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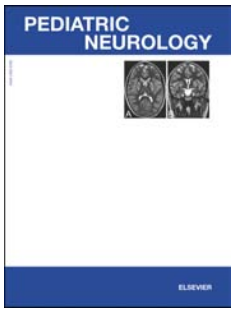

A bedside measure of body composition in Duchenne muscular dystrophy

Sarah A. Elliott, PhD Zoe E. Davidson, PhD Peter S.W. Davies, PhD Helen Truby, PhD

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Authors:

Sarah A Elliott, PhD, Children's Nutrition Research Centre, Queensland Children's Medical Research Institute, The University of Queensland, Herston, QLD 4029, Australia.

Zoe E Davidson, PhD, Department of Nutrition and Dietetics, Southern Clinical School, Monash University, Clayton, VIC 3168, Australia.

Peter SW Davies, PhD, Children's Nutrition Research Centre, Queensland Children's Medical Research Institute, The University of Queensland, Herston, QLD 4029, Australia.

Helen Truby, PhD, Department of Nutrition and Dietetics, Southern Clinical School, Monash University, Clayton, VIC 3168, Australia.

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Corresponding Author:

Professor Helen Truby
Department of Nutrition and Dietetics
Southern Clinical School
Monash University
Level 1, 264 Ferntree Gully Road
Notting Hill, Victoria, 3168
Tel: +61 3 9902 4270
Email: helen.truby@ monash.edu

Introduction: In clinical practice, monitoring body composition is a critical component of nutritional assessment and weight management in boys with Duchene muscular dystrophy (DMD). We aimed to evaluate the accuracy of a simple bedside measurement tool for body composition, namely bioelectrical impedance analysis (BIA), in boys with DMD.

Methods: Measures of fat free mass (FFM) were determined using a BIA machine and compared against estimations obtained from a reference body composition model. Additionally, the use of raw impedance values were analysed using three existing predictive equations[1-3] for the estimation of FFM. Accuracy of BIA was assessed by comparison against the reference model by calculation of biases and limits of agreement.

Results: Body composition was measured in ten boys with DMD, mean age 9.01 ± 2.34 years. The BIA machine values of FFM were on average 2.3 ± 14.1 kg higher than reference values. Limits of agreement (based on 95 % CI of the mean) were -7.4 to 2.9 kg. There was a significant correlation between the mean FFM and difference in FFM between the BIA machine and the reference model ($r = -0.86$, $p= 0.02$) suggesting that the bias was not consistent across the range of measurements. The most accurate predictive equation for the estimation of FFM using raw impedance values was Pietrobelli's[3]; mean difference -0.7 kg, 95 % limits of agreement (-3.5 to 2.0 kg).

Conclusion: In a clinical setting, where a rapid assessment of body composition is advantageous, the use of raw impedance values, combined with the Pietrobelli[3] equation, is recommended for the accurate estimation of FFM, in boys with DMD.

Key words Duchenne muscular dystrophy, bioelectrical impedance, body composition, fat mass, fat free mass

INTRODUCTION

Duchenne muscular dystrophy (DMD) is the most common of the genetically inherited neuromuscular diseases in males, affecting one in every 3500 live male births.[4] The disease process follows a predictable course, altering body composition via progressive muscle wasting and degeneration resulting from the replacement of muscle with fat and fibrous tissue.[5] The body composition changes observed in DMD are unique, therefore, it is vital that accurate and acceptable techniques to assess body composition, and body composition change in boys with DMD are available to clinicians which could be used to monitor disease progression.

While laboratory based body composition methods such as hydrostatic weighing, isotope dilution and multi compartment models, are often more accurate, each has inherent practical limitations, which render them unsuitable for routine use in clinical practice. Ideally, body composition measurement techniques in children with chronic diseases need to be quick, non-invasive and acceptable for repeated measures. The prediction of total body water (TBW) by bioelectrical impedance analysis (BIA) and in turn, body composition, is an inexpensive technique which has applicability across a range of chronic diseases where standard body composition models are inaccurate[6,7]. Diseases such as juvenile rheumatoid arthritis and crohn's disease, in which chronic inflammation and sub clinical malnutrition combined with use of corticosteroids is an example where BIA with disease specific equations has proven to be a useful means of tracking nutritional status overtime[8,9].

