$. In contrast, the lowest correlation coefficients were

246   observed for TR_{FFM}: $R=0.88$, $R^2=0.78$, and adjusted $R^2=0.76$. A total of five independent variables were

247   entered for the TR_{FFM} ERE: ZI_{TR}, weight, height, age, and R_{TR} (Table 3).

248

249   Cross-Validation between BIA and DXA about FFM

250   *Linear regression, total error, and line of identity*: Linear regression analyses were used to calculate the

251   correlation between BIA estimates based on the final segmental ERE and standard DXA measurements.

252   In the total error (*TE*) calculation (Equation 3), Y−Y′ represents the difference between the DXA

253   measurement (Y) and the BIA estimate (Y′), while N represents the sample size.

$$TE = \sqrt{\sum (Y - Y')^2 / N} \qquad \text{(Equation 3)}$$

254   The *TE* for the comparison between DXA and BIA values for FFM was 5.4 (kg), with a correlation of

255   0.84 ($p<0.05$) (Fig. 1).

256   Residuals and bias in Bland–Altman plots: To evaluate the validity of the final ERE, the residuals (i.e.,

257   the difference between BIA-estimated and DXA-measured FFM) and means of the two methods were

258   assessed using the Bland–Altman plot (Fig. 2). The bias of the difference between the two FFM

259   measurements was –4.60 kg. BIA estimates obtained using the ERE tended to be higher than those

260   obtained using DXA values. Furthermore, there was a tendency for the residuals of the bias to be evenly

261   distributed, and increased bias tended to be associated with decreased residuals.

262

263   DiscussionIn the present study, we aimed to develop sERE for BIA in APTs using DXA measurements as

264   the reference standard. Given the correlation (r=0.85), *TE* (5.4 kg), and coefficient of determination

265   (R^2=71%) between FFM values obtained using Sum_sEREs and DXA, our findings confirmed that the

266   sERE developed in this study could assess FFM in APTs.

267   Based on findings obtained in previous studies, gender differences should be considered in the selection

268   of independent variables to obtain more accurate estimates (Beaudart et al. 2020). However, this study

269   was conducted on only male APTs excluding female because of a difference in the gender ratio within the

270   total APTs recruited; hence, representativeness of gender could not be achieved. Furthermore, data from

271   the Multidimensional Body–Self Relations Questionnaire suggest that women experience significantly

272   higher level of dissatisfaction with their bodies than do men following amputation (Holzer et al. 2014).

273   For the above reasons, Thus, our sEREs were developed using data from male APTs only.

274   The process of developing sERE was carried out by applying the FFM of DXA on both the dominant

275   limb and the same limb BIA variables or vice versa. Therefore, the dominant and non-dominant limbs

276   were not considered specifically. Additionally, in the process of validation of the developed sERE for

277   each limb, the same limb variables were applied.

278   There are examples for various types of amputation for uni-, bi-, and multi-lateral APTs. However, in this

279   study, for the development of a basic sERE, only unilateral APTs were included to control the length

280   variable of the amputated site. Given the difficulty in controlling for the BPL among multilateral APTs,

281   we also restricted our participants to unilateral APTs.

282   The measurement range of the InBody S10 device (Biospace, Korea) extends from 5 to 1000 kHz (1, 5,

283   50, 250, 500, and 1000 kHz). MFBIA can more accurately measure intra- and extracellular body water

284   than SFBIA of 50 kHz. However, SFBIA of 50 kHz is better for measuring cell membrane properties

285   through Xc. Because, it provides equivalence of information for the function of Xc at 50 kHz

286   versus other frequencies (Piccoli et al. 2005). For developing basic sERE of APTs, we carefully

287   analyzed the cell membrane state of the APT rather than analyzing between the intra- and extracellular

288   body water properties (Kyle et al. 2004b) (Heymsfield et al. 2005). Only an SFBIA of 50 kHz was used

289   for the calculation of total body water, on which estimations for FFM are based using proprietary

290   equations (Achamrah et al. 2018).

291   Previous BIA studies have excluded APTs as well as patients with joint deformation, hemi-paralysis, and

292   uncommonly large/small bodies (Dogan et al. 2012) (Kyle et al. 2004e) (Mialich et al. 2014). Tanaka et

293   al. (2007) proposed using a tetrapolar 4-point BIA to measure BC in such individuals (Mialich et al.

294   2014) (Tanaka et al. 2007).

295   However, in a 4-point BIA with the tetrapolar system, the electrodes are attached manually by connecting

296   electrodes to measure the segments according to manufacturer's instructions. Four-point estimates are

297   derived based on the characteristics of the flow of current on one side of the body, allowing for the

298   calculation of BC for the specific body part, following which total estimates are obtained. Thus, the

299   measurements had substantial systematic errors, including underestimation or overestimation of accuracy

300   (Janssen et al. 2000) (Tanaka et al. 2007). Given the errors of the tetrapolar 4-point BIA, Foster and

301   Lukaski (1996) highlighted the need for further research regarding the use of such measurements for the

302   analyses of BC (Lukaski 1996). To avoid these issues, we utilized the tetrapolar 8-point segmental BIA

303   (sBIA) as InBody S10, in which the pairs of electrodes are attached to measure the different body

304   segments.

305   Before 1980, the ERE for FFM only included the resistance index (height^2/resistance). ZI was calculated

306   by taking into account the variables for the whole-body height in ERE of non-APTs. However, the sERE

307   of APT developed in this study was analyzed by applying ZI_{BPL} and BIA parameters considering the

308   length of each body part, including the residual limbs and condition of this amputated region.

309   In five sEREs for each part, the redundancy of variables was considered through VIF and the highly

310   accurate sEREs calculated through a meticulous analysis process based on the BIA characteristics that

311   affect the APT.

312   The BIA variables applied to the sEREs are R, Xc, and Z as shown in Table 3. we were to confirm that a

313   complex quantity composed of (R) which is caused by TBW, and capacitance of the cell membrane

314   related to (Xc), and obstruction to the flow of an alternating current (Z) that was dependent on the

315   frequency of the applied current based on the theoretical basis of BIA in the limbs of APT (Kyle et al.

316   2004a; Kyle et al. 2004c) (Khalil et al. 2014).

317   Generally, assuming our body as a cylinder, both arms and legs are attached to the body, and the whole-

318   body height is used for the ZI (ZI=Height^2/Z). However, in this APT study, the length of each limb was

319   substituted for the ZI of each limb (ZI_{BPL}=BPL^2/Z) in order to make the estimation equation of the

320   segmental limb considering amputated extremity. For example, in the process of developing estimating

321   equations, such as the FFM of each of the right upper and left upper limbs etc., the final model with a low

322   statistical error and a high estimating power was selected using the length and BIA variables of the same

323   body part. The APTs had imbalance between the left and right limbs, similar to that reported in a previous

324   study (Sherk et al. 2010), showing muscle and fat imbalance between the injured and sound limb;

325   consequently, there was a difference in variables entered in the final sERE obtained from the final model

326   selected as the criterion of the significance level (P<0.05), VIF (<10), SEE, and R^2.

