Introduction

Evaluation of body composition characteristics is important in public health nutrition research. The last National Health and Nutrition Survey, 2012 (ENSANUT in Spanish acronym) reported high prevalence of obesity and related disorders in Mexican populations, particularly among adults (Gutiérrez et al. 2013). Remarkably high prevalence of overweight (39.6%) and obesity (31.6%) among adults from the southern region of the country have been reported in the survey. Yucatan is one of the Southern Peninsular States of Mexico where prevalence of excess weight (overweight and obesity) was very high (80%) among adults that exceeded the national average (71.3%). Prevalence of obesity was higher in the urban regions (34%) than in the rural areas (26.5%) of Yucatan (Gutiérrez et al. 2013).

Nutritional status assessment procedures are summarized by the mnemonic ABCD: anthropometric measurements, biochemical parameters, clinical diagnosis, and dietary habits (Dwyer et al. 1993). In anthropometric evaluation of nutritional status and body fatness using skinfold caliper, measurement errors could be high among excess weight individuals, particularly among centrally obese people (Sebo et al. 2017). Despite some limitations on the assumption of hydration factor and body geometry of severely obese individuals, bioelectrical impedance analysis (BIA) is reliable to evaluate body composition characteristics (body fat and fat free mass) with less intra-observer error than anthropometry (Coppini et al. 2005; Macias et al. 2007; Yamashita et al. 2012).
simple and non-invasive technique; the portable equipment is relatively non-expensive and provides accurate results (Bohm and Heitmann 2013; Ricciardi and Talbot 2007). The bioelectrical impedance (BI) instruments are different in number of electrodes and the models have in-built software programs (specific algorithm of the device that are unknown to the researcher) to estimate body composition characteristics by age, sex, and activity patterns etc. Several models of BI instruments are available in the market and quality assessment and validation are very important (Macias et al. 2007; Ramirez et al. 2012). The results obtained using BIA are also required to be validated with reference to the results obtained through dual-energy X-ray absorptiometry (DEXA or DXA) that is often considered as a gold standard for the evaluation of body composition characteristics (Khalil et al. 2014; Scafoglieri and Clarys 2018).

In this background, instead of using the direct values of body composition characteristics obtained from the BIA, Macias et al. (2007) developed a prediction equation to estimate fat free mass (FFM) for the Mexican adults using the resistance and reactance (ohms) values obtained through BIA and cross-validated the proposed equation with reference to the results obtained through air displacement plethysmography (ADP). In that study, the participants were 20 to 50-year-old men and women from Sonora, a northern State in Mexico. The authors reported that the equation, based on two-compartment model was accurate, precise, and free of bias. They also reviewed the literature to justify that ADP was a reliable and valid method and as good as hydrostatic weighing for the evaluation of body composition characteristics. Till date, the prediction formula developed by Macias et al. (2007) is a reliable one that is available in Mexico to estimate FFM of adult individuals from the resistance and reactance values obtained through BIA and the article has been cited by many authors stating the usefulness of the formula (Balas-Nakash et al. 2010; Caicedo-Eraso et al. 2019; Lu et al. 2016; Mbada et al. 2015; Schifferli et al. 2011; Schifferli Castro and Zuniga 2017; Wada and Tekin 2007). This raised my interest to use the same formula for Mexican adults (Macias et al. 2007) and to find the agreement and bias between the estimated values of FFM (BIA-based and resistance and reactance-based) in a sample of adults from Merida, Yucatan, Mexico.

The objectives of the present study were to estimate body composition characteristics (body fat mass and fat free mass) of young adult men using the BIA, and to test the agreement and proportional bias in the estimation of fat free mass (FFM) obtained through BIA and that derived from the resistance and reactance values using in a formula for Mexican adults.

Participants and Methods

The present cross-sectional study recruited 60 male students aged 21 to 23 years from the Universidad Modelo, a private University in Merida, Yucatan. The participants were selected through non-probability sampling (convenience sample) from a group of students of the Faculty of Medicine. Ethical clearance was obtained from the institutional committee and the participants signed the informed consent form as per guideline.

