



## Development and Validation of a Simple Equation to Predict Fat-Free Mass in the Adult Population

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(Received 12 Oct 2021; accepted 19 Dec 2021)

### Abstract

**Background:** Estimating Fat-Free Mass (FFM) is an integral part of Body composition measurements, so obtaining an accurate estimation for evaluating FFM is critical for researchers and specialists. We aimed to develop and validate a simple equation for predicting FFM in the adult population.

**Methods:** Participants were 1996 adults (1085 men and 911 women), and 18 to 69 years old from Ahvaz City, southern Iran. They were randomly divided into the derivation (n=1396) and the validation (n=600) groups with no significant differences from Jan 2018 to Feb 2020. FFM was measured by Bioelectrical Impedance Analyzer (BIA) (InBody 770©; Biospace, Seoul, South Korea). Based on the demographic variables retrieved from the Derivation group, 8 FFM predictive equations were developed using multiple regression; finally, the most accurate model (using the coefficient of determination (R<sup>2</sup>)) was chosen and then validated on the Validation group for more evaluation.

**Results:** The best equation derived from demographic characteristics was: "FFM= 0.28 × Weight (kg) + 0.57×Height (cm)+7.35×Sex (M=1, F=0)+0.03×Age (years)-70.61"; where sex = 1 for male and 0 for female. R=0.94, R<sup>2</sup>=0.89, standard error of the estimate=4.04 kg.

**Conclusion:** Our developed and cross-validated anthropometric prediction equation for fat-free mass estimation using BIA attained a high coefficient of determination, a low standard error of the estimate, and the lowest coefficient of variation. Predictive equations may be reliable and valuable alternative methods for the clinical evaluation of fat-free mass in the adult population.

**Keywords:** Equations; Estimate; Fat-free mass; Bioimpedance; Body composition

### Introduction

In recent decades, the number of obese or overweight people has been rising gradually all around

the world (1). The statistics represent that there are more than 2 billion people (30% of the world



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population) in the world suffering from obesity (2), while the WHO's plan is to abate obesity rates by 2025 (3). This disorder can lead to several problems such as heart disease, stroke, and cancers. Since obesity is a significant risk factor for many chronic diseases, there is an increasing demand for accurate body composition assessments (1).

Body composition assessments estimate the proportions of fat mass (FM) and fat-free mass (FFM). Fat mass includes fat from brain tissue, the skeleton, and adipose tissue; while FFM comprises the body's water, protein, and mineral components. Body composition assessments also measure the fat distribution and body water (intracellular and extra-cellular) (4). Body composition measurements help monitor the risk of developing chronic diseases since the proportion and distribution of body fat is a significant component of the metabolic load (5). Obesity is often measured using body mass index (BMI), but this method does not distinguish between FM and FFM and cannot measure changes in body composition (6).

Several methods and instruments have been designed for body composition assessment, such as Magnetic Resonance Imaging (MRI), Deuterium Oxide dilution (D2O), Computed Tomography (CT), and Dual-energy X-ray Absorptiometry (DXA), sophisticated, expensive, and time-consuming (4, 5). In addition, Bioimpedance analysis (BIA) is an accurate method that has been studied in various Reliability and Agreement studies with gold standard methods such as DXA, D2O for FFM evaluation (7-9).

Many studies have developed anthropometric prediction equations to estimate FFM and FM in adults in the past years. The advantages of using equations are their accessibility and convenience. Different studies have used BIA as a reference method for the development and validation of equations (10-12).

Due to the importance of body composition assessment and considering that to date, little study has been conducted to develop simple equations for the adult population. This study was conduct-

ed to develop and validate a simple equation for predicting FFM in the adult population.

## Materials and Methods

### *Subjects*

From Jan 2018 to Feb 2020, this cross-sectional study was conducted. Overall, 2015 adults (1095 men and 916 women) from a wider area of Ahvaz City in Iran were asked to participate in the survey. Any factor that disturbed the hydration status was considered as an exclusion criterion, such as hypertension, renal disease, oedema, pregnancy, lactation, and diuretics. Twelve subjects (seven men and five women) were excluded from the study based on the exclusion criteria. Study subjects were randomly divided into two subgroups of derivation and validation; the derivation group included two-thirds of the total samples, and the validation group included one-third of the total (10, 11). Afterward, the FFM predictive equation was derived from the derivation group and then validated in the second group. The equation was validated on all data to ensure the correctness of the predictive equation.

