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PII: S1094-6950(18)30267-1
DOI: https://doi.org/10.1016/j.jocd.2019.03.002
Reference: JOCD 1120

To appear in: *Journal of Clinical Densitometry*

Received date: 20 December 2018
Revised date: 12 March 2019
Accepted date: 12 March 2019

Please cite this article as: Li-Wen Lee , Yu-San Liao , Hsueh-Kuan Lu , Kuen-Chang Hsieh , Ching-Chi Chi , Performance of bioelectrical impedance analysis in the estimation of bone mineral content in healthy children aged 6-12 years, *Journal of Clinical Densitometry* (2019), doi: https://doi.org/10.1016/j.jocd.2019.03.002

Performance of bioelectrical impedance analysis in the estimation of bone mineral content in healthy children aged 6-12 years

Running title: Bioelectrical impedance analysis and bone health in children

Li-Wen Lee[1,2], Yu-San Liao[2,3], Hsueh-Kuan Lu[4], Kuen-Chang Hsieh[5,6,*], Ching-Chi Chi[7,8,*]

[1]Department of Diagnostic Radiology, Chang Gung Memorial Hospital, Chiayi, Taiwan

[2]Department of Nursing, Chang Gung University of Science and Technology, Chiayi Campus, Taiwan

[3]Department of Diagnostic Radiology, Chang Gung Memorial Hospital, Yunlin, Taiwan

[4]Sport Science Research Center, National Taiwan University of Sport, Taichung, Taiwan

[5]Office of Physical Education and Sport, National Chung Hsing University, Taichung, Taiwan

[6]Research Center, Charder Electronic Co, Ltd, Taichung, Taiwan

[7]Department of Dermatology, Chang Gung Memorial Hospital, Linkou, Taoyuan, Taiwan

[8]College of Medicine, Chang Gung University, Taoyuan, Taiwan

*These authors contributed equally to this work

Correspondence author

Prof. Ching-Chi Chi

E-mail: chingchi@cgmh.org.tw; chingchichi@gmail.com

Abstract

[Background] Bioelectrical impedance analysis (BIA) is a widely available tool which provides mineral estimate. However, BIA is not currently recognized as a bone mineral measuring method. This study aimed to explore the ability of BIA to predict bone mineral content (BMC) in children, using dual-energy X-ray absorptiometry (DXA) as a gold standard.

[Methods] Healthy children aged 6-12 years (n=176) were recruited for BIA and DXA measurements. Predictive models were generated using basic indices (age, height, weight, waist circumference, hip circumference, etc.) and BIA parameters (minerals, fat mass and fat free mass).

[Results] The root-mean-square deviation and R^2 for the total BMC predictive model

were 0.089 kg and 0.926, respectively using height and weight as predictors whereas 0.113 kg and 0.886, respectively using minerals by BIA. The root-mean-square deviation and R^2 for the subtotal BMC predictive model were 0.080 kg and 0.935, respectively using height and weight as predictors whereas 0.098 kg and 0.906, respectively using minerals by BIA. The best predictive models included basic indices and BIA parameters as predictors, but they had only slightly better performance over simple models.

[Conclusions] Mineral content by BIA was good predictor of total and subtotal BMC in healthy children but with similar overall model performance compared to basic indices. More complex models combined all the predictive variables gave better prediction power, but of little improvement to these simple models. The BIA instrument does not appear to be useful in estimating BMC in healthy children as basic indices are more widely available measures but provide comparable performance. Future studies are needed to determine the clinical usefulness of the more complex prediction model in children with disease or children in other subgroups.

Keywords: bioelectrical impedance analysis, Dual-Energy X-ray Absorptiometry Scan, bone mineral content, children

