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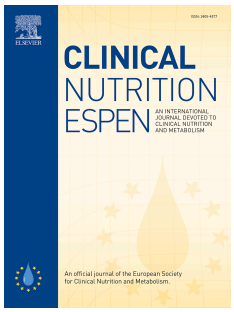

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Urinary Creatinine Based Equations for Estimation of Fat Free Mass in Patients with Intestinal Insufficiency or Intestinal Failure

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Abstract

Background & Aims: Assessment of body composition is an important aspect of disease management in patients with intestinal insufficiency (INS) or intestinal failure (IF). However, in daily clinical settings most body composition methods are too expensive or impractical, leaving body composition to be assessed by less reliable methods such as skin fold thickness. *The aim* of this study was to investigate and validate the use of an equation for the estimation of fat-free mass (FFM) with bioelectrical impedance analysis (BIA) as reference method.

Methods: A literature search for identification of urinary creatinine-based FFM-prediction equations was carried out a long side the creation of an equation by multiple linear regression. The correlation of each equation with FFM (measured by BIA in 277 patients with either INS or IF) was done by Pearson's correlation. Further investigation and validation of performance was done for the equations with the strongest correlation by Bland-Altman analysis, determination of root mean square error (RMSE), and intraclass correlation (ICC). The validation was carried out in a new group of 37 patients with either INS or IF.

Results: A total of 11 prediction equations were correlated with FFM measured by BIA. The equation called FFMmultiple and FFM-5 had the strongest correlation ($r = 0.969$, $p < 0.01$ and $r = 0.950$, $p < 0.01$, respectively). FFMmultiple was superior to FFM-5 regarding Bland-Altman analysis, RMSE, and ICC in the study group (Mean bias ± Standard Deviation = 0.042 ± 2.352 versus 0.309 ± 3.196; 95% limits of agreement = [-4.568 ; 4.651] versus [-5.955 ; 6.578]; RMSE =

0.158 versus 0.236; ICC = 0.969 versus 0.948). Cross-validation resulted in a Bland-Altman analysis with a statistically significant difference between FFMmultiple and FFM by BIA. FFM-5 showed wide 95% limits of agreement ([-6.977 ; 6.421]).

Conclusions: Two urinary creatinine-based equations (FFMmultiple and FFM-5) showed promising results as possible substitutes to BIA, however further investigation and cross validation revealed inauspicious results. Thus, the present study cannot recommend the use of a prediction equation instead of BIA for the assessment of FFM in patients with INS and IF.

Keywords: Intestinal insufficiency, Intestinal failure, Fat-free mass, Urinary creatinine, Bioelectrical impedance analysis, Prediction equation

1. Introduction

Intestinal insufficiency (INS) and intestinal failure (IF) are both conditions characterized by a reduced function or a physical loss of the intestine that leads to a decreased absorption of macronutrients and/or fluids and electrolytes (1). Patients that can maintain health and growth by use of oral/enteral nutritional (ON) supplements are defined as having INS, while patients requiring parental nutrition (PN) and/or intravenous fluids are defined as having IF (1,2). INS and IF may affect any age, it may have an acute onset, or be the slow, progressive development of a chronic disease, and may be reversible or irreversible (chronic) (1,3). Both INS and IF can result in malnutrition which again can cause sarcopenia and osteoporosis; a risk that recently has been found to be immense in patients with INS and IF (4–10). Thus, assessment and monitoring of nutritional status including body composition is therefore an important aspect of disease management in INS or IF (3,11).

The most common and widely used body composition (BC) model, is the two-component (or two-compartment) model that separates the body mass into fat mass and fat-free mass (FFM) (12–14). Technologies such as dual energy X-ray absorptiometry (DXA), computed tomography (CT), magnetic resonance imaging (MRI), and bioelectrical impedance analysis (BIA) have been recommended for body composition (BC) assessment by the Global Leadership Initiative on Malnutrition (GLIM) (15). Though, a common limitation to these methods are the acquisition of technical equipment, that in most settings of nutritional assessment throughout the world, are

neither available nor affordable (15,16). As an alternative to the above-mentioned methods, GLIM proposes the use of anthropometric measurements such as skin fold thickness. Although, being an easy, inexpensive, fast, and portable method, it requires precise and consistent measuring technique, and if not obtained, results in a high inter- and intra-observer variation (10,17,18). I.e., there is a need for easily accessible and less costly body composition approaches that does not compromise reliability.

A preliminary study (16) found that urinary creatinine correlated well with FFM measured by BIA (FFM-BIA) and that it was independent of patients being INS- or IF-patients though, further investigation was needed (16). Thus, the purpose of the present study was to identify an equation with urinary creatinine as a variable that could estimate FFM in patients with INS and IF with BIA as reference method. Such an equation would be valuable in clinical settings without advanced body composition instruments as well as being quick and economic. Hence, it would be in the interest of both clinicians, patients, and society.

2. Materials and Methods

2.1 Study design

The present study was a retrospective, cross-sectional, database study of consecutively recruited clinically stable patients with INS or IF on respectively, ON or home PN. The study was conducted at the Center for Nutrition and Bowel Disease (CET), Aalborg University Hospital, Denmark, during the period of September to December 2019. The study was approved by the Danish Data Protection Agency, Northern Denmark Region (journal no.: 2019-49). The study consisted of 5 steps:

- ***Step 1.*** Literature search for the identification of FFM-prediction equations with urinary creatinine as a variable.
- ***Step 2.*** Multiple linear regression for the creation of an equation with a strong fit to FFM-BIA.
- ***Step 3.*** Correlation of equations with FFM-BIA.
- ***Step 4.*** Analysis of agreement, precision, and reliability of the equations with the best correlation.
- ***Step 5.*** Cross-validation of the analyzed equations.

2.2 Study population

The study population consisted of a study group and a cross-validation group. Informed consent was not obtained for any patients in the two groups since all information was collected from a pseudonymized data base. The data base consisted of routine assessment measurements of consecutively recruited INS- and IF-patient admitted to the CET at Aalborg University Hospital, Denmark. Metabolically stable patients were selected based on having a complete data set of nutritional tests, blood samples, and a 24-hour urine sample. Patients with missing or invalid data (C-reactive protein > 100 mg/L, or compromised BIA results due to amputation, overhydration/dehydration, edema, lymphoedema, BMI <16 kg/m^2 or BMI >34 kg/m^2, or implants) were excluded. The study group consisted of 277 patients recruited during the period 2010-2019 while the cross-validation group consisted of 37 patients recruited during the period of 2019.

2.3 Measurements

Height, body weight, BMI, plasma creatine, 24-hour urinary creatinine, FFM-BIA, FFM by identified equations as well as age and sex were all data recorded for the use in the present study. All measurements for both the study group and cross-validation group originated from tests done as part of the routine assessment of the patient's health and nutrition status and thus, performed in the same way. Anthropometric measurements and BIA were done on the same day and by the same researcher in order to ensure accuracy. Analysis of blood and urine samples were done by the Department of Clinical Biochemistry at Aalborg University Hospital within 1 month of the anthropometric measurements and BIA. The following sections elaborate on the method of measurements used in the present study.

2.3.1 Anthropometric assessment

The standing height was measured barefooted to the nearest 0.1 cm by a wall-mounted stadiometer (Seca 222).

Body weight (BW) was measured using a digital electronic scale (Seca 701) that measured to the nearest 0.1 kg. Patients were measured wearing light indoor clothing and no shoes.

BMI was derived by the measurements of body weight and height (kg/m^2).

2.3.2 Biochemical assessment

Plasma creatinine was measured from a blood sample while urinary creatinine concentration was determined based on a 24-hour urine collection. All measurements were done using Roche-Cobas 6000/8000 (Roche Diagnostics, Basel, Switzerland) and analyzed by standard methods. Patients were instructed on how to collect 24-hour urine according to the guide from the Department of Clinical Biochemistry at Aalborg University Hospital.

3.3.3 FFM measured by BIA

BIA was performed using a multi-frequency analyzer BioScan 920-II (Maltron, Essex, UK) in order to estimate FFM. The patients were measured in a state of at least 4 hours fasting (water was allowed until 2 hours before the measurement), 8 hours retaining from physical activity, emptied bladder, and laying down in a supine position at a non-conducting bed or couch for 10 min before measurement. The patients were laying with legs separated approximately 45° and arms approximately 30° away from the torso. Adhesive electrodes were placed on the patients' right side, on the surface of the dorsal hand, wrist, foot, and ankle in a standard tetra-polar electrode arrangement. FFM was calculated according to the undisclosed proprietary equation developed by the manufacture of the multi-frequency analyzer BioScan 920-II (Maltron, Essex, UK).

3.3.4 FFM calculated by equations

FFM was calculated according to the identified equations found in the literature and the equation used in the preliminary study (16) (Table 1).

3.4 Literature search of FFM-predictions equations

In step 1 a literature search was conducted in the period of September to November 2019 to identify equations estimating FFM with urinary creatinine as a variable. The database PubMed (US National Library of Medicine, National Institutes of Health) was systematically searched for search words;

intestinal failure, intestinal insufficiency, urinary creatinine, 24-hour creatinine, creatinine, creatinine kinetics, predictive equation, equation, predicting, bio marker, marker, bioelectrical impedance analysis, fat-free mass, fat free mass, lean body mass, body composition, and nutritional status in every possible combination and Mesh-terms were applied where possible. The search was restricted to English and Danish languages. Initial selection of articles was based on title, second selection was based on abstract and finial selection was based on reading the full articles.

