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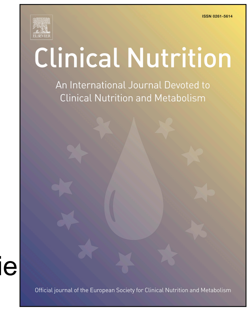


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# Usability of classic and specific bioelectrical impedance vector analysis in measuring body composition of children

Submission type: Research article

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**Key words:** children; body composition; bioelectrical impedance vector analysis; BIVA; DXA; BIS

**Running head**: *Bioelectrical impedance vector analysis in measuring body composition of children*

**Abstract**

In this study, we aimed to analyse the relationship between body composition and bioelectrical variables in children and adolescents.

The sample was composed of 6801 individuals (4035 males; 2766 females) aged 8-20 years included in the National Health and Nutrition Examination Survey (NHANES) years 1999-2004.

Classic and *specific* bioelectrical impedance vector analysis (BIVA) were applied and compared with dual-energy X-ray absorptiometry (DXA) for the evaluation of fat mass (FM) and fat-free mass (FFM), and bioimpedance spectroscopy (BIS) for the evaluation of intra-cellular water (ICW), extra-cellular water (ECW), and total body water (TBW). Fat-free mass index (FFMI) was calculated. Spearman's correlation, regression, and depth-depth analyses were applied.

The evaluation of body composition with BIVA agreed well with that of DXA or BIS, independently of sex, age, and ethnicity: classic BIVA was mostly sensitive to differences in TBW, ECW/ICW, whereas *specific* BIVA to differences in %FM, FFMI, and ECW/ICW. The depth-depth analysis confirmed the associations of classic BIVA (coeff. 0.500, $p < 0.001$), and *specific* BIVA (coeff. 0.512, $p < 0.001$), also considering the significant effect of age ($p < 0.001$). In classic BIVA the associationslightly stronger in females (by 0.03, $p=0.042$) and among Blacks (0.06, $p=0.002$), whereas in *specific* BIVA it was stronger by 0.06 ($p<0.001$) in females and similar among ethnic groups.

The combined use of the two BIVA approaches represents a valuable tool for complete evaluation of body composition in growth studies, for the prevention and monitoring of malnutrition, and the monitoring of the performance in young athletes.

**Introduction**

Children and adolescents change their body composition as they grow, with major differences in the pubertal period (1). Changes in the content and distribution of fat mass (FM), muscle mass and body fluids can also happen as a consequence of lifestyle, primarily due to alterations in nutrition and physical activity (2).

Hence, the screening and monitoring of body composition is highly relevant for evaluation of appropriate growth in children, and for the prevention or early detection of unsafe conditions (e.g. malnutrition). Indeed, a disequilibrium in the content and distribution of body compartments can impede healthy physical, cognitive, and psychosocial growth (3,4). The evaluation of body composition is also useful in the context of sport applications, where it allows for the evaluation of particular physical characteristics, in relation to muscle, fat and water quantity, distribution and bilateral symmetry, for the monitoring of training effects and reducing injury risk among young athletes (5).

Methods for evaluating body composition range from the simple, non-invasive and inexpensive techniques such as anthropometry and single-frequency bioelectrical impedance analysis (SF-BIA) to more accurate, but more complicated and expensive techniques, such as dual-energy X-ray absorptiometry (DXA) and the four-compartment model at the molecular level, and computed tomography or magnetic resonance imaging at the tissue level of body composition analysis (6). In particular, DXA is a commonly used technique because of its accuracy and minimal invasiveness; however, the high cost, non-trasportability and limited feasibility in subjects with a large body size (obese or athletes) reduce the possibilities of application. Undoubtedly, anthropometry and SF-BIA are the most suitable for applications in large samples and heterogeneous contexts. However, anthropometry alone is not able to differentiate the contribution of different body compartments and to recognise particular but increasingly common conditions, such as normal weight obesity (7). The traditional SF-BIA approach, although able to quantify FM, fat-free mass (FFM) or total body water (TBW) compartments, is hampered by the possible error of standard assumptions on body composition, such as TBW equal to 73.2% of FFM (8), and to the application of regression equations that could be inappropriate for the whole sample under study.