BIA measures the impedance of the body to the flow of an alternating current.[10] The intra cellular and extra cellular fluids offer resistance to the flow, while cell membranes act as capacitors and thus offer reactance to the flow. As a result, impedance can be directly related

to total body water (TBW). BIA is utilised in a range of clinical conditions to routinely measure body composition. Clinical trials have shown the use of BIA as a non-invasive diagnostic tool to evaluate nutritional status, determine the prognosis of clinical patients, and evaluate the influence of therapeutic agents in disease management[7,11-13].

Currently, there is limited information to inform and direct nutritional intervention in boys with DMD. The goal of weight management in children with this chronic disease is to preserve FFM whilst managing excess weight gain. However, standard anthropological measures such as BMI and skin fold measures provide only blunt measures and are invalid for use in this population[14-17]. Boys, who may appear 'normal weight' according to their BMI, may have significantly increased fat mass. Similarly, changes in body composition as a result of disease progression or therapeutic treatment may not be observed using just BMI as an indicator or nutritional status. Assessment of body composition variables such as fat mass and FFM are consequently fundamental components of clinical management in boys with DMD. Furthermore, while the early introduction of corticosteroid treatment has led to significant improvements in physical ability and pulmonary function, side effects such as weight gain and changes in body composition require immediate and ongoing attention from clinicians.

Little is known about the use of BIA and its ability to accurately assess body composition in boys with DMD, particularly in those who now commence steroids very early in life. Furthermore, the anomalous body composition changes associated with DMD may alter body water distributions, and consequently may negate the basic assumptions made in the BIA calculations.

As body composition in boys with DMD is of interest to clinicians and dietitians, as an indicator of disease progression, and / or management success, it is vital that techniques used to measure body composition are validated accordingly in boys with DMD. Consequently, the aim of this study was to evaluate the use of a clinical tools such as BIA for the estimation of FFM against estimations obtained from a reference three component (3C) model[18]. Additionally, the use of raw bioelectrical impedance values were analysed using three existing FFM predictive equations[1,3,19] for the estimation of FFM in steroid treated ambulatory boys with DMD.

MATERIALS AND METHODS

Subjects

Ambulatory boys with DMD were recruited from two neuromuscular clinics in Australia ("Montrose Access" - a community centre providing therapies for boys with DMD, and the Children's Neuroscience Centre at the Royal Children's Hospital, Melbourne). Diagnosis was defined as documentation of a deletion or duplication in the dystrophin gene, or absence of dystrophin on muscle biopsy, in conjunction with phenotypic evidence based on characteristic clinical symptoms or signs by nine years of age (i.e. proximal muscle weakness, waddling gait, and Gowers' manoeuvre), an elevated serum creatine kinase, and ongoing difficulty with ambulation. All boys were receiving corticoid steroid treatment (Prednisolone™ 0.12 – 0.65 mg/kg/day or Deflazacort™ 0.83mg/kg/day).

Ethics

The experimental protocol was approved by the Royal Children's Hospital Brisbane (2007/119), the Royal Children's Hospital Melbourne (29075B), and the University of Queensland Human Ethics Committee (2007000797). Written informed consent was obtained from parents and assent from the child prior to the commencement of the study.

Anthropometry

Height was measured to the last completed millimetre using a wall-mounted stadiometer (Holtain Instruments Limited Crymych UK) and weight was measured to the nearest 0.05kg using calibrated electronic scales (Tanita BWB-600 Wedderburn Scales Australia). BMI was calculated as weight divided by the square of height (m). Height, weight and BMI were converted to Z-scores using Centers for Disease Control and Prevention reference values for children.[20] Pubertal status was recorded by a paediatric endocrinologist as Tanner stages.[21]

Body Composition

Bioelectrical impedance was measured using a hand to foot multi-frequency tetrapolar device, (BodyStat 1500 MD; BodyStat, Isle of Man, UK) adhering to standard operating procedures with the subject's gender, age, height, and weight entered into the device which enables FFM to be directly calculated from the internal algorithm (the default equation being Houtkooper et al.[22]). FFM was further calculated directly from the impedance index (ZI) as described by Kushner et al.[23]. The ZI was then used to calculate FFM, using three different equations[1-3] that were derived from children with a similar age range as our study group (Table 1).

