Assessment of fat-free mass from bioelectrical impedance analysis in men and women with Prader-Willi syndrome: cross-sectional study

Giorgio Bedogni, Graziano Grugni, Gabriella Tringali, Sofia Taminò, Paolo Marzuolo, and Alessandro Sartorio

ABSTRACT
We have recently shown that population-specific formulae are required to estimate fat-free mass (FFM) from bioelectrical impedance analysis (BIA) in obese women with Prader-Willi syndrome (PWS) matched by age and percent fat mass (FM) to non-PWS women. The present cross-sectional study was aimed at developing generalised BIA equations that could be used in PWS subjects independently of sex and FM. We used dual-energy X-ray absorptiometry to measure FFM and BIA to measure whole-body impedance at 50 kHz (Z_50) in 34 women and 21 men with PWS. The impedance index, that is, height (cm)^2/Z_50 (X), explained 77% (BCa-bootstrapped 95% CI 65 to 85%) of the variance of FFM. BIA can be used to estimate FFM in obese and non-obese PWS men and women by means of population-specific equations.

Introduction
Prader-Willi syndrome (PWS) is a multi-systemic genetic disorder caused by lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region (Angulo et al. 2015). PWS has an estimated prevalence of 1/10,000-1/30,000 and childhood-onset obesity is among its most prominent clinical features.

The fat-free mass (FFM) of obese subjects with PWS is characteristically lower than that of obese subjects without PWS (Forbes 1997; Theodoro et al. 2006; Bedogni et al. 2015b; Orsso et al. 2017). Even if the energy expenditure of PWS subjects is similar to that of non-PWS subjects when it is standardised on FFM (Butler et al. 2007), there is much interest in knowing whether the lower FFM of PWS subjects does contribute to their burden of disease (Butler et al. 2007; Lloret-Linares et al. 2013; Bridges 2014).

Bioelectrical impedance analysis (BIA) is a simple and portable method for the assessment of body composition (BC) that relies on the use of prediction formulae (Guo et al. 1996). The portability of BIA makes it attractive for performing multi-centre studies of BC in PWS subjects (Bedogni et al. 2015a). Such multi-centre studies are important to advance research on PWS because of the generally low number of subjects with PWS who can be enrolled at a single centre (Bedogni et al. 2014).

We have recently shown, using dual-energy X-ray absorptiometry (DXA) as a comparator (Shepherd et al. 2017), that obese PWS women have on average 7 kg less of FFM than non-PWS women with the same impedance index and require BIA population specific-formulae (Bedogni et al. 2015a).

The aim of this cross-sectional study was to test whether generalised BIA equations can be developed for obese and non-obese PWS men and women. The present study differs from our previous study (Bedogni et al. 2015a) because here we enrolled PWS patients of both sexes and independently from their obesity status.

Materials and methods
Study design
We performed a cross-sectional study on PWS patients followed at the Division of Auxology of the...
Istituto Auxologico Italiano (Piancavallo, Verbania, Italy). The study protocol was approved by the local Ethical Committee and all subjects gave their written informed consent. The study was performed in accordance with the Declaration of Helsinki and with the 2005 Additional Protocol to the European Convention of Human Rights and Medicine concerning Biomedical Research.

Subjects
PWS men and women were consecutively enrolled in the study between January 2015 and January 2017. Inclusion criteria were: (1) genetically confirmed diagnosis of PWS; (2) Caucasian origin; (3) age ≥ 18 years. The only exclusion criterion was weight > 140 kg (as the employed DXA scanner could not accommodate heavier subjects).

Sample size
Sample size was calculated from the data made available by our previous study (Bedogni et al. 2015a). Fifty-five subjects are needed to detect a slope of 0.6 with a power of 99% at an alpha level of 0.05 assuming a standard deviation (SD) of 10 for the response variable (FFM, kg) and one of 10 for the predictor variable (ZI50, Ω).

Measurements
Anthropometry, DXA and BIA were performed on the same day by the same trained operators as described below.