Introduction

Bioelectrical impedance analysis (BIA) is a two-compartment body composition method based on impedance measurements of biological tissues. The measured impedance is proportional to the volume of body water and therefore, total body water (TBW) can then be determined. In the presence of a constant TBW to fat free mass (FFM) ratio, impedance can be converted into FFM [*1, 2*]. Hydration of FFM in adults is assumed to be approximately 73% [*3*], but in reference children aged 6-12 years it is 75.1% to 77.6% [*4-6*]. Fat mass (FM) can be determined by calculating the difference between total body weight and FFM. In addition to FFM and FM, modern BIA devices can also estimate the total body mineral content, assuming a constant proportion of minerals in FFM. In adults, mineral fraction of the FFM is 6.8% and 6.2% for men and women, respectively [*6, 7*]. In children, mineral fraction of the FFM is relatively small compared with those of adults (5.1%, 5.4% and 5.7% in boys aged 7-9, 9-11 and 11-13 years, respectively vs. 4.9%, 5.2% and 5.5% in girls aged 7-9, 9-11 and 7-13 years, respectively) [*4-6, 8*]. Although many studies have validated the precision and accuracy of FM, FFM and percentage body fat (PBF) by BIA, less is known about mineral content. Currently, BIA is not an established method for estimating minerals in biological tissues.

In the human body, minerals may exist in bone, referred to as bone mineral

content (BMC), and in body fluids, referred to as non-osseous mineral content [*4*]. Bone quantity and quality are important health issues in children as they are at an important stage for building up bone mass and strength for later life [*9*]. The ability to quantify BMC is valuable for monitoring nutritional status, growth delay and fracture risks in children. The only direct way to assess body mineral content in humans is through chemical analysis of cadavers [*10*]. Alternative, *in vivo* BMC can be analysed by dual-energy X-ray absorptiometry (DXA) and non-osseous mineral content can be derived by assuming a constant ratio of osseous to non-osseous minerals [*11*]. Currently, DXA is used as the criterion method for measuring BMC in the 4-compartment and 5-compartment body composition methods [*12*]. BMC is generally assumed to be approximately 80% of the total body mineral in both adults and children [*7, 13*]. In children, DXA is also the recommended method for assessing BMC and the preferred skeletal sites of measurements are the spine and total body less head, according to 2013 Official Pediatric Positions of the international Society for Clinical Densitometry [*9*].

BIA is a simple, practical and inexpensive tool which provides an estimate of total body mineral, a combined weight of the osseous and non-osseous mineral masses. However, a major drawback with BIA method is that it is based on a number of assumptions; violations of these assumptions may affect the accuracy of BIA

assessment. Indeed, the assumptions are not always true as biological variation may occur and change the TBW, protein and mineral ratios in FFM. In children, the proportion of minerals to TBW is known to vary by age, sex and body fatness [*6, 13*]. Moreover these age- and sex-specific mineral fractions in the FFM are developed from reference children in the 50th percentile height and body fatness, and the results may not be applicable to children with a wide range of body composition. To the best of our knowledge, there is only one discussion in the literature regarding the predictive ability of BIA in BMC, that of Patil et al. [*14*], who reported the prediction ability of BMC in adult subjects.

DXA is the reference standard for measuring BMC, exposing subjects to only daily background radiation. However, a concern remains about the health effects of low dose radiation in children. BIA is a widely available instrument that does not use ionizing radiation to provide an estimate of mineral content. Therefore, we aimed to explore the ability of BIA to predict bone mineral in children, using DXA as a gold standard. To do so, different regression models of BMC were generated using a variety of BIA parameters and simple measurements in healthy children aged 6-12 years. The fit and prediction error of the models were compared.

Materials and Methods

Design, sample, and setting

This cross-sectional prospective study was approved by the local Institutional Review Board, and written informed consents were provided by the subjects and their parents. A total of 176 healthy Taiwanese children aged 6-12 years were recruited between February 2015 and February 2016. None of the subjects were pregnant, had amputations, implants, or chronic illnesses, or were prescribed regular medication. Participants were instructed to fast for at least 2 h before measurements. Vigorous activity and alcohol were avoided for a minimum of 48 h before the study day. Girls were not given appointments during their menstrual cycle. Measurements were performed after urination and change into a hospital gown.