3C body composition model (Reference method).

This requires the measurement of TBW body volume (BV) and weight. FFM can be calculated by rearrangement of the Fuller equation,[18] equation using BV, TBW and WT;

$$\text{FFM} = (2.465 \times \text{WT}) - (2.220 \times \text{BV}) + (0.764 \times \text{TBW}) \quad (1)$$

Where weight (WT) is in kg, and BV and TBW are in litres.

TBW: TBW was obtained from isotopic dilution[24] involving two isotopes (deuterium and $_{18}$Oxygen). A baseline urine sample was collected for the determination of the background isotope enrichment level. Participants were then given a weighed mixture of doubly labelled water (DLW) ($^{2}H_2O$ and $H_2{}^{18}O$) and spot urine samples were collected post dose after 5hrs. The analysis of the isotopic enrichment was determined with an Isoprime Dual Inlet Stable Isotope Ratio Mass Spectrometer (MassLynx 4.0i Software, Isoprime, Manchester, U.K.) coupled in-line with a Multiprep-Gilson autosampler. All samples were analyzed in duplicate

and laboratory standards were calibrated using the international suite of waters SMOW, SLAP and GISP. Results were reported in ‰ (delta units) relative to SMOW. The zero-time intercepts were used to determine the dilution space (N) at the time of the dose using the equation of Halliday and Miller.[25] Total body water (TBW) was then calculated as the mean of the $^{2}H_2$ and ^{18}O dilutions spaces (^{2}H dilution space/1.04 and ^{18}O dilution space/1.01, respectively).[26]

Body Volume: Air-displacement plethysmography measurements were performed using the BodPod® (Life Measurement Inc., Concord, CA, USA; software version 1.69), calibrated prior to each measurement[27] and completed twice or until the body volume (BV) measurements were within 150 ml or 0.2% of each other. The average of the two successful measurements was taken. Raw BV was adjusted for thoracic gas volume and correction for isothermal-like effects of the air near the skin, surface area artefact adhering to the methods of Fields et al[28].

Statistical Analysis

Mean, SD and range were used to describe the study sample. Shaprio Wilk and Kolmogorov-Smirnov tests determined distribution normality. FFM estimates from BIA using predictive equations were compared with the reference method (3C) by analysis of variance (ANOVA). Post hoc comparisons of means were performed where appropriate using Dunnett's post hoc test. The mean (± 1.96 SD) limits of agreement for the difference between methods was calculated according to Bland and Altman[29]. The bias was then tested for significance from zero by using Student's t-test. The consistency of the bias was assessed by calculating the correlation between the mean and difference of the measured values[30]. Level of significance set at 5 % was used for all comparisons. Results are expressed as means ± SD. Statistical

computation was performed using the SPSS for Windows (Version 18.0; SPPS Inc, Chicago, IL.).

RESULTS

Data were obtained from ten ambulatory participants, all of whom attended regular school. All were receiving steroids (prednisolone 0.12 mg/kg/day to 0.65 mg/kg/day) and had a pubertal status of stage one (according to Tanner[21]). Weight and height Z scores indicated that the boys were short for their age, but of similar weight to the reference population, which was reflected in the mean BMI Z score of 1.58 ± 0.83, presented in Table 2.

Mean (± SD) FFM measured by BIA (BodyStat 1500 MD) (23.7 ± 5.2 kg) was statistically different (assessed using paired t-tests, t = -2.72, p = 0.01) to FFM as calculated from the 3C reference model (21.4 ± 3.07 kg) (Table 3). Bland-Altman analysis showed that the bias between methods for FFM was 2.3 ± 2.6 kg. Limits of agreement (based on 95 % CI of the mean) were -7.4 to 2.94 kg. A plot of the difference (bias) between the two methods against mean (± SD) FFM for the two methods is presented in Figure 1. A significant negative correlation (r = -0.81, p = 0.00) between the differences and means for FFM was seen. Percentage FM was underestimated using the BodyStat1500 MD in comparison to the 3C reference model (29.9 ± 7.9 kg vs. 34.2 ± 11.6 kg, respectively).