### *Ethics Approval*

The study was performed according to the Helsinki Declaration; the conditions and manner of conducting the study were fully explained to the participants then all of them signed the Informed consent form. The Ethical Committee of Ahvaz Jundishapur University of Medical Sciences approved this descriptive-analytical study (Ethics Code: IR.AJUMS.REC.1399.422).

### *Anthropometric measurements*

Anthropometric assessments were performed based on international recommendations by a trained nutritionist (13). Participants were weighed in light clothing and barefoot by a mechanical scale (Seca, Hamburg, Germany) at the nearest 0.1 kg, height was measured to the nearest 0.1 cm using a stadiometer portable scale (Seca, Hamburg, Germany). BMI was calculated by the Quetelet formula (body weight (kg) divided

by the square of the height (m<sup>2</sup>) (14). All measurements were taken during the morning and in a fasting status, then repeated by another nutritionist.

### ***FFM assessment***

Body composition and FFM were measured by a segmental multifrequency bioelectrical impedance analyzer (InBody 770©; Biospace, Seoul, South Korea). Body composition was measured based on three different frequencies with high accuracy and reliability.

All instructions for accurate measurement of BIA (Bioelectrical Impedance Analysis) were followed carefully. Instructions included the following: at least 8 h of fasting status before measurement, no strenuous exercise for 12 h before measurement, no walking at least 3 h beforehand, empty bladder, no alcohol or energy drinks or caffeine beforehand, absence of menstruation for women and freedom of any metal subjects (15).

DXA is the reference method for measuring FFM. There were significant correlations in FM and FFM measurements between BIA and DXA in adults (7-9).

### ***Statistical analysis***

Descriptive statistics were presented as mean and standard deviation (SD) for continuous variables and proportions for categorical variables. The Kolmogorov–Smirnov test was used to assess the normality distribution of variables. An independent sample t-test was used to compare the results between the derivation and validation groups. Pearson's correlation was calculated between the dependent variable and weight, height, BMI, sex, and age.

Simple linear regression of each variable against fat-free mass estimation by BIA was performed to select the most appropriate variables for the multivariate analysis. The previous step was based on the strength of each association. Multiple linear regressions were used to generate equations to predict fat-free mass based on the FFM calculated by BIA. Considering the clinical and routine practice, the following variables: age, weight, height, sex, BMI considered relevant.

Additionally, we searched for the highest R and R<sup>2</sup> value and the lowest standard error of the estimate (SEE) values of each set of stepwise regression.

The degree of concordance regressions with the reference method (BIA) was analyzed quantitatively by calculating the Pearson's correlation coefficient (r) and Lin's concordance correlation coefficient (CCC), and graphically, with the Bland-Altman method. Lin's concordance correlation coefficient was computed with a 95% confidence interval calculated using Fisher's z-transformation (16).

Bland Altman plots (17) were used to explore the distributions of systematic and random errors and determine agreement levels between the predicted and observed fat-free mass. The most straightforward derived predictive equation was validated in the Validation group. The standard error of the estimate was used to define the accuracy of predictive equations. Further, we investigated the limits of agreement between the BIA measurement and predictive equation. We adopted a confidence interval of 95.0%. Scatter plots were created to evaluate the correlation coefficient between observed and predicted fat-free mass. The coefficient of variation was calculated to explore the predicting dispersion. We analyzed the difference between the BIA-estimated and predicted fat-free mass by one-sample t-tests. Paired-samples t-tests were used to investigate the agreement between the BIA-estimated and predicted fat-free mass. Moreover, the mean difference and Limits of Agreement (LOA) calculated between the predicted and observed fat-free mass. In addition, 95% LOA were calculated as the mean difference  $\pm$  1.96 standard deviations. The IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis.