Alternative bioimpedance approaches, such as bio-impedance spectroscopy (BIS), 50-kHz phase angle (PhA) and bioelectrical impedance vector analysis (BIVA) can overcome these limitations, as the analysis is based on raw data, without the need to use regression equations to predict body components. BIS is multifrequency bioimpedance approach based on measurements across a range of frequencies, suitable for evaluating body fluid status (extra-cellular water, ECW, intra-cellular water, ICW, and TBW) and with great application in clinical and scientific area (9). The classic BIVA approach has been proposed by Piccoli et al. (10) and the analytical variant named *specific* BIVA by Buffa et al. (11) and Marini et al. (12). In both cases the analysis simultaneously considers resistance (R), and reactance (Xc) measured at 50kHz, and their derived measure of impedance (vector length) and PhA. The difference between the two BIVA approaches is related to the adjustment of R and Xc. In classic BIVA, bioelectrical values are standardised for height (R/H, Xc/H), in order to reduce the effect of the conductor length, whereas in *specific* BIVA, in agreement with the Ohm's law, values are standardised for height and cross-sectional area (Rsp, Xcsp), thus increasing the control of body dimensions' effect and hence the ability to recognise the nature of the conductor. The values of PhA are unaffected by the adjustment method and are equal across the two approaches. The suitability of BIVA in measuring body composition has been verified by different studies (11-17), showing that compared to reference techniques, classic BIVA is able to detect TBW (variations mainly associated with vector length, Z/H) (13,14), whereas *specific* BIVA is related to FM or %FM (vector length, Zsp), and both approaches (PhA) are sensitive to ICW/ECW ratio (11,13,14). On the contrary, classic BIVA has revealed weak associations in the analysis of FM or %FM (11,12,14,16,17), and *specific* BIVA in the analysis of TBW (14). Studies have been conducted in samples of European young adult athletes (classic and *specific* BIVA (14); *specific* BIVA (15); classic BIVA (13)), US young adults (classic and *specific* BIVA (11); classic BIVA (18)), and Italian elderly (classic and *specific* BIVA (12)). The evidence in juveniles is scantier. In fact, to the best of our knowledge, the results of BIVA have been compared to those of more accurate techniques in only one sample of nearly 300 European children and adolescents (classic BIVA (16); *specific* BIVA (17)).

In order to verify the usefulness of classic and *specific* BIVA in children, the aim of this study was to analyse the relationship between body composition and bioelectrical variables in a large sample, using DXA as a reference technique for fat and fat-free mass, and BIS for body fluid status.

**Materials and methods**

*Sample and variables*

The sample examined in this study is composed of 6801 children (4035 males; 2766 females) aged 8-20 years. It derives from the National Health and Nutrition Examination Survey (NHANES) years 1999-2004; this is the most recent NHANES surveys that include both bioimpedance and DXA data.

The National Center for Health Statistics (NCHS) Research Ethics Review Board approved the data survey, and the written informed consent was obtained as a first step of the procedure. The DXA examination was conducted first, followed by BIS. All measurements were taken by a trained technician.

Anthropometric measurements (height, weight, waist, arm, and calf circumferences) were taken on the right side of the body, using a Toledo digital scale, Seca electronic stadiometer and a steel measuring tape, following standard protocols (19). Body mass index (BMI) was calculated as weight / height$^2$ (kg/m$^2$).

The z-scores for a child's sex and age for height, weight, and BMI were calculated based on the CDC growth charts (ages 0 to <20 years).

DXA measurements (FM, kg; FFM, kg; FM percentage, %FM) of the whole body were performed with a Hologic QDR-4500A fan-beam densitometer (Hologic, Inc., Bedford, Massachusetts), using the APEX software and following standard procedures (20). FFM index (FFMI) was calculated using the formula: FFM/height$^2$ (kg/m$^2$) (21).

Bioimpedance measurements (R and Xc, ohm, at 50 kHz; ICW and ECW, kg, derived from BIS device software) were taken with a HYDRA ECF/ICF Bio-Impedance Spectrum Analyzer (Model 4200) manufactured by Xitron Technologies, Inc., San Diego, California. The BIS device measures impedance at 50 frequencies logarithmically spaced from 5KHz to 1 MHz (22), and the device software fits the spectral data to the Cole model and then applies the model terms to complex equations based in part on Hanai mixture theory (23,24).