327   In the present study, we utilized a forward stepwise multiple regression analysis that included diverse

328   independent variables such as residual limb length. When developing an ERE, approximately 20

329   participants are required for each independent variable entered. Given that our study included 75

330   participants, four or fewer independent variables are considered appropriate (Heyward & Wagner 2004).

331   These independent variables included ZI_{BPL}, height, weight, age, onset, and segmental BIA factors.

332   Table 3 shows the final EREs. The number of variables entered in each sERE ranged from two to five.

333   Although previous studies have specified that only four variables should be included, based on our

334   sample size, it was necessary to consider factors, such as R^2, multicollinearity (tolerance and VIF), and

335   SEE, to develop the most ideal model. Nonetheless, the sERE for the trunk was the only equation to have

336   been developed using five variables.

337   BIA estimates of FFM are based on TBW measurements, which are derived from Z values obtained by

338   passing microcurrents throughout the human body. In this calculation, the human body is assumed to be

339   cylindrical (TBW = ρ x height^2/Z, ρ=constant). Based on the theory that TBW comprises 73% of FFM

340   (TBW = FFM × 0.73), the estimates of FFM can be obtained using the following equation: FFM =

341   TBW/0.73. Factors, such as race, age, sex, and medical history, influence the unique conduction constant

342   (ρ) as well as the correlation between TBW and ZI, making it necessary to include several independent

343   variables in the TBW calculation (Kyle et al. 2004b). Therefore, in this study, we included additional

344   independent variables, such as onset and characteristics of the body part amputated.

345   Several BIA studies conducted outside of Korea have included patients with hemi-paralysis(Kafri et al.

346   2014) (Nalepa et al. 2019) (Yoo et al. 2016), pediatric scoliosis (Matusik et al. 2016), or Turner's

347   syndrome (i.e., abnormally small body)(Guedes et al. 2010). However, no such studies have been

348   conducted on the APTs. Despite this, studies have recommended that BIA measurements be obtained in

349   the non-amputated limb (Kyle et al. 2004e) (Mialich et al. 2014).

350   However, to estimate the whole-body FFM, the amputated body parts must be considered. In our study,

351   the sEREs for FFM did not exhibit a close relationship with PA_{BPL}, and we did not include PA as an

352   independent variable for any ERE in Table 3, similar to models developed using data from the general

353   population (Mialich et al. 2014). Meanwhile, Xc has been utilized in numerous EREs for FFM in studies

354   conducted outside Korea (Lieberman 1993) (Kyle et al. 2001) (Roubenoff et al. 1997) (Stolarczyk et al.

355   1994).

356   In accordance with previous findings, our sERE for FFM exhibited a close relationship with Xc, whereas

357   onset did not appear to exert a significant impact on FFM estimates. Although changes in BC occur over

358   time following amputation, FFM can be maintained with systematic rehabilitation and BC management

359   during the first postoperative year. This was noted by a previous study of those for whom 2 to 15 years

360   had passed since amputation (Eckard et al. 2015; Renstrom et al. 1983b).

361   In our study, the final sEREs were selected by considering factors, such as R^2, tolerance, VIF, and SEE.

362   As shown in Figure 2, the correlation for FFM values was high (R=0.84), with an R^2 of 71%. This

363   correlation is higher than the standard of 0.80 (R^2=64%) suggested by Heyward and Wagner (Heyward &

364   Wagner 2004) for validity research. A bivariate linear regression equation (Y = aX ± b) was used to

365   confirm the accuracy of Sum_sEREs as well as the correlation. The slope (a) and y-intercept (b) were

366   used to analyze the correlation between DXA and BIA measurements. The slope of the bivariate linear

367   regression equation was 0.89, whereas the y-intercept was 1.60, yielding a simply positive correlation of y

368   = x. However, the slope of 0.89 exhibited a positive correlation that was close to the standard of 1.

369   Furthermore, the y-intercept was close to the ideal standard of 0. Taken together, these results suggest the

370   possibility of sERE for APT.

371   Individual errors for DXA and BIA results are shown in the Bland–Altman plot in Figure 3. The average

372   value representing the difference between the two methods (i.e., Bias) was -4.60 kg. When the bias

373   approaches 0 (y=0), there is no mean difference in the measurement values with an ideal validity.

374   However, bias did not approach 0 in our study. BIA tended to overestimate FFM, relative to the value

375   obtained using DXA. Ainsworth (1997) suggested that the results are ideal if the proportion that exceeds

376   the standard of overestimation and underestimation is less than 30% (Ainsworth et al. 1997). In our study,

377   the proportion of APTs who exceeded the standard of bias was 50.7%, suggesting a need for further

378   validity studies.

379

380   Limitation

381

382   We used 50kHz to characterize the cell membrane. We will proceed with the MFBIA study including 1,

383   50, 250, 500, 1000kHz, and over frequencies, as a future research project. In this study, limitations were

384   placed on the use of only 50kHz frequency. In this study, a single frequency of 50 kHz was used to

385   explain the characteristics of homeostasis for cell membrane well, but ICF and ECF have limitations in

386   reflecting the characteristics at a normal level. In this study, limitations were placed on the use of only 50

387   kHz frequency. In validation procedure, we recognized importance of validating method through a split

388   group design, K-fold or LOOV-type. However, without external cross-validation through group of

389   predictive power test, only the sEREs were developed. We confirmed only cross-validation between DXA

390   and Sum_sEREs of FFM values as a limitation. This study was considered to be possible study of APT's

391   sERE for the first time, and we would plan to conduct a validation study by recruiting more APTs in

392   future studies. In this study, we did not perform a thoughtful analysis of the PA, but it will be done for the

393   healthcare of the APTs in the future.

394

395

396   Conclusions

397

398   In the present study, we utilized multiple regression analysis to develop an sERE for FFM in APTs, using

399   DXA as the reference standard. There were bias (-4.598), and LOA (-10.26~1.06) as deficiencies in the

400   results of this study. However, the minimal clinical feasibility may be confirmed based on the coefficient

401   of determination (R^2=71%), and TE (5.40 kg). The accuracy is not high, but the possibility of using it has

402   been confirmed. We processed meaningful study as a sERE of APTs excluded subjects from the past. It is

403   believed to be basically possible research of APT's sERE.

404   In addition, we identified several independent variables that should be considered while developing such

405   sERE for APT. Further studies are required to determine the validity of our sERE and the most

406   appropriate BIA frequencies for measuring FFM in APTs.

