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RESEARCH PAPER

Assessment of fat-free mass from bioelectrical impedance analysis in obese women with Prader-Willi syndrome

Giorgio Bedogni[1,2], Graziano Grugni[3,4], Gabriella Tringali[4], Fiorenza Agosti[4], and Alessandro Sartorio[3,4]

[1]*Liver Research Center, Basovizza, Trieste, Italy,* [2]*International Center for the Assessment of Nutritional Status (ICANS), University of Milano, Milano, Italy,* [3]*Istituto Auxologico Italiano, IRCCS, Division of Auxology and Metabolic Diseases, Verbania, Italy, and* [4]*Istituto Auxologico Italiano, IRCCS, Experimental Laboratory for Auxo-Endocrinological Research, Milano and Verbania, Italy*

Abstract

Background: Fat-free mass (FFM) is lower in obese subjects with Prader-Willi syndrome (PWS) than in obese subjects without PWS. FFM prediction equations developed in non-PWS subjects may, thus, not work in PWS subjects.
Aim: To test whether the estimation of FFM from bioelectrical impedance analysis (BIA) in PWS subjects requires population-specific equations.
Methods: Using dual-energy X-ray absorptiometry, this study measured FFM in 27 PWS and 56 non-PWS obese women and evaluated its association with the impedance index at 50 kHz (ZI_{50}), i.e. the ratio between squared height and whole-body impedance at 50 kHz.
Results: At the same level of ZI_{50}, PWS women had a lower FFM than non-PWS women. However, when PWS-specific equations were used, FFM was accurately estimated at the population level. An equation employing a dummy variable coding for PWS status was able to explain 85% of the variance of FFM with a root mean squared error of 3.3 kg in the pooled sample ($n = 83$).
Conclusion: Population-specific equations are needed to estimate FFM from BIA in obese PWS women.

Keywords

Bioelectrical impedance analysis, body composition, dual energy X-ray absorptiometry, Prader-Willi syndrome, prediction equations

History

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Introduction

Prader-Willi syndrome (PWS) is a genetic disorder due to the absent expression of the paternally active genes in the PWS critical region of chromosome 15. PWS has an estimated incidence of 1 in 25 000 live births and childhood-onset obesity is among its most prominent clinical features (Cassidy et al., 2012).

The body composition (BC) of PWS subjects is peculiar in that, together with a higher fat mass (FM), they have a lower fat-free mass (FFM) (Brambilla et al., 1997). Even if energy expenditure per unit of FFM is similar in PWS and non-PWS subjects (Schoeller et al., 1988), the lower FFM of PWS subjects may contribute to their risk of disease (Bridges, 2014). One of the main aims of growth hormone treatment in PWS is indeed to increase FFM (Bridges, 2014; Coupaye et al., 2013).

Reference techniques for the assessment of BC are available in few centres worldwide and more affordable methods such as dual-energy X-ray absorptiometry (DXA) are not always available (Lee & Gallagher, 2008). Moreover, DXA scanners from different manufacturers give different BC estimates and the same often happens when different software versions are used to analyse the same data (Plank, 2005). These problems are exacerbated when a rare disease such as PWS is studied, as the contribution of many centres is needed to obtain a reasonably high number of patients (Brambilla et al., 2011; Grugni et al., 2013).

Bioelectrical impedance analysis (BIA) is a widely employed method for the assessment of BC that relies on the use of prediction equations (Deurenberg, 1994; Guo et al., 1996). Such equations are presently obtained mostly by cross-validating BIA against DXA (Bedogni et al., 2013). BIA equations are based on the impedance index (ZI), i.e. the height^2/impedance (Z) ratio. The use of ZI is based on the assumption that the human body behaves like a cylindrical isotropic electrical conductor (Heymsfield & Wang, 1994). Although this assumption is false, ZI works well in practice, but is sensitive to changes in height and Z. Moreover, population-specific equations are needed to assess BC in obese subjects (Bedogni et al., 2013; Sartorio et al., 2005). Height is lower in PWS than in non-PWS subjects (Cassidy et al., 2012) and Z is expected to be higher in PWS patients because of their lower FFM (Heymsfield & Wang, 1994). This implies that BIA equations developed in non-PWS subjects may not be accurate when applied to PWS subjects. Even though this was hypothesized at least 20 years ago

Correspondence: Dr. Giorgio Bedogni, Clinical Epidemiology Unit, Liver Research Center, Building Q, AREA Science Park, Strada Statale 14 km 163.5, 34012 Basovizza, Trieste, Italy. E-mail: giorgiobedogni@gmail.com

(Davies & Joughin, 1992), BIA has not yet undergone a formal cross-validation in PWS subjects.