Classic and *specific* BIVA were applied (10-12) (figure 1). In classic BIVA, R and Xc are standardised for height (R/H, ohm/m; R/H, ohm/m). *Specific* bioelectrical values (Rsp, Xcsp; ohm·cm ) are multiplied by a correction factor (A/L). The A value is estimated as: 0.45 arm area + 0.10 trunk area + 0.45 calf area (cm$^2$); arm, trunk and calf area are calculated as $C^2/4\pi$, where C (cm) is the circumference of the segment. The length is calculated as L = 1.1H, where H is the height in cm. In both approaches, impedance (vector length, Z/H or Zsp) is calculated as $(Radj^2 + Xcadj^2)^{0.5}$ ohm/m and ohm·cm, respectivelyand PhA (arctn Xc/R · 180/π, degrees).

As proposed by Piccoli et al. (10), the impedance vector (characterised by its length and inclination, i.e., by PhA) is projected on the Cartesian plane (R/Xc graph) and analysed in relation to the values of the reference population (through tolerance ellipses).

According to classic BIVA, individuals that fall towards the upper pole of the ellipse are characterised by a tendency towards dehydration, while those falling towards the lower pole tend to be oedematous; on the right side of the diagram are positioned the vectors of individuals with less cellular mass and on the left side are those with greater cellular mass. Furthermore, according to Piccoli et al. (10), athletes are characterised by vectors that tend to fall in the upper left quadrant, while individuals with obesity are characterised by vectors in the lower left quadrant.

As in classic BIVA, the minor axis of *specific* tolerance ellipses refers to PhA variability, and is indicative of the ICW/ECW ratio, with lower values of body cell mass (particularly muscle mass) toward the right lower area. In contrast to classic BIVA, the major axis of *specific* tolerance ellipses refers to variations of %FM, with increasing values towards the upper pole.

*Statistical analysis*

The agreement between body composition estimated by DXA (%FM, FFMI) or BIS (TBW, ECW/ICW) and bioelectrical values (vector length, PhA) obtained with the classic or *specific* BIVA was evaluated using univariate and multivariate approaches.

Considering deviations from normality, evaluated by means of the Shapiro-Wilk test, the association between variables was analysed separately by sex using Spearman's correlation. The possible effect of age, and ethnicity was evaluated using standard regression analysis.

The multivariate comparison between techniques was realised using a depth-depth analysis (25,26), considering the effect of age, sex, and ethnicity. This multivariate statistical analysis is appropriate to study the relationship between two groups of variables associated with each other, such as the bioelectrical variables characterising BIVA (PhA and vector length) and those of body composition obtained with DXA or BIS (%FM, FFMI, TBW, ECW/ICW). The selection of body composition variables to be tested with depth-depth analysis was done considering the results of univariate statistics. According to depth statistics, the compatibility of a single multivariate observation with the rest of the sample is proportional to the depth value that can be measured for each sample based on all samples. The greater the depth, the less different is the individual sample from the rest. The two sets of BIVA and DXA/BIS measures with unknown multivariate distributions led to two corresponding sets of depth measures. The sets of depths measures derived from different techniques were compared using Spearman's correlation. If the correlation coefficients were positive and significant, then the subjects measured with specific BIVA and DXA or BIS were concordant in depth, i.e., the techniques provided similar information on body composition.

Statistical analyses were performed using the free software R (http://www.R-project.org) with the ddalpha library for depth calculation, classic BIVA (27), and s*pecific* BIVA (www.specificbiva.unica.it).

**Results**

The sample was quite homogenously distributed by sex (suppl. table); all age classes and ethnic groups were well represented, with the only exception of the 'Other' people group, which represents a minority (figure 2). Body composition measurements show wide variability, overall characterised by high values of BMI and %FM in all groups, that increase in mean with age (suppl. table, figure 3).

In both sexes, classic vector length was negatively correlated with TBW and FFMI, and positively correlated with ECW/ICW ($p<0.001$); *specific* vector length was positively correlated with %FM, FFMI, and TBW, and negatively correlated with ECW/ICW ($p<0.001$). In both approaches, PhA was positively correlated with FFMI and TBW, and negatively associated with ECW/ICW (table 1, figure 4 showing the strongest and weakest associations) ($p<0.001$). The relationship was not always linear, as the sensitivity of Zsp increases with increasing %FM values, and the sensitivity of Z/H with decreasing values of TBW and FFMI. A significant correlation was also observed between Z/H and %FM (table 1, figure 4), with an opposite direction in the two sexes (positive in males and negative in females), and between PhA and %FM (positive in females and negative in males) (table 1).