Three equations were also used to estimate FFM from the measured ZI using the BodyStat1500 MD. Table 4 illustrates the mean difference between the methods, 95 % confidence intervals, statistical significance of the bias, as well as the correlation between the differences and means for FFM of the two methods and its statistical significance. No statistically significant bias between either of the predictive equations and the 3C reference model were seen. Pietrobelli's[3] equation showed the least amount of bias between the two methods. Additionally, no proportional bias between the differences and means for FFM

estimated from the 2003 Pietrobelli[3] equation and the 3C reference model was apparent, as illustrated by a lack of correlation.

Impedance index was significantly correlated with FFM ($r = 0.88$, $p = 0.00$) and TBW ($r = 0.93$, $p = 0.00$) obtained from the reference method, as seen in and Figure 2 and Figure 3.

DISCUSSION

Compared to complex criterion methods to evaluate body composition, BIA offers a rapid, non-invasive, and cheap, bedside method which can be routinely used to measure and monitor body composition in children. BIA potentially, offers a more informative adjunct measure than standard anthropometry.

The impact of early introduction of steroids has been examined in populations such as juvenile arthritis[8,31] and Crohn's disease[32]. With chronic use, glucocorticoids have known impacts on metabolism such as promoting energy intake (increased appetite) and storage (gain in FM and reduction in FFM) although the exact mechanisms of action are not fully elucidated. In children with chronic diseases who receive steroids the side effects such as weight gain may override the perceived benefits, leading to their withdrawal. A simple measure of body compartment change provides a tool for clinicians to monitor changes in body composition.

This study was the first to provide an evaluation of the accuracy of BIA for the estimation of FFM, using a 3C model as the reference method in ambulatory boys with DMD. The use of the BodyStat1500 MD for estimating body composition in boys with DMD was hindered by the significant proportional bias seen when examining the relationship between the mean FFM values and the difference in FFM values from the 3C and BIA method. Whilst there was significant bias between the two methods, there was also a significant negative trend apparent, which indicated that the bias was not consistent across the range of FFM found in the participants studied here. As FFM decreased, the BIA error in estimation increased (Figure 1). These results are similar to those observed by McDonald et al.[33] and could have significant repercussions if the BodyStat1500 MD is to be used to assess body composition in

boys with DMD in a clinical setting. In contrast, Mok et al.[17] found that in comparison to deuterium dilution (reference method), BIA estimates of FM did not differ significantly to those obtained by the reference method, and the authors suggested that BIA should be considered as a viable option for estimating body composition in boys with DMD. More recently, Mok et al.,[34] also evaluated the use of BIA to asses *change* in body composition in a group of 26 ambulatory boys with DMD aged three to 11 years old. Estimated FFM from BIA was not significantly different from the reference method at baseline (BIA: 17.8 ± 4.1 vs. reference: 15.5 ± 3.7 kg) or at five months follow-up (BIA: 18.1 ± 3.8 vs. reference: 15.8 ± 3.6 kg). However, the different reference methods used in these studies were associated with limitations in accurately describing body composition in boys with DMD, which may have contributed to this discrepancy.

Furthermore, the impedance which is an output from the BodyStat1500 MD can be utilised (along with height), as it is a good predictor of TBW, and hence FFM[19]. Here, the use of three different predictive equations which incorporate ZI for the estimation of FFM were investigated. These results showed that the most accurate equation for the estimation of FFM using ZI was the 2003 age specific equation of Pietrobelli et al.[3] which was able to estimate FFM with negligible bias and tight confidence intervals. There was little evidence of a relationship between the bias and amount of FFM ($r = 0.20$, ns) when using the Pietrobelli[3] equation. Similarly, no association was seen using the De Lorenzo[2] and Bedgoni[1] equations; however, clinicians should be aware of the larger biases and wider confidence intervals observed when using these two equations, and note that that should not be used interchangeably.