Anthropometry
Weight and height were measured following the Anthropometric Standardization Reference Manual (Lohman et al. 1991). BMI was calculated as weight (kg)/height (m)² and classified according to the NIH guidelines (National Institutes of Health 1998).

Dual-energy X-ray absorptiometry
Body mass (BM), fat mass (FM), lean tissue mass (LTM), bone mineral content (BMC) and FFM (i.e. LTM + BMC) were measured using a GE-Lunar Prodigy scanner with GE Encore software version 8.80 (GE Medical Systems, Milwaukee, WI) (Shepherd et al. 2017). In our laboratory, the within-day coefficient of variation (CV) of percent FM, that is FM/BM, as determined by two repeated DXA measurements of five obese adults is 2.3% (Bedogni et al. 2013).

Bioelectrical impedance analysis
Whole-body impedance was measured at a frequency of 50 kHz (Z50) using a 4-polar impedance-meter (Human-IM Plus, DS-Medica, Milan, Italy) following international guidelines (Deurenberg 1994). BIA was performed in the fasting state and after 15 min of resting in the supine position. The impedance index (ZI50) was calculated as the ratio between height (cm)² and Z50 (Ω). In our laboratory, the within-day CV of Z50 as determined by 10 repeated BIA measurements of 10 obese adults is 2% (Bedogni et al. 2013).

Statistical analysis
Continuous variables are reported as means and SDs. Between-sex comparisons of continuous variables were performed using Student’s t-test and those of categorical variables using Pearson’s chi-squared test. We evaluated the influence of sex on the FFM-ZI50 relationship using three pre-specified linear regression models. The response variable of all the three models was FFM (continuous, kg). The predictors of Model 1 were ZI50 (continuous, cm²/Ω), sex (discrete; 0 = female, 1 = male) and a ZI50-sex (continuous-discrete) interaction; those of Model 2 were ZI50 and sex; the only predictor of Model 3 was ZI50. The linearity of the ZI50-sex interaction was checked using plots and multivariable fractional polynomials with interaction (Royston and Sauerbrei 2004). Standard diagnostic plots were used to evaluate model fit (Weisberg 2014). The adjusted coefficient of determination (R²_adj) and the root mean squared error of the estimate (RMSE) were used as measures of model fit. The 95% confidence intervals (95% CI) of the regression coefficients, R²_adj and RMSE were calculated using bias-corrected accelerated (BCa) bootstrap on 1,000 random samples of 55 subjects (Efron 1994). The bootstrap offers an efficient way of correcting for overoptimism and is presently considered the best method for performing internal cross-validation (Harrell 2015). Statistical analysis was performed using Stata 15.1 (Stata Corporation, College Station, TX).

Results
Table 1 gives the measurements of the 34 women and 21 men with PWS. As the genetic diagnosis of PWS is concerned, 41 patients had an interstitial deletion of
the proximal long arm of chromosome 15 (del15q11.2-q13) and 14 had maternal uniparental disomy of chromosome 15.

PWS women were lighter and shorter than PWS men. Although the mean (SD) difference in FFM between men and women was −7.6 (1.9) kg, it was only −2.7 (1.7) % when standardised on BM.

Table 2 gives the 3 regression models used to evaluate the influence of sex on the FFM-ZI50 relationship in PWS.

Model 1 shows that ZI50 and sex do not interact, that is, the FFM-ZI50 regression lines of PWS men and women are parallel.

Model 2, obtained by removing the ZI50-sex interaction from Model 1, shows that sex has no statistically significant effect on the FFM-ZI50 relationship, that is, the FFM-ZI50 regression lines of PWS men and women can be considered as superimposed. It should nonetheless be noted that the lower limit of the 95% CI of the coefficient of sex in Model 2 is −0.01 kg and its upper limit is 4.90 kg. This 95%CI is compatible with an independent and possibly biologically relevant effect of sex on the FFM-ZI50 relationship but larger sample sizes are required to detect it with enough precision.

Model 3, obtained by removing sex from Model 2, is equivalent to Model 2 in terms of accuracy, leading one to conclude that sex does not improve the prediction of FFM from BIA in our sample of PWS adults.