3.5 Statistical analysis

Descriptive statistics for the study population and cross-validation group were expressed as number and percentages or as mean ± standard deviation (SD) where appropriate. A level of 0.05 and 95% confidence intervals were used as statistically significant level for all statistical tests, i.e., stating statistical significance at the $p = < 0.05$ level.

In step 2 a multiple linear regression was carried out to identify other important variables other than urinary creatinine for the estimation of FFM. Variables being investigated included: age, height, BW, and sex. The assumption of independence of observations (residuals) was assessed by Durbin-Watson statistic with a value of 2 indicating no correlations between residuals (19). Collectively linearity between independent variables and the dependent variable was assessed by visual inspection of a scatterplot of the studentized residuals against the unstandardized predicted values. The same scatterplot was used to investigate the assumption of homoscedasticity by looking for scatter points exhibiting no pattern and approximately constant spread (19). Linearity between each independent variable and the dependent variable was determined by visual inspection of partial regression plots. Multicollinearity was assessed by checking that the Tolerance value of each dependent variable was less than 0.1 (19). Unusual points such as outliers, highly influential points, and high leverage points were evaluated respectively by assessing cases' standardized residual for a value greater than ± 3 as well as studentized deleted residual for a value greater than ± 3 SD, indicating an outlier, leverage points for a value less than 0.2, indicating being safe, and Cook's Distance value for a value above 1, indicating the need for further investigation (19). Normality was assessed by a probability-probability plot of standardized residuals. The fit of the multiple model was reported by the adjusted coefficient of determination (adj. R^2) together with the statistical significance of the model. Constant and coefficient of independent variables along with statistical significance were reported as well as expressed in a regression equation.

In step 3 a Pearson's product correlation was carried out between FFM-BIA and each equation. Assumptions of linear relationship and identification of outliers were assessed by visual inspection. Normality was determined by Shapiro-Wilk's test with a statistical significance of $p < 0.05$ indicating violation of normality. Non-normal distribution of data was further investigated for skewness and kurtosis. A statistical significance level of 0.01 was accepted, corresponding to a z-score of ±2.58, i.e., a z-score within this interval indicating a normal distribution. Pearson's correlation coefficient was reported together with the statistical significance.

In step 4 the agreement, precision, and reliability were assessed for the equation with the strongest correlation with FFM-BIA and for the regression equation created in step 2. Agreement, precision, and reliability were assessed by Bland-Altman analysis, root mean square error (RMSE), and Intraclass correlation coefficient (ICC), respectively. The Bland-Altman plot was made of the difference between FFM-BIA and the selected equation against the average of both measures. The magnitude and direction of bias was assessed by a line in the plot representing the mean difference or mean error (i.e., the average of differences between the paired measurements) in relation to the line corresponding to zero differences (i.e., an y value of 0. The closer the mean bias is to zero the higher is the agreement between the paired measurements) (20). The precision of the mean bias was assessed by calculating the 95% CI of the mean bias. Statistically significant difference was determined if the line of equality, i.e. the line of $y = 0$, was not in the interval (20). Limits of agreement was defined as $\pm 1.96 \times \text{SD}$ around the mean bias, with narrow limits indicating high precision and wide limits indicating low precision (21). The accuracy was assessed by the root mean square error (RMSE) calculated as square root of the mean squared differences (22). Intraclass correlation coefficient (ICC) was calculated to determine the reliability of measurements. The ICC estimates and 95% confident intervals were calculated based on a single rater, consistency, two-way mixed-effects model. The ICC value was interpreted as: "poor reliability" for values less than 0.5, "moderate reliability" for values between 0.5 and 0.75, "good reliability" for values between 0.75 and 0.90, "excellent reliability" for values greater than 0.90 (23).

In step 5 a cross-validation was performed by comparing results of Pearson's product correlation, Bland-Altman analysis, RMSE, and ICC for the validation group with results of the same tests for the study group.

All assumptions for statistical test and analyses were fulfilled otherwise stated. All statistics were done using the software IBM SPSS Statistics version 26 for Windows (SPSS Inc, Chicago, IL).

3. Results

3.1 Step 1. Literature search

In total 5 equations for the estimation of FFM with urinary creatinine as a variable were identified and 4 of them were found in an alternative version, thus 9 equations were selected for the use in the study from a total number of 11 articles. Table 1 lists the equations together with their respective references. Besides the equations identified through literature search, the table also includes the equation used in the preliminary study.

Equation from preliminary study	References	Equations
(FFM-calc)	Original source: Welle et al., 1996 (24) Referred by: Køhler, Olesen & Rasmussen, 2019 (16)	$FFM(kg) = 23.3 \times uCrea(g/day) + 21.2$
Equations from literature search	**References**	**Equations**
Equation 1 **(FFM-1)**	Original source: Keshaviah et al., 1994 (25) based on Forbes & Bruining, 1976 (26) Referred by: Avesani et al., 2004 (27) and Bhatla et al., 1995 (28)	a) $FFM(kg) = 0.029 \times daily\ creatinine\ production\ (mg/dl) + 7.38$ b) $Daily\ creatinine\ production\ (mg/dl) = uCrea(mg/day) + MD(mg/dl)$ c) $MD(mg/dl) = 0.38 \times pCrea(mg/dl) \times BW(kg)$
Equation 1 Alternative **(FFM-1A)**	Keshaviah et al., 1994 (25) based on Forbes & Bruining (26) Lo et al., 1994 (29)	a) $FFM(kg) = 0.029 \times daily\ creatinine\ production\ (mg/dl) + 7.38$ b) $Daily\ creatinine\ production\ (mg/dl) = uCrea(mg/day) + MD(mg/dl)$ c) $MD(mg/dl) = 0.418 \times BW(kg)$
Equation 2 **(FFM-2)**	Miller & Blyth, 1952 (30)	$FFM(kg) = 20.97 + 0.5161 \times uCrea(mg/hour)$
Equation 3 **(FFM-3)**	Virgili et al., 1994 (31)	$FFM(kg) = 27.4 \times uCrea(g/day) + 14.0$
Equation 3 Alternative **(FFM-3A)**		Equation for males: $FFM(kg) = 24.1 \times uCrea(g/day) + 19.4$ Equation for females: $FFM(kg) = 22.7 \times uCrea(g/day) + 18.2$
Equation 4 **(FFM-4)**	Original source: Forbes & Bruining, 1976 (26) Kawasaki et al., 1993 (32) Referred by: Narumi et al., 2015 (33)	a) $FFM(kg) = 7.38 + 0.02908 \times uCreaCalc1(\mathrm{mg}/day)$ b) $uCreaCalc1(mg/day) = 2.04 \times age + 14.89 \times BW(kg) + 16.14 \times height(cm) - 2244.45$
Equation 4 Alternative		$FFM(kg) = 7.38 + 0.02908 \times uCrea(\mathrm{mg}/day)$

(FFM-4A)		
Equation 5 **(FFM-5)**	Ix et al., 2011 (34) Jassal et al., 2015 (35)	a) $FFM(kg) = 13.0 + 0.03 \times uCreaCalc2(mg/day)$ b) $uCreaCalc2(mg/day) =$ $879.89 + 12.57 \times BW(kg) - 6.19 \times age\ (-379.42\ if\ female)$
Equation 5 Alternative **(FFM-5A)**		$FFM(kg) = 13.0 + 0.03 \times uCrea(mg/day)$
BW = body weight; FFM = fat-free mass; MD = metabolic degradation; pCrea = plasma creatinine; uCrea = urinary creatinine; uCreaCalc1 = number 1 equation for calculation of urinary creatine; uCreaCalc2 = number 2 equation for calculation of urinary creatinine.		

Table 1. List of equations for the estimation of fat-free mass.

3.2 Demographics and clinically characteristics of study group

In total 277 Caucasian INS- and IF-patients were included in the study group. Demographics and clinically characteristics are presented in table 2.

Variable	**Patients**
Demographics	
Total number	277 (100%)
Male	121 (43,7%)
Female	156 (56.3%)
Age (years)	59.5 ± 15.2
Height (cm)	169.0 ± 9.3
Weight (kg)	63.5 ± 15.3
Biochemistry	
pCrea (µmol/day)	95.9 ± 91.6
uCrea (mmol/day)	8.4 ± 3.4
Body composition	
FFM-BIA (kg)	46.2 ± 9.5
FFM-calc (kg)	43.3 ± 9.0
FFM-1 (kg)	35.7 ± 11.2
FFM-1A (kg)	35.7 ± 11.3
FFM-2 (kg)	41.4 ± 8.3
FFM-3 (kg)	40.0 ± 10.5
FFM-3A (kg)	41.0 ± 9.7
FFM-4 (kg)	52.5 ± 9.7
FFM-4A (kg)	35.0 ± 11.2
FFM-5 (kg)	45.9 ± 10,2
FFM-5A (kg)	41.5 ± 11.5
FFMmultiple (kg)	46.1 ± 9.2

FFM-BIA = fat-free mass measured by bioelectrical impedance analysis;
FFMmultiple = fat-free mass calculated by multiple linear regression equation;
pCrea = plasma creatinine; uCrea = 24-hour urinary creatinine

Table 2. Demographics and clinically characteristics of patients in the study group. Data are presented as mean ± Standard Deviation (SD) or percent (%) of total number of patients in study group.