## **Results**

### ***General characteristics***

Overall, 1996 participants (1085 men and 911 women) entered the study. No significant differ-

ences were observed between the derivation and validation group, in terms of all variables such as

sex distribution, age, height, weight, BMI, and FFM (Table 1).

**Table 1:** General characteristics and FFM values in all participants, Derivation and Validation groups

<i>Variables</i>	<i>ALL</i>	<i>Derivation</i>	<i>Validation</i>	<i>P-value*</i>
	<i>(n=1996)</i>	<i>group</i>	<i>group</i>	
	Mean (SD) or n (%)			
SEX				0.21
Males (%)	1085(54.4)	771(55.2)	314(52.3)	
Females (%)	911(45.6)	625(44.8)	286(47.7)	
Age (years)	35.19(8.93)	35.00(8.97)	35.63(8.84)	0.22
<30 yr (%)	503(25.2)	342(24.5)	161(26.8)	0.07
30–40 yr (%)	998(50.0)	684(49.0)	314(52.3)	
40–50 yr (%)	332(16.6)	242(17.3)	90(15.0)	
> 50 yr (%)	163(8.2)	128(9.2)	35(5.8)	
Height (cm)	170.07(9.78)	170.21(9.83)	169.73(9.67)	0.32
Weight (kg)	84.15(17.62)	84.03(17.69)	84.44(17.45)	0.63
BMI (kg/m <sup>2</sup> )	29.06(5.40)	28.95(5.29)	29.32(5.64)	0.14
Underweight (%)	19(1.0)	10(0.7)	9(1.5)	0.06
Normal weight (%)	407(20.4)	227(19.8)	130(21.7)	
Overweight (%)	860(43.1)	590(42.3)	270(45.0)	
Obesity (%)	710(35.6)	519(37.2)	191(31.8)	
FFM (kg)	56.16(12.43)	56.44(12.50)	55.50(12.29)	0.12

Abbreviations: n, number of participants; SD, standard deviation, FFM, Fat Free Mass measured by BIA; BMI: Body Mass Index. \*t-Test between derivation and validation group,  $P < 0.05$  were set as significant.

**Check the correlations of variables**

The correlation between FFM measured by BIA and affecting variables such as weight, height,

BMI, sex, and age was calculated using bivariate correlation in both derivation and validation groups and all subjects (Table 2).

**Table 2:** Correlation between variables and Fat Free Mass (FFM) in all participants, Derivation and Validation Groups

<i>Variable</i>	<i>ALL</i>	<i>Derivation</i>	<i>Validation Group</i>
		<i>Group</i>	
Weight (kg)	0.72**	0.71**	0.73*
Height (cm)	0.84**	0.85**	0.83*
BMI (kg/m <sup>2</sup> )	0.27**	0.25**	0.30*
Sex	0.76**	0.77*	0.73*
Age	-0.10**	-0.11*	-0.06

Abbreviations: FFM, fat-free mass (kg); BMI, body mass index. \*\*Significant correlation of  $P < 0.001$  (2-tailed).

Based on the retrieved data, the correlation between all variables and FFM was significant ( $P<0.001$ , 2-tailed), except for the age variable in the validation group. Among the variables, the highest coefficient correlation with FFM was related to height (0.84), sex (0.76), weight (0.72), and then age (- 0.10), respectively.

**Derivation of equation**

Linear regressions of each variable against fat-free mass were evaluated based on descriptive characteristics and then equation for estimating FFM was designed, using BMI, weight, height, sex, and age in the derivation group. This equation was: "  $FFM = 0.28 \times \text{Weight (kg)} + 0.57 \times \text{Height (cm)} + 7.35 \times \text{Sex (M=1, F=0)} + 0.03 \times \text{Age (years)} - 70.61$ "

**Validation of the equation**

The derived equation was tested and validated on other groups (Validation and all subjects). The correlation and Lin's concordance correlation coefficient were analyzed between FFM estimated by equation and referenced FFM (measured by BIA) (Table 3); Scatter plots were also drawn from all three groups for further evaluation (Fig. 1). According to gender, BMI classification, and

age category, the linear correlations showed a high degree of correlation in all categories ( $R=0.94$  in all participants). Lin's concordance coefficient was also calculated ( $CCC=0.94$  in all participants). Therefore, there was a strong correlation between FFM derived from the equation and the reference (Table 3).