All the above associations remained significant also when considering the effect of age and ethnicity.

The depth-depth analysis showed good agreement between the results of classic and *specific* BIVA with DXA and BIS. In particular, classic BIVA showed results consistent with BIS in relation to TBW and ECW/ICW and with DXA in relation to FFMI (coeff. 0.500, $p < 0.001$), with a slightly stronger relationship in females (by 0.03, $p=0.042$) and among Blacks (0.06, $p=0.002$), and an increase with age ($p < 0.001$). The Spearman's correlation between depths was 0.69 (95% CI: 0.68 - 0.71; $p<0.0001$). *Specific* BIVA showed similar results to DXA and BIS in relation to %FM, FFMI and ECW/ICW (coeff. 0.512, $p < 0.001$). The relationship was stronger in females as in males it was lower by 0.06 ($p<0.001$), and increased with age ($p<0.001$), whereas it was similar among ethnic groups. The Spearman's correlation between depths was 0.65 (95% CI: 0.64-0.67; $p<0.0001$).

**Discussion**

This research showed that BIVA agrees well with DXA or BIS in the evaluation of body composition in a large and variable sample of children and adolescents, independently of sex, age and ethnicity: classic BIVA was particularly sensitive to differences in the quantity and distribution of body fluids (TBW, ECW/ICW) and to FFMI (with a pattern different from the expected according to classic BIVA), whereas *specific* BIVA was sensitive to %FM, FFMI and ECW/ICW.

These results confirm the theoretical expectations of *specific* BIVA and partly those of classic BIVA, and are consistent in the two sexes, extending previous studies. In particular, the negative correlation between Z/H and TBW has been previously observed by Marini et al. (14), Campa et al. (13), Heavens et al. (18); however, according to this last study, the detection of hydration was not accurate in a sample of 9 individuals. Similarly, previous studies have observed the positive correlation of Zsp with %FM (as in Buffa et al. (11); Marini et al. (12); Campa et al. (28); Stagi et al. (15), both at total and segmental level; Wells et al. (17), considering FM)); and the negative correlation between PhA and ECW/ICW in both classic and *specific* BIVA (as in Buffa et al. (11); Campa et al. (13); Chertow et al. (29); Marini et al. (14)). *Specific* BIVA was also observed to be sensitive to FFMI, mostly due to the positive correlation with PhA, with vectors of individuals with higher values migrating toward the left upper quadrants, as observed in similar samples (Buffa et al. (11), using skeletal muscle index; Stagi et al. (15), using FFMI). Classic BIVA detected variations in FFMI (likely because of its correlation with TBW) that were positively related to PhA and negatively correlated with Z/H in both sexes (as similarly observed by Wells et al. (16), considering FFM, and consistently with the results of Campa et al. (30)). However, the highly significant association with classic vector length indicates that vectors of individuals with high FFMI values migrate towards the left lower part of the RXc graph, thus falling into the position attributed to obesity on the basis of the classic BIVA theoretical paradigm. Furthermore, classic BIVA did not accurately identify %FM. In fact, the correlation between vector length and %FM was negative, consistent with the expectations of classic BIVA, in females, but positive in males; a similar difference between sexes was observed by Marini et al. (14) in a different sample. The weak association with %FM and the relation with FFMI which does not follow the expectations of classic BIVA, have been previously reported in the literature (Buffa et al. (11); Castizo et al. (31); Marini et al. (12); Marini et al. (14); Wells et al. (16)) and needs to be better investigated.

The above results support the interpretation of body composition following the standard BIVA procedure, as exemplified by the case of females 18-19 years old, showing the decreasing values of TBW and the increasing values of %FM along the major axis of classic and specific tolerance ellipses, respectively (figure 5).

The main limitation of this research is the lack of a gold standard reference methodology for estimating TBW and ECW/ICW. However, to the best of our knowledge, no other large samples with both bioelectrical impedance and body water compartment variables are available in the literature. Furthermore, the present results are consistent with those obtained in smaller samples of adults using dilution techniques (Campa et al. (13); Marini et al. (14)). On the other hand, this study has the strength to be the first one to consider the consistency between BIVA and DXA or BIS results in a large sample of children and adolescents of both sexes, using a bivariate approach.

