A Comparison of Multiple Frequency versus Single Frequency Bioelectrical Impedance Techniques for the Assessment of Body Composition

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A COMPARISON OF MULTIPLE FREQUENCY VERSUS SINGLE FREQUENCY BIOELECTRICAL IMPEDANCE TECHNIQUES FOR THE ASSESSMENT OF BODY COMPOSITION

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Purpose. The bioelectric impedance analyzer (BIA) is a device commonly used to assess body composition; however, there are questions concerning the reliability of single- and multiple-frequency machines across multiple testing days and their relative accuracies. The purpose of the present study was to compare a single- versus multiple-frequency BIA results across two testing sessions and to compare results for each session to plethysmography as represented by the Bod Pod. Our hypotheses were that results for both BIA units would be consistent between testing days and no significant differences would be seen among the three testing methods. Methods. Twenty young adults (MEAN±SD, age 24.1±3.7 years) were randomly tested on the three devices to determine their reliability and validity. Results. Repeated measures ANOVA, Pearson correlations, Bland-Altman analyses and Chronbach’s alpha confirmed the consistencies of measurement between days for each BIA device and among the three body composition techniques. Conclusion. Single- and multiple-frequency BIA each provide reliable results across testing sessions and these devices and the Bod Pod can be expected to provide consistent results in healthy, young adults. Key Words: BODY COMPOSITION, PLETHYSMOGRAPHY, BIOELECTRICAL IMPEDANCE ANALYZER
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Chapter 1

Introduction

Obesity is a major health epidemic with rising prevalence. In 2012, 34.9% of all American adults were obese\textsuperscript{67}. This can contribute to many cardiovascular, pulmonary, and metabolic diseases. Recent evidence on mortality has questioned the use of body mass index (BMI) as a criterion for categorizing obesity given the lack of distinction between lean body mass and body fat in its computation\textsuperscript{27}. Body composition has been established as a better indicator of body fat than BMI\textsuperscript{27}. Body composition assessments are used to quantify fat mass and fat-free mass in subjects for health purposes, to determine weight loss goals, and to improve athletic performance\textsuperscript{48}. However, these techniques are usually expensive, require considerable expertise to administer, and in many cases are time intensive. There are many modalities used to assess body composition including: hydrostatic weighing (HW), dual-energy X-ray absorptiometry (DXA), air displacement plethysmography (Bod Pod), bioelectrical impedance analysis (BIA), skinfold measurements, and circumference ratios. These methods vary drastically in accuracy, time requirements, and cost.

BIA devices are becoming more commonplace as assessment tools for determining body composition\textsuperscript{67,71,76}, since they are relatively inexpensive, non-invasive, require little technical expertise, and can be performed with subjects of all ages and body types\textsuperscript{33}. Different types of BIA equipment on the market include single-frequency and multi-frequency devices, which vary drastically in price. Studies have reported conflicting findings concerning the validity of BIA\textsuperscript{39,51,76}, and the comparative validities of single- and multi-frequency BIA devices has also been questioned\textsuperscript{88}. Further research
is required to compare the validity and reliability of single and multiple frequency BIA devices.

The current study had two purposes. The first is to determine the reliability of a single-frequency BIA versus a multi-frequency BIA unit across two testing sessions; and second, to compare results for each device on each testing day to Bod Pod results. The research hypotheses were that in a sample of young adults, 18-35 years of age, 1) there would be consistent results for each BIA assessment method across testing days; and, 2) there would be consistent values for body fat percentage among Bod Pod results and BIA results on each testing day.
Chapter 2

Background

*Bod Pod.* There are two regularly administered laboratory techniques that utilize displacement to determine body composition. The technique with the longer research history is hydrostatic weighing (HW), which was the gold standard in body composition testing prior to the development of such technologically advanced techniques as DXA and ultrasound. This method of body composition testing was first established by Goldman and Buskirk, and later modified by Akers and Buskirk\(^1,30\). The HW method uses Archimedes principle, which states that volume of water displaced by a submerged object equals the volume of that object\(^48\). The difference between an individual’s mass in air and mass in water, after correcting for water density using the temperature of the water, allows computation of body density\(^48\).