Measurements

All measurements were completed on the same morning, with a total study time of approximately 1 hour. One measurement per subject was performed using each instrument. Body height and weight were measured with subjects wearing no shoes using a digital scale (Super-View, HW-3050, Taipei, Taiwan). Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. The z-scores for height and BMI were computed using the WHO AnthroPlus software according to the WHO Reference 2007 for the period 5-19 years (https://www.who.int/growthref/tools/en/). The z-score of weight reference was not

derived, as the WHO growth chart did not provide data for children older than 10 years. Waist circumference (WC) was measured at the midpoint between the lowest rib and the iliac crest. Hip circumference (HC) was measured around the widest portion of the buttocks, with the tape parallel to the floor. Whole body DXA was performed using a Hologic Delphi A scanner (Hologic, Bedford, MA, USA) and analyzed using software version 12.5 with pediatric whole-body analysis mode. Total body composition estimates, including BMC_{DXA}, FFM_{DXA} (lean mass + BMC), FM_{DXA}, and PBF_{DXA} were measured. Subtotal (total body less head) BMC_{DXA} was calculated by excluding the head from total body measurements. A multi-frequency (20 kHz and 100 kHz) BIA device using an 8-point tactile electrode system (Inbody 230, Biospace Corp., Seoul, Korea) was used to measure TBW_{BIA}, mineral content ($mineral_{BIA}$), FFM_{BIA}, and FM_{BIA} using the in-build equations. The hydration of FFM was calculated by dividing TBW_{BIA} by the FFM_{BIA}. The mineral fraction in FFM was calculated by dividing $mineral_{BIA}$ by the FFM_{BIA}. The DXA and BIA measurement details have been described previously [*15*].

Statistical analysis

Student's t-test was used to compare means of two samples using IBM SPSS Statistics version 22.0 (IBM Corp., NY, USA). An alpha of 0.05 was used as the cut off for significance. Prediction models were implemented in the Waikato Environment for

Knowledge Analysis (WEKA) software version 3.8.2 [*16*]. Pearson's correlation coefficients (r) were obtained to evaluate the strength of linear correlation between predictors and each bone estimate. The dataset was partitioned into training and test sets using 5-fold cross-validation. The best-first search method combined with the wrapper approach was applied to reduce the number of predictors [*17*]. Then, linear modeling was applied to predict bone estimates in total and subtotal body regions using different groups of variables. The model which best predicted the DXA measurement and achieved the highest adjusted R^2 (R^2) and minimal root-mean-square deviation (RMSE) was identified as the best performing prediction. Nine basic indices included subject characteristics such as age, sex, height, weight, BMI, height z-score, BMI z-score, WC and HC. BIA parameters included $mineral_{BIA}$, FM_{BIA} and FFM_{BIA}. There were five groups of models in this study. Variables entered into group 1 were age and sex; those used in group 2 were basic indices; those used in group 3 were $mineral_{BIA}$; those used in group 4 were $mineral_{BIA}$ and basic indices; and those used in group 5 were FM_{BIA}, FFM_{BIA} and basic indices. According to Green's formula [*17*], the effective sample size with a power of 0.8 (alpha = 0.05) was calculated as: 50 + 8 x number of predictors. The number of candidate predictors was 13 in this study, resulting in an effective sample size of 154. Data were expressed as mean ± standard deviation (SD).

Results

A total of 90 girls and 86 boys aged 6-12 years completed the study. Demographic data and body composition measurements for the study group are presented in **Table 1**. There were no significant gender differences, except for a significantly higher FFM hydration in boys and a higher mineral fraction in the FFM in girls. Mean (± SD) FFM hydration was 73.3±0.2 % and 73.4±0.3 % in girls and boys, respectively ($p < 0.001$). Mean mineral fraction in the FFM ratio was 7.3±0.3 % and 7.0±0.3 % in girls and boys, respectively ($p < 0.001$). Total weight measured by DXA was significantly higher than that measured by BIA (35.7±13.5 kg by DXA vs. 34.9±13.2 kg by BIA, $p < 0.001$). However, correlation between DXA-measured body weights and BIA-measured body weights were excellent ($R^2 = 0.9998$, RMSE = 0.181 kg). Mean height and BMI z-score were greater than zero in both sexes. The mean PBF_{DXA} was 29.5±7.4 % in girls and 26.9±10.7% in boys. Mean BMC_{DXA} was 1.210±0.300 kg in girls and 1.257±0.347 kg in boys. Mean $mineral_{BIA}$ was 1.795±0.439 kg in girls and 1.834±0.523 kg in boys.