Anthropometric measurements were recorded by the author, following international protocol (Lohman et al. 1988). Height (cm) was measured to the nearest tenth of a centimeter using a standard stadiometer with platform (Seca, Germany). Body weight (kg) was recorded to the nearest 0.05 kg using an electronic scale (Tanita Co., Tokyo-Japan). Data of age (years), sex, and the anthropometric measurements were necessary for bioelectrical impedance analysis (BIA).

Body composition characteristics (body fat mass or FM and fat free mass or FFM) were estimated using a multifrequency whole body bioelectrical impedance (BI) analyzer Tanita MC 180 MA (Tanita Co., Tokyo-Japan), following a standard protocol (BIA 1994) and manufacturer’s instruction. The equipment had eight pairs of tactile electrodes for foot pad and hand grips. Previous studies reported reliability of the obtained results using a similar model of Tanita (number 305) that mentioned best-fit algorithm of the device (Jebb et al. 2000). Participants were in light clothing, without jewelry and metal objects in the body. They were asked to abstain from foods (fasting for last 12 hours), moderate or vigorous exercise, alcohol consumption and any medicine intake in 24 hours prior to the evaluation. Assessment was done in the morning, between 7 and 8 A.M. Data collection was done in the University with prior appointments. The participants were apparently healthy and did not report any health complications at least in three months prior to the evaluation.

The FFM was also estimated using a formula for Mexican adults (Macias et al. 2007). FFM (kg) = 0.7374 *(Height²/Resistance in ohms) + 0.1763 *(Body weight) - 0.1773 *(Age) + 0.1198 *(Reactance in ohms) - 2.4658. The dimensions for age, height, and weight were years, cm, and kg respectively. The resistance and reactance values obtained from the Tanita MC 180 MA (Tanita Co., Tokyo-Japan) have been used in the formula to estimate FFM. Body mass index (BMI) might be an interest for the readers that has been calculated as weight (kg) divided by height in meter square. Evaluation of BMI-based nutritional status of adult men followed standard guideline (WHO 1995). Fat mass (FM) (kg and %) was obtained from BIA and was also calculated from FFM and body...
weight. Fat mass index (FMI) was calculated as FM (kg) divided by height (m²).

The SPSS® statistical software (version 15.00) was used for data analysis. Mean and standard deviation values of age, anthropometric (height, body weight, BMI) and body composition characteristics (FM and FFM) were calculated. Normality in the distribution of variables followed the principle of Shapiro-Wilk test (p > 0.05). The values of FFM, obtained directly from the BIA and that derived from the formula (Macias et al. 2007) were compared and statistical tests to find agreement between the estimates and proportional bias were run. Intraclass correlation coefficient, t-test (one sample and paired), linear regression analysis, and Bland-Altman plots (Bland and Altman 1986) were used to estimate the agreement and bias. Level of significance was set at α = 0.05 in all analyses.

**Results**

The participants were young adult men (age 21.88 ± 0.85 years). Mean value of height (167.63 cm) ranged between 159.0 cm and 188.0 cm. Mean value of body weight (62.65 kg) also had wide ranging minimum (48.10 kg) and maximum (80.70 kg) values. The participants had moderate BMI (22.59 ± 2.69 kg/m²) and 23.33% of them were overweight. No participant was obese. Mean values of fat free mass (FFM) obtained directly from BIA (41.44 ± 6.08 kg) was marginally different from that derived using formula (Macias et al. 2007) (41.36 ± 5.91 kg). Estimated values of fat mass (FM) (BIA-based = 22.21 ± 4.89 kg, formula-based = 21.29 ± 4.59 kg) and fat mass index (FMI) (both BIA and formula-based = 0.13) were marginally different or similar, respectively that were obtained using the two methods. Estimated mean values of percentage body fat (PBF) using BIA (33.37 ± 5.83%) and that derived from the FFM value obtained using formula (33.87 ± 5.18%) were also marginally different (Table 1).