The Bod Pod uses the same principles as HW, but rather than using water displacement, it uses air displacement to determine body density. Although the Bod Pod cannot be regarded as an industry standard, a considerable volume of research indicates that it is a reliable\(^61\) and valid method of determining body composition when compared to HW\(^9,21,23,45,54,58,59\) and DXA\(^26\) in adults, and also has been shown to be strongly correlated with HW, BIA and DXA\(^46\). Many subjects have reported that they prefer the Bod Pod to HW since submersion in water is inconvenient\(^21\).

Despite the accuracy of the Bod Pod, specific factors should be considered that will increase both the validity and reliability of results. For example, one study indicated that water consumption, water retention, and dehydration can affect body composition results produced by the Bod Pod\(^89\), and therefore affect the accuracy and reliability of the
data. Other research indicates that Bod Pod results are not comparable to other methods in an obese population samples. One study showed that there was a significant difference in body fat percentage between Bod Pod and DXA, and BIA and DXA in obese women\(^7\). A second study demonstrated that the Bod Pod and BIA underestimated body fat percentage compared to DXA in obese children and adolescents\(^42\).

The research regarding athletes produced mixed results. A study examining body composition in NCAA DIII wrestlers indicated that BIA, Bod Pod, and skinfolds showed significant correlations \((r = 0.80-0.96; \ p < 0.01)\) with HW\(^23\); and another study found the Bod Pod to be a valid method of body composition assessment in athletic and nonathletic women when compared to DXA as a standard measure\(^4\). However, a third study suggested that the Bod Pod significantly overestimated body fat percentage by 8% in female athletes and by 16% for a leaner subjects compared with HW\(^87\).

Research also suggests gender differences. One study demonstrated that the average body fat percentage determined by Bod Pod was similar to that by HW for adults, but the study also reported a significant gender difference with the Bod Pod generating body fat results 16% lower in males and 7% higher in females compared to HW\(^46\). Although the Bod Pod and DXA were strongly correlated \((r=0.94)\), the mean difference of 2.2% was significant in men\(^3\).

*Body Fat Percentage Equations.* Both HW and Bod Pod utilize equations to covert body density (BD) to body fat percentage (BF%) using either the Brozek equation:

\[
BF\% = (4.570/BD) - 4.142 \quad 15,47
\]

Or Siri equation:

\[
BF\% = (4.950/BD) - 4.50 \quad 47
\]
Brozek and Keys divided the body into four compartments: water, protein, bone and fat\textsuperscript{40}, whereas, most other researchers used a two compartment model which included fat and fat-free mass\textsuperscript{15,40}. Fat-free mass is assumed to have a constant density of 1.1 g\textbullet;cc\textsuperscript{-1} at 37\degree C\textsuperscript{8,15,40} as compared to fat mass at 0.9 g\textbullet;cc\textsuperscript{-1} at 37\degree C\textsuperscript{8,15,55,56}. These assumptions were formulated and later verified by Behnke, along with other assumptions related to hydration and bone to muscle ratios\textsuperscript{8,55,56}. The mathematical relationships between these two divisions were computed by Brozek and Keys\textsuperscript{15,40}; however, Siri stated that the protein: mineral ratio from Brozek and Keys could lead to 2.1\% variation in body fat percentage\textsuperscript{40,78}. Siri estimated that body fat percentage could vary 2.7\% due to hydration levels and questioned the effects of the variability of bone density and water content on body composition as stated by Brozek\textsuperscript{78}. Lohman calculated that based on these discrepancies, the margin of error could be 3-4\%\textsuperscript{2,47}. Both equations yield results within 1\% of each other\textsuperscript{1,16,47,57}, however, some research indicates a margin of error as great as 2.5\%\textsuperscript{47}. Siri also overestimates fat percentages in subjects with body fat percentages greater than 30\%\textsuperscript{47}.