For more reliability and increased accuracy, the equation was evaluated by other statistical tests. According to Table 4, mean differences and limits of agreement between FFM derived from the equation and referenced FFM were calculated in all three groups and subgroups according to gender, BMI classification, and age groups. Bland and Altman plots between FFM prediction equation and referenced FFM in validation group, derivation group, and all participants are given in Fig. 2a-c; the distributions of systematic and random errors and limits of agreements were evaluated in all three groups. Totally, the outcome was satisfactory; the low value of the mean difference (- 0.04 in all subjects) and narrow range of limit of agreement (7.97, -8.05 in all subjects) showed that the estimated equation works to a highly accurate degree; it also confirmed the results of previous Validations (Tables 5,6).

**Table 3:** Models for estimating FFM based on Descriptive characteristics

	$\beta$					<i>(Constant)</i>	<i>R</i>	<i>R</i> <sup>2</sup>	<i>SEE</i>
	BMI	Weight	Height	Sex	Age				
MODLE 1	0.58**	-	-	-	-	39.22	0.25	0.06	12.08
MODLE 2	0.64**	-	-	-	- 0.21**	45.33	0.30	0.09	11.92
MODLE 3	0.71**	-	-	19.94**	-	24.56	0.83	0.69	6.93
MODLE 4	0.74**	-	-	19.77**	- 0.14**	28.90	0.83	0.70	6.80
MODLE 5	-	0.28**	0.83**	-	-	-110.22	0.92	0.85	4.76
MODLE 6	-	0.28**	0.86**	-	0.08**	-117.10	0.92	0.85	4.70
MODLE 7	-	0.28**	0.55**	7.53**	-	-66.65	0.94	0.89	4.05
MODLE 8	-	0.28**	0.57**	7.35**	0.03*	-70.61	0.94	0.89	4.04

Abbreviations: BMI, body mass index; Sex, male=1, female=0;  $\beta$ , beta coefficient;  $R^2$ , adjusted coefficient of determination; SEE, standard error of the estimate.

\*Significant correlation of  $P<0.05$  (2-tailed).

\*\*Significant correlation of  $P<0.001$ (2-tailed)



**Table 4:** Determination of equation accuracy in participants using linear correlation and Lin's concordance correlation coefficient

Variable	All participants		Derivation Group		Validation Group	
	R	CCC	R	CCC	R	CCC
Total	0.94	0.94	0.94	0.94	0.94	0.94
Male	0.86	0.84	0.86	0.84	0.86	0.84
Female	0.86	0.86	0.87	0.86	0.86	0.85
Underweight	0.90	0.84	0.93	0.84	0.90	0.91
Normal	0.93	0.92	0.92	0.92	0.93	0.94
Overweight	0.94	0.93	0.94	0.93	0.94	0.91
Obese	0.94	0.94	0.95	0.94	0.94	0.94
<30 yr	0.95	0.95	0.95	0.95	0.95	0.95
30–40 yr	0.93	0.93	0.93	0.93	0.93	0.92
40–50 yr	0.94	0.94	0.94	0.94	0.94	0.94
> 50 yr	0.95	0.95	0.94	0.95	0.95	0.96

Abbreviations: R, Pearson linear correlation coefficient; CCC, Lin's concordance correlation coefficient