Paired t-tests between BIA-based and formula-based estimates, e.g., FFM (t = 1.37, p = 0.18) and PBF (t = 1.43, p = 0.16) did not show significant differences of mean values. Intraclass correlation coefficient (ICC) between the two estimates of FFM was significant (Cronbach’s alpha = 0.99, p<0.0001). ICC of the estimated PBF using two methods also showed similar value that was calculated in case of FFM. The difference in the values of two estimates of FFM (BIA-based and formula-based) was normally distributed (p-value = 0.63). One sample t-test for the difference of FFM values (BIA and formula-based) was not significant (t = 1.37, mean difference -0.02, p = 0.18). A simple linear regression model predicting BIA-based FFM from the formula-based FFM after adjusting for age and sex was significant (regression coefficient = 1.03, standard error = 0.009, t = 110.79, p<0.0001, R² and adjusted R² = 0.99), showing interrelationships between the variables (Fig. 1).

In addition, BMI showed significant correlation (p<0.001) with both BIA-based and formula-based FM (r = 0.52), FFM (r = 0.60), and FMI (r = 0.60). However, correlation between BMI and PBF (either BIA-based or

**Table 1.** Descriptive statistics of age, anthropometric and body composition characteristics of young adult male university students (n = 60)

| Variables         | Mean (SD) | Minimum | Maximum |
|-------------------|-----------|---------|---------|
| Age (years)       | 21.88 (0.85) | 21.45   | 23.87   |
| Height (cm)       | 167.63 (7.17) | 159.00  | 188.00  |
| Weight (kg)       | 62.65 (8.33) | 48.10   | 80.70   |
| BMI (kg/m²)       | 22.59 (2.69) | 19.85   | 28.81   |
| FM_BIA (kg)       | 22.21 (4.89) | 11.71   | 33.09   |
| FM-FOR (kg)       | 21.29 (4.59) | 12.47   | 32.53   |
| FMI_BIA (kg/m²)   | 0.13 (0.03) | 0.07    | 0.19    |
| FMI_FOR (kg/m²)   | 0.13 (0.03) | 0.08    | 0.19    |
| FFM_BIA (kg)      | 41.44 (6.08) | 31.12   | 54.82   |
| FFM_FOR (kg)      | 41.36 (5.91) | 31.36   | 54.71   |
| PBF_BIA (%)       | 33.37 (5.83) | 23.37   | 45.45   |
| PBF_FOR (%)       | 33.87 (5.18) | 24.90   | 44.80   |
| Resistance (BIA) (ohms) | 524.82 (45.99) | 405.00   | 645.00 |
| Reactance (BIA) (ohms) | 59.79 (6.68) | 45.60   | 71.40   |

SD: Standard deviation; BMI: Body mass index; FM_BIA: Fat mass (BIA-based); FM_FOR: Fat mass (formula-based) (Macias et al. 2007); FMI_BIA: Fat mass index (BIA-based); FMI_FOR (FMI formula-based) (Macias et al. 2007); FFM_BIA: Fat free mass (BIA-based); FFM_FOR: Fat free mass (formula-based) (Macias et al. 2007); PBF_BIA: Percentage Body fat (BIA-based); PBF_FOR: Body fat (%) (formula-based) (Macias et al. 2007).
The result of Bland–Altman test for agreement or proportional bias between the estimated FFM using two methods (BIA and formula-based) is presented in Fig. 2. In the resulting scatter plot, Y axis shows the difference of two paired FFM estimates (BIA and formula-based) and X axis presents the average of the measures. Mean value of the difference of paired estimates of FFM (BIA and formula-based) (Y axis) was -0.02 that had minimum (-0.90) and maximum (0.95) values, standard error of estimate 0.06, and standard deviation 0.44. The average of the measures (BIA and formula-based FFM) in the X axis had mean (41.40 kg), minimum (31.34 kg), maximum (54.77 kg) values, standard error of estimate (0.77), and standard deviation (5.99). The Bland-Altman plot shows almost all data points lie within 95% confidence interval limits and the points were equally distributed above and below the line of mean difference (upper limit = 0.90, lower limit = -0.89) (Fig. 2) that indicates no proportional bias in the estimates of FFM using two methods.