**Bioelectrical Impedance Analyzers.** BIA is another modality utilized to determine body composition\textsuperscript{70,71,76} that is a relatively inexpensive as compared to HW, Bod Pod, and DXA, non-invasive, and can be performed on subjects of all ages and body types\textsuperscript{33}. One disadvantage of this method is that the accuracy of the results are associated with hydration levels. Using this method to measure conductive volume is not a new concept, since it has been used to assess other biological functions such as pulsatile blood flow\textsuperscript{51,63} and total body water\textsuperscript{15,41,51,52,71,76,82,83}. BIA measures conductance through a subject’s body tissues by applying a constant, low level, alternating, frequency-dependent
electrical current\textsuperscript{48,51,88}. The proposition that BIA can measure fat-free mass is based upon the impedance being affected by geometric shape, cross-sectional area, and specific signal frequency\textsuperscript{48,51}. Fat-free mass, which includes a protein matrix and contains mostly water and electrolytes, has a greater conductivity than fat mass\textsuperscript{48,51,69}. Additionally, intracellular and extracellular fluid, which has a high electrolyte concentration, is a good conductor of electricity, while cell membranes due to their phospholipid bilayer structure have low conductance, and therefore high capacitance\textsuperscript{48,51}. Low frequency currents pass through extracellular fluid only, while higher frequencies pass through both intracellular and extracellular fluid\textsuperscript{48,51,63,64}.

Many other electrical aspects as they relate to the body had to be determined in the development of BIA devices. Nyboer hypothesized that biological volumes were inversely related to resistance and reactance\textsuperscript{48,65}. It was also determined that resistance is a better predictor of impedance than reactance because reactance magnitude is smaller than resistance magnitude\textsuperscript{15,48,52}. Researchers found a strong inverse relationship between impedance and body water\textsuperscript{15}, and a strong positive relationship between conductance and fat-free mass\textsuperscript{51,52}. Nyboer also established statistically significant relationships between conductance and body composition utilizing HW results without residual volume measurements\textsuperscript{66}. This relationship was used to develop a linear regression equation for fat-free mass, total body water, and potassium based on conductance\textsuperscript{15,35,52,66,76,82,83}. However, Segal et al. could not verify this regression equation as a tool for quantifying fat-free mass\textsuperscript{76}. In contrast, Nyboer was able to implement the measurement of body
water as a method to determine body composition. Based on the theory that a relationship exists between conductance and body composition, the tetrapolar technique of BIA was developed to minimize skin-electrode impedance. BIA was found to be a valid and reliable, fat-free mass predictor in men and women; however, a 2% variability has been observed due to differences in body water content.

*Single vs. Multiple Frequency BIA.* With the advancements in technology, the number of contact electrodes in BIA equipment has increased from four to eight. BIA equipment can now measure not only total body water and body fat percentage, but also lean mass and fat distribution within the whole body and individual segments. There are a number of BIA units on the market that vary in the number of electrodes used, the range of frequencies employed and the cost. Some studies have demonstrated the validity of single frequency BIA, while others suggest that it overestimates body fat percentage in athletes and underestimates it in obese subjects. The multi-frequency systems are believed to correct this error by using low and high frequencies that calculate intercellular water, extracellular water, and total body water. A study by Bedogni et al. supported the use of a multi-frequency system by demonstrating the accuracy and precision of the InBody 720 for total body water estimates.

Völgyi et al. performed a comparison between single frequency and multi-frequency BIA systems. Using the single frequency Tanita BC 418 MA (Arlington Heights, Illinois), and multi-frequency InBody 720 (Biospace Co., Cerritos, CA). Both devices underestimated body fat percentage by 2-6% when compared to DXA. Völgyi et al. presented a number of explanations for their results. First, they noted that different algorithms are used for athletes and non-athletes in the Tanita BC 418 MA due to
differences in physiological and hydration values between these groups. The Tanita BC 418 MA algorithms also include age, height, gender, and impedance, while the InBody 720 only utilizes electrical properties. Second, the variances seen with the InBody 720 were specific to subjects with higher BMI (>25) and this could possibly be corrected by including age in the algorithm used. Third, age and gender algorithms could have accounted for differences in results between the BIA devices. Fourth, physical activity level may alter BIA results if muscle distribution is disproportional to activity level because this method measures resistance regardless of body fat percentage and the appendages have 85% of total impedance but a total volume of only 35%. This last explanation appears to be specific to the InBody 720 since it displayed significant differences in results due to physical activity level, while the Tanita BC 418 MA did not. BIA devices can have a wide range of error by underestimating body fat percentage in lean subjects and overestimating in obese subjects. The validity for both of these BIA devices therefore requires greater scrutiny and further comparative assessments in different population samples are suggested.