407

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411

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413   References

414   Achamrah N, Colange G, Delay J, Rimbert A, Folope V, Petit A, Grigioni S, Déchelotte P, and
415   Coëffier M. 2018. Comparison of body composition assessment by DXA and BIA
416   according to the body mass index: A retrospective study on 3655 measures. *PLoS ONE*
417   13:e0200465. 10.1371/journal.pone.0200465
418   Ainsworth BE, Stolarczyk LM, Heyward VH, Berry CB, Irwin ML, and Mussulman LM. 1997.
419   Predictive accuracy of bioimpedance in estimating fat-free mass of African-American
420   women. *Med Sci Sports Exerc* 29:781-787. 10.1097/00005768-199706000-00008
421   Beattie P, Isaacson K, Riddle DL, and Rothstein JM. 1990. Validity of derived measurements of
422   leg-length differences obtained by use of a tape measure. *Phys Ther* 70:150-157.
423   10.1093/ptj/70.3.150
424   Beaudart C, Bruyère O, Geerinck A, Hajaoui M, Scafoglieri A, Perkisas S, Bautmans I, Gielen E,
425   Reginster JY, and Buckinx F. 2020. Equation models developed with bioelectric
426   impedance analysis tools to assess muscle mass: A systematic review. *Clin Nutr*
427   *ESPEN* 35:47-62. 10.1016/j.clnesp.2019.09.012
428   Brantlov S, Ward LC, Jødal L, Rittig S, and Lange A. 2017. Critical factors and their impact on
429   bioelectrical impedance analysis in children: a review. *J Med Eng Technol* 41:22-35.
430   10.1080/03091902.2016.1209590
431   Buckinx F, Landi F, Cesari M, Fielding RA, Visser M, Engelke K, Maggi S, Dennison E, Al-
432   Daghri NM, Allepaerts S, Bauer J, Bautmans I, Brandi ML, Bruyère O, Cederholm T,
433   Cerreta F, Cherubini A, Cooper C, Cruz-Jentoft A, McCloskey E, Dawson-Hughes B,
434   Kaufman JM, Laslop A, Petermans J, Reginster JY, Rizzoli R, Robinson S, Rolland Y,
435   Rueda R, Vellas B, and Kanis JA. 2018. Pitfalls in the measurement of muscle mass: a
436   need for a reference standard. *J Cachexia Sarcopenia Muscle* 9:269-278.
437   10.1002/jcsm.12268
438   Buckinx F, Reginster JY, Dardenne N, Croisiser JL, Kaux JF, Beaudart C, Slomian J, and
439   Bruyère O. 2015. Concordance between muscle mass assessed by bioelectrical

440 impedance analysis and by dual energy X-ray absorptiometry: a cross-sectional study.
441 *BMC Musculoskelet Disord* 16:60. 10.1186/s12891-015-0510-9
442 Bukowski EL. 2006. Atlas of Amputations and Limb Deficiencies: Surgical, Prosthetic, and
443 Rehabilitation Principles, ed 3. *Phys Ther* 86:595-596. 10.1093/ptj/86.4.595
444 Campa F, and Toselli S. 2018. Bioimpedance Vector Analysis of Elite, Subelite, and Low-Level
445 Male Volleyball Players. *Int J Sports Physiol Perform* 13:1250-1253. 10.1123/ijspp.2018-
446 0039
447 Centomo H, Amarantini D, Martin L, and Prince F. 2008. Differences in the coordination of
448 agonist and antagonist muscle groups in below-knee amputee and able-bodied children
449 during dynamic exercise. *J Electromyogr Kinesiol* 18:487-494.
450 10.1016/j.jelekin.2006.11.008
451 Charatsi AM, Dusser P, Freund R, Maruani G, Rossin H, Boulier A, Le Bourgeois M,
452 Chedevergne F, de Blic J, Letourneur A, Casimir G, Jais JP, and Sermet-Gaudelus I.
453 2016. Bioelectrical impedance in young patients with cystic fibrosis: Validation of a
454 specific equation and clinical relevance. *J Cyst Fibros* 15:825-833.
455 10.1016/j.jcf.2016.05.004
456 Chin T, Sawamura S, Fujita H, Nakajima S, Oyabu H, Nagakura Y, Ojima I, Otsuka H, and
457 Nakagawa A. 2002. Physical fitness of lower limb amputees. *Am J Phys Med Rehabil*
458 81:321-325. 10.1097/00002060-200205000-00001
459 Coffey L, Gallagher P, and Desmond D. 2014. Goal pursuit and goal adjustment as predictors of
460 disability and quality of life among individuals with a lower limb amputation: a
461 prospective study. *Arch Phys Med Rehabil* 95:244-252. 10.1016/j.apmr.2013.08.011
462 Colica C, Di Renzo L, Gualtieri P, Romano L, Costa de Miranda R, De Lorenzo A, and Purificato
463 I. 2018. Development and cross-validation of predictive equation for estimating total
464 body lean in children. *Ann Ist Super Sanita* 54:20-27. 10.4415/ann_18_01_06
465 Dogan MH, Karadag B, Ozyigit T, Kayaoglu S, Ozturk AO, and Altuntas Y. 2012. Correlations
466 between sarcopenia and hypertensive target organ damage in a Turkish cohort. *Acta*
467 *Clin Belg* 67:328-332. 10.2143/ACB.67.5.2062685
468 Eckard CS, Pruziner AL, Sanchez AD, and Andrews AM. 2015. Metabolic and body composition
469 changes in first year following traumatic amputation. *J Rehabil Res Dev* 52:553-562.
470 10.1682/JRRD.2014.02.0044
471 Gallagher P, Allen D, and Maclachlan M. 2001. Phantom limb pain and residual limb pain
472 following lower limb amputation: a descriptive analysis. *Disabil Rehabil* 23:522-530.
473 10.1080/09638280010029859
474 Guedes AD, Bianco B, Lipay MV, Callou EQ, Castro ML, and Verreschi IT. 2010. A specific
475 bioelectrical impedance equation to predict body composition in Turner's syndrome. *Arq*
476 *Bras Endocrinol Metabol* 54:24-29. 10.1590/s0004-27302010000100005
477 Hackenberg L, Hierholzer E, Potzl W, Gotze C, and Liljenqvist U. 2003. Rasterstereographic
478 back shape analysis in idiopathic scoliosis after posterior correction and fusion. *Clin*
479 *Biomech (Bristol, Avon)* 18:883-889. 10.1016/s0268-0033(03)00169-4
480 Hecht F, MedicineNet.com, and Shiel WC. 2003. Webster's New World Medical Dictionary, 3rd
481 Edition.
482 Heymsfield S, Lohman T, Wang ZM, and Going SB. 2005. Human Body Composition. 2nd ed.
483 *Champaign IL Human Kinetics*.
484 Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J, and Pierson RN, Jr. 1990.
485 Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. *Am J*
486 *Clin Nutr* 52:214-218. 10.1093/ajcn/52.2.214
487 Heyward VH, and Wagner DR. 2004. *Applied body composition assessment*. Leeds: Human
488 Kinetics.
489 Heyward VH, Wagner, D. R. 2004. *Applied body composition assessment*: Human Kinetics,
490 Champaign, IL.