**Table 5:** validation of FFM derived from the equation on the all participants and subgroups

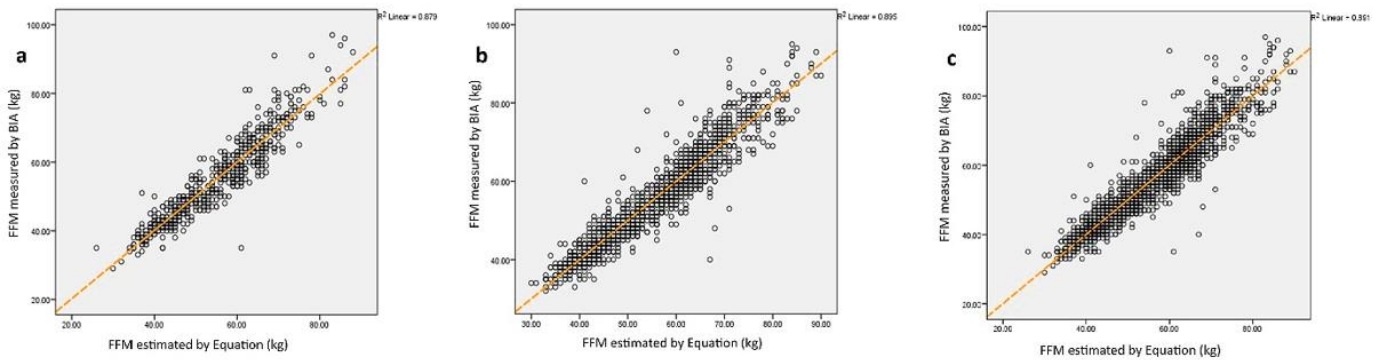
Variables	Derivation group			Validation group			All participants		
	Mean ± SD BIA <sup>1</sup>	Mean ± SD Equation <sup>2</sup>	*P-Value	Mean ± SD BIA <sup>1</sup>	Mean ± SD Equation <sup>2</sup>	*P-Value	Mean ± SD BIA <sup>1</sup>	Mean ± SD Equation <sup>2</sup>	*P-Value
Total	56.44 ± 12.50	56.47 ± 11.83	0.79	55.50 ± 12.29	55.58 ± 11.58	0.63	56.16 ± 12.44	56.20 ± 11.76	0.63
Male	65.14 ± 9.15	65.16 ± 0.27	0.86	64.13 ± 9.50	64.54 ± 7.35	0.12	64.85 ± 9.26	64.98 ± 7.47	0.33
Female	45.72 ± 6.11	45.75 ± 5.89	0.82	46.03 ± 6.80	45.75 ± 6.0	0.17	45.82 ± 6.33	45.73 ± 5.95	0.51
Underweight	40.78 ± 6.94	43.10 ± 8.41	0.14	40.54 ± 6.63	42.93 ± 8.11	0.004	40.66 ± 6.61	43.02 ± 8.04	0.009
Normal	52.22 ± 10.28	53.03 ± 10.26	0.06	51.80 ± 11.26	52.68 ± 11.37	0.09	52.08 ± 10.59	52.92 ± 10.61	0.07
Overweight	55.98 ± 12.20	55.59 ± 11.07	0.10	55.46 ± 11.23	55.40 ± 10.34	0.83	55.82 ± 11.90	55.53 ± 10.84	0.42
Obese	59.34 ± 13.04	59.56 ± 12.65	0.84	58.79 ± 13.37	58.41 ± 12.62	0.22	59.33 ± 13.13	59.26 ± 12.64	0.62
<30 yr	56.44 ± 13.59	56.83 ± 13.03	0.06	55.06 ± 13.80	54.95 ± 12.36	0.73	56.00 ± 13.66	56.23 ± 12.87	0.19
30–40 yr	57.97 ± 12.04	57.83 ± 11.0	0.37	56.56 ± 11.68	56.98 ± 10.78	0.10	57.53 ± 11.94	57.56 ± 10.97	0.80
40–50 yr	55.45 ± 12.39	55.01 ± 12.14	0.09	54.15 ± 11.14	53.55 ± 12.27	0.13	55.09 ± 12.06	54.61 ± 12.17	0.29
> 50 yr	50.17 ± 9.69	51.01 ± 9.81	0.30	51.47 ± 12.27	51.23 ± 11.42	0.65	50.45 ± 10.27	51.05 ± 10.14	0.15