3.3 Step 2. Multiple linear regression

To identify other important variables other than urinary creatinine for the estimation of FFM a multiple linear regression was performed. In addition to urinary creatinine the following co-variables were included; *age, height, BW, and sex* to predict FFM-BIA. Visual inspection of a plot of the studentized residuals against the unstandardized prediction values revealed a trend towards heteroscedasticity by having an increasing funnel shape. Two cases (i.e., patients) were detected as outliers and two other cases were detected as potential outliers. The regression was carried out despite of the described violations. The multiple linear regression model statistically significantly predicted FFM (kg), $F(5, 271) = 835.221$, $p < 0.005$, adj. $R^2 = 0.938$. Regression coefficients, standard errors, significance, and 95% confidence interval are given in table 5 in "*Supplementary material*". The regression equation was written as:

$$FFM\ (kg) = -18.548 + (1.300 \times uCrea(\text{g/day}) + \left(-0.079 \times age(years)\right) + \left(0.266 \times height(cm)\right) + \left(0.327 \times BW(kg)\right) + (5.591 \times sex(where\ female\ is\ 0\ and\ male\ is\ 1)).$$

The multiple regression equation will in the remaining part of the article be referred to as FFMmultiple.

3.4 Step 3. Correlation of FFM-BIA and prediction equations

All variables (FFM-BIA, FFM-calc, FFM-1, FFM-1A, FFM-2, FFM-3, FFM-3A, FFM-4, FFM-4A, FFM-5, FFM-5A, and FFMmultiple) were found to be positive skewed with a z-score ranging from 2.59-5.81. Pearson's correlation was carried out regardless of violation of the assumption of normal distribution as reported above. FFM-5 and FFMmultiple showed the strongest correlation with a correlation coefficient of r = 0.950, $p < 0.01$ and r = 0.969, $p < 0.01$, respectively (see figure 1 and

2). The remaining results of the Pearson's correlation are shown in table 6 in "*Supplementary material"* while figure 7-15 in "*Supplementary material"* shows the results graphically.

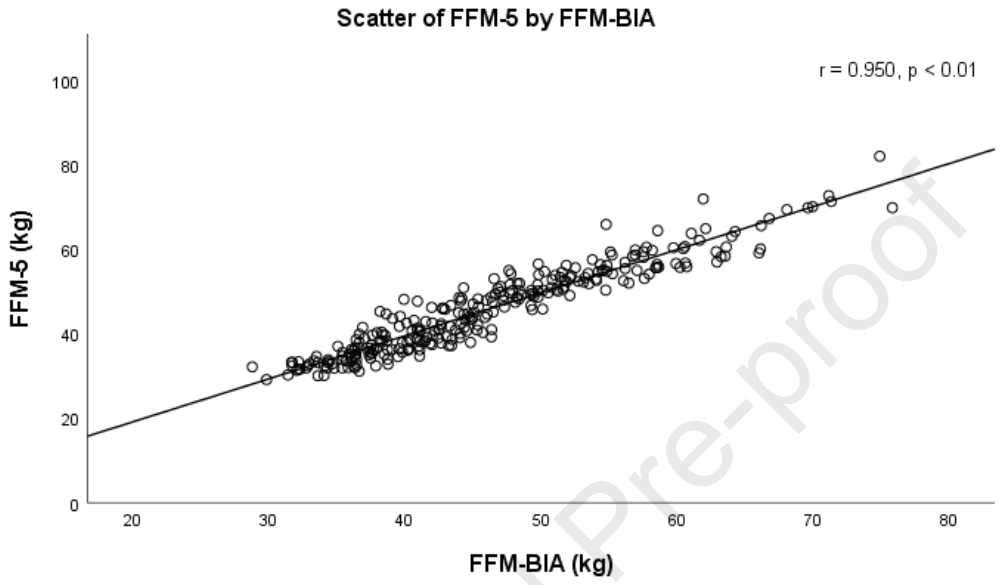

Figure 1. Scatter plot of fat-free mass calculated by the estimated urinary creatinine equation of Ix et al., 2011, and Jassal et al., 2015, (FFM-5) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

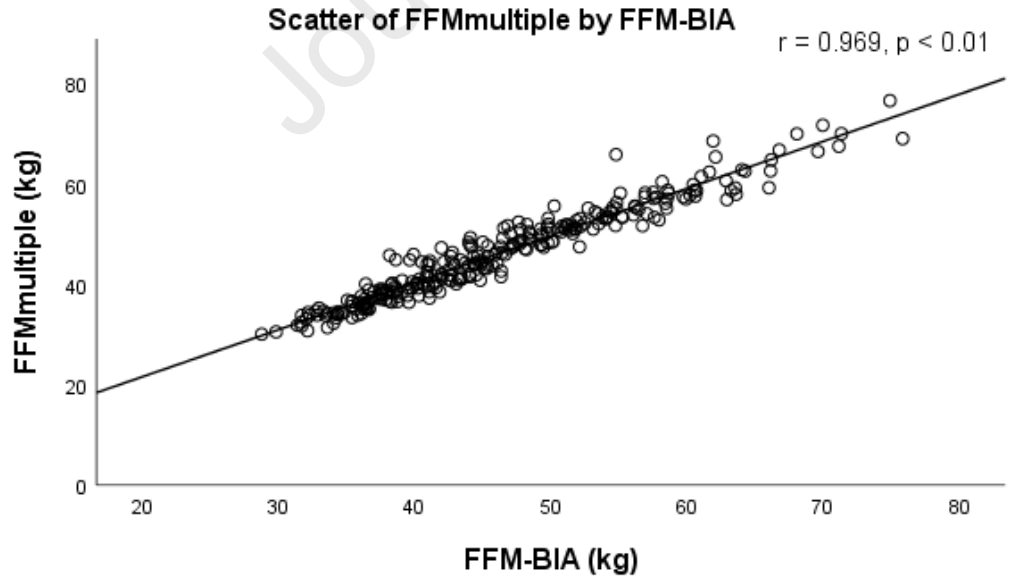

Figure 2. Scatter plot of fat-free mass calculated by multiple linear regression equation (FFMmultiple) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

3.5 Step 4. Agreement, precision, and reliability of FFMmultiple and FFM-5

The Bland-Altman analysis of FFM-BIA and FFMmultiple showed a high agreement as indicated by a mean bias ± SD of 0.042 ± 2.352 kg. A lower agreement was found for FFM-BIA and FFM-5 by a mean bias ± SD of 0.309 ± 3.196 kg. The 95% CI of the mean bias for FFM-BIA and FFMmultiple was [-0.236 ; 0.320] and for FFM-BIA and FFM-5 it was [-0.069 ; 0.687] thereby, confirming that the difference between FFM-BIA and FFMmultiple and the difference between FFM-BIA and FFM-5 was not statistically significance. The Bland-Altman plot for FFM-BIA and FFMmultiple revealed a minor positive trend towards greater differences at higher measurements (i.e., proportional bias). A high precision was found for FFM-BIA and FFMmultiple by narrow 95% limits of agreements ranging from -4.568 kg to 4.651 kg while a slightly higher range of -5.955 kg to 6.578 kg was found for FFM-BIA and FFM-5 (figure 3-4).

A RMSE of 0.158 indicated a high accuracy for FFM-BIA and FFMmultiple and an excellent reliability was found by an ICC of 0.969 with a 95% confidence interval of [0.960 ; 0.975]. A lower accuracy was found for FFM-BIA and FFM-5 by a RMSE of 0.236 kg and an excellent reliability as indicated by an ICC of 0.948 with a 95% confidence interval of [0.934 ; 0.959].

It must be stated that the difference between FFM-BIA and FFMmultiple as well as the difference between FFM-BIA and FFM-5 were not normally distributed ($p < 0.01$). The z-scores for skewness and kurtosis were respectively -3.55 and 7.77 for the difference between FFM-BIA and FFMmultiple, and -2.92 and 1.28 for the difference between FFM-BIA and FFM-5.

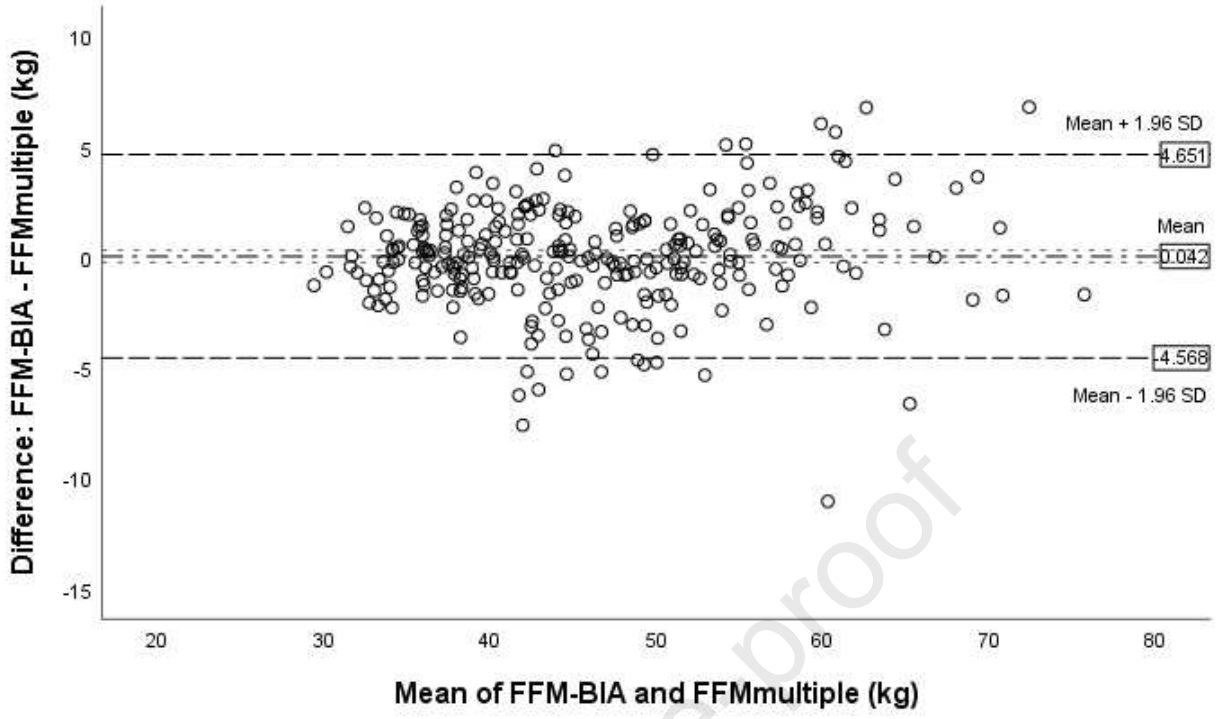

Figure 3. Bland-Altman plot comparing fat-free mass (FFM) measured by bioelectrical impedance analysis (FFM-BIA) and calculated by FFMmultiple. y = 0 indicates zero difference; (– - –) indicates mean bias (y = 0.042) with 95% lower and upper confidence interval (- - -); (– – –) indicates lower and upper limits of 95% agreement (y = -4.568 and y = 4.651).