491   Holzer LA, Sevelda F, Fraberger G, Bluder O, Kickinger W, and Holzer G. 2014. Body image
492   and self-esteem in lower-limb amputees. *PLoS ONE* 9:e92943.
493   10.1371/journal.pone.0092943
494   Isakov E, Burger H, Gregoric M, and Marincek C. 1996. Stump length as related to atrophy and
495   strength of the thigh muscles in trans-tibial amputees. *Prosthet Orthot Int* 20:96-100.
496   10.3109/03093649609164425
497   Jamaluddin S, Sulaiman AR, Imran MK, Juhara H, Ezane MA, and Nordin S. 2011. Reliability
498   and accuracy of the tape measurement method with a nearest reading of 5 mm in the
499   assessment of leg length discrepancy. *Singapore Med J* 52:681-684.
500   Janssen I, Heymsfield SB, Baumgartner RN, and Ross R. 2000. Estimation of skeletal muscle
501   mass by bioelectrical impedance analysis. *J Appl Physiol (1985)* 89:465-471.
502   10.1152/jappl.2000.89.2.465
503   Janssen I, Heymsfield SB, and Ross R. 2002. Application of simple anthropometry in the
504   assessment of health risk: implications for the Canadian Physical Activity, Fitness and
505   Lifestyle Appraisal. *Can J Appl Physiol* 27:396-414. 10.1139/h02-021
506   Jeon KC, Kim SY, Jiang FL, Chung S, Ambegaonkar JP, Park JH, Kim YJ, and Kim CH. 2020.
507   Prediction Equations of the Multifrequency Standing and Supine Bioimpedance for
508   Appendicular Skeletal Muscle Mass in Korean Older People. *Int J Environ Res Public*
509   *Health* 17. 10.3390/ijerph17165847
510   Kafri MW, Potter JF, and Myint PK. 2014. Multi-frequency bioelectrical impedance analysis for
511   assessing fat mass and fat-free mass in stroke or transient ischaemic attack patients.
512   *Eur J Clin Nutr* 68:677-682. 10.1038/ejcn.2013.266
513   Kaysen GA, Zhu F, Sarkar S, Heymsfield SB, Wong J, Kaitwatcharachai C, Kuhlmann MK, and
514   Levin NW. 2005. Estimation of total-body and limb muscle mass in hemodialysis patients
515   by using multifrequency bioimpedance spectroscopy. *Am J Clin Nutr* 82:988-995.
516   10.1093/ajcn/82.5.988
517   Khalil SF, Mohktar MS, and Ibrahim F. 2014. The theory and fundamentals of bioimpedance
518   analysis in clinical status monitoring and diagnosis of diseases. *Sensors (Basel)*
519   14:10895-10928. 10.3390/s140610895
520   Kopelman PG. 2000. Obesity as a medical problem. *Nature* 404:635-643. 10.1038/35007508
521   Kriemler S, Puder J, Zahner L, Roth R, Braun-Fahrländer C, and Bedogni G. 2009. Cross-
522   validation of bioelectrical impedance analysis for the assessment of body composition in
523   a representative sample of 6- to 13-year-old children. *European Journal of Clinical*
524   *Nutrition* 63:619-626. 10.1038/ejcn.2008.19
525   Kulkarni J, Adams J, Thomas E, and Silman A. 1998. Association between amputation, arthritis
526   and osteopenia in British male war veterans with major lower limb amputations. *Clin*
527   *Rehabil* 12:348-353. 10.1191/026921598672393611
528   Kurdibaylo SF. 1996. Obesity and metabolic disorders in adults with lower limb amputation. *J*
529   *Rehabil Res Dev* 33:387-394.
530   Kwak SG, and Kim JH. 2017. Central limit theorem: the cornerstone of modern statistics.
531   *Korean J Anesthesiol* 70:144-156. 10.4097/kjae.2017.70.2.144
532   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-
533   Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C, and Composition
534   of the EWG. 2004a. Bioelectrical impedance analysis--part I: review of principles and
535   methods. *Clin Nutr* 23:1226-1243. 10.1016/j.clnu.2004.06.004
536   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-
537   Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AMWJ, and Pichard C. 2004b.
538   Bioelectrical impedance analysis - Part I: Review of principles and methods. *Clinical*
539   *Nutrition* 23:1226-1243. 10.1016/j.clnu.2004.06.004
540   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-
541   Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AMWJ, and Pichard C. 2004c.