Abbreviations: FFM, Fat free Mass (kg); BIA, bioelectrical impedance analysis. <sup>1</sup>mean ± standard deviation of Fat Free Mass measured by BIA (kg); <sup>2</sup> mean ± standard deviation of Fat Free Mass estimated by the equation (kg). \*Mean measured-predicted value (significance at 0.05 level)

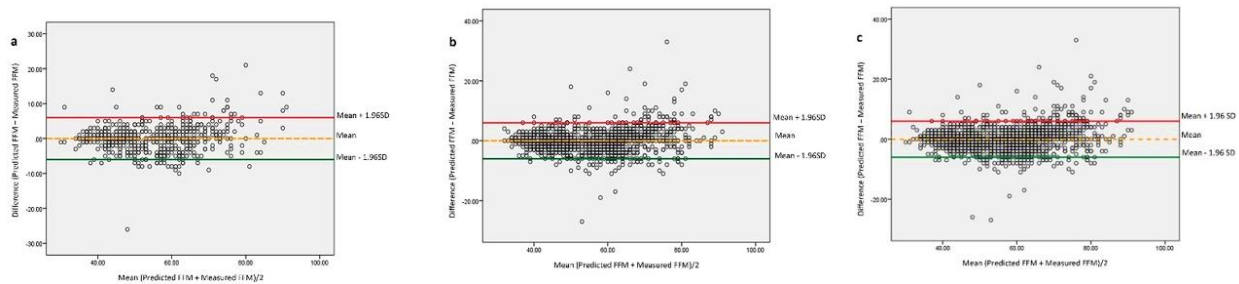
**Table 6:** Mean difference and Limits of agreement between FFM derived from equation and referenced FFM\*

Variables	Derivation group		Validation group		All participants	
	Mean Difference $\pm$ SD (kg)	Limits of Agreement (kg) (Lower, Upper)	Mean Difference $\pm$ SD (kg)	Limits of Agreement (kg) (Lower, Upper)	Mean Difference $\pm$ SD (kg)	Limits of Agreement (kg) (Lower, Upper)
Total	-0.02 $\pm$ 4.03	7.88, -7.93	-0.08 $\pm$ 4.21	8.18, -8.34	-0.04 $\pm$ 4.08	7.97, -8.05
Male	0.02 $\pm$ 4.69	9.16, -9.22	0.27 $\pm$ 3.49	8.92, -9.74	-0.13 $\pm$ 4.71	9.09, -9.37
Female	-0.02 $\pm$ 3.03	5.93, -5.98	-0.41 $\pm$ 4.76	7.12, -6.57	0.06 $\pm$ 3.19	6.32, -6.18
Underweight	-2.32 $\pm$ 4.61	6.71, -11.37	-2.39 $\pm$ 1.80	1.15, -5.93	-2.35 $\pm$ 3.48	4.46, -9.18
Normal	-0.81 $\pm$ 4.03	7.09, -8.72	-0.87 $\pm$ 3.73	6.44, -8.20	-0.83 $\pm$ 3.93	6.88, -8.55
Overweight	0.38 $\pm$ 3.90	8.04, -7.26	0.05 $\pm$ 4.40	8.68, -8.57	0.28 $\pm$ 4.06	8.25, -7.69
Obese	-0.03 $\pm$ 4.10	8.00, -8.07	0.37 $\pm$ 4.24	8.69, -7.64	0.07 $\pm$ 4.14	8.19, -8.04
<30 yr	-0.39 $\pm$ 3.96	7.37, -8.16	0.10 $\pm$ 4.09	8.14, -7.92	-0.23 $\pm$ 4.01	7.62, -8.09
30–40 yr	0.14 $\pm$ 4.15	8.29, -8.01	-0.41 $\pm$ 4.47	8.36, -9.18	-0.03 $\pm$ 4.26	8.33, -8.39
40–50 yr	0.44 $\pm$ 4.11	8.50, -7.62	0.59 $\pm$ 3.77	7.99, -6.79	0.48 $\pm$ 4.02	8.36, -7.39
> 50 yr	-0.83 $\pm$ 3.13	5.29, -6.97	0.24 $\pm$ 3.11	6.34, -5.86	-0.60 $\pm$ 3.14	5.56, -6.78