Figure 1 gives a scatterplot of FFM vs. ZI50 in PWS women and men with the common regression line estimated from Model 3.

Discussion

We have recently shown that obese PWS women have less FFM than age- and FM-matched non-PWS women with the same level of the impedance index and require population-specific BIA formulae (Bedogni et al. 2015a). The present study was aimed at extending our previous findings by testing whether generalised BIA equations can be developed for PWS patients of both sexes and independently of their obesity status.

We found that ZI50 explained 77% (BCa-bootstrapped 95% CI 65 to 85%) of the variance of FFM with an RMSE of 3.7 kg (BCa-bootstrapped 95% CI 3.2 to 4.5 kg). By dividing the RMSE of 3.7 kg for the mean FFM (39.6 kg, n = 55) one obtains a percent RMSE of 9%, which is acceptable for estimating FFM at the population level.

Our previous study was focussed on obese PWS women (Bedogni et al. 2015a). Because our aim there was to quantify the effect of PWS on the FFM-ZI50 relationship, we compared obese PWS with obese non-PWS subjects matched for sex (female), age and percent FM. We found that at the same level of ZI50, the FFM of obese PWS women was on average 7 kg

### Table 1. Measurements of the study subjects.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Women (n = 34)</th>
<th>Men (n = 21)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (kg)</td>
<td>30 (8)</td>
<td>32 (9)</td>
<td>0.43</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.3 (21.5)</td>
<td>97.6 (20.5)</td>
<td>0.040</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.50 (0.07)</td>
<td>1.56 (0.06)</td>
<td>0.002</td>
</tr>
<tr>
<td>BMI (kg / m²)</td>
<td>38.2 (10.5)</td>
<td>39.9 (8.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>BMI class (NIH)</td>
<td>Normal</td>
<td>5 (15%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>3 (9%)</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Obesity class 1</td>
<td>5 (15%)</td>
<td>4 (19%)</td>
<td></td>
</tr>
<tr>
<td>Obesity class 2</td>
<td>8 (24%)</td>
<td>4 (19%)</td>
<td></td>
</tr>
<tr>
<td>Obesity class 3</td>
<td>13 (38%)</td>
<td>11 (52%)</td>
<td></td>
</tr>
<tr>
<td>ZI50 (cm² / Ω)</td>
<td>594 (102)</td>
<td>551 (86)</td>
<td>0.12</td>
</tr>
<tr>
<td>ZI50 (cm² / Ω)</td>
<td>39 (7)</td>
<td>46 (8)</td>
<td>0.002</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>43.6 (14.3)</td>
<td>46.7 (13.1)</td>
<td>0.42</td>
</tr>
<tr>
<td>FM / BM (%)</td>
<td>53.2 (6.3)</td>
<td>50.5 (6.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>36.7 (6.9)</td>
<td>44.7 (7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FFM / BM (%)</td>
<td>46.8 (6.3)</td>
<td>49.5 (6.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>LTM (kg)</td>
<td>35.3 (7.0)</td>
<td>42.8 (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LTM / BM (%)</td>
<td>44.8 (5.8)</td>
<td>47.7 (5.8)</td>
<td>0.078</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>1.5 (0.3)</td>
<td>1.6 (0.3)</td>
<td>0.23</td>
</tr>
<tr>
<td>BMC / BM (%)</td>
<td>2.0 (0.7)</td>
<td>1.8 (0.4)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Student’s t-test for continuous variables and Pearson’s chi-squared for categorical variables. Values are means and standard deviations. BMI: body mass index; NIH: National Institutes of Health; ZI50: impedance at 50 kHz; ZI50*: impedance index at 50 kHz; FM: fat mass; BM: body mass; FFM: fat-free mass; LTM: lean tissue mass; BMC: bone mineral content.