542   Bioelectrical impedance analysis - Part II: Utilization in clinical practice. *Clinical Nutrition*
543   23:1430-1453. 10.1016/j.clnu.2004.09.012
544   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gomez J, Lilienthal
545   Heitmann B, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, A MWJS, and Pichard
546   C. 2004d. Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr*
547   23:1430-1453. 10.1016/j.clnu.2004.09.012
548   Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gomez J, Lilienthal
549   Heitmann B, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, A MWJS, Pichard C,
550   and Espen. 2004e. Bioelectrical impedance analysis-part II: utilization in clinical practice.
551   *Clin Nutr* 23:1430-1453. 10.1016/j.clnu.2004.09.012
552   Kyle UG, Genton L, Karsegard L, Slosman DO, and Pichard C. 2001. Single prediction equation
553   for bioelectrical impedance analysis in adults aged 20--94 years. *Nutrition* 17:248-253.
554   10.1016/s0899-9007(00)00553-0
555   Lee BJ, Cha HG, and Lee WH. 2016. The effects of sitting with the right leg crossed on the
556   trunk length and pelvic torsion of healthy individuals. *J Phys Ther Sci* 28:3162-3164.
557   10.1589/jpts.28.3162
558   Lee SY, Ahn S, Kim YJ, Ji MJ, Kim KM, Choi SH, Jang HC, and Lim S. 2018. Comparison
559   between Dual-Energy X-ray Absorptiometry and Bioelectrical Impedance Analyses for
560   Accuracy in Measuring Whole Body Muscle Mass and Appendicular Skeletal Muscle
561   Mass. *Nutrients* 10. 10.3390/nu10060738
562   Lee SY, and Gallagher D. 2008. Assessment methods in human body composition. *Curr Opin*
563   *Clin Nutr Metab Care* 11:566-572. 10.1097/MCO.0b013e32830b5f23
564   Lieberman LS. 1993. Advances in body composition assessment. By Timothy G. Lohman. vii +
565   150 pp. Champaign, IL: Human Kinetics Publishers, 1992. $18.00 (paper). *American*
566   *Journal of Human Biology* 5:593-593. 10.1002/ajhb.1310050514
567   Lohman TG. 1992a. *Advances in Body Composition Assessment*: Human Kinetics, Champaign,
568   IL.
569   Lohman TG. 1992b. Advances in Body Composition Assessment.
570   Lukaski HC. 1996. Biological indexes considered in the derivation of the bioelectrical impedance
571   analysis. *Am J Clin Nutr* 64:397s-404s. 10.1093/ajcn/64.3.397S
572   Matusik E, Durmala J, and Matusik P. 2016. Association of Body Composition with Curve
573   Severity in Children and Adolescents with Idiopathic Scoliosis (IS). *Nutrients* 8:71.
574   10.3390/nu8020071
575   Mialich MS, Sicchieri JMF, and Junior AAJ. 2014. Analysis of Body Composition: A Critical
576   Review of the Use of Bioelectrical Impedance Analysis. *International Journal of Clinical*
577   *Nutrition* 2:1-10.
578   Mishra SC, Chhatbar KC, Kashikar A, and Mehndiratta A. 2017. Diabetic foot. *Bmj* 359:j5064.
579   10.1136/bmj.j5064
580   Mulasi U, Kuchnia AJ, Cole AJ, and Earthman CP. 2015. Bioimpedance at the bedside: current
581   applications, limitations, and opportunities. *Nutr Clin Pract* 30:180-193.
582   10.1177/0884533614568155
583   Nalepa D, Czarkowska M, Zaluska W, Jakubowska K, and Chrusciel P. 2019. Electrical
584   bioimpedance in patients after ischemic stroke, a civilization disease. *Ann Agric Environ*
585   *Med* 26:46-50. 10.26444/aaem/84849
586   Neelly K, Wallmann HW, and Backus CJ. 2013. Validity of measuring leg length with a tape
587   measure compared to a computed tomography scan. *Physiother Theory Pract* 29:487-
588   492. 10.3109/09593985.2012.755589
589   Nguyen QD, Fusch G, Armbrust S, Jochum F, and Fusch C. 2007. Impedance index or
590   standard anthropometric measurements, which is the better variable for predicting fat-
591   free mass in sick children? *Acta Paediatr* 96:869-873. 10.1111/j.1651-
592   2227.2007.00272.x

593   O'Sullivan SB, Schmitz TJ, and Fulk G. 2013. Physical Rehabilitation.
594   Piccoli A, Pastori G, Guizzo M, Rebeschini M, Naso A, and Cascone C. 2005. Equivalence of
595   information from single versus multiple frequency bioimpedance vector analysis in
596   hemodialysis. *Kidney Int* 67:301-313. 10.1111/j.1523-1755.2005.00083.x
597   Rahman M, and Berenson AB. 2010. Accuracy of current body mass index obesity classification
598   for white, black, and Hispanic reproductive-age women. *Obstet Gynecol* 115:982-988.
599   10.1097/AOG.0b013e3181da9423
600   Renstrom P, Grimby G, and Larsson E. 1983a. Thigh muscle strength in below-knee amputees.
601   *Scand J Rehabil Med Suppl* 9:163-173.
602   Renstrom P, Grimby G, Morelli B, and Palmertz B. 1983b. Thigh muscle atrophy in below-knee
603   amputees. *Scand J Rehabil Med Suppl* 9:150-162.
604   Robbins CB, Vreeman DJ, Sothmann MS, Wilson SL, and Oldridge NB. 2009. A review of the
605   long-term health outcomes associated with war-related amputation. *Mil Med* 174:588-
606   592. 10.7205/milmed-d-00-0608
607   Rosenberg DE, Turner AP, Littman AJ, Williams RM, Norvell DC, Hakimi KM, and Czerniecki
608   JM. 2013. Body mass index patterns following dysvascular lower extremity amputation.
609   *Disabil Rehabil* 35:1269-1275. 10.3109/09638288.2012.726690
610   Ross CA. 1972. Guidelines for measurement of amputation stump length. *Bulletin of Prosthetics
611   Research*:67-82.
612   Roubenoff R, Baumgartner RN, Harris TB, Dallal GE, Hannan MT, Economos CD, Stauber PM,
613   Wilson PW, and Kiel DP. 1997. Application of bioelectrical impedance analysis to elderly
614   populations. *J Gerontol A Biol Sci Med Sci* 52:M129-136. 10.1093/gerona/52a.3.m129
615   Sadeghi H, Allard P, and Duhaime PM. 2001. Muscle power compensatory mechanisms in
616   below-knee amputee gait. *Am J Phys Med Rehabil* 80:25-32. 10.1097/00002060-
617   200101000-00007
618   Sang Gyu K, lt, sup, gt, lt, sup, gt, Sung-Hoon P, lt, sup, gt, lt, sup, and gt. 2019. Normality Test
619   in Clinical Research. *Journal of Rheumatic Diseases* 26:5-11. 10.4078/jrd.2019.26.1.5
620   Sardinha LB, Correia IR, Magalhães JP, Júdice PB, Silva AM, and Hetherington-Rauth M. 2020.
621   Development and validation of BIA prediction equations of upper and lower limb lean
622   soft tissue in athletes. *Eur J Clin Nutr* 74:1646-1652. 10.1038/s41430-020-0666-8
623   Sherk VD, Bemben MG, and Bemben DA. 2010. Interlimb muscle and fat comparisons in
624   persons with lower-limb amputation. *Arch Phys Med Rehabil* 91:1077-1081.
625   10.1016/j.apmr.2010.04.008
626   Silveira EA, Barbosa LS, Rodrigues APS, Noll M, and De Oliveira C. 2020. Body fat percentage
627   assessment by skinfold equation, bioimpedance and densitometry in older adults. *Arch
628   Public Health* 78:65. 10.1186/s13690-020-00449-4
629   Stolarczyk LM, Heyward VH, Hicks VL, and Baumgartner RN. 1994. Predictive accuracy of
630   bioelectrical impedance in estimating body composition of Native American women. *Am
631   J Clin Nutr* 59:964-970. 10.1093/ajcn/59.5.964
632   Stone PA, Flaherty SK, Aburahma AF, Hass SM, Jackson JM, Hayes JD, Hofeldt MJ, Hager
633   CS, and Elmore MS. 2006. Factors affecting perioperative mortality and wound-related
634   complications following major lower extremity amputations. *Ann Vasc Surg* 20:209-216.
635   10.1007/s10016-006-9009-z
636   Suk S, Bom PS, and Do KS. 2001. Assessment of Quality of Life in Lower Limb Amputees
637   Using Short-Form 36. *Ann Rehabil Med* 25:505-513.
638   Tanaka NI, Miyatani M, Masuo Y, Fukunaga T, and Kanehisa H. 2007. Applicability of a
639   segmental bioelectrical impedance analysis for predicting the whole body skeletal
640   muscle volume. *J Appl Physiol (1985)* 103:1688-1695. 10.1152/japplphysiol.00255.2007
641   Toselli S, Marini E, Maietta Latessa P, Benedetti L, and Campa F. 2020. Maturity Related
642   Differences in Body Composition Assessed by Classic and Specific Bioimpedance