Abbreviations: FFM, fat-free mass (kg), SD, standard deviation. \*Fat Free Mass measured by BIA (kg)



**Fig. 1:** Scatter plots between FFM estimated by Equation and FFM measured by BIA in Validation group (a), Derivation group (b) and All participants (c), which showed a high correlation between the predictor equation and the reference



**Fig. 2:** Bland and Altman plots between FFM prediction equation and FFM measured by BIA in Validation group (a), Derivation group (b) and All participants (c)

## Discussion

We aimed to obtain a practical and straightforward equation for the accurate estimation of FFM in the adult population. Our study was conducted on a substantial population (n=1996). This is the first study in this field conducted on the Iranian population. Participants logged in the study included all BMI categories (underweight to obese adults) and all adult age groups [18 to 60]. This study evaluated the designed equation with comprehensive and various statistical methods to test its precision. We tested the results of the derived equation on the validation group and whole participants. Then, the results were compared with FFM measured by BIA. Lin's concordance and Pearson's correlation between measured FFM and FFM derived from the equation was significant ( $r=0.94$ ,  $P<0.05$ ,  $CCC=0.94$ ). To increase the accuracy and further validation, we also calculated the limit of agreement between FFM and FFM estimated by the equation; the results were also acceptable.

BMI is one of the easiest methods for evaluating and determining the anthropometric status and classifying individuals as underweight, normal, overweight, and obese. It is easy to use, inexpensive and fast (18). BMI is significantly correlated with %BF, but on the other hand, BMI does not estimate body composition and gives us only a general view with a weak ability to predict other components of body composition (19).

Only limited studies estimate FFM based on anthropometric measurements without the need for bio-impedance values such as resistance and reactance. Most studies (20-22) have used resistance or reactance to obtain the FFM estimation equation, which requires the use of bio-impedance to measure it, so these equations are sophisticated. They need advanced measuring tools and finally cannot be used as a simplified equation without the need for high-level tools.

The study by Diniz et al. (10) was one of the few similar studies performed on 209 patients with

chronic hepatitis C (CHC), done in Brazil to obtain a simplified equation for evaluating FFM.

Independent variables such as sex, height, weight, and waist circumference were used in this study, while the age variable was not presented in the predicted equation. This study was designed for CHC patients; so, it cannot be used in a healthy population.

Lyra et al. (12) conducted a study to obtain the FFM predictive equation in 218 Brazilian adolescents aged 10 to 16 yr with healthy weight based on body circumferences. The significant drawbacks of this study were the lack of a validation group and the lack of using height, weight, and BMI variables in designing the estimation equation, although these independent variables have a high correlation coefficient with FFM (10, 11). It is also developed for normal-weight adolescents, so it may not be generalizable to other age groups.

The main limitation of our study was the lack of DXA measurement as the standard gold method for measuring FFM. Although BIA is not considered as a gold standard for assessing FFM, several studies have shown high accuracy and reliability of body composition measured by BIA in comparison with DXA (7-9), in the general population as well as the clinical setting, and to be used as a reference method (10-12).

Based on this information, our study was more comprehensive than previous studies; the number of participants, target population, variables used in the derived equation, the number, and variety of statistical analyses are the strengths of this study, which made it a comprehensive study of its kind; something we did not find in similar studies.

## Conclusion

Our study was conducted to explore an accurate and easy-to-use Equation that can estimate FFM with a high degree of agreement. There is no need for expensive, time-consuming, or advanced tools; it can be used by health professionals (e.g.,



medical doctors, nutritionists, dieticians), especially in clinical settings (e.g., outpatient clinics) where sophisticated instruments such as MRI, CT, DXA, and BIA are not available.

## Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Acknowledgements

This work was financially supported by a grant (99s46) from the Vice-chancellor for Research Affairs of Ahvaz Jundishapur University of Medical Sciences. We thank all participants of the study for their cooperation. We would also like to thank the School of Allied Medical Sciences Ahvaz staff for their valuable contributions.

## Conflict of interest

The authors declare that there is no conflict of interests.

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