The current study had two purposes: to determine the reliability of a single-frequency BIA versus a multi-frequency BIA devices across two testing sessions, and to compare results for each device on each testing day to Bod Pod results in a sample of young adults, 18-35 years of age.
Chapter 3

Methods

Participants. Twenty participants, male (n=13) and female (n=7), participated in this study. Participants were recruited using flyers approved by the University of Miami’s Institutional Review Board. The flyers were posted throughout the University of Miami’s Coral Gables campus. Interested participants were given information via phone or email including: explanation of study purpose, testing protocol, inclusion and exclusion criteria, duration of the testing, proper attire, and directions to the Max Orovitz and Merrick laboratories and the Herbert Wellness Center.

Design. This study incorporated a randomized design utilizing three testing modalities on all participants. A consort diagram showing the study design is presented in Figure 3.1. Inclusion criteria for participation in the study included: being between the ages 18 and 35 and having no known cardiovascular or pulmonary issues. Exclusion criteria included: inability to speak fluent English, and for women, not having menstruated within the past two weeks. Tests were performed during three separate sessions. During session 1, informed consent and photography and video release forms were completed and BIA testing on both devices occurred. During session 2, both BIA tests were repeated. During session 3, Bod Pod testing occurred. There was no consumption of food or drink or vigorous physical activity allowed for 3 hours prior to testing, but adequate hydration was to be maintained by drinking water frequently for the twenty-four hour period prior to the 3-hour fast. Multi-frequency BIA was conducted in
the Merrick Laboratory. Single frequency BIA was conducted in the Max Orovitz Laboratory. Bod Pod measures were performed in the Wellness Suite within the Herbert Wellness Center.

**Equipment.** The equipment used for this study included: Bod Pod (Cosmed USA, Inc., Concord, CA), Tanita BC 418 single-frequency BIA (Tanita Corp., Arlington Heights, IL), and InBody 520 multi-frequency BIA, (Biospace Co., Cerritos, CA).

**Testing Protocol**

**Bod Pod Procedure**

Participants were told to arrive at the laboratory in a "balanced" hydration status (this was accomplished by drinking water frequently the day prior to testing). They were also instructed to empty their bladders just prior to testing and to remain completely relaxed, dry, and at a normal body temperature by minimizing unnecessary movements. They were also instructed to remove all jewelry. During testing participants wore spandex (sports bra and shorts for females, compression shorts for males) and a swim cap. The test was repeated three times and body density was computed using the average of the three trials. Body fat is calculated using the appropriate equation (Siri for general population and Brozek for Black subjects)

**BIA procedure:**

Participant information including gender, age, and height were entered as required into the device. The participant removed excess clothing, jewelry, socks, and shoes. Hands and feet were wiped with an alcohol prep pad to reduce surface resistance due to particulate matter and body oil. The participant then stepped on the BIA machine
to be weighed. The participant then held handles on the machine and remained still throughout duration of test.

*Statistical Analyses*

A 3-way (body composition assessment device) within subjects, repeated measures ANOVA with effect size ($n^2_p$) and Chronbach’s alpha were performed to examine the differences between the modalities. For the ANOVA, Bonferoni *post hoc* tests were used to determine the sources of the differences. These analyses were performed using the SPSS statistical package 19 (IBM Corp., Armonk, NY). Regression analyses and Bland-Altman plots used for comparisons between the BIA devices and sessions were calculated using SigmaPlot 12 (Systat Software Inc., San Jose, CA).
Chapter 4

Results

Participant characteristics including age, height, and weight are provided in Table 4.1. Participants were healthy, active undergraduate and graduate college students.

Validity. Figure 4.1 illustrates the body fat percentage (Mean±SE) for the single-frequency BIA (15.910±1.70), multiple-frequency BIA (17.795±2.03), and Bod Pod (17.285±1.88). The 3-way, repeated measures ANOVA, revealed no significant difference among the three devices ($F_{2,38}= 2.203$, $p>.124$, $n^2_p=.104$).