643   Vector Analysis among Male Elite Youth Soccer Players. *Int J Environ Res Public Health*
644   17. 10.3390/ijerph17030729
645 Ustun TB, Chatterji S, Bickenbach J, Kostanjsek N, and Schneider M. 2003. The International
646   Classification of Functioning, Disability and Health: a new tool for understanding
647   disability and health. *Disabil Rehabil* 25:565-571. 10.1080/0963828031000137063
648 Walter-Kroker A, Kroker A, Mattiucci-Guehlke M, and Glaab T. 2011. A practical guide to
649   bioelectrical impedance analysis using the example of chronic obstructive pulmonary
650   disease. *Nutrition journal* 10:35. 10.1186/1475-2891-10-35
651 Ward LC. 2019. Bioelectrical impedance analysis for body composition assessment: reflections
652   on accuracy, clinical utility, and standardisation. *Eur J Clin Nutr* 73:194-199.
653   10.1038/s41430-018-0335-3
654 Wickramasinghe VP, Lamabadusuriya SP, Cleghorn GJ, and Davies PSW. 2008. Assessment
655   of body composition in Sri Lankan children: validation of a bioelectrical impedance
656   prediction equation. *European Journal of Clinical Nutrition* 62:1170-1177.
657   10.1038/sj.ejcn.1602835
658 Woodrow G. 2009. Body composition analysis techniques in the aged adult: indications and
659   limitations. *Curr Opin Clin Nutr Metab Care* 12:8-14. 10.1097/MCO.0b013e32831b9c5b
660 Yoo C, Kim J, Yang Y, Lee J, and Jeon G. 2016. Bioelectrical impedance analysis for severe
661   stroke patients with upper extremity hemiplegia. *J Phys Ther Sci* 28:2708-2712.
662   10.1589/jpts.28.2708-2712
663 Yoo S. 2014. Complications following an amputation. *Phys Med Rehabil Clin N Am* 25:169-178.
664   10.1016/j.pmr.2013.09.003
665 Zachariah SG, Saxena R, Fergason JR, and Sanders JE. 2004. Shape and volume change in
666   the transtibial residuum over the short term: preliminary investigation of six subjects. *J*
667   *Rehabil Res Dev* 41:683-694. 10.1682/jrrd.2003.10.0153
668 Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, and Brookmeyer R. 2008.
669   Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys*
670   *Med Rehabil* 89:422-429. 10.1016/j.apmr.2007.11.005
671

Figure 1

The testing postures and the electrode placements

(A) InBody S10 in the supine position [permitted from the manufacture] (B) Electrode placements of sound limbs (C) Electrode placements of residual limbs

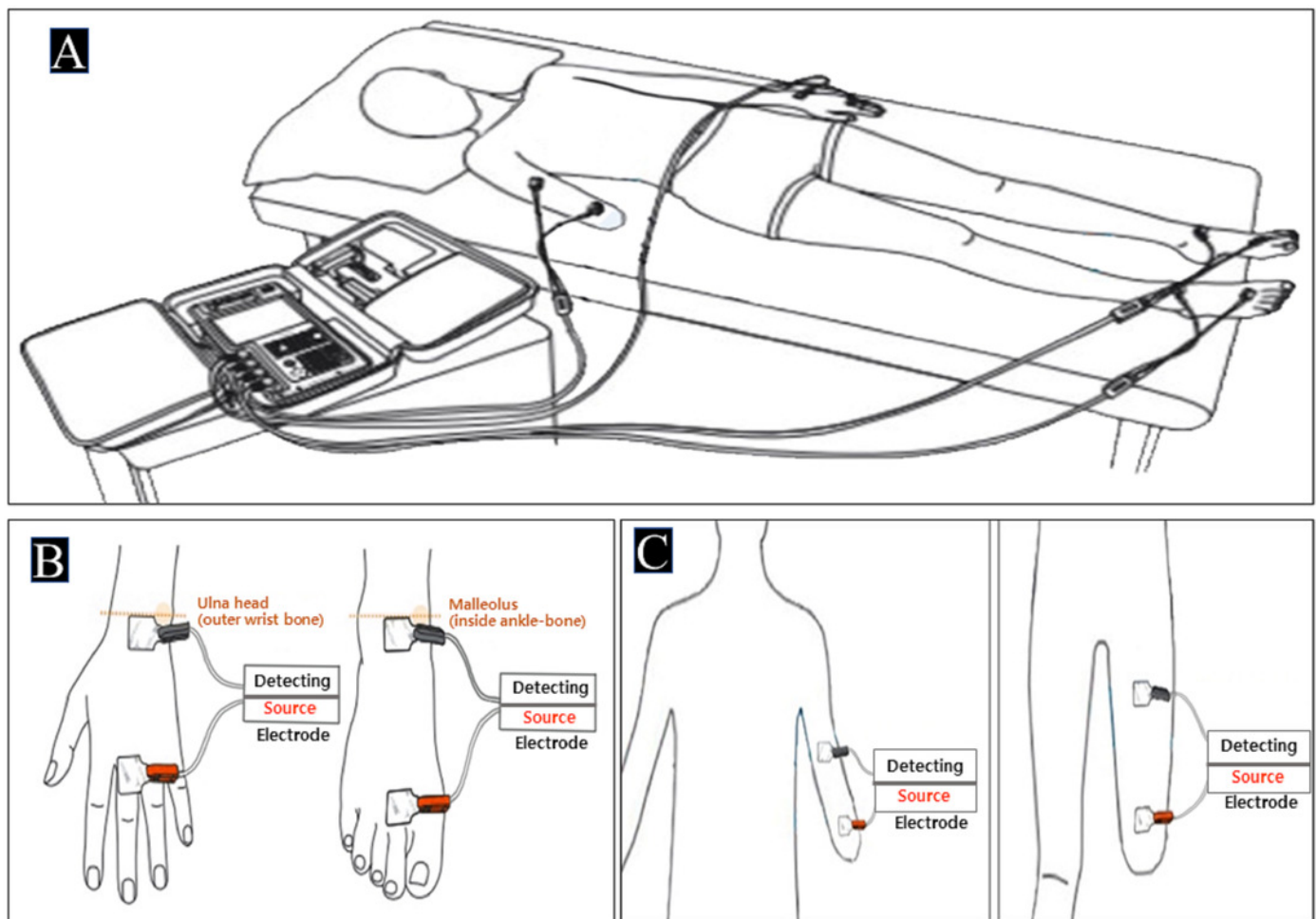



Figure 2

Bivariate linear regression for FFM values obtained using DXA and sEREs

FFM: fat-free mass (kg), sEREs: segmental estimated regression equations, TE: total error, r: validity coefficient, DXA: dual-energy X-ray absorptiometry