A linear regression analysis using the difference of FFM values as dependent variable and the average of the measurements as the independent variable showed no significant interrelationship ($t = 1.52$, p-value 0.14, and standard error 0.002) that accepted the null hypothesis. Age was adjusted in the model and regression coefficient was close to zero (0.003) with 95% confidence interval limits (lower -0.001, upper 0.008) that indicated no proportional bias and appeared to have an agreement between the measures (estimated FFM using BIA and that derived from formula, proposed by Macias et al. 2007).

**Discussion**

It is evident from the present study that the prediction formula proposed by Macias et al. (2007) to estimate FFM using the resistance and reactance values of BIA is reproducible and that has been successfully used in the present study. No significant difference between the BIA-based and formula-based FFM values was observed in the present study. A consistent agreement between the estimates was clear from correlation coefficients, regression models, and Bland-Altman plot. These results confirm the reliability of the use of resistance and reactance values to estimate body composition characteristics when the in-built algorithm of the BIA device to estimate the same is not known. The participants were young adults and values of their body composition characteristics were wide ranging: FFM (31 to 55 kg) and PBF (23 to 45%). Interestingly, BMI of the participants was not very high in the sample (mean value 22.59 kg/m²). In addition, BMI showed significant correlation with FM, FFM, and FMI (height adjusted FM) but not with PBF. The results indicate body fat in kilogram (FM) and height adjusted values (FMI) are more important indicators of adiposity than a generalized value of PBF in relation to BMI. Considering high prevalence of BMI-based overweight and obesity in Mexican adults as reported earlier (Gutiérrez et al. 2013), the present sample of young adults (n= 60) showed relatively low frequency of overweight (23%) and nobody was obese. This result may be due to a non-probability sample that neither represent the population of this age-group from Merida, nor the State of Yucatan. In the previous study from Sonora, a northern Mexican State, mean values of BMI were also not very high (men 26.2 kg/m², women 25.4 kg/m²) in the sample of 20 to 50-year-old adults (Macias et al. 2007).

Mexico is a country with huge ethnic and genetic diversity (Moreno-Estrada et al. 2014). We understand that human biology of the populations in the north of the country is different from that in the Southern Peninsular Mexico. Mean values of BIA-based PBF (25.8%) and FFM (57.9 kg) among adult male participants in the previous report (Macias et al. 2007) were different from that recorded in the present study (PBF 33.37%, FFM 41.44 kg). The adult men in the present study showed higher PBF and lower FFM in comparison with that reported in the previous study (Macias et al. 2007). This may be interpreted as differences in body composition characteristics between the two sub-populations of Mexico.

A standard multifrequency whole body bioelectrical impedance (BI) analyzer Tanita MC 180 MA (Tanita Co. Tokyo-Japan) was used in the present study that was reported to be similar in the reliability of another model.
Body composition characteristics were evaluated using a different equipment (Model BIA-103, RJL Systems Detroit, MI) in the previous study by Macias et al. (2007). When the in-built software programs for body composition assessment are not known, it seems that the resistance and reactance values obtained from the equipment used in the present study worked satisfactorily. The resistance and reactance-based prediction formula to estimate FFM for adults (Macias et al. 2007) is pioneering of its kind in Mexico. In their study, the authors cross-validated the results obtained using the equation with reference to results from the air displacement plethysmography (ADP). Mean values of BIA-based and ADP-based FFM were same (57.9 kg) among men in that study. In the present study, mean value of BIA-based FFM (41.44 kg) was marginally different from the derived one obtained using the resistance and reactance values applied in the prediction equation (41.36 kg) proposed earlier (Macias et al. 2007). However, the difference in the mean values was not significant (p > 0.05) as evaluated using paired t-test.