Table 4.2 presents the results from the Bland-Altman analyses examining comparisons between the Bod Pod and single and multiple frequency BIA devices on each BIA testing day. For the comparisons between the single-frequency BIA and Bod Pod results, mean values for each day were -0.510±4.418 (95% CI -2.586, 1.566), and -0.435±4.554 (95% CI -2.575, 1.705), respectively. These results are presented graphically in Figures 4.2 and 4.3, respectively. Internal consistencies for comparisons of day 1 and day 2 for the single frequency BIA compared to Bod Pod calculated using Chronbach’s alpha were $\alpha=.875$ and $\alpha=.854$, respectively. Bland-Altman plots for these comparisons are presented in Figures 4.2 and 4.3, respectively. The plots provide a visual confirmation of the agreement between the single-frequency BIA results on days 1 and 2 and the Bod Pod results.

Bland-Altman analyses comparing days 1 and 2 for the multiple-frequency BIA compared to the Bod Pod yielded similar results. Mean values were 1.375±4.762 (95% CI –0.862, 3.612) for day 1, and 1.260±4.799 (95% CI -.995, 3.515) for day 2.
Figures 4.4 and 4.5 provide each Bland-Altman plot, and Chronbach’s alpha values were $\alpha = .828$ and $\alpha = .932$ for days 1 and 2, respectively.

Reliability. Table 4.3 provides Bland-Altman analyses and Chronbach’s alpha scores for the single frequency and multiple frequency devices across testing sessions. For the single frequency device, the mean value was -0.075±1.684 (95% CI –.866, .716) (see Figure 4.6) and the agreement score as computed using Chronbach’s alpha was $\alpha = .984$. For the multiple frequency device, the mean value was 0.115±0.780 (95% CI –0.251, 0.481) (see Figure 4.7) Chronbach’s alpha for the multiple frequency device across testing days was $\alpha = .995$.

The final two analyses indicate the reliability of the single frequency and multiple frequency devices on each testing day. Table 4.3 provides Bland-Altman analyses and Chronbach’s alpha scores for these analyses. For the day 1, as illustrated in Figure 4.8, the mean value was 1.885±3.096 (95% CI 0.431, 3.340). Chronbach’s alpha for testing day 1 between devices was $\alpha = .946$. For the day 2, as illustrated in Figure 4.9, the mean value was 1.695±3.341 (95% CI 0.126, 3.265). Chronbach’s alpha for testing day 2 between devices was $\alpha = .919$. 
Chapter 5

Discussion

Body composition testing is an important tool in determining an individual’s health and disease risk. The search for a technique that is easily executed, non-invasive, and affordable has resulted in the development of a family of BIA devices that employ single and multiple frequency currents to assess body composition. The hypotheses presented in the current study were that in a sample of young adults, 18-35 years of age, 1) there would be consistent results for each BIA assessment method across testing days; and, 2) there would be consistent values for body fat percentage among Bod Pod results and BIA results on each testing day.

Validity for Single Frequency BIA. BIA device validity was tested using repeated measures ANOVA to compare means, Bland-Altman plots for bias, and Chronbach’s α for internal consistency. For single frequency BIA day 1 as compared to the Bod Pod, there was a statistically significant internal consistency (r=.875, p<.0001) with a 4.152% mean range with a tendency to underestimate the values. For single frequency BIA day 2 as compared to the Bod Pod, there was a very good, statistically significant internal consistency (r=.854, p<.0001) with a 4.280% mean range with a tendency to underestimate the values. The results of this study support a very strong correlation between single frequency BIA and Bod Pod.

Prior research that is in agreement with these results has shown that single frequency BIA is a valid method for body composition testing in healthy participants. Those studies were conducted by Segal et al. in 1985 and 1988. The earlier study conducted by Segal et al.,76 in men (age=30.3±11.0 years, BMI=26.9±4.5 kg•m²;
Mean±SD) and women (age=32.3±11.4 years, BMI=30.5±9.2 kg•m²; Mean±SD) using four terminal impedance analyzer (R. J. L. Systems, Detroit, MI). The relationship between HW and BIA for fat-free mass was determined using sex-specific equations provided with the equipment. The researchers found very strong correlations between BIA and HW methods when comparing fat-free mass (Rxx =0.912, SEE=4.43%)76. The later study conducted by Segal et al.,77 in men (age=34.0±8.0 years, BF%=22.0±7.0; Mean±SD) and women (age=24.0±5.0 years, BF%=28.0±6.0; Mean±SD) used a four terminal impedance analyzer (R. J. L. Systems, Detroit, MI). After performing stepwise multiple regression and quadruple cross-validation, these researchers found strong correlations between HW and BIA when comparing body fat percentage in men and women (Rxx=.809, SEE=4.44%; Rxx=.852, SEE=3.98%, respectively )77.