The ZI was highly correlated ($r = 0.88$, $p \leq 0.05$) with FFM obtained from the 3C reference method, as might be expected, suggesting the usefulness of the impedance method in boys with DMD. With any predictive equation, its use is a function of the population in which it was developed. The equations tested here were developed for healthy children, in whom the fundamental assumptions pertaining to the hydration and density of FFM and its internal compartments are valid. Changes in the distribution of water between the intracellular and extracellular compartments may cause significant increases in the percentage of ECW and in the ICW:ECW ratio. Moreover, the prediction of FFM by many impedance models is based on the assumption that the FFM is 73.2 % TBW. This percentage is based on adult studies and varies with age. Therefore, changes in the extracellular water volume may result in an over estimation of FFM in boys with DMD[33]. The use of BIA in conjunction with other techniques such as ultra sound imaging and electrical impedance myography, may help elucidate the effects of steroids on muscles and allow a better determination of FFM composition.

The relevance of measuring FFM in boys with DMD in the clinical setting was recently highlighted by Vuillerot et al [35]. Their longitudinal research suggested that the increase or maintenance of FFM in steroid treated boys with DMD was associated with halting the deterioration of motor function. The authors suggested that body composition measures (in particularly FFM) could be a convenient outcome measure for future clinical trials assessing the use of therapeutic agents in boys with DMD.

As obesity occurs early in the disease process, and amplifies the burden on already weakened muscles, close monitoring of body composition is pertinent to the management of boys with DMD. As standard nutritional indexes, such as BMI, are misleading (in this population), it is

important that accurate, non-invasive, and rapid measures of body composition are available for use in clinical practice. As with many body composition prediction equations, those used with BIA will be population specific. Equations developed and validated in healthy children and adolescents should be applied with caution in children where body composition is affected by the disease process, and long term use of therapeutic agents such as corticosteroids.

It is recognised study is not without its limitations. Similarly to Mok et al.[17] it was not designed to evaluate the absolute validity of the BodyStat1500 MD, or a specific BIA equation, but to investigate the use and limitations of BIA in boys with DMD, as a potential bedside tool to assess body composition in clinical practice.

CONCLUSION

This study demonstrated the relative shortness, and high body fat of young steroid treated ambulatory boys with DMD. It is recognised that the use of the BodyStat 1500 MD internal algorithm for the estimation of FFM in ambulatory boys with DMD is inadequate. However, the ZI resulting from BIA could be used in the estimation of FFM when imported into the Pietrobelli[3] equation.

BIA provides a rapid, non-invasive and relatively inexpensive hand held tool to measure and monitor body composition in a clinical setting. Yet, before it could be recommended to be used routinely in clinical practice, it is imperative that longitudinal studies be carried out to enable the development of a DMD population specific predictive equation for the estimation of FFM, at various stages of the disease progression.

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FIGURE CAPTIONS

Figure 1. The difference in FFM determined using predicted FFM (BodyStat 1500) and measured FFM (3C model) against the mean FFM using both methods. The bold line represents the correlation between the difference in FFM and the mean FFM from the two methods.

Figure 2. Correlation between FFM (measured by the 3C model) and the Impedance Index

Figure 3. Correlation between TBW (measured by the 3C model) and the Impedance Index

TABLES

Table 1. Predictive equations for the estimation of fat free mass.

Source	Sex	Age range (y)	Equation
Bedgoni et al[1]	M/F	7-13	FFM = 4.8 + 0.7 ZI
De Lorenzo et al[2]	M/F	7-13	FFM = 2.330 + 0.588 ZI + 0.211WT
Pietrobelli et al[3]	M	7-14	FFM = 0.6375 ZI + 5.9913

ZI, Impedance Index; HT, Height (cm); WT, Weight (kg); FFM, fat free mass (kg).

Table 2. Physical characteristics of boys with DMD (n = 10).

	Mean ± SD	Range
Age (y)	9.01 ± 2.34	5.88 - 13.59
Height (cm)	123.7 ± 6.0	116.7 - 132.9
Height Z score	-1.30 ± 1.55	-3.52 - 1.28
Weight (kg)	34.6 ± 9.6	22.5 - 49.2
Weight Z score	0.76 ± 1.27	-1.56 - 2.49
BMI (kg/m^2)	22.3 ± 5.0	15.9 - 31.4
BMI Z score	1.58 ± 0.83	0.23 - 2.49

BMI, body mass index.

Table 3. Body Composition estimated from 3C model and BodyStat1500.