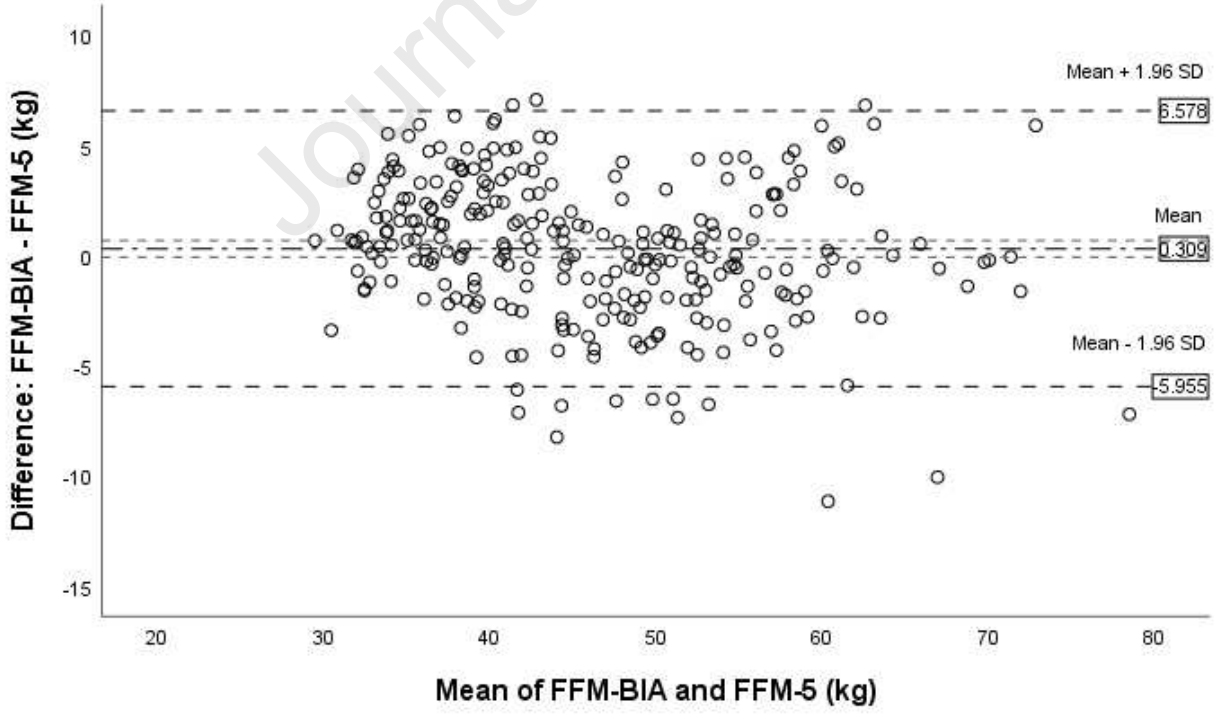

Figure 4. Bland-Altman plot comparing fat-free mass (FFM) measured by bioelectrical impedance analysis (FFM-BIA) and calculated by the estimated urinary equation of Ix et al., 2011, and Jassal et al., 2015, (FFM-5). y = 0 indicates zero difference; (– - –) indicates

the mean bias (y = 0.309) with 95% lower and upper confidence interval (- - -); (– – –) indicates lower and upper limits of 95% agreement (y = -5.955 and y = 6.578).

3.6 Step 5. Cross-validation of FFMmultiple and FFM-5

3.6.1 Demographics and clinically characteristics of cross-validation group

In total 37 Caucasian INS- and IF-patients were included in the cross-validation group. Demographics and clinically characteristics are presented in table 3.

Variable	**Patients**
Demographics	
Total number	37 (100%)
Male	12 (32.4%)
Female	25 (67.7%)
Age (years)	63 ± 12
Height (cm)	165.81 ± 8.66
Weight (kg)	62.04 ± 13.40
Biochemistry	
uCrea (mmol/day)	8.03 ± 2.52
Body composition	
FFM-BIA (kg)	43.05 ± 7.45
FFMmultiple (kg)	43.83 ± 7.70
FFM-5 (kg)	43.33 ± 8.77
FFM-BIA = fat-free mass measured by bioelectrical impedance analysis; FFMmultiple = fat-free mass calculated by multiple linear regression equation; FFM-5 = fat-free mass calculated by equation found in reference 34 and 35; uCrea = 24-hour urinary creatinine	

Table 3. Demographics and clinically characteristics of patients in the cross-validation group. Data are presented as mean ± Standard Deviation (SD) or number and percent (%) of total number of patients.

3.6.2 Correlation of FFM-BIA with FFMmultiple and FFM-5

Preliminary analyses indicated that FFM-BIA was not normally distributed (p = 0.038) with a z-score for skewness and kurtosis of respectively 1.510 and -0,838. Pearson's correlation was carried out regardless of this violation. The correlation coefficient for FFMmultiple was r = 0.960, $p < 0.01$, thereby being 0.09 lower than the coefficient for FFMmultiple in the study group. The correlation coefficient for FFM-5 was r = 0.924, $p < 0.01$ which was 0.026 lower than the coefficient for FFM-5 in the study group. The results of the Pearson's correlation are shown graphically in figure 16-17 in *"Supplementary material"*.

3.6.3 Agreement, precision, and reliability

In the cross-validation group, the mean bias was lowest for FFM-5 when compared to FFMmultiple (-0.278 versus -0.777) however, the SD was higher for FFM-5 than FFMmultiple (±3.428 versus ±2.146). Also, the 95% CI of the mean bias was wider for FFM-5 when compared to FFMmultiple ([-1.417 ; 0.862] versus [-1.492 ; -0.061]) though, the 95% CI of the mean bias for FFMmultiple was outside the line of zero difference thereby, confirming that the difference between FFM-BIA and FFMmultiple was statistically significant. The 95% limits of agreement were furthermore widest for FFM-5 versus FFMmultiple ([-6.977 ; 6.421] versus [-4.983 ; 3.429]). These results can be visually interpreted in figure 18 and figure 19, respectively, in *"Supplementary material"*. The accuracy was found to be highest for FFM-5 with a RMSE of 0.278 versus a RMSE of 0.585 for FFMmultple. In contrast to this, the reliability was highest for FFMmultiple with an ICC of 0.960 and 95% confidence interval of [0.924;0.979] when compared to FFM-5 with an ICC of 0.912 and 95% confidence interval of [0.835;0.954]. These results are listed in table 4 together with the results of the Bland-Altman plot as well as the difference between the results obtained for the same equations in the study group.

	Mean bias	**SD**	**95% CI of mean bias**		**95% limits of agreement (mean bias ± 1.96 SD)**		**RMSE**	**ICC**	**95% CI of ICC**	
			Lower	**Upper**	**Lower**	**Upper**			**Lower**	**Upper**
FFMmultiple (Cross-validation group) (kg)	-0.777	2.146	-1.492	-0.061	-4.983	3.429	0.585	0.960	0.924	0.979
FFMmultiple (Study group)	0.042	2.352	-0.236	0.320	-4.568	4.651	0.158	0.969	0.960	0.975
Difference	**-0.819**	**-0.206**	**-1.256**	**-0.381**	**-0.415**	**-1.222**	**0.427**	**-0.009**	**-0.036**	**0.004**
FFM-5 (Cross-validation group)	-0.278	3.418	-1.417	0.862	-6.977	6.421	0.278	0.912	0.835	0.954
FFM-5 (Study group)	0.309	3.196	-0.069	0.687	-5.955	6.578	0.236	0.948	0.934	0.959
Difference	**-0.587**	**0.222**	**-1.348**	**0.175**	**-1.022**	**-0.157**	**0.042**	**-0.036**	**-0.081**	**-0.005**

Table 4. Results of Bland-Altman analysis, root mean square error (RMSE), and interclass correlation (ICC) for fat-free mass (FFM) calculated by multiple linear regression equation (FFMmultiple) in cross-validation group and study group, and by the estimated urinary creatinine equation of Ix et al., 2011, and Jassal et al., 2015, (FFM-5) in cross-validation group and study group, together with difference in results between the cross-validation group and study group. All numbers are in kg.