BIA may provide a simple method to perform multi-centre studies of BC, which are essential to understand the functional significance of the lower FFM of PWS patients (Bridges, 2014; Müller, 2013). Thus, the aim of the present study was to evaluate the accuracy of BIA for the prediction of FFM in PWS vs non-PWS obese women.

Methods

Subjects

Twenty-seven PWS women and 56 non-PWS women were consecutively enrolled into the study at the Divisions of Metabolic Diseases and Auxology of the Istituto Auxologico Italiano (Piancavallo, Verbania, Italy). Inclusion criteria for all women were: (1) age $\geq$ 18 years, (2) body mass index (BMI) $\geq$ 30 kg/m^2 and (3) weight $\leq$ 140 kg (as the DXA scanner employed for the study could not accommodate heavier subjects). PWS women underwent a mini-mental state examination (MMSE), as per standard practice at our Centre (Capodaglio et al., 2011). Physical activity was evaluated by interview. Anthropometry, DXA and BIA were performed within 24 hours by the same trained operators as described below. The study protocol was approved by the local Ethical Committee and written informed consent was obtained by the women and/or by their parents.

Anthropometry

Weight and height were measured following international guidelines (Lohman et al., 1988). BMI was calculated as weight (kg)/height (m)^2 (WHO, 2000).

Dual-energy X-ray absorptiometry

DXA was performed using a GE-Lunar Prodigy scanner (GE Medical Systems, Milwaukee, WI). A head-to-toe scan was performed in the default mode with the subject lying supine on the scanner's bed. DXA scans were analysed using GE Encore software version 8.80 (GE Medical Systems, Milwaukee, WI). The three-compartment DXA model separates body mass (BM) into FM, lean tissue mass (LTM) and bone mineral content (BMC), with the sum of LTM and BMC representing FFM (Pietrobelli et al., 1996). Percentage FFM, LTM, BMC and FM were calculated as FFM/BM, LTM/BM, BMC/BM and FM/BM, respectively.

Bioelectrical impedance analysis

Whole-body impedance was measured at a frequency of 50 kHz (Z_{50}) using a 4-polar impedance-meter (Human-IM Plus, Dietosystem, Milan, Italy) (Bedogni et al., 2003). Current-injecting electrodes were placed on the dorsal surfaces of the hand and foot proximal to metacarpal-phalangeal and metatarsal-phalangeal joints, respectively, and voltage-sensing electrodes were placed between the wrist and the ankle (Deurenberg, 1994). BIA measurements were performed in the fasting state ($\geq$ 8 hours) and after 15 minutes of resting in the supine position (Deurenberg, 1994). In our laboratory, the within-day coefficient of variation of Z_{50} is 2%, as determined by two repeated measurements of 10 obese adults (Bedogni et al., 2013).

Statistical analysis

Coarsened exact matching (CEM) was used to match PWS and non-PWS women on age (Iacus et al., 2011; Bedogni et al., 2014). Continuous measurements of PWS and non-PWS women were compared using a generalized linear model (GLM) with PWS status (0 = no; 1 = yes) as predictor, a Gaussian variance and an identity link (Hardin & Hilbe, 2012). The need for population-specific equations in PWS women was evaluated using a GLM employing FFM_{DXA} (continuous, kg) as outcome and the following predictors: (1) Z_{50} (continuous, Ω), height (continuous, m) or ZI_{50} (continuous, cm^2/Ω); (2) PWS status (discrete, 0 = no; 1 = yes); and (3) a PWS*predictor interaction (discrete*continuous). The coefficient of determination (R^2), the root mean square error of the estimate (RMSE) and the Akaike information criterion (AIC) were calculated for each model. Standard diagnostic tests and plots were used to check model fit (Hardin & Hilbe, 2012). The Bland-Altman method was used to calculate the limits of agreement (LOA) between FFM estimated by ZI_{50} (FFM_{ZI50}) and FFM measured by DXA (FFM_{DXA}) (Bland & Altman, 1999; Carstensen, 2010). Statistical analysis was performed using Stata 13.1 (Stata Corporation, College Station, TX), together with the user-written CEM command (Blackwell et al. 2009).