	3C Model		**BodyStat 1500**	
	Mean	SD	Mean	SD
TBW(L)	17.0	3.4	18.0	3.8
FFM (kg)	21.4	3.1	23.71*	5.2
% Fat	34.2	11.6	29.91*	7.9

The difference in FFM determined using predicted FFM (BodyStat 1500) and measured FFM (3C model) against the mean FFM using both methods.

SD, standard deviation; 3C, 3 component model; TBW, total body water; FFM, fat free mass.

* Significantly different to 3C model (paired t-test).

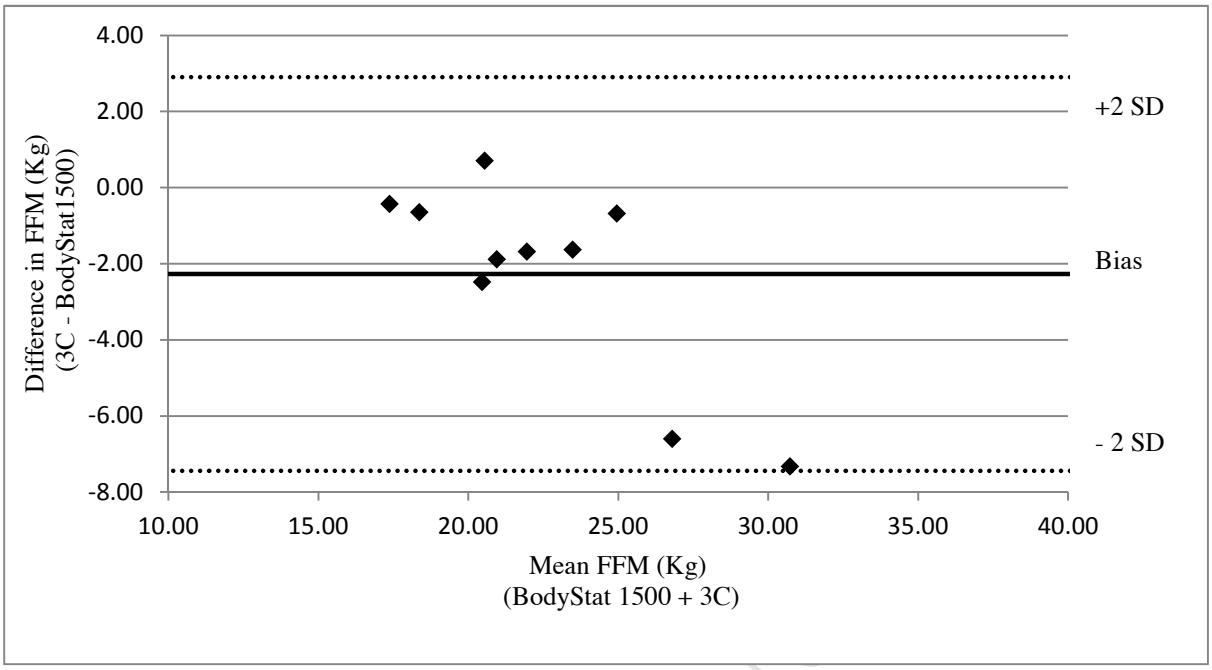
Table 4. Bland-Altman analysis of the bias between measured and predicted fat free mass.