4. Discussion

To the authors knowledge this is the first study investigating the determination of a urinary creatinine-based equation for the estimation of FFM in INS- and IF-patients with BIA as reference method. In total, 11 urinary creatinine-based equations were correlated with FFM measured by BIA. The study clearly demonstrated that equations with multiple parameters in addition to urinary creatinine correlated better with FFM-BIA. Of these, especially two equations (i.e., FFMmultiple and FFM-5) resulted in a superior correlation and were therefore further investigated. This revealed that FFMmultiple had the best agreement, accuracy, and reliability. However, cross-validation, showed a statistically significant difference between FFM-BIA and FFMmultiple thus, compromising the utility of the equation. Since no significant difference was found between FFM-BIA and FFM-5 in the cross-validation, the FFM-5-equation seems instinctively more attractive despite having an inferior agreement, accuracy, and reliability in the study group as compared to the FFMmultiple equation.

4.1 Parameters influencing FFM

All equations in the present study assume that urinary creatinine is an estimate of FFM. This assumption is well documented (12,36) however, it is a simplification of the human body. Thus, regarding the present study, it was not surprising that equation FFMmultiple resulted in the strongest correlation since it not only took urinary creatinine into consideration, but also age, sex, height, and BW.

FFMmultiple indicated a negative correlation between FFM and age – a result in line with previously reported data by Kyle et al., 2001, who conducted a large study with 433 healthy persons aged 18-94 years. The study found that FFM decreased with age after it had reached a relative stable level during maturity and more specifically that the change happened at a faster rate after 60 years of age (37). Thus, FFMmultiple may be less suited for very young patients (because their FFM increases with age) and very old patients (because their FFM decreases at a faster rate with age).

The influence of sex was furthermore studied by Kyle et al., 2001, who reported that FFM for males was higher than FFM for females though, the age-related loss of FFM was greater in males than in females (37). A study by Obisesan et al., 2005, also reported a higher FFM for males as compared

to females. Moreover, they found that FFM peaked earlier in males (at age 51-54 years) than in females (at age 55-59 years) (38). Collectively these studies indicate that the sex related influence on FFM is of a dynamic and complex character; a character that is not reflected fully in FFMmultiple since the equation solely assigns each sex a fixed number.

When it comes to height and BW, these 2 parameters were found to be strong positive determinants of FFM in the FFMmultiple equation. This result has previously been documented by Hume, 1966, who predicted FFM from height and BW in males and females (39). The predictions were cross-validated in 2015 by Carnevale et al., resulting in the conclusion that Hume's equations could be consequently used in clinically stable patients with no recent weight changes when DXA measurements were not available (40).

Despite the stronger correlation of FFMmultiple in compare to the correlation of the other equations, it is important to mention that the superiority of FFMmultiple cannot be attributed to the inclusion of multiple parameters only. FFMmultiple was derived from the study group of the present study whereas the equations found in the literature, were extracted from other study groups with other techniques than BIA as reference method. This is an essential difference in the underlaying criteria of the equations that must be considered when interpreting the results.

Finally, a comment must also be given about the term correlation. Just because the present study has found strong correlation between FFM and age, sex, height, BW, and urinary creatinine as parameters, it is not to be confused with a causal relationship, i.e., correlation does not presume causality. Thus, the present study does not imply that FFM is sorely caused by age, sex, height, BW, and urinary creatinine.

4.2 Determination of the best equation for estimation of FFM in INS- and IF-patients

The investigation of FFMmultiple and FFM-5 showed that FFMmultiple had a better agreement with BIA than FFM-5 as well as a better RMSE and ICC. Despite this, the cross validation revealed that the difference between FFM-BIA and FFMmultiple was statistically significant. Thus, FFMmultiple cannot be recommended as a substitute of FFM-BIA. This could automatically lead to the conclusion that FFM-5 is the best alternative to FFM-BIA because the equation did not result in a statistically significant difference in the cross validation. However, this would be a simplified deduction since FFM-5 showed 95% limits of agreement of [-6.977 ; 6.421]. This may be too wide

an interval to accept the equation as a useful tool in the daily clinical monitoring of INS- and IF-patients.

Furthermore, the results in the present study were based on the use of BIA as reference method. Heymsfield et al., 1997, has stated that an important quest in body composition research is to find the best reference method. The selected method must be appropriate for the body composition level and component under investigation (41). Hence, the choice of BIA as reference in the present study is appropriate in terms of composition level and component because BIA estimates FFM at the molecular level just like the component (i.e., urinary creatinine) under investigation. Though, BIA is a so-called type I body composition method and is therefore not regarded as an acceptable reference standard because it is developed against established reference methods (41). This means that error from the reference method used for developing the regression equation for use in BIA, is included in the BIA results, thus adding to additionally error besides the one caused by measuring bioelectrical impedance. It would therefore have been more appropriate if a type II body composition method (e.g., hydro densitometry or isotope dilution method) had been used as reference, though such a method was not available in the present study. Adding to further possible errors in the estimation of FFM is the use of the default equation of the BIA device. This equation is an undisclosed proprietary equation developed by the manufacture and it may have been derived from a population that differs from the one under investigation in the present study. Thus, the results should be interpreted with caution and with the chosen reference method in mind.

4.3 Generalization versus specification

The present study did not make any differentiation between patients with INS and patients with IF. This decision was made on basis of a preliminary study (16) that found that the correlation of urinary creatinine with FFM-BIA was independent of patients having INS or IF. Thus, disease specification was opted out in favor of generalization. However, since INS and IF are conditions that can be caused by many different diseases (1), the present study cannot rule out the possibility that a disease specific prediction equation would be more favorable in some disease cases. Also, the preliminary study (16) as well as the present study was based on a population of patients with INS and IF who was metabolic stable. Thus, it is unclear how well any of the equations in the present study will perform in patients who are metabolic unstable.

4.4 Clinical usefulness of a urinary creatinine-based prediction equation in INS- and IF-patients

There is no doubt that the concept of measuring FFM based on a prediction equation is attractive; it is less time consuming, more practical, and more economical than high-tech methods. Thus, it seems intuitive to embrace a urinary creatinine-based prediction equation for the estimation of FFM in INS- and IF-patients however, one must not forget to address its clinical usefulness.

The collection of a 24-hour urine sample can be difficult for some patients and inconvenient. Patients with frequent bowel moment during urination or patients who tends to have diarrhea (e.g., patients with short bowel syndrome) may be unable to collect a sample without feces. The contamination with feces may affect the measurement of creatinine (42) and thus affect the FFM calculation. In the present study no problems were reported regarding the 24-hour urine collection, but it may constitute a challenge in specific subgroups of INS- and IF-patient.

Furthermore, the use of urinary creatinine as a determinant of FFM assumes that a constant relationship exists between skeletal muscle mass and urinary creatinine output. Though, studies have shown that this is not entirely true. Multiple factors have been found to affect the amount of urinary creatinine thus questioning its validity (12). These factors include dietary creatine, menstrual cycle, infection, fever, trauma, and of course renal function (43,44). In the present study these factors were not investigated though, one may argue that some of the patients in the study group had renal dysfunction since the mean ± SD of plasma creatinine was 95.9 ± 91.6 μmol/day. In case of renal failure, urinary creatinine will not reflect the true relationship between skeletal muscle mass and urinary creatinine output, thereby confounding the FFM result. This is an important clinical limitation to be aware of, especially since renal failure is frequently seen in patients with IF on long-term home parenteral nutrition (45).

Another important aspect to mention is the interpretation of FFM. FFM is the total measure of essential lipids, total body water, protein, carbohydrates, soft tissue minerals, and bone minerals in the body. The value of FFM can be interpreted on a patient/subject specific level or on a population specific level. So far, few population specific reference values have been published (46), thus for now in the clinic, an FFM-prediction equation will show most benefit as a monitoring-tool for body development on a patient level only.

4.5 Study limitations

The present study is not without limitations. These include the use of prediction equations that were not developed in INS- and IF-patients. Thus, differences in study population characteristics may account for the seen results. Urinary creatinine was determined from a single 24-hour urine collection without any diet restrictions, due to practical reasons. However, there are evidence of dietary influence on urinary creatinine and recommendation of using 3 consecutive 24-hour urine collections in order to obtain a representative creatinine excretion (12,44). FFM was calculated according to the manufacture of the BIA device instead of using a population specific regression equation. Furthermore, BIA was used as reference method, ignoring the fact that BIA is a type II property-based body composition method. In addition to this measurement errors may have occurred, though necessary precautions had been made.

4.6 Recommendations for future studies

For future studies it is recommended that a prediction equation is developed based on a multiple linear regression with use of urinary creatinine, age, height, BW, and sex. The dependent variable should be FFM, preferably measured by a type II property-based method or a multicomponent body composition model. The prediction equation should along with BIA be compared to a type II property-based method. This would allow for the identification of which of the 2 measurements, i.e., the predication equation or BIA, that estimates FFM most accurate and precisely according to a "gold standard". Furthermore, BIA should not calculate FFM by manufacture's equation and patient's renal status should be evaluated.

5. Conclusion

The present study demonstrated that urinary creatinine a long with additionally components such as age, sex, height, and body weight, correlated strongly with FFM measured by BIA in INS- and IF-patients. Two equations (FFMmultiple and FFM-5) showed promising results as possible substitutes to BIA, however further investigation and cross validation revealed inauspicious results. Thus, the present study cannot recommend the use of a prediction equation instead of BIA for the assessment of FFM in patients with INS and IF.

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Statement of authorship

Nanna Dyhre-Petersen: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing – Original draft, Visualization. **Marianne Køhler:** Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing – Review & Editing, Co-supervision. **Henrik Højgaard Rasmussen:** Conceptualization, Methodology, Writing – Review & Editing, Supervision, Project administration.