**Conclusion**

In summary, the present study extends the results on the utility of classic and *specific* BIVA for the evaluation of body composition in children and adolescents in a sample representative of the US population and characterised by a wide variability in body composition. The sensitivity of the two BIVA approaches to partly different variables, with classic BIVA adequate for estimating TBW, ECW/ICW, and *specific* BIVA for evaluating %FM, FFMI and ECW/ICW, suggests the utility of their combined use for a whole evaluation of body composition. The two approaches can give a detailed picture of a child's growth and may prove to be a valuable tool for preventing and monitoring malnutrition, and for monitoring body composition in sports sciences applications.

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**Competing Interests**

The authors have no competing interests to declare.

**Author contributions**

Conceptualization (SS, AMS, CE, EM); Data curation (SS, FJ, CE); Formal analysis (SS, SC, EM); Writing - review & editing (all the authors).

**Figure legends**

Figure 1. Tolerance ellipses of classic and *specific* BIVA. R/H, classic resistance; Xc/H, classic reactance; Rsp, *specific* resistance; Xcsp, *specific* reactance.

Figure 2. Sample distribution by age and ethnicity. N, sample size.

Figure 3. Sample distribution of fat mass percentage.

Figure 4. Correlation between bioelectrical and body composition values. Z/H, classic impedance, ohm/m; Zsp, *specific* impedance, ohm·cm; %FM, fat mass percentage; FFMI, fat-free mass index,

kg/m$^2$; TBW, total body water, kg; ECW, extracellular water, kg; ICW intracellular water, kg; M, males; F, females.

Figure 5. Distribution of 5$^{th}$ (white dots), 95$^{th}$ (black dots), and all deciles (increasing red intensity with increasing deciles) for total body water (TBW, kg) and fat mass percentage (%FM) in classic (left) and *specific* (rigth) tolerance ellipses of the sample of females 18-19 years old. Values of TBW increase towards the lower pole of the ellipses, whereas those of %FM increase towards the upper pole.