Results

Table 1 gives the measurements of the 27 PWS and 56 age-matched non-PWS women.

As expected, PWS women were lighter and shorter and had a lower FFM than non-PWS women ($p < 0.001$ for all comparisons). Coherently with their lower FFM, PWS women had a higher Z_{50} ($p < 0.01$). All PWS women had a

Table 1. Measurements of the obese women with and without Prader-Willi syndrome.

	PWS women ($n = 27$)	Non-PWS women ($n = 56$)
Age (years)	30 (27–33)	31 (28–33)
Weight (kg)	90.1 (83.6–96.6)	108.9 (105.8–111.9)**
Height (m)	1.47 (1.45–1.50)	1.61 (1.59–1.63)**
BMI (kg/m^2)	41.5 (38.5–44.6)	41.8 (40.9–42.8)
FM_{DXA} (kg)	47.4 (43.4–51.5)	54.4 (52.3–56.6)*
FM_{DXA} (%)	55.6 (54.1–57.1)	51.0 (50.0–52.1)**
FFM_{DXA} (kg)	37.4 (35.0–39.7)	52.1 (50.5–53.7)**
FFM_{DXA} (%)	44.4 (42.9–45.9)	49.0 (47.9–50.0)**
LTM_{DXA} (kg)	36.0 (33.6–38.3)	49.6 (48.1–51.2)**
LTM_{DXA} (%)	42.7 (41.3–44.1)	46.6 (45.6–47.7)**
BMC_{DXA} (kg)	1.4 (1.3–1.5)	2.5 (2.4–2.6)**
BMC_{DXA} (%)	1.7 (1.5–1.9)	2.4 (2.2–2.5)**
Z_{50} (Ω)	578 (539–616)	509 (488–530)*
ZI_{50} (cm^2/Ω)	39 (36–42)	52 (50–54)**

*$p < 0.01$, **$p < 0.001$ vs PWS women.

Values are means and 95% confidence intervals estimated from a generalized linear model (see text for details).

PWS, Prader-Willi syndrome; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FFM, fat-free mass; LTM, lean tissue mass; BMC, bone mineral content; Z_{50}, whole-body impedance at 50 kHz; ZI_{50}, whole-body impedance index at 50 kHz.

MMSE > 24, which is associated with normal daily activities (Capodaglio et al., 2011). All PWS and non-PWS women were engaged in less than 2 hours per week of structured physical activity.

Table 2 gives the GLMs used to test whether the FFM-BIA association differed between PWS and non-PWS women.

The PWS*predictor interaction was not significant for any predictor (models not shown). However, PWS status was significant in all models ($p < 0.001$), with PWS women having on average −11.8 kg, −10.1 kg and −7.0 kg of FFM at the same values of Z_{50} (model A), height (model B) and ZI_{50} (model C) of non-PWS women. A comparison of models A, B and C in Table 2 on the basis of R^2, RMSE and AIC, reveals that ZI_{50} is more accurate than Z_{50} and height at predicting FFM. Taking into account PWS status, ZI_{50} explained 85% of FFM variance with an RMSE of 3.3 kg and an AIC of 439 in the pooled sample ($n = 83$; model C).

Figure 1(A) plots the relationship between FFM_{DXA} and ZI_{50} in PWS and non-PWS women for a domain of ZI_{50} comprised between 26–56 Ω. Such a domain was chosen because it covers the values of ZI_{50} common to PWS and non-PWS women. The plot is a visual representation of the regression model including the PWS*ZI_{50} interaction. As explained above, because such interaction was not significant, it was removed from the model leaving a constant between-group difference of −7.0 (95% CI = −9.2 to −4.8 kg) of FFM for PWS vs non-PWS women (Model C of Table 2).

Figure 1(B) shows a Bland-Altman plot of the agreement between FFM_{ZI50} estimated from model C of Table 2 and FFM_{DXA} in the pooled sample. The mean (SD) difference between FFM_{ZI50} and FFM_{DXA} was 0.01 (3.40) kg. As there was no evidence of proportional bias ($r = -0.21$, $p = 0.07$), the LOA (−6.66 to 6.68 kg) can be used to evaluate agreement. As nearly always happens for BC techniques based on prediction equations (Deurenberg, 1994; Guo et al., 1996), the LOA are wide and discourage the use of these equations at the individual level (Bedogni et al., 2013). However, these equations can still be used to test hypotheses and make inferences at the population level (Bedogni et al., 2013). Interestingly, the Bland-Altman plot showed that the overall agreement was similar in PWS and non-PWS women, despite the lower FFM of the former.