Conflicts of interest and funding sources

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Supplementary material

Variable	Unstandardized Coefficients		Standardized Coefficients	Sig.	95.0% Confidence Interval for *B*	
	B	***SE_B***	***β***		**Lower Bound**	**Upper Bound**
Intercept	-18.548	4.232		.000	-26.881	-10.216
uCrea (g/day)	1.300	0.492	0.052	.009	0.331	2.269
Age (years)	-0.079	0.010	-0.127	.000	-0.099	-0.059
Height (cm)	0.266	0.026	0.260	.000	0.216	0.317
BW (kg)	0.327	0.013	0.525	.000	0.302	0.352
Sex	5.591	0.456	0.292	.000	4.693	6.488

B = unstandardized regression coefficient; SE_B = Standard error of the coefficient; *β* = standardized coefficient, Sig. = significance, uCrea = urinary creatinine; BW = body weight.

Table 5. Summary of results for coefficients of the multiple linear regression analysis.

	FFM-calc (kg)	FFM-1 (kg)	FFM-1A (kg)	FFM-2 (kg)	FFM-3 (kg)	FFM-3A (kg)	FFM-4 (kg)	FFM-4A (kg)	FFM-5 (kg)	FFM-5A (kg)	FFM multiple (kg)
FFM-BIA (kg)	0.657**	0.669**	0.664**	0.657**	0.657**	0.711**	0.934**	0.657**	0.950**	0.657**	0.969**
** Correlation is significant at the 0.01 level (2-tailed).											

Table 6. Pearson's correlation coefficient for the correlation of fat-free mass measured by bioelectrical impedance analysis with prediction equations.

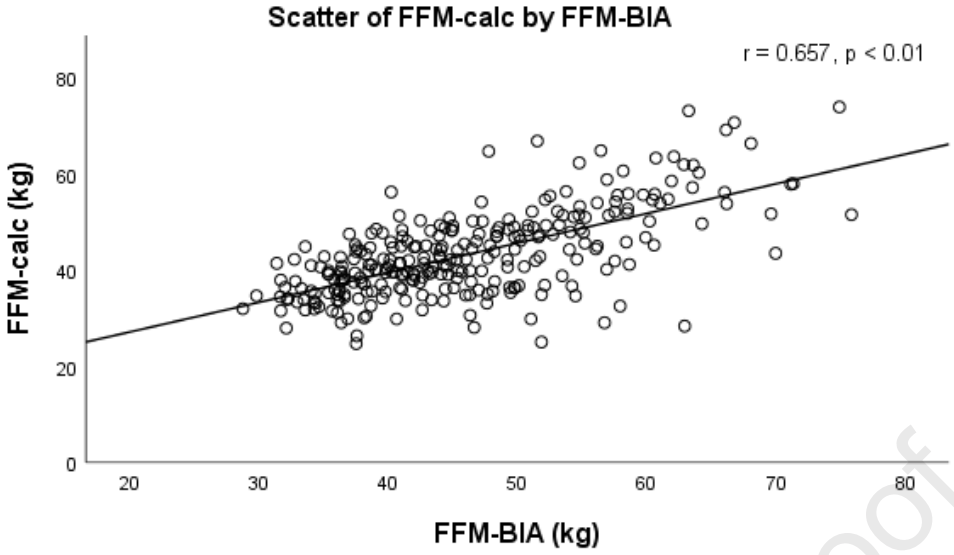

Figure 7. Scatter plot of fat-free mass calculated by the equation of Welle et al., 1996, (FFM-calc) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

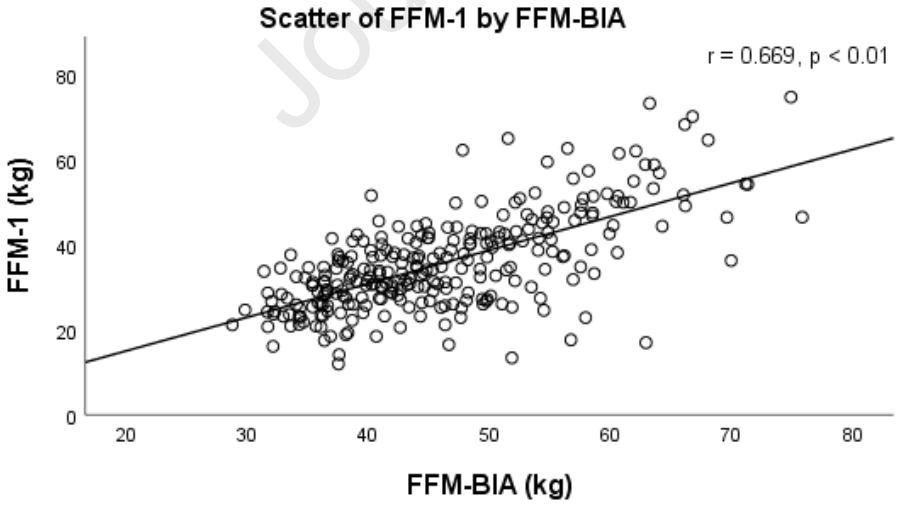

Figure 8. Scatter plot of fat-free mass calculated by the equation of Keshaviah et al., 1994, (FFM-1) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

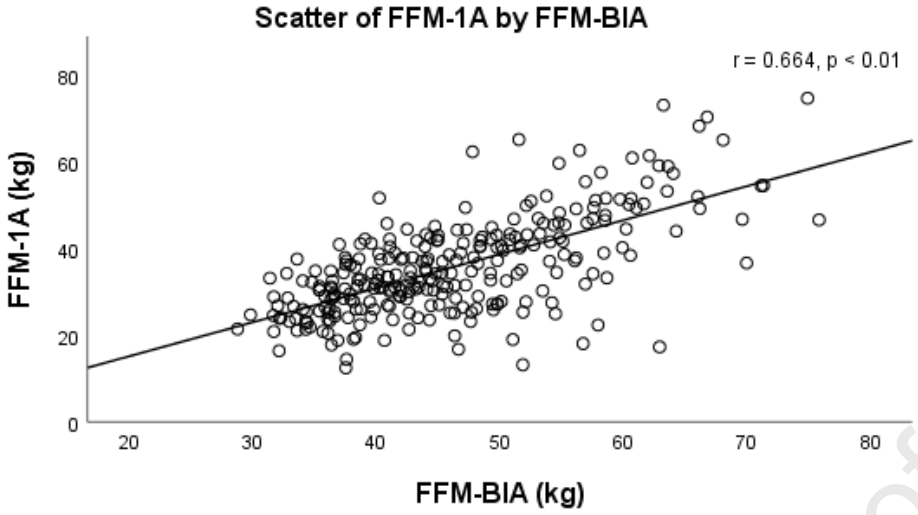

Figure 9. Scatter plot of fat-free mass calculated by the equation of Keshaviah et al., 1994, and Lo et al., 1994, (FFM-1A) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

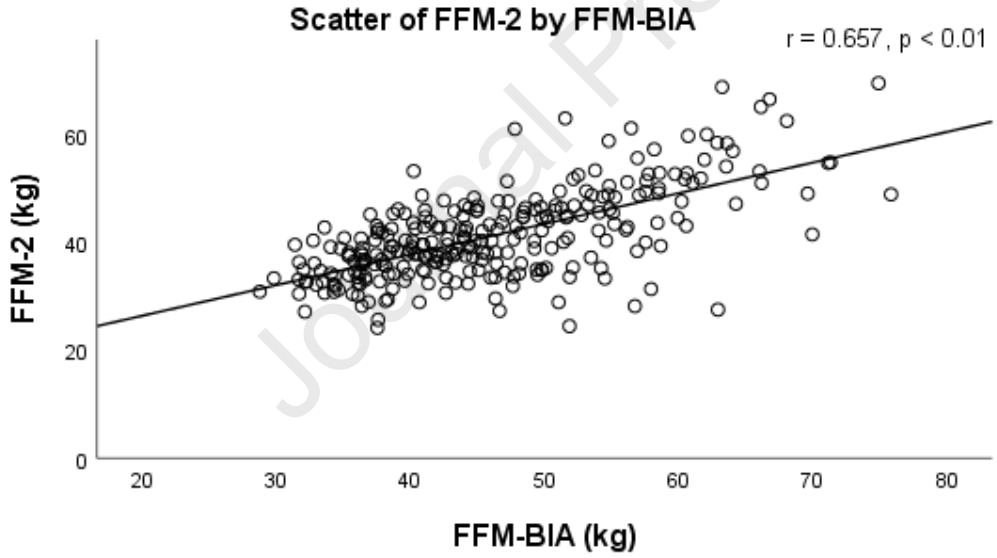

Figure 10. Scatter plot of fat-free mass calculated by the equation of Miller & Blyth, 1952, (FFM-2) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

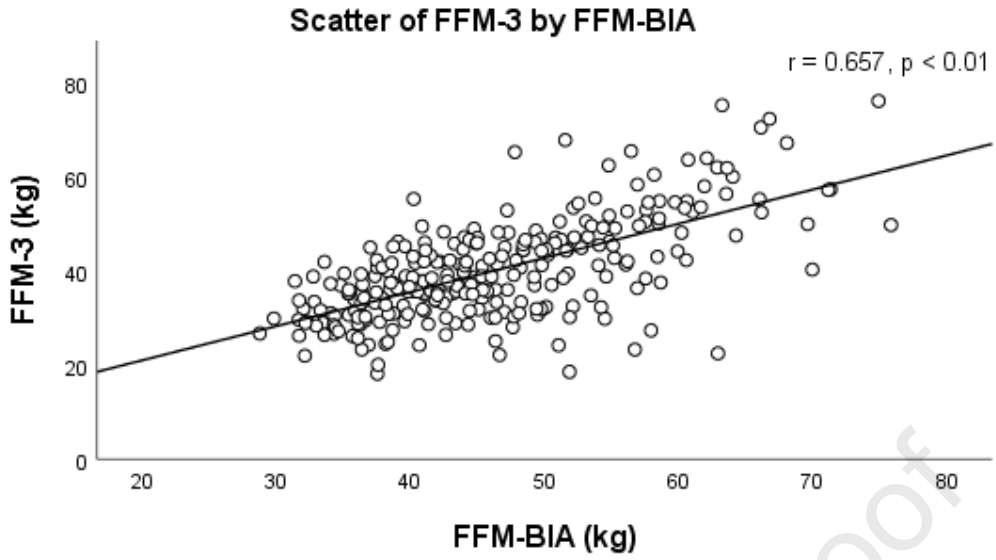

Figure 11. Scatter plot of fat-free mass calculated by the equation of Virgili et al., 1994, (FFM-3) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