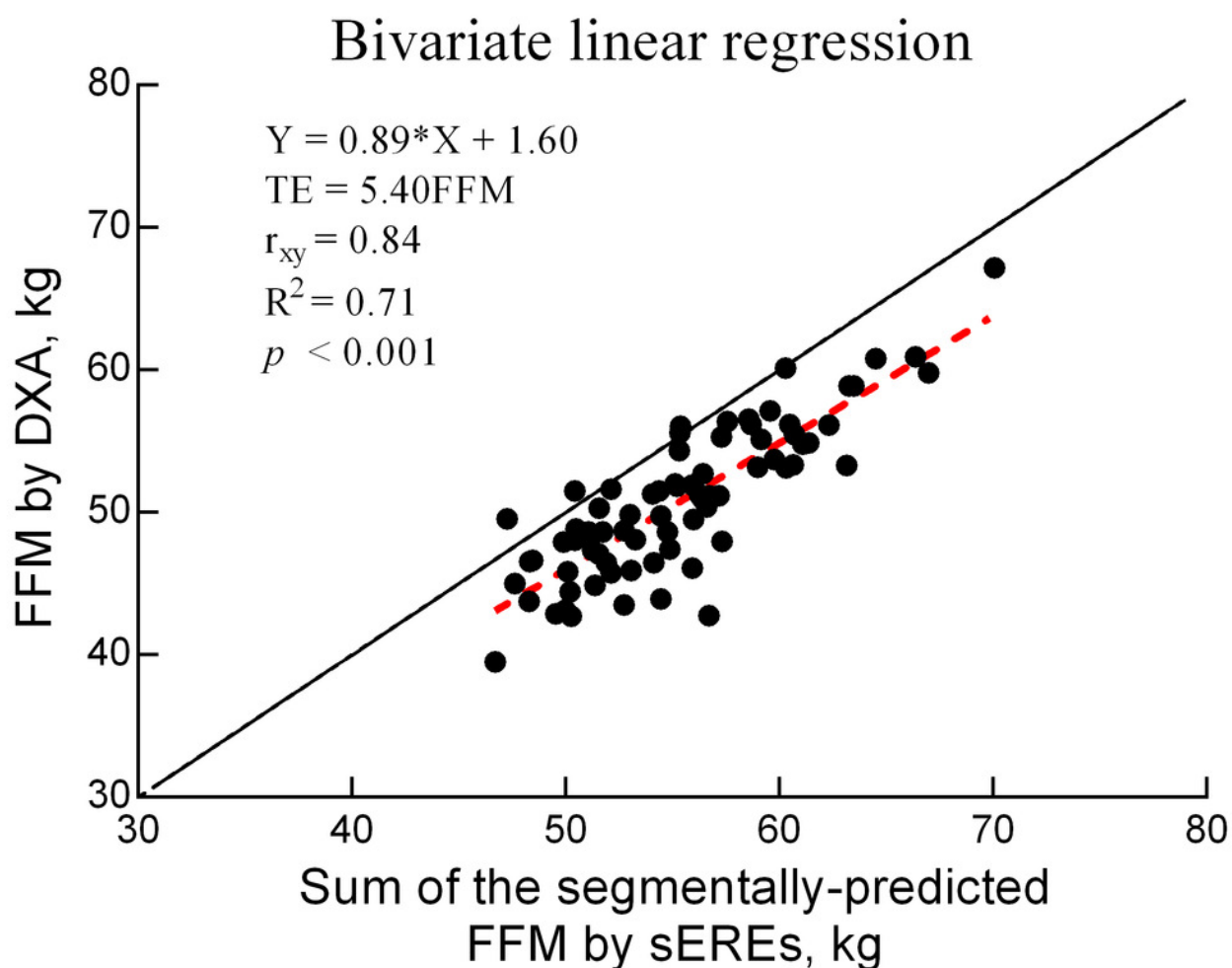



Figure 3

Bland–Altman plot

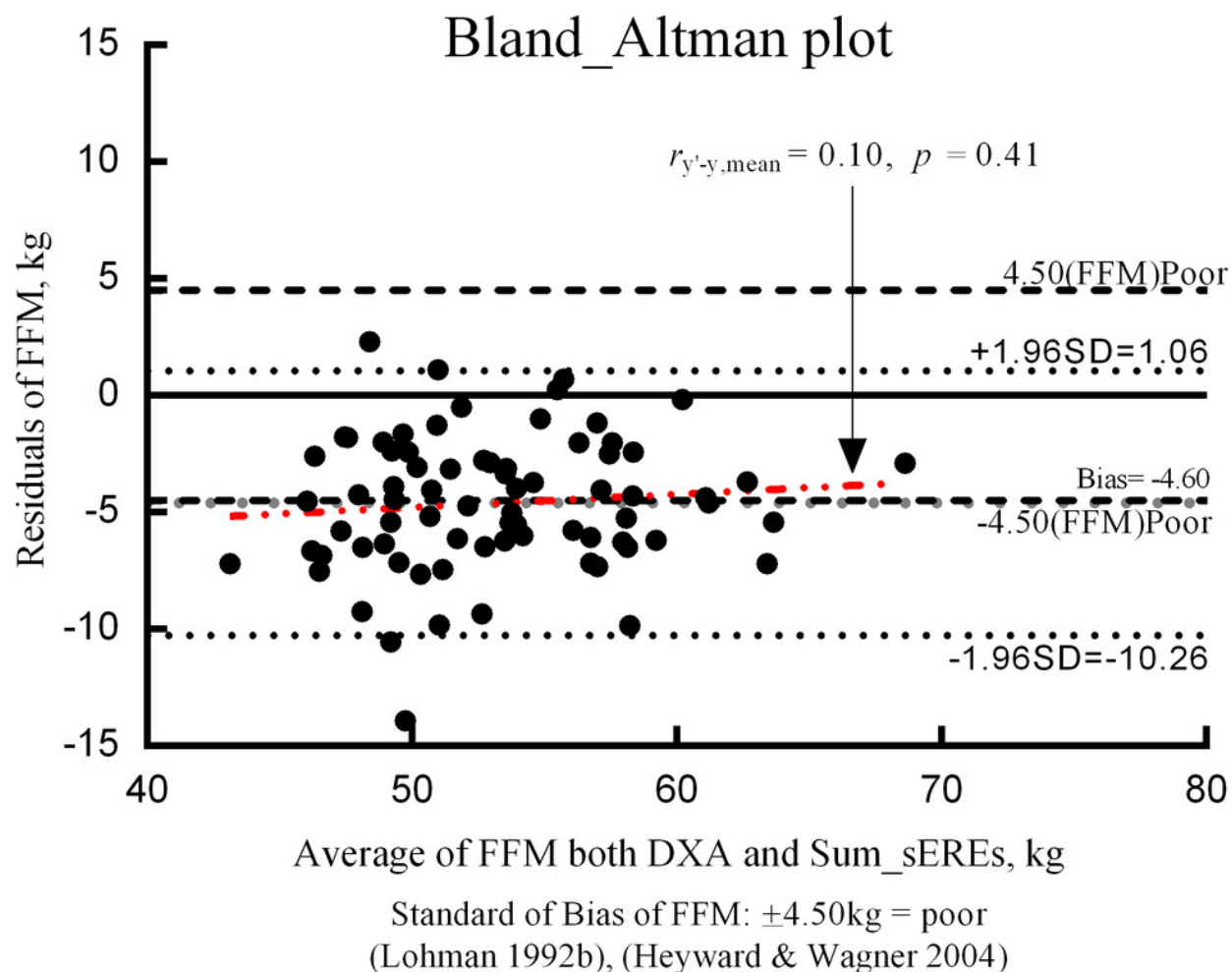
Bias: mean of DXA-BIA value, ±4.5(FFM) poor = "poor" standard for evaluating prediction errors, FFM: fat-free mass (kg), DXA: dual-energy X-ray absorptiometry, sEREs: segmental estimated regression equations