Discussion

In the present study, we tested the hypothesis that BIA requires population-specific equations to estimate FFM in obese PWS women. Although it is well accepted that obese non-PWS subjects need population-specific equations (Bedogni et al., 2013; Das et al., 2003; Jiménez et al., 2012; Sartorio et al., 2005), the hypothesis that obese PWS subjects may require population-specific equations because of their lower FFM has remained untested for the last 20 years (Davies, 1999). Our finding that obese PWS women have a lower FFM at the same ZI_{50} of obese non-PWS women has important implications for the use of BIA in population studies of PWS subjects.

We used DXA as a criterion method for cross-calibrating BIA. Although DXA is not a reference method in the strict sense, it provides a reproducible ranking of soft and mineral tissues (Bedogni et al., 2013). DXA is also gaining increasing popularity in epidemiological studies (Kelly et al., 2009) and is presently the technique of choice for cross-calibrating non-invasive BC techniques (Kriemler et al., 2009, 2010). However, DXA scanners from different manufacturers provide different BC estimates (Plank, 2005). DXA is also not widely available and these problems are exacerbated when a rare disease such as PWS is studied. The contribution of many centres is in fact needed to enrol a number of PWS patients great enough to allow reliable inferences. In our experience (Brambilla et al., 2011; Grugni et al., 2013), most of these centres do not have a DXA facility and those having such facilities often employ scanners from different manufacturers.

Contrarily to DXA, BIA is widely available and this explains why it is probably the most used indirect BC technique. As all indirect BC techniques, BIA relies on the use of prediction equations, which are often accurate at the population level but are rarely accurate at the individual level (Bedogni et al., 2013; Deurenberg, 1994; Guo et al., 1996). However, when this fact is taken into account and proper cross-calibration is performed, BIA becomes a great resource

Table 2. Comparison of models to predict fat-free mass from bioelectrical impedance analysis in obese women with and without Prader-Willi syndrome.

	FFM_{DXA} (kg)		
	Model A	Model B	Model C
Z_{50} (Ω)	−0.04 [−0.05 to −0.03]*	–	–
PWS (1 = yes; 0 = no)	−11.80 [−14.26 to −9.35]*	−10.07 [−13.38 to –6.76]*	−7.00 [−9.24 to −4.76]*
Height (m)	–	32.58 [16.34 to 48.83]*	–
ZI_{50} ($cm^2/Ω$)	–	–	0.57 [0.46 to 0.68]*
Intercept	72.56 [65.99 to 79.14]*	−0.61 [−26.83 to 25.62]	22.07 [16.45 to 27.7]*
n	83	83	83
R^2	0.751	0.675	0.853
RMSE (kg)	4.36	4.98	3.34
AIC	482	505	439

*$p < 0.001$.

Values are regression coefficients and 95% confidence intervals estimated from a generalized linear model (see text for details).

FFM, fat-free mass; DXA, dual-energy X-ray absorptiometry; PWS, Prader-Willi syndrome; Z_{50}, whole-body impedance at 50 kHz; ZI_{50}, whole-body impedance index at 50 kHz; n, number of subjects; R^2, coefficient of determination; RMSE, root mean squared error; AIC, Akaike information criterion.