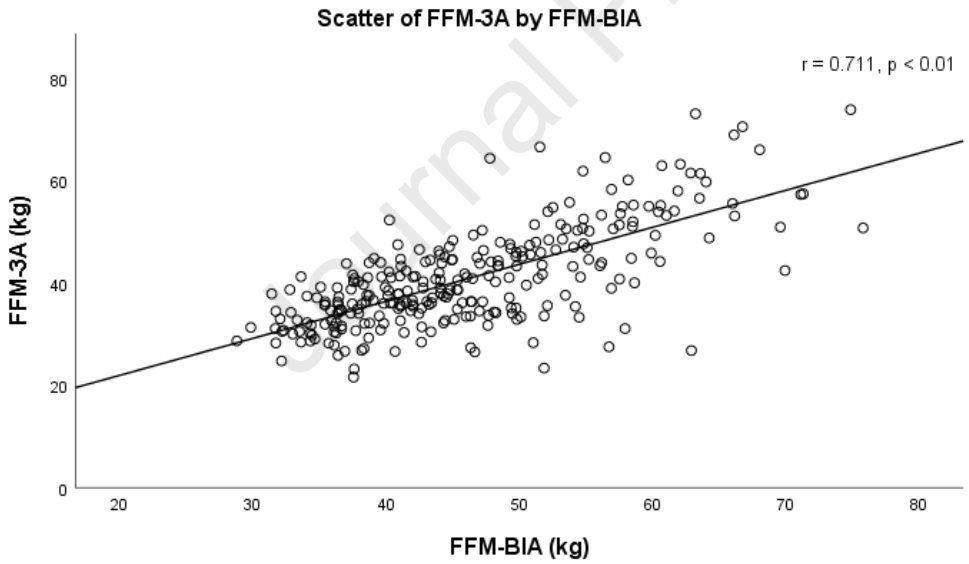

Figure 12. Scatter plot of fat-free mass calculated by the gender specific equation of Virgili et al., 1994, (FFM-3A) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

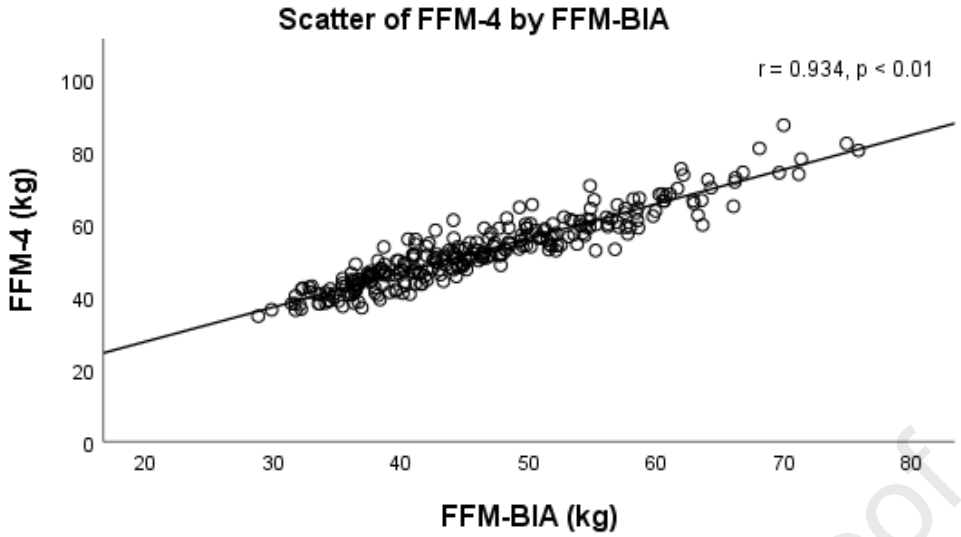

Figure 13. Scatter plot of fat-free mass calculated by the estimated urinary creatinine equation of Forbes & Bruining 1976; Kawasaki, 1993, (FFM-4) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

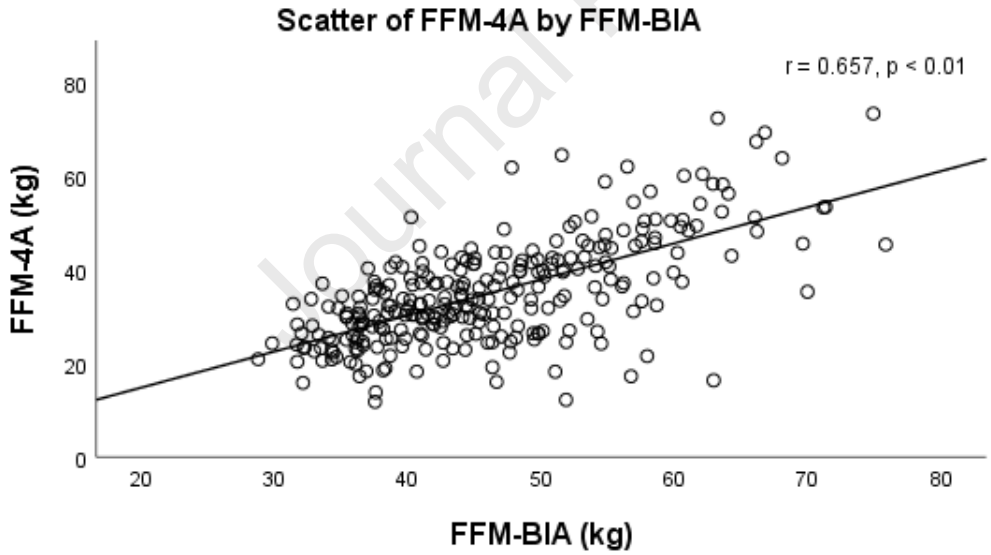

Figure 14. Scatter plot of fat-free mass calculated by the equation of Forbes & Bruining 1976; Kawasaki, 1993, with use of 24-hour measured urinary creatinine (FFM-4A) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

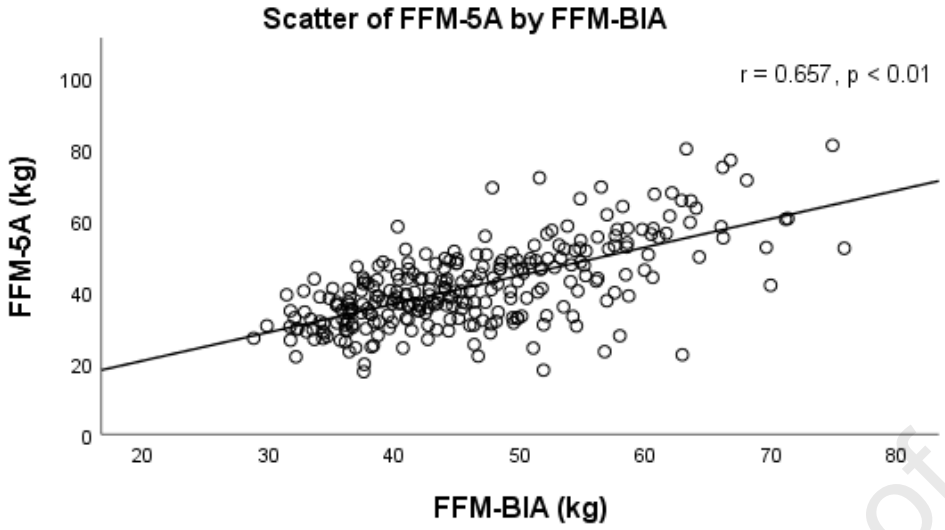

Figure 15. Scatter plot of fat-free mass calculated by the equation of Ix et al., 2011, and Jassal et al., 2015, with the use of 24-hour measured urinary creatinine (FFM-5A) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA). Line represents mean regression line.

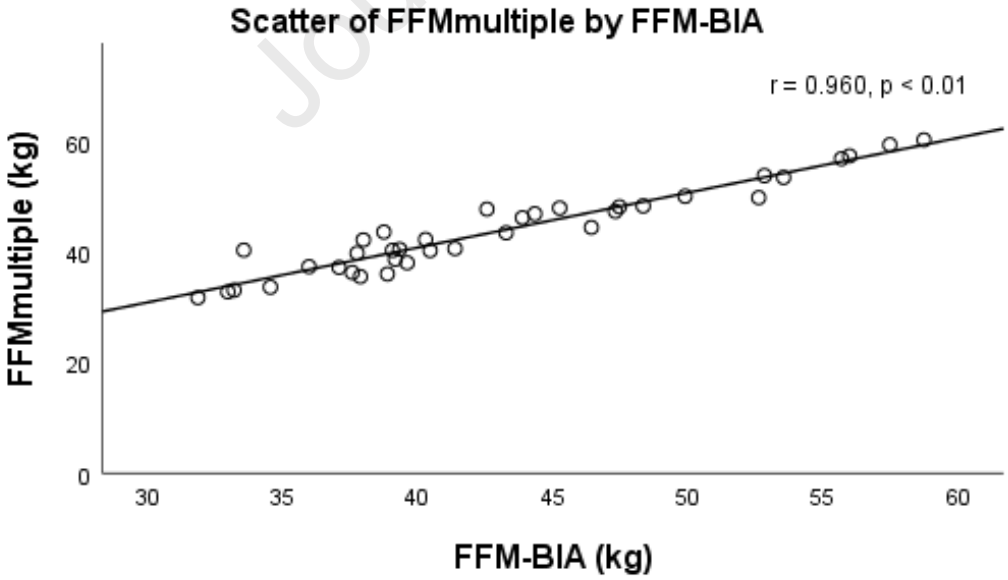

Figure 16. Scatter plot of fat-free mass calculated by multiple linear regression equation (FFMmultiple) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA) in cross-validation group. Line represents mean regression line.