The correlations between bone estimates (BMC_{DXA} and subtotal BMC_{DXA}) and 13 candidate independent variables are presented in **Table 2**. In general, the correlation coefficients were greater for subtotal than for whole body estimates. Among basic

indices, height and weight had the strongest association with BMC estimates (r = 0.894-0.924). $Mineral_{BIA}$, TBW_{BIA} and FFM_{BIA} had similar strengths of association with each bone estimate, indicating that the BIA device assumed a constant proportion of mineral and water content in FFM. Since $mineral_{BIA}$ and FFM_{BIA} were derived from TBW_{BIA}, TBW_{BIA} were excluded from the candidate independent variables.

The predictive equations obtained from linear regression modelling are shown in **Table 3**. For each model, the best performing prediction with the smallest RMSE is presented.. When basic indices were entered, bone estimate predictions were more accurate. Compared to age and sex, basic indices gave better predictive performance for subtotal BMC_{DXA} (R^2 = 0.587, RMSE = 0.199 kg in group 1 vs. R^2 = 0.935, RMSE = 0.0780 kg in group 2). Interestingly, inclusion of sex, WC, HC, BMI, height z-score and BMI z-score did not improve the prediction performance.

The ability of $mineral_{BIA}$ to predict bone estimates by DXA is presented in group 3. In general, basic indices gave better predictive performance compared to $mineral_{BIA}$. The predictive performance of $mineral_{BIA}$ for subtotal BMC_{DXA} was R^2 = 0.906, RMSE = 0.098 kg and was R^2 = 0.886, RMSE = 00.113 kg for total BMC_{DXA} . Inclusion of $mineral_{BIA}$ increased the prediction accuracy compared to anthropometric indices alone, but the model performance improved only slightly. Among all groups, group 5 models provided the smallest RMSE, with age, height,

FFM_{BIA} and FM_{BIA} as predictors. In group 5 models, the predictive performance was R^2 = 0.939, RMSE = 0.078 kg for subtotal $BMC_{DXA;}$ and R^2 = 0.932, RMSE = 0.086 kg for total BMC_{DXA}. For subtotal BMC_{DXA}, the best fit model was with $mineral_{BIA}$ and basic indices (R^2 = 0.959).

Discussion

This study showed that the value of total body minerals provided by BIA was a good predictor for total and subtotal BMC with a high adjusted R^2 value and low RMSE. Basic indices such as age, height, weight, BMI, WC and HC along with age-adjusted height, weight or BMI are simple, practical and inexpensive measurements taken during regular health checks. If these measurements showed a close correlation with BMC, they could be useful biomarkers for predicting bone health. This study showed that basic subject characteristics such as height and weight were comparative to $mineral_{BIA}$ in predicting BMC. Although a combination of BIA and body measurements predicted BMC better than body measurement or BIA measurement alone, improvements were small. This study showed a high ability of basic indices to predict subtotal BMC_{DXA} (R^2 = 0.935, RMSE = 0.080 kg). The prediction performance was in close agreement with two previous studies which reported R^2 = 0.936 and standard error of estimate (SEE) = 0.16 kg in children aged 5-18 years [*18*] and R^2 =

0.959, SEE = 0.091 kg in children aged 6-18 years [*19*]. Basic indices have also been used to predict BMC z-score, but with less predictive performance than for BMC. The predictive power was R^2 = 0.33-0.43 for BMC in children aged 5-20 years [19]. As basic index models use lower-cost predictors and are widely available, BIA models may have limited value in bone estimates in healthy children.

Healthy bodies remain a strict water and electrolyte balance such that the electrical properties are mainly determined by the tissue water content [*20*]. Based on this assumption, most BIA models estimate the amount of FFM using a regression equation with tissue electrical measurements and other parameters such as weight, age, gender and anthropometric measurements [*21*]. In BIA, mineral content is estimated by assuming a constant proportion of water, protein and minerals in FFM according to reference bodies, because bone is a low water content tissue which cannot be differentiated using BIA [*20*]. In DXA, the principle for measuring BMC is based on the difference in mass attenuation coefficients of bone and soft tissue at two different X-ray energies. Despite the different designs of the two methodologies, our results showed that the $mineral_{BIA}$ was strongly correlated with the BMC by DXA. Patil et al. reported that the predictive power of BIA was R^2 = 0.6275 for BMC in healthy adults aged 23-81 years [*22*]. In their study, including anthropometric parameters clearly improved the predictive power from R^2 = 0.6275 to 0.8237. In our

study, the inclusion of anthropometric parameters to $mineral_{BIA}$ improved the prediction power of subtotal BMC from R^2 = 0.906 to 0.959. Our study showed a better prediction power of BIA and a smaller increase in prediction power after anthropometric parameters were included into the regression model. The difference in results between our study and the previous one was not due to different designs of BIA as both studies used multi-frequency BIA devices from the same manufacturer. The discrepancy may arise from the different age groups of our respective subjects.