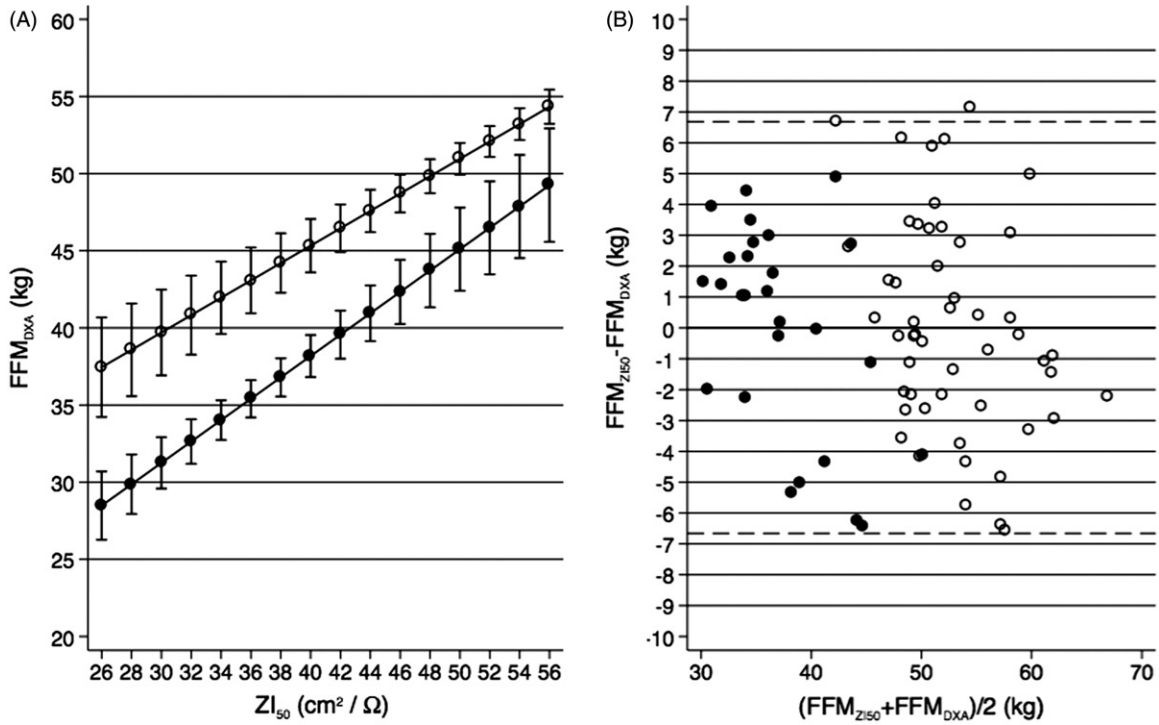

Figure 1. Relationship between fat-free mass measured by dual-energy X-ray absorptiometry and the whole-body impedance index. (A) The relationship between fat-free mass and the impedance index in the women with and without Prader-Willi syndrome. Values are means and 95% confidence intervals estimated from a generalized linear model including a Prader-Willi syndrome*impedance index interaction. The domain of the impedance index (26–56 Ω) was chosen to cover the values of the impedance index common to the women with and without PWS. Black points represent women with PWS and white points women without PWS. (B) A Bland-Altman plot depicting the mean bias (continuous line) and the limits of agreement (dashed lines) of fat-free mass estimated from bioelectrical impedance analysis using Model C of Table 2. Black points represent women with PWS and white points women without PWS. FFM, fat-free mass; DXA, dual-energy X-ray absorptiometry; ZI_{50}, impedance index at 50 kHz.

to perform population studies of BC and its functional correlates. Because BIA equations rely on ZI, i.e. height^2/Z, we were not surprised to find that PWS women needed population-specific equations. Compared to non-PWS women, PWS women are in fact shorter and have a lower FFM, which is inversely proportional to Z. While Z_{50} offered a reasonably accurate assessment of FFM, ZI_{50} was more accurate, proving that ZI_{50} is a useful predictor in PWS-associated obesity.

FFM was expectedly lower in PWS than in non-PWS women. Cognitive impairment may be partly responsible for the lower physical activity of PWS subjects, but it is an unlikely contributor to the lower FFM of our PWS women as they had MMSE scores > 24. Also, our PWS and non-PWS women were engaged in similarly low levels of physical activity, i.e. < 2 hours per week.

Although this is the first study to evaluate the accuracy of BIA to assess BC in PWS subjects, it has several limitations. First, we studied only Caucasian PWS women and our results may not generalize to non-Caucasian ethnic groups and to men. Second, we performed BIA only at a frequency of 50 kHz, but the use of higher frequencies may allow a better prediction of FFM (Malavolti et al., 2003). Third, mostly because of the rarity of PWS, we could assess the BC of a relatively low number of PWS patients ($n = 27$). Fourth, although we were able to confirm that obese PWS women need BIA population-specific equations (Davies & Joughin, 1992), such equations must be cross-validated in external samples before they can be employed for research purposes (Bedogni et al., 2013).

In conclusion, BIA may provide a practical means to perform multi-centre studies of BC in PWS patients, provided that population-specific equations are used.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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