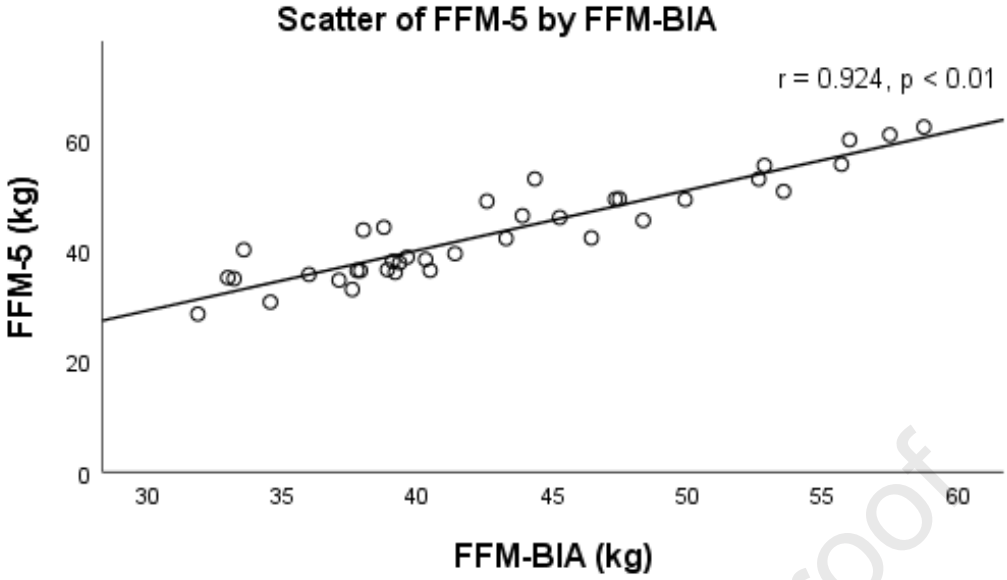

Figure 17. Scatter plot of fat-free mass calculated by the estimated urinary creatinine equation of Ix et al., 2011, and Jassal et al., 2015, (FFM-5) and fat-free mass measured by bioelectrical impedance analysis (FFM-BIA) in the cross-validation group. Line represents mean regression line.

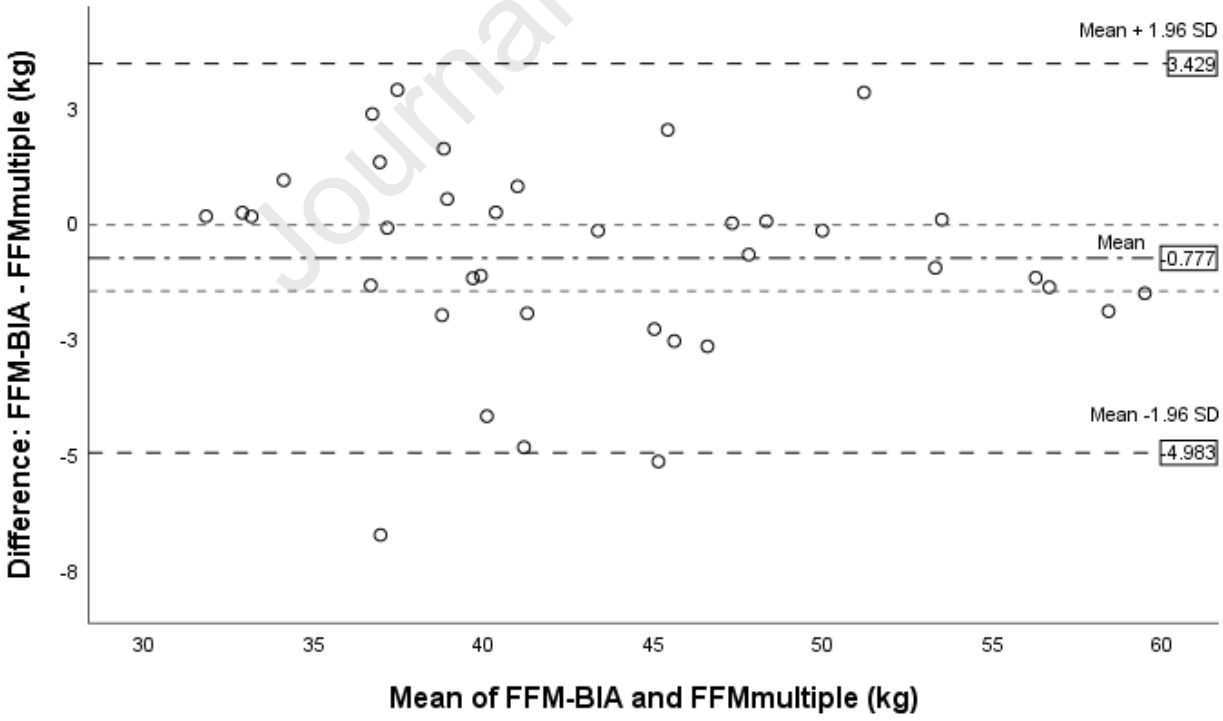

Figure 18. Bland-Altman plot comparing fat-free mass (FFM) measured by bioelectrical impedance analysis (FFM-BIA) and calculated by FFMmultiple in cross-validation group. y = 0 indicates zero difference; (– - –) indicates mean bias (y = -0.777) with 95% lower and upper confidence interval (- - -); (– – –) indicates lower and upper limits of 95% agreement (y = -4.983 and y = 3.429).

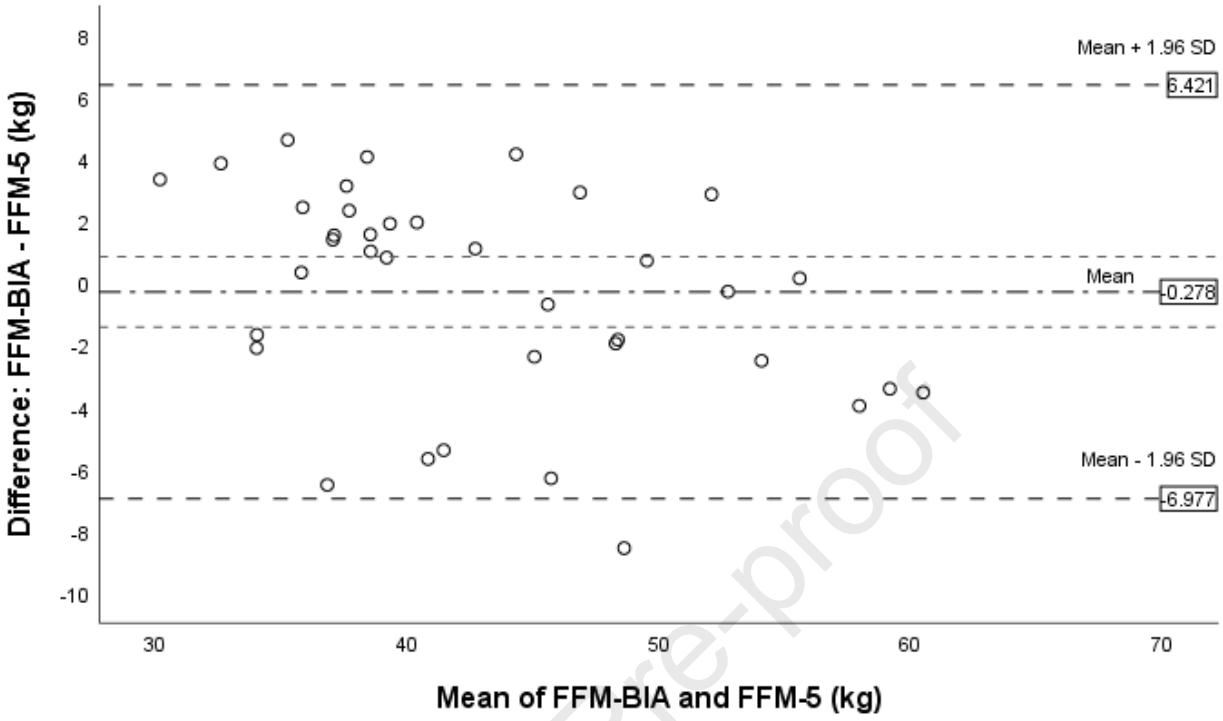

Figure 19. Bland-Altman plot comparing fat-free mass (FFM) measured by bioelectrical impedance analysis (FFM-BIA) and calculated by the estimated urinary equation of Ix et al., 2011, and Jassal et al., 2015, (FFM-5) in cross-validation group. y = 0 indicates zero difference; (– - –) indicates the mean bias (y = -0.278) with 95% lower and upper confidence interval (- - -); (– – –) indicates lower and upper limits of 95% agreement (y = -6.977 and y = 6.421).