Another study conducted by Gray et al.,31 in men and women (age=41±1 years, BMI=32.6±.9 kg•m²; Mean±SEM) using a portable tetrapolar impedance analyzer (R. J. L. Systems, Detroit, MI). Using generalized and fat-specific equations for BIA compared to HW data from Segal et al.77, researchers found very strong correlations between single frequency BIA and HW (Rxx=0.94-0.99)31. Other studies investigated validity and standard error for estimating (SEE). One such study conducted in 1986 by Lukaski et al., in men (age=26.9±8.0 years, BF%=16.2±7.0; Mean±SD) and women (age=27.0±6.4 years, BF%=25.1±6.6; Mean±SD) using a four terminal impedance plethysmograph (model 101, R. J. L. Systems, Detroit, MI). Correlation coefficients were calculated by comparing the observed and predicted fat-free mass values using a double cross-validation procedure. These researchers found very high correlations between single
frequency BIA and HW for men and women for fat-free mass ($R_{xx}=0.981$, $R_{xx}=0.953$, respectively) with a SEE of 2-3%\textsuperscript{51}.

The previous 1985 Segal et al.\textsuperscript{76} study also compared the validity of the BIA to HW by transforming the results to percent fat units to calculate the zero-order correlation and SEE. The researchers found an excellent correlation but high SEE between BIA and HW ($R_{xx}=.934$, $\text{SEE}=6.10\%$). There is conflicting data about the difference in SEE for BIA devices between those two studies for several reasons including algorithm differences, which can contribute to the variance\textsuperscript{39}. Lukaski et al.\textsuperscript{51} used gender-specific equations, whereas Segal et al.\textsuperscript{76} used empirically derived equations. Furthermore, both of these studies had differential changes in total body water\textsuperscript{39}, which is a major confounding variable for BIA devices. Even though these studies had conflicting SEE results, they still support the validity of single frequency BIA devices as illustrated by the strong correlations.

The previously mentioned study conducted by Jackson et al.\textsuperscript{37} is also in agreement with the results of this study confirming the validity of BIA assessments. Using cross-validation for the BIA equations and HW-determined body composition, these researchers found strong correlations for body fat percentage between BIA and HW in men and women ($R_{xx}=0.71$, $R_{xx}=0.76$, respectively). The previously mentioned study conducted by Tyrell et al.\textsuperscript{84} also displayed results that are in agreement with this study for validity. Pearson's correlation analyses were used to test the relationship between these devices. These researchers found that the correlations between single frequency BIA and DXA were very strong for fat-free mass, fat mass, and body fat percentage ($R_{xx}=0.98$, $R_{xx}=0.98$, $R_{xx}=0.94$, respectively). They determined that single frequency BIA is a valid
method for calculating body composition in children. Across varying sample populations, BIA validity was observed but results were inconclusive for SEE.

A study by Lazzer et al. investigated the interchangeability of single frequency BIA and DXA in overweight adolescents in boys (age=14.1±1.4 years, BMI=27.2±4.9 kg•m²; Mean±SD) and girls (age=15.2±1.4 years, BMI=28.4±3.5 kg•m²; Mean±SD) using Tanita BF-625 (Tanita Corp., Arlington Heights, IL). Using t-tests, ANOVA, and Bland-Altman analyses, these researchers found that the BIA underestimated fat mass by 23.7±3.6 kg in boys and by 20.5±2.0 kg girls. These results are not in agreement with the results of the current study, most likely due to the overweight sample population. Another study by Hosking et al. also investigated the interchangeability of single frequency BIA and DXA in children (age=8.9±0.3 years; Mean±SD) for boys and girls (BMI=17.7 and 18.0 kg•m², respectively) using the Tanita Body Composition Analyzer TBF-300M (Tanita UK Ltd, West Drayton, Middlesex, UK). Using Bland-Altman analyses and linear regression, these researchers found that the BIA overestimated fat-free mass by 2.4% and 5.7%, and underestimated fat mass by 6.5% and 10.3% in boys and girls, respectively. BIA also calculated lower body fat percentages than DXA for boys and girls (4.8% and 12.8%, respectively). Researchers determined that BIA and DXA were not interchangeable, however, the smaller stature of the children versus adults could have been a contributing factor.