Abbreviations and concepts

Table 1. Abbreviations and concepts

Abbreviation	Description
General parameters:	
BC	Body composition
BIA	Bioimpedance analysis
BIS	Bioimpedance spectroscopy
BPL	Body part length
DXA	Dual-energy X-ray absorptiometry
ERE	Estimated regression equations
MFBIA	Multi-frequency bioimpedance analysis
ROI	Regions of interest
sEREs	Segmental estimated regression equations
SFBIA	Single-frequency bioimpedance analysis
Sum_sEREs	Sum of the segmentally-predicted FFM values by sEREs
Subjects parameters:	
APTs	Amputees
LA, RA, LL, RL, TR	Left arm (LA), Right arm (RA), Left leg (LL), Right leg (RL), Trunk (TR)
Physiological parameters:	
BMC	Bone mineral content (kg)
FFM	Fat-free mass (kg)
FM	Fat mass (kg)
SLTM	Soft lean tissue mass (kg)
TBW	Total body water (L)
BIA parameters:	
PA	Phage angle (°)
R	Resistance (Ohm, Ω)
Xc	Reactance (Ohm, Ω)
Z	Impedance (Ohm, Ω).
ZI	Impedance index ($ZI = Height^2/Z$)
Statistical analysis parameters:	
LOA	Limits of agreement
R^2	Coefficient of determination
SEE	Standard error of estimate
TE	Total error

VIF	Variance inflation factor

Participant characteristics

*Mean ± SD; APTs: amputees; BMI: body mass index; SD: standard deviation

Table 2. Participant characteristics				
	Upper-limb APTs (n=17)		Lower-limb APTs (n=58)	
	Trans-humeral (n=5)	Trans-radial (n=12)	Trans-femoral (n=32)	Trans-tibial (n=26)
Age (year)	42.6 ± 5.7*	50.4 ± 11.6	41.1 ± 13.3	43.6 ± 10.5
Height (cm)	174.6 ± 4.2	168.3 ± 7.2	172.0 ± 5.9	171.4 ± 5.6
Weight (kg)	78.7 ± 4.0	74.2 ± 7.6	73.7 ± 13.7	73.2 ± 12.5
BMI (kg/m²)	25.9 ± 2.3	26.2 ± 2.0	24.8 ± 3.8	24.9 ± 3.6
Residual limb Length (cm)	21.4 ± 3.1	42.7 ± 4.9	34.6 ± 6.2	62.2 ± 6.7
Onset (year)	13.6 ± 7.9	16.8 ± 11.8	13.2 ± 11.9	18.2 ± 13.9

*Mean ± SD; APTs: amputees; BMI: body mass index; SD: standard deviation

The Final segmental estimated regression equations for FFM (kg)

FFM: fat-free mass (kg); ZI_{BPL}: body part length impedance index; Z_{BPL}: impedance; Xc_{BPL}: reactance; R_{BPL}: resistance; LA: left arm; RA: right arm; TR: trunk; LL: left leg; RL: right leg; Adj.: Adjusted; VIF: variation inflation factor; SEE: standard error estimate (kg)

Table 3. The Final segmental estimated regression equations for FFM (kg)

LA_{FFM}	$y = -3.759 + 0.204(ZI_{LA}) + 0.410(Xc_{LA}) + 0.019(\text{height}) - 0.007(\text{age})$ R = .948 R² = .898 Adj. R ² = .892 SEE = 0.286 VIF: ZI_{LA} = 1.286, Xc_{LA} = 1.193, height = 1.378, age = 1.274
RA_{FFM}	$y = -1.370 + 0.212(ZI_{RA}) + 0.054(Xc_{RA})$ R = .858 R² = .736 Adj. R² = .729 SEE = 0.402 VIF: ZI_{RA} = 1.002, Xc_{RA} = 1.002
LL_{FFM}	$y = -4.089 + 0.162(Xc_{LL}) + 0.143(ZI_{LL}) + 0.039(\text{weight}) + 0.006(R_{LL})$ R = .953 R² = .908 Adj. R² = .902 SEE = 0.909 VIF: Xc_{LL} = 4.712, ZI_{LL} = 1.593, weight = 1.451, R_{LL} = 4.465
RL_{FFM}	$y = -3.715 + 0.009(R_{RL}) + 0.152(ZI_{RL}) + 0.139(Xc_{RL}) + 0.031(\text{weight})$ R = .968 R² = .937 Adj. R² = .933 SEE = 0.739 VIF: R_{RL} = 8.942, ZI_{RL} = 1.460, Xc_{RL} = 8.415, weight = 1.166
TR_{FFM}	$y = -12.061 + 0.046(ZI_{TR}) + 0.073(\text{weight}) + 0.212(\text{height}) - 0.419(R_{TR}) + 0.041(\text{age})$ R = .880 R² = .775 Adj. R² = .758 SEE = 1.510 VIF: ZI_{TR} = 2.441, weight = 1.649, height = 1.823, R_{TR} = 1.709, age = 1.294

FFM: fat-free mass (kg); ZI_{BPL}: body part length impedance index; Z_{BPL}: impedance; Xc_{BPL}: reactance; R_{BPL}: resistance; LA: left arm; RA: right arm; TR: trunk; LL: left leg; RL: right leg; Adj.: Adjusted; VIF: variation inflation factor; SEE: standard error estimate (kg)