Dual-energy X-ray absorptiometry (DEXA or DXA) is considered as a gold standard for the evaluation of body composition characteristics (Laskey 1996; Scafoglieri and Clarys 2018). Magnetic resonance imaging and DXA are highly precise methods to estimate body composition characteristics (Taing et al. 2017). However, the instruments are expensive, invasive, not portable, and time consuming for the evaluation process. In large-scale surveys, BIA provides satisfactory results and advanced models are capable of segmental analysis of body composition: whole body and regional (extremities). Studies reported higher reliability of the results from whole body BIA than segmental BIA when the values were compared with the results obtained using DXA (Bracco et al. 1996; Demura et al. 2004; Erselcan et al. 2000; Ricciardi and Talbot 2007). In this background, BIA-based prediction formula for FFM on a two-compartment model was reported to be accurate, precise, and free of bias (Macias et al. 2007).

A study from Mexico among 86 postmenopausal women aged 42 to 74 years with metabolic syndrome (Balas-Nakash et al. 2010), estimated body fat using DXA and compared the results with the derived values using the formulas proposed in a study based on the data of National Health and Nutrition Examination Survey III (NHANES III) (Sun et al. 2005) of the U.S.A and by another study from Mexico (Macias et al. 2007). The DXA-based PBF (44.54%) had different levels of agreement (Bland-Altman plot) with the values derived from the formula proposed in a NHANES III-based study (41.93%) (Sun et al. 2005) and that estimated using the equation proposed by Macias et al. 2007 (43.32%). The NHANES III-based formula and prediction equation by Macias et al. 2007 underestimated PBF. However, the report (Balas-Nakash et al. 2010) raised doubt about the use of prediction equation to estimate FFM that was proposed by Macias et al. 2007 because age of many women participants in that study was higher than 50 years. In the reference study (Macias et al. 2007), age of women participants was between 22 and 48 years.

A study from China, developed BIA-based prediction equations to estimate FFM for adult men and women using two different BI instruments (hand-to-hand and hand-to-foot) and DXA was the criterion method (Lu et al. 2016). Both types of BIA-based prediction equations demonstrated similar reliability and the hand-to-hand BIA model was found to be more practical for the assessment of body composition characteristics. The study suggested importance of separate prediction equations for men and women. Another study among indigenous Australian adults aged 36 to 60 years developed prediction equation to estimate FFM and the reference method was DXA (Hughes et al. 2015).

A study from Brazil reported that mean values of BIA-based PBF (38.0%) and formula-based PBF (38.7%) were similar; BIA overestimated PBF in overweight and underestimated the same among obese adult women (Pimentel et al. 2010). The formula was developed in an earlier study (Segal et al. 1988). Low sensitivity of BIA to identify individuals with high BMI and body fat was reported by several authors (Macias et al. 2007; Pimentel et al. 2010). Another study among adults of 18 to 64 years of age from Chile (Schifferli Castro and Zuniga 2017) compared BIA-based estimate of FFM with that derived from a prediction formula for FFM reported earlier (Schifferli et al. 2011). The previous study (Schifferli et al. 2011) developed prediction equation for BIA-based FFM, taking DEXA as the criterion or reference method. BIA overestimated PBF in men and underestimated in women compared to the corresponding values obtained using the prediction formula (Schifferli Castro and Zuniga 2017). Therefore, it is evident that BIA-based estimates of FFM may be different in men and women and vary with the levels of BMI and body fatness. This raised concern for separate formulas for men and women and selection of a precise criterion method like DXA. In addition, this issue reflects the limitation of the use of the BIA-based estimates where the in-built algorithm of the device (that varies with the models also) is not known.

A cross-sectional study among university students aged 18 to 24 years from Colombia developed single-frequency BIA-based prediction equations for total body water and FFM, where BI spectroscopy for three compartment model was used as the criterion method (Caicedo-Eraso et al. 2019). In both methods, resistance and reactance values were used. Estimated values of total body water and FFM that were obtained using the two methods did not show...
significant differences. The results of regression models showing low standard error of estimates and increase in R² were like that reported in Macias et al. 2007.

A study from the U.S. (Wada and Tekin 2007) used BIA-based FFM data from NHANES III and applied the prediction equations reported earlier from the same country for men and women to derive FFM (Sun et al. 2003). The study reported huge number of prediction formulas for FFM and lean body mass (LBM). The authors (Wada and Tekin 2007) also cited articles to show the conversion of LBM to FFM using formulas. Therefore, prediction formulas for FFM had received better technical precision.