**Validity for Multiple Frequency BIA.** For multiple frequency BIA day 1 as compared to the Bod Pod, there was a statistically significant internal consistency (r=.828, p<.0001) with a 4.474% mean range with a tendency to overestimate the values. For multiple frequency BIA day 2 as compared to the Bod Pod, there was excellent,
statistically significant, internal consistency (r=.932, p<.0001) with a 4.510% mean range with a tendency to overestimate the values. The results of this study support a very strong correlation between multiple frequency BIA and Bod Pod. When comparing the mean difference between the three test modalities, there were non-significant differences of 1.885% between single frequency and multiple frequency BIA, 1.375% between single frequency BIA and Bod Pod, and 0.510% between multiple frequency BIA and Bod Pod. The results of this study support the validation among all three methods.

Even though these differences are not statistically significant, previous research demonstrates different findings. The multiple frequency BIA is hypothesized to alleviate some of the single frequency BIA issues by using low and high frequency electric currents which can test for extracellular, intracellular, and total body water\textsuperscript{6,88}. A recent study in 2008 conducted by Völgyi et al.\textsuperscript{88} investigated these claims in men (age=54.2±11 years, BMI=26.5±3.2 kg \textsuperscript{m^2}; Mean±SD) and women (age=56.1±11.7 years, BMI=25.5±4.6 kg \textsuperscript{m^2}; Mean±SD) using single frequency BIA (Tanita BC 418 MA, Tanita Corp., Arlington Heights, IL) and multiple frequency BIA (InBody 720, Biospace Co., Cerritos, CA). Using comparative means and Bland-Altman analyses between both BIA devices and DXA, the researchers found that DXA had statistically higher fat mass estimates (2–5%, p < 0.001) in normal men and in normal women as compared to both BIA devices. Both BIA devices calculated similar fat mass and fat-free mass for normal men and women. The multiple frequency BIA estimated statistically higher fat-free mass (p<0.01), but lower fat mass estimates than DXA of almost 6% in normal men and women\textsuperscript{88}. The single frequency BIA calculated lower estimates for all categories but not statistically different than DXA. The fat-free mass algorithm used by the single frequency
BIA incorporates age, height, gender, and impedance as variables; while the multiple frequency BIA used only the electrical properties obtained during testing which can cause a non-systematic bias. Age and gender contributed to these differences, but when age was adjusted, there was no statistically significant difference between the BIA devices for men and women (p>0.05). The single frequency BIA also uses different algorithms for athletes and non-athletes since hydration levels and fat mass versus fat-free mass distribution differ between these two groups. However, the categorization for athlete versus non-athlete is subjective and is not limited to an individual currently on a collegiate or professional sports team. The results of the study by Völgyi et al. demonstrate a wide margin of individual error when calculating fat mass estimates; therefore, questioning the validity of single and multiple frequency BIA devices as compared to DXA. However, similar results were found between the single and multiple frequency BIA devices.

Another study was conducted by Sun et al. that compared multiple frequency BIA to DXA in men (age=39.65±12.5 years, BMI=27.53±4.39 kg\(\cdot\)m\(^2\); Mean±SD) and women (age=42.66±9.7 years, BMI=26.03±5.00 kg\(\cdot\)m\(^2\); Mean±SD) using multiple frequency, tetrapolar BIA (QuadScan 4000; Bodystat, Douglas, United Kingdom). Using paired t-tests, Pearson’s correlation, ANOVA, Bland-Altman analyses, and multivariate regression to calculate results, researchers found strong correlations between BIA and DXA for men and women (\(R_{xx}=0.78\), \(R_{xx}=0.85\), respectively), as well as, for the entire sample population (\(R_{xx}=0.88\)). Mean body fat percentage was statistically lower for BIA (32.89±8.00; Mean±SD) than it was for DXA (34.72±8.66; Mean±SD). In men, BIA overestimated body fat percentage by 3.03% when body fat percentage was <15%; and
underestimated by 4.32% when body fat percentage was >25%. In women, BIA overestimated body fat percentage by 4.40% when body fat percentage was <25%; and underestimated by 2.71% when body fat percentage was >33%79. Even though BIA calculated a 2% lower body fat percentage compared to DXA, there was a very strong correlation between the methods. Both of these studies used a much larger and older sample size, and did not control for hydration, exercise, or menstruating in women which could all influence the results.