BIA methods are developed and validated against criterion techniques such as DXA, isotope dilution or multi-compartment models. However, the reference techniques may not provide accurate measurements if the underlying assumptions are violated [*23*]. Since the value of mineral content by BIA is mainly derived from FFM, an appropriate TBW measurement and accurate FFM partition are essential. In our study, the mean FFM hydration was 73.3% for girls and 73.4% for boys, and the mean mineral fraction in the FFM was 7.3% for girls and 7.0% for boys, indicating that the BIA device used in this study may predict pediatric FFM and mineral using the adult mode. This study investigated the correlation but not the agreement of mineral content by BIA and BMC by DXA, and results may therefore be affected by the BIA assumption to a lesser extent. Another concern is that the mean percentage body fat of the current study was 29.5% in girls and 26.9% in boys, which was

approximately 10% higher than that of the reference children in the literature [*4-6*]. A significantly higher FFM hydration has been reported in children with PBF more than 30% compared to that in normal-weighted children [*13*]. This may explain why models developed with FM_{BIA} and FFM_{BIA} in the regression equations performed better in predicting bone estimates than those with $mineral_{BIA}$.

There has been growing interest for the application of BIA in body composition measurement with the introduction of multi-frequency and standing posture models. However, most of the validation studies focus on the FFM, FM and PBF estimates, and less is known about the prediction ability of bone estimates. This study explored the predictive ability of BIA as well as other simple methods to provide bone estimates, which fills in the knowledge gap of the application of BIA to bone health. Our study showed that mineral content by BIA may have a limited role over basic measurements in making bone estimates in healthy children.

There are some limitations in the current study. First, this study did no chemical analysis to measure BMC. However, DXA is the *in vivo* gold standard for bone measurements and has been widely used in the multi-compartment models of body composition analysis. Second, our results may not be applicable to the general pediatric population or to diseased children. Third, our study used chronological age and did not include measures of skeletal maturation such as puberty stage or bone

age. Further study is needed with inclusion of bone age into the BMC prediction model. Fourth, the exact BIA equation for $mineral_{BIA}$ was not available from the manufacture's manual and over-fitting of the regression equation may occur if the calculation of $mineral_{BIA}$ already uses body indices as independent variables. Finally, BIA does not provide BMC measurements, so we could only examine the correlations but not agreements between $mineral_{BIA}$ and BMC_{DXA}.

To conclude, both $mineral_{BIA}$ and basic indices are good predictors of total and subtotal BMC in healthy children aged 6-12 years with similar overall model performance. More complex models that combined all predictive variables gave better prediction power, but little clinical value over simple predictive models. The BIA instrument does not appear to be useful in estimating BMC in healthy children as basic indices are more widely available measures but provide comparable performance. Future study is needed to explore whether the more complex model with higher predictive accuracy could better predict BMC in children with disease or other subgroups of children.

Acknowledgements:

We would like to thank the Chang Gung Medical Foundation for providing the funding (Grant number CMRPG6D0353 and CMRPG3G1541).

Conflict of Interest:

One of the authors (KCH) is employed by Charder Electronic Co, Ltd. This company did not provide KCH financial support in executing this study. Nor did the company have any additional role in the research funding, study design, data collection and analysis, decision to publish, or preparation of the manuscript. There are no patents, products in development nor marketed products to be declared. The other authors declare no conflict of interest.

Funding:

This study was funded by the Chang Gung Medical Foundation (Grant number CMRPG6D0353 and CMRPG3G1541).

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Table 1. Subjects characteristics.