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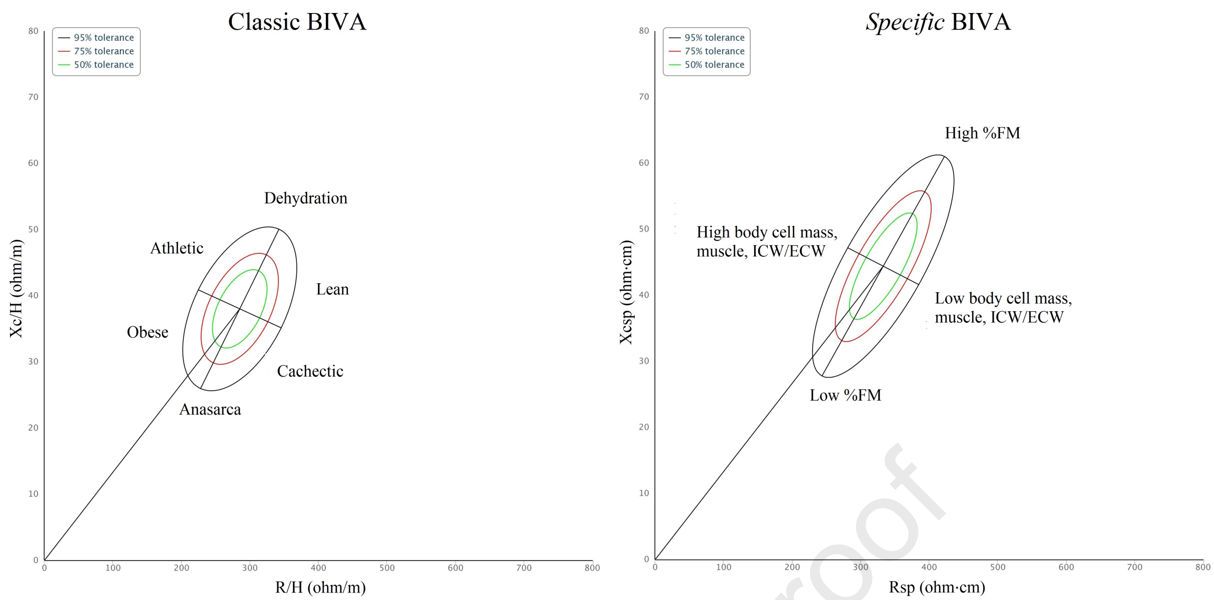
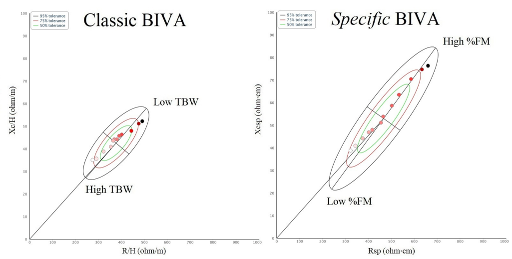
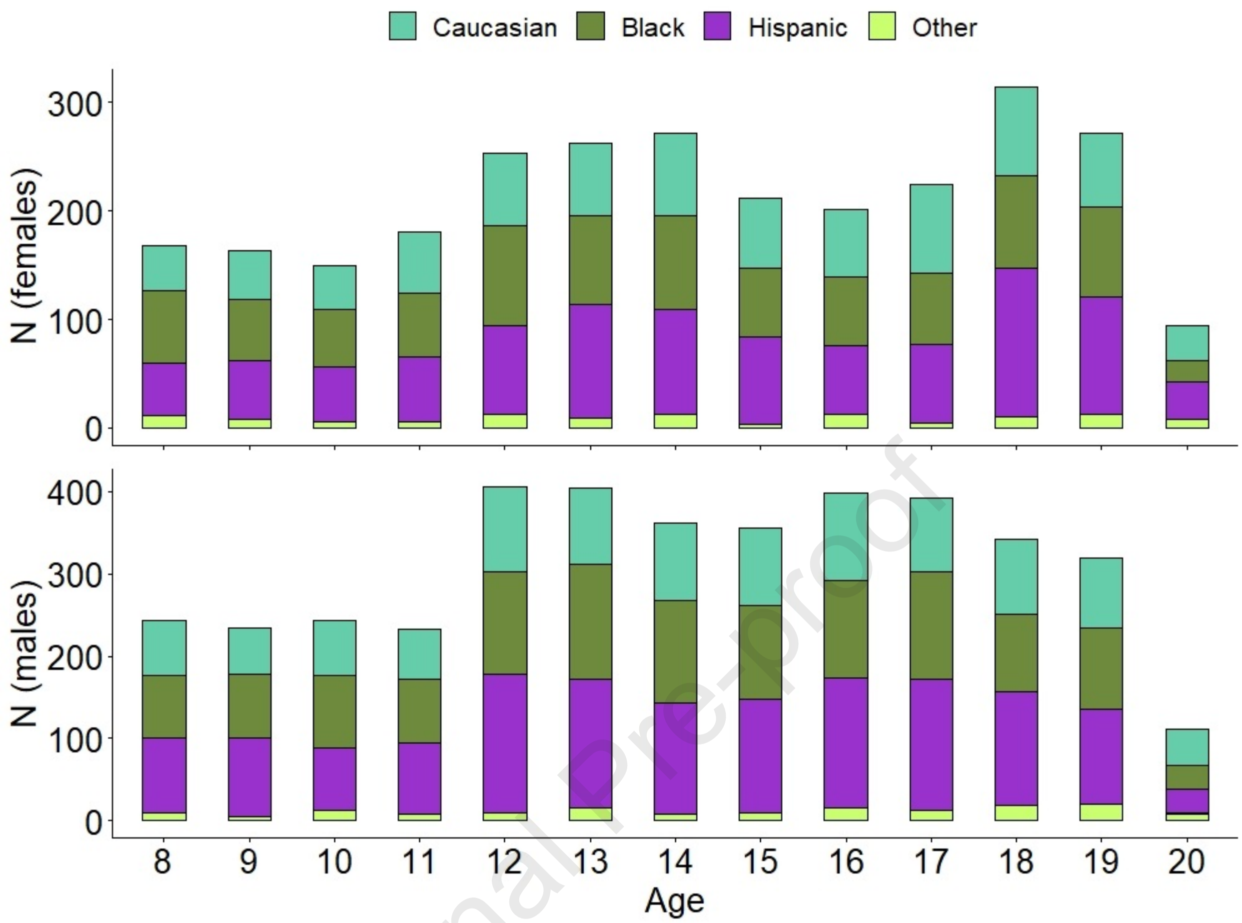
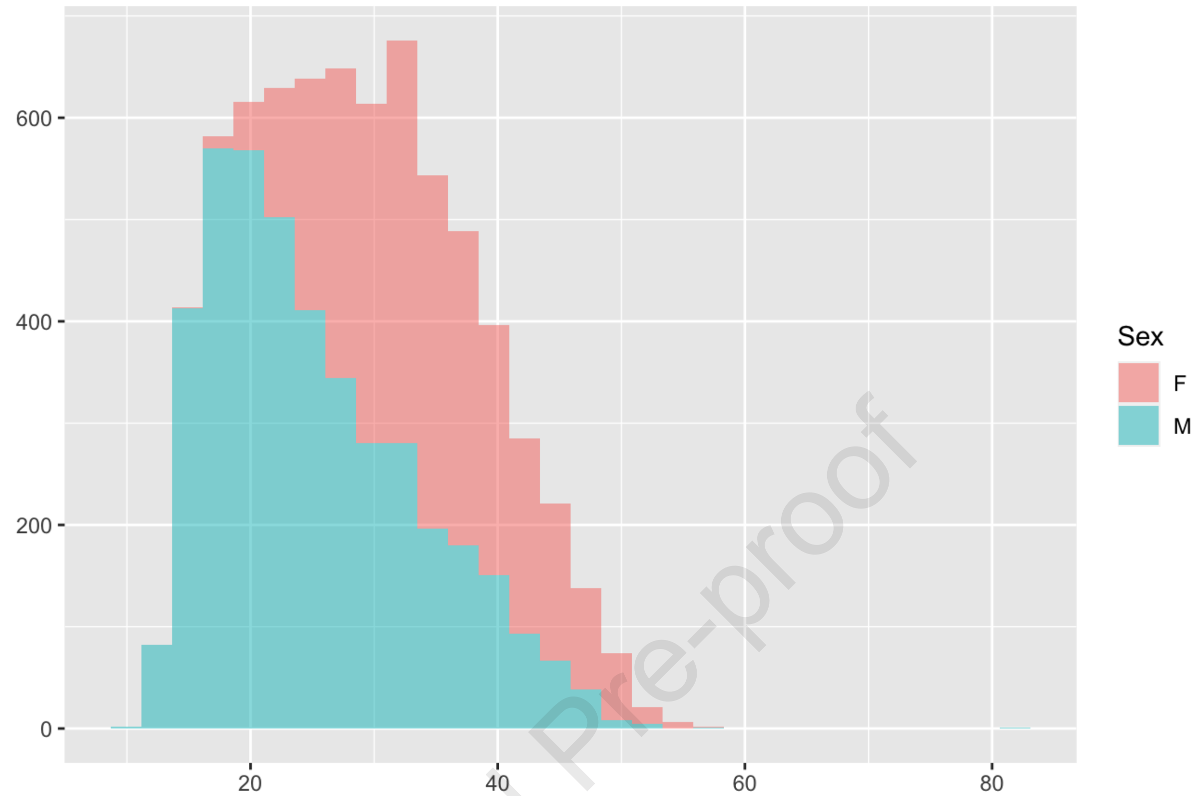
| Males | | | | |
|---|---|---|---|---|
| | TBW | ECW/ICW | %FM | FFMI |
| Z/H (ohm/m) | -0.972** | 0.640** | 0.095** | -0.894** |
| Zsp (ohm·cm) | 0.224** | -0.063** | 0.837** | 0.367** |
| PhA (°) | 0.732** | -0.962** | -0.246** | 0.707** |
| **Females** | | | | |
| | TBW | ECW/ICW | %FM | FFMI |
| Z/H (ohm/m) | -0.940** | 0.424** | -0.354** | -0.837** |
| Zsp (ohm·cm) | 0.521** | -0.251** | 0.898** | 0.619** |
| PhA (°) | 0.582** | -0.927** | 0.174** | 0.536** |