Reliability for Single Frequency BIA. BIA device reliability between days was tested using Bland-Altman plots for bias, and Chronbach’s $\alpha$ for internal consistency. For the single frequency BIA, there was a statistically significant internal consistency ($r=.984$, $p<.0001$) with a 1.582% mean range with a slight tendency to underestimate the values. Therefore, the results of this study support the hypothesis that single frequency BIA devices would show a high level of reliability across testing sessions.

Our results are in agreement with a number of studies that examined the reliability of single frequency BIA devices. An early study was conducted by Jackson et al.39 in men (age=36.7±9.5 years, BMI=23.2±3.1 kg•m²; Mean±SD) and women (age=28.5±5.7 years, BMI=20.8±3.9 kg•m²; Mean±SD) for a four terminal impedance analyzer (model BIA103B, R. J. L. Systems, Detroit, MI). Using a generalizability analysis of variance approach, reliability coefficients, and proportions of error variance were computed across days, testers, subjects, and all interactive terms. These researchers found that the BIA device had excellent reliability for both men and women ($R_{xx}=0.957$, $R_{xx}=0.967$, respectively), and compared favorably to HW ($R_{xx}=0.972$, $R_{xx}=0.973$, respectively). Jackson et al.39 also found that the generalizability reliability coefficients to be nearly
identical between males and females. Additionally, BIA was found to have larger error than HW of >3% and -2%, respectively. They also reported that the BIA error variance was very small; however, they noted that more than 60% of these small errors were due to the body water changes within subjects. Another study conducted by Macfarlane, in men (age=21.8±2.4 years, BMI=21.7±2.1 kg•m²; Mean±SD) and women (age=22.7±2.8 years, BMI=19.7±2.4 kg•m²; Mean±SD) for three single frequency, bipolar foot-to-foot BIA devices (models: UM-022, BF-350, and TBF-410, Tanita Corp., Japan). Using intra-class correlations, these researchers calculated excellent within-day reliability for body mass and body fat percentage for all three devices (ICC=1.000)\textsuperscript{53}.

A number of BIA reliability studies were conducted on children and adolescents which all exhibited excellent reliability. A study was conducted by Wu et al\textsuperscript{90}, in boys (age=12.7±2.7 years, BF%=16.1±7.6; Mean±SD) and girls (age=11.6±1.6 years, BF%=20.1±6.2; Mean±SD) for two single frequency BIA devices (model BU-103, R. J. L. Systems, Detroit, MI; model BMR-2000, Berkeley Medical Research, Berkeley, CA). Using intra-class correlations, these researchers calculated excellent between-week reliability for fat-free mass for the R. J. L. and Berkeley models (ICC=0.987, ICC=0.997, respectively). Another study was conducted by Gutin et al.\textsuperscript{32}, in boys (age=10.33±0.58 years, BMI=18.64±3.46 kg•m²; Mean±SD) and girls (age=10.27±0.63 years, BMI=18.97±3.86 kg•m²; Mean±SD) using a RJL-l01 analyzer (RJL Systems, Mt. Clemens, MI). Using intra-class correlations, these researchers calculated excellent within-day reliability for body fat percentage (ICC=0.995). In a later study, Tyrell et al.\textsuperscript{84} examined the reliability of a single frequency Tanita BIA (Stellar Innovations Inc., Tokyo, Japan) across two consecutive testing sessions in eighty-two European, New
Zealand Maori and Pacific Island children, aged 4.9 ± 10.9 years. Intra-class correlations were used to evaluate the reliability of the two BIA measurements. Similar to the results of our study, the foot-to-foot analyzer in their study showed reliability for impedance measures of r=0.995. Although BIA was used in diverse populations, reliability was consistently observed.

Reliability for Multiple Frequency BIA. The multiple frequency BIA also demonstrated statistically significant internal consistency (r=.995, p<.0001) with a .732% mean range with a tendency to overestimate the values. Therefore, the results of this study support the hypothesis that multiple frequency BIA devices would show a high level of reliability across testing sessions.

Due to the relatively new development