Sex	Girl (n=90)		Boy (n=86)	
	Mean	Standard deviation	Mean	Standard deviation
Basic indices				
Age (years)	9.4	1.6	9.5	1.8
Height (cm)	137.5	11.4	137.6	12.2
Weight (kg)	33.6	10.8	36.1	15.3
BMI (kg/m^2)	17.4	3.5	18.4	5.0
Height z-score	0.4	0.9	0.4	1.0
BMI z-score	0.2	1.3	0.4	1.7
WC (cm)	61.6	9.1	64.8	15.0
HC (cm)	75.2	9.5	75.4	12.1
DXA measurements				
Total weight	34.444	11.023	37.051	15.650
BMC_{DXA} (kg)	1.210	0.300	1.257	0.347
Subtotal BMC_{DXA} (kg)	0.904	0.290	0.923	0.328

FFM_{DXA} (kg)	23.813	6.027	25.834	7.711
FM_{DXA} (kg)	10.630	5.939	11.217	9.107
PBF_{DXA} (%)	29.5	7.4	26.9	10.7
BIA measurements				
Total weight	33.7	10.8	36.1	15.3
TBW_{BIA} (kg)	18.156	4.597	19.169	5.662
$Mineral_{BIA}$ (kg)	1.795	0.439	1.834	0.523
FFM_{BIA} (kg)	24.771	6.257	26.103	7.707
FM_{BIA} (kg)	8.941	5.541	10.043	8.801
PBF_{BIA} (%)	25.1	7.5	24.3	10.8
FFM hydration (%)***	73.3	0.2	73.4	0.3
Mineral in FFM (%)***	7.3	0.3	7.0	0.3

[1] Note: ***, $p < 0.001$

[2] Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; BMC_{DXA}, bone mineral content as measured by dual-energy X-ray absorptiometry (DXA); FFM_{DXA}, fat-free mass as measured by DXA; FM_{DXA}, body fat mass as measured by DXA; PBF_{DXA}, percent body fat as measured by DXA; TBW, total body water as measured by bioelectrical impedance analysis (BIA); FFM_{BIA}, fat-free mass as measured by BIA; FM_{BIA}, body fat mass as measured by BIA; PBF_{BIA}, percent body fat as measured by BIA.

Table 2. Correlation coefficients between bone estimates and the independent variables.

Variables	BMC_{DXA}	Subtotal BMC_{DXA}
Sex	0.073	0.031
Age	0.778	0.768
Height	0.894	0.899

Weight	0.921	0.924
BMI	0.756	0.753
Height z-score	0.495	0.517
BMI z-score	0.561	0.564
WC	0.740	0.751
HC	0.873	0.874
TBW_{BIA}	0.946	0.952
$Mineral_{BIA}$	0.942	0.952
FFM_{BIA}	0.946	0.953
FM_{BIA}	0.754	0.754

Table 3. Regression model list.

Estimate	Equation	Adjusted R^2	RMSE
Group 1: Sex, Age			
BMC_{DXA}	0.151×Age – 0.190	0.603	0.204
Subtotal BMC_{DXA}	0.142×Age – 0.425	0.587	0.199
Group 2: Basic indices: Sex, Age, Ht, Wt, BMI, height z-score, BMI z-score, WC, HC			
BMC_{DXA}	0.012×Ht + 0.014×Wt – 0.946	0.926	0.089
Subtotal BMC_{DXA}	0.012×Ht + 0.013×Wt – 1.185	0.935	0.080
Group 3: $Mineral_{BIA}$			
BMC_{DXA}	0.634×$Mineral_{BIA}$ + 0.083	0.886	0.113
Subtotal BMC_{DXA}	0.611×$Mineral_{BIA}$ – 0.195	0.906	0.098
Group 4: $Mineral_{BIA}$, Basic indices			
BMC_{DXA}	0.114×$Mineral_{BIA}$ + 0.010×Ht + 0.012×Wt – 0.726	0.927	0.090
Subtotal BMC_{DXA}	0.173×$Mineral_{BIA}$ + 0.079×Ht + 0.010×Wt – 0.853	0.959	0.079
Group 5: FFM_{BIA}, FM_{BIA}, Basic indices			
BMC_{DXA}	0.023×Age + 0.006×Ht + 0.011×FM_{BIA} + 0.023×FFM_{BIA} – 0.461	0.932	0.086
Subtotal BMC_{DXA}	0.009×Ht + 0.010×FM_{BIA} + 0.022×FFM_{BIA} –	0.939	0.078

	0.901