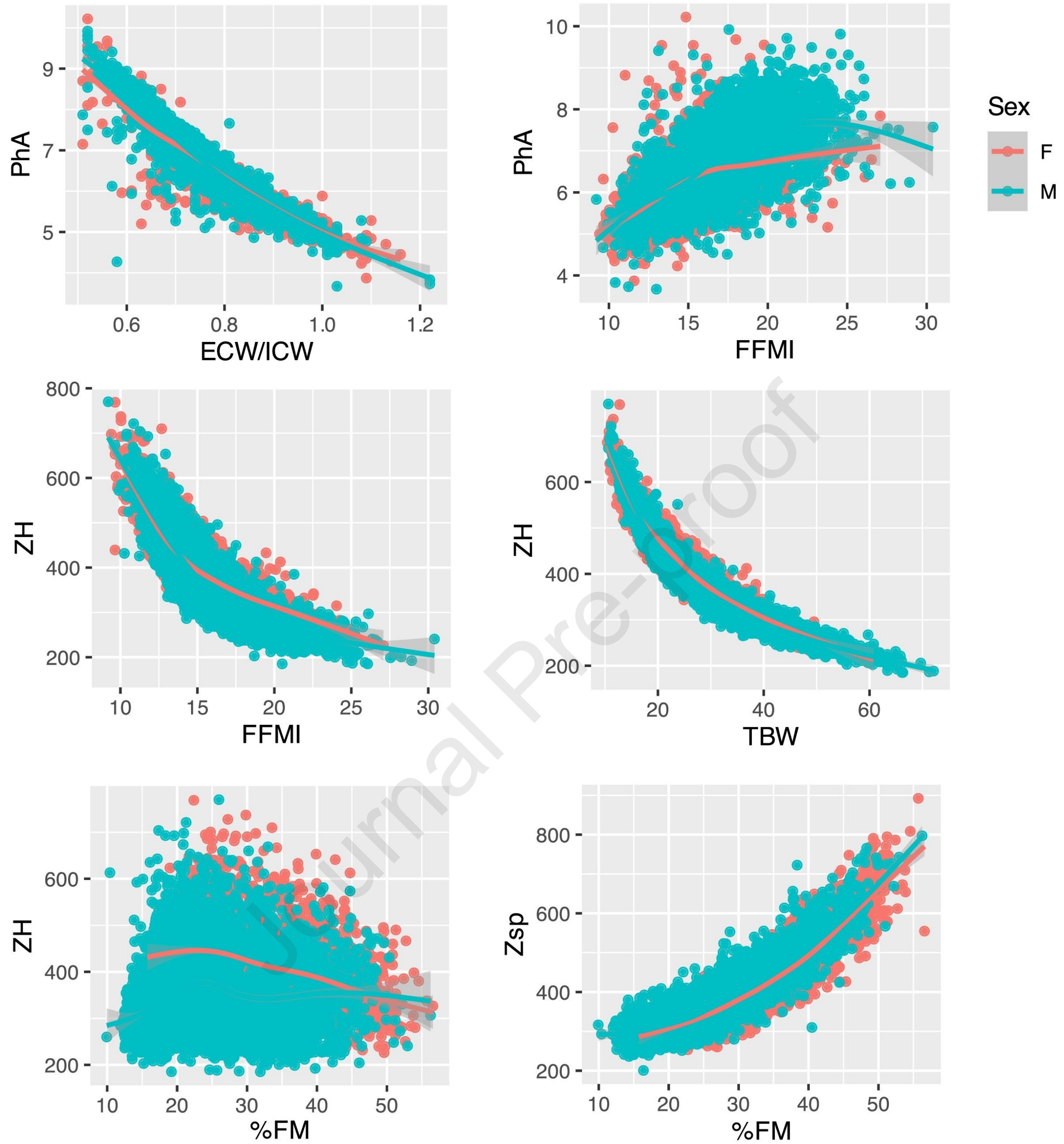
TBW, total body water; ECW, extracellular water; ICW intracellular water; %FM, fat mass percentage; FFMI, fat-free mass index; Z/H, classic impedance; Zsp, *specific* impedance.


Classic BIVA
95% tolerance
75% tolerance
50% tolerance
Dehydration
Athletic
Lean
Obese
Cachectic
Anasarca
Xc/H (ohm/m)
R/H (ohm/m)
Specific BIVA
High %FM
High body cell mass, muscle, ICW/ECW
Low body cell mass, muscle, ICW/ECW
Low %FM
Xcsp (ohm·cm)
Rsp (ohm·cm)


Classic BIVA
Low TBW
High TBW
Xc/H (ohm/m)
R/H (ohm/m)
Specific BIVA
High %FM
Low %FM
Xcsp (ohm·cm)
Rsp (ohm·cm)


Caucasian
Black
Hispanic
Other
N (females)
300
200
100
0
N (males)
400
300
200
100
0
8
9
10
11
12
13
14
15
16
17
18
19
20
Age


600
400
200
0
20
40
60
80
Sex
F
M


PhA
ECW/ICW
PhA
FFMI
Sex
F
M
ZH
FFMI
ZH
TBW
ZH
%FM
Zsp
%FM