### Table 2. Relationship between fat-free mass and the impedance index in PWS adults.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZI50 (cm² / Ω)</td>
<td>0.88 * [0.64 to 1.11]</td>
<td>0.81 * [0.66 to 0.96]</td>
</tr>
<tr>
<td>Male</td>
<td>8.16 [−3.60 to 21.32]</td>
<td>2.35 [−0.01 to 4.90]</td>
</tr>
<tr>
<td>ZI50 Male</td>
<td>−0.14 [−0.45 to 0.16]</td>
<td>−</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.59 [−6.50 to 11.06]</td>
<td>5.16 [−0.40 to 10.86]</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.79 [0.66 to 0.85]</td>
<td>0.79 [0.68 to 0.85]</td>
</tr>
<tr>
<td>( N )</td>
<td>55</td>
<td>55</td>
</tr>
</tbody>
</table>

95% bias-corrected accelerated (BCa) bootstrapped confidence intervals in brackets. Values are regression coefficients and measures of model fit. ZI50: impedance index at 50 kHz; RMSE: root mean squared error; \( R^2 \): adjusted coefficient of determination.

* \( p < 0.001. \)
lower than that of obese non-PWS women, clearly showing the need of population-specific formulae for PWS women. While in our previous study (Bedogni et al. 2015a), we treated sex and percent FM as founders that had to be controlled for to obtain a less biased estimate of the effect of PWS on the FFM-ZI50 relationship, sex and percent FM were left free to vary in the present study because our aim was to develop generalised BIA equations not linked to specific levels of sex and percent FM.

Twenty-four percent of the PWS women and 10% of the PWS men studied here were not obese as determined by BMI (Table 1). Using BMI as a proxy for percent FM, only a minority of the PWS subjects were not obese, as is to be expected from a sample of PWS subjects recruited in adult age (Angulo et al. 2015). More importantly, and in keeping with our intentions, the range of percent FM as measured by DXA (33 to 61%) was higher than in our previous study (Bedogni et al. 2015a).

A sexual dimorphism in the BC of men and women starts to be apparent at puberty and is always detectable thereafter (Wells 2007). Such dimorphism acts as a proxy for selected diseases such as osteoporosis and cardiovascular disease. It is presently unknown whether a sexual dimorphism exists in the BC of PWS subjects and whether it is associated with their burden of disease (Bedogni et al. 2015b). At similar levels of age and BMI, our PWS women had a lower FFM than our PWS men on absolute grounds. However, the mean (SD) difference was only −2.7 (1.7) % when FFM was standardised on BM, which cannot be considered biologically relevant. Although our main interest here toward sex was to evaluate its independent contribution to the FFM-ZI50 relationship in PWS, our data do not support the idea of a biologically important dimorphism in the BC of PWS men and women.

The present study has some limitations. First, 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). Second, although PWS affects males and females with similar frequencies (Angulo et al. 2015), more women (62%) than men were available in our series of consecutively enrolled patients. This is important also in view of the fact that, albeit not statistically significant, the effect of sex on the FFM-ZI50 relationship may change into a biologically relevant one after increasing sample size. Third, although we used the presently accepted reference method to perform internal cross-validation, that is, the bootstrap (Kriemler et al. 2009; Harrell 2015), the BIA equation developed in the present study need to be cross-validated in external samples before it can be employed for research purposes.

**Conclusion**

In conclusion, BIA can be used to estimate FFM in obese and non-obese PWS men and women by means of population-specific equations. The BIA equations that we have developed need external cross-validation before they can be employed for research purposes.

**Disclosure statement**

The authors declare that they have no competing interests.
**Funding**

The study was supported by Progetti di Ricerca Corrente, Istituto Auxologico Italiano, Verbania and Milan, Italy.

**ORCID**

Giorgio Bedogni [http://orcid.org/0000-0002-1495-9306](http://orcid.org/0000-0002-1495-9306)
Graziano Grugni [http://orcid.org/0000-0003-4708-2544](http://orcid.org/0000-0003-4708-2544)
Sofia Tamini [http://orcid.org/0000-0002-2503-5117](http://orcid.org/0000-0002-2503-5117)
Paolo Marzullo [http://orcid.org/0000-0003-3215-5747](http://orcid.org/0000-0003-3215-5747)
Alessandro Sartorio [http://orcid.org/0000-0002-9620-4133](http://orcid.org/0000-0002-9620-4133)

**References**