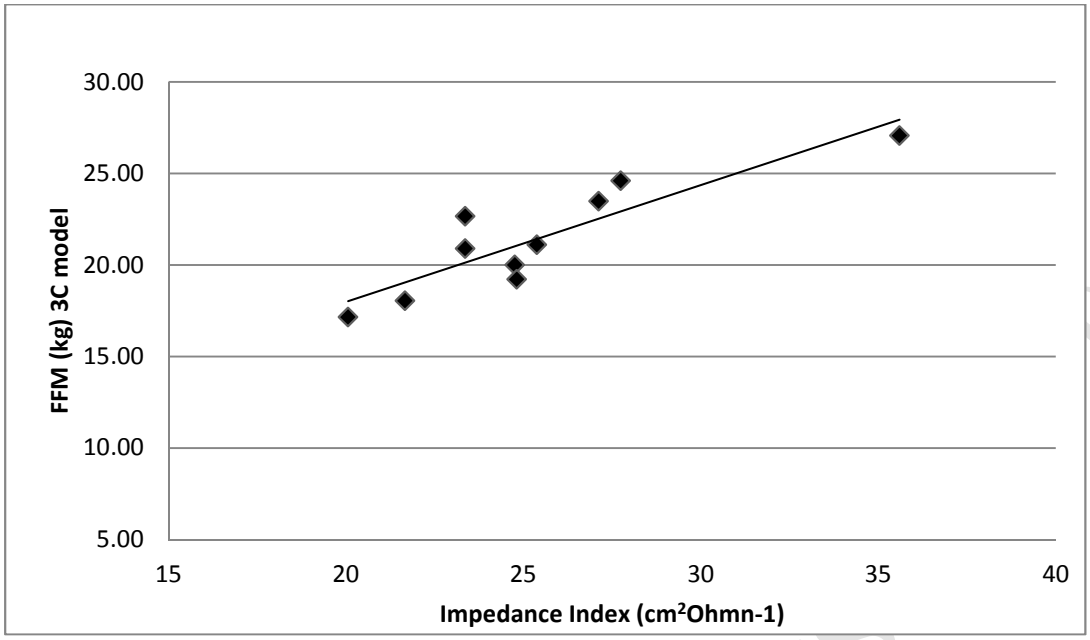
Source	**FFM**				
	Bias (kg)	95% Confidence Interval	p^*	$r^\#$	p^+
Bedgoni et al. [1]	- 1.1	-10.0,7.97	ns	0.02	ns
De Lorenzo et al. [2]	- 3.1	-2.0,8.2	ns	-0.40	ns
Pietrobelli et al.[3]	- 0.7	-3.5,2.0	ns	0.20	ns

p^* Two-sided p value from t-test between the two methods.

$r^\#$ Product-moment correlation coefficient between the difference and mean FFM of the two methods.

p^+ p value corresponding to the r values for the product-moment correlation coefficient between the difference and mean FFM of the two methods.





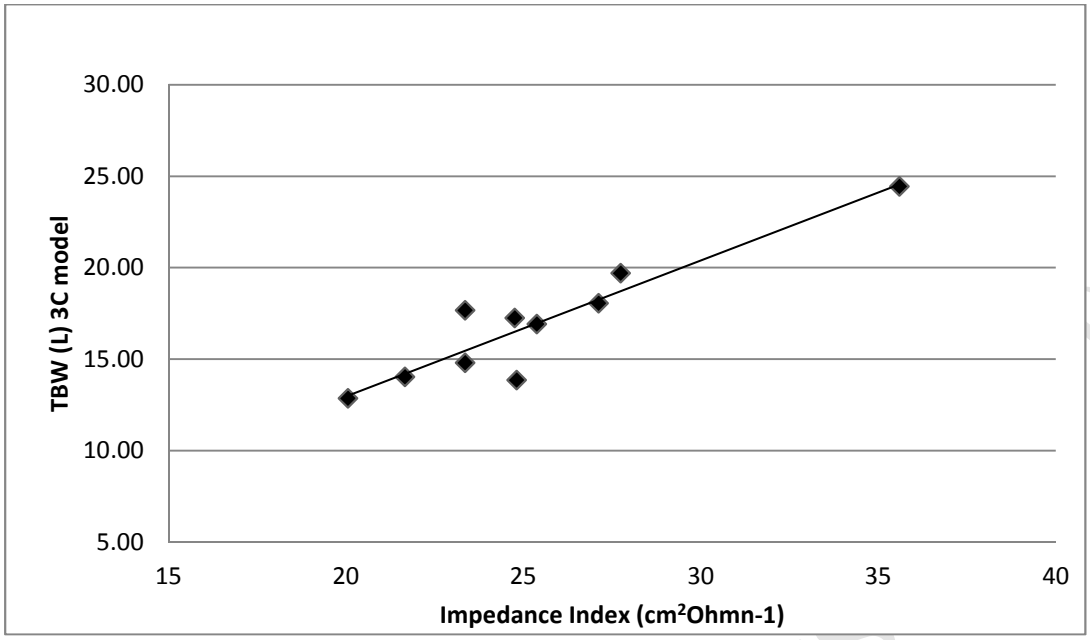


Figure 3. Correlation between TBW (measured by the 3C model) and the Impedance Index