The prediction formula by Macias et al. 2007 is common for adult men and women. Another study also reported single prediction equation to estimate FFM for adult men and women aged 20 to 94 years of age (Kyle et al. 2001). Separate prediction formulas for men and women were reported from the U.S. (Sun et al. 2003); validation and cross-validation samples of whites and blacks were taken into consideration. A report from China also reported separate equations for adult men and women (Lu et al. 2016). Higher adiposity in women compared to men was reported in previous studies from Nigeria (Mbada et al. 2015) and Mexico (Macias et al. 2007). High correlation between BIA-based PBF and skinfold-based values was observed instead of overestimation of PBF by the former method (Mbada et al. 2015).

Accuracy of the results obtained using BIA depends on the specific algorithm of the device (Jaffrin and Bousbiat 2014). Therefore, validation and cross-validation of the results obtained from an equipment are important before use. Comparison of the results with that obtained through more precise method like DXA is important. In spite of several limitations of the use of BIA as mentioned before (Coppini et al. 2005), the advantages include portability, safety, non-invasive nature of application, less intra-observer error in the estimation of body composition characteristics, segmental analysis of body fat and fat free mass, relatively moderate cost, and reliable results etc. However, care should be taken for the hydration factor and body geometry of severely obese individuals, variation of body water during menstruation and other health conditions. Another important issue will be the use of the terms FFM and LBM that are not interchangeable. The LBM consists of FFM and essential body fat that may vary between 2% and 10% (Scafoglieri and Clarys 2018; Segal et al. 1988).

In this background, it is justified to mention the limitations of the present study and new call for improved research. In the present study, the term FFM has been used to maintain uniformity with the reference study (Macias et al. 2007). Previous reports on the use of BIA, authors mentioned LBM prediction instead of FFM (Tagliabue et al. 2001). Another report also cited articles on how to convert prediction formulas for LBM to FFM (Wada and Tekin 2007). This issue should be considered in future research. Other limitations of the present study were non-probability sampling, relatively small sample size from a short age-range of 21 to 23-year-old adult men. Neither of the two samples (Macias et al. 2007 and the present study) were probabilistic. The present report shows the results of male participants. Therefore, sample size and age might be the other important factors that call for future research to explore the results in wider age-group and in the representative samples of the populations from different regions of Mexico where individuals with BMI-based nutritional status of normal, overweight, and obesity will be present. It is also important to estimate the differential agreement and bias of the obtained values of FFM using two methods at the levels of BMI. Perhaps in future research, separate formulas for men and women will give more precise results as reported in other studies that were also compared with the results obtained through DXA (Sun et al. 2003). Separate prediction equations for men and women may show differential degree of agreement between the BIA-based FFM and the corresponding value derived from the resistance and reactance-based equations.

Despite such limitations, the present study showed significant concordance of the estimated values of FFM using BIA with that estimated one using the prediction formula (resistance and reactance value-based). The results further established the reproducibility of the prediction formula for Mexican adults proposed earlier (Macias et al. 2007).

From the above discussion, it seems that the estimation of FFM using the BIA has certain limitations because the in-built algorithm of the device is not known and that also may vary in different models. Moreover, the formulas used in the program of the device may differ by age, sex, activity patterns, and population backgrounds. On the other hand, the prediction formulas to estimate FFM using the resistance and reactance values obtained from BIA has several advantages over the problems of unknown algorithm of the device. Studies reported agreement of the results obtained using such formulas with those obtained using a gold standard method like DXA. However, some reports are also available on the disagreement of results (over and underestimation of FFM) at the levels of high BMI (overweight and obesity) and body fat. Differential agreements were also found in men and women.

In conclusion, the present study has shown significant agreement of the estimated FFM using the bioelectrical impedance analyzer (Tanita MC 180 MA, Tanita Corporation, Tokyo, Japan) with the estimated FFM using the resistance and reactance values obtained from the BIA that has been used in a prediction formula proposed...
earlier for Mexican adults (Macias et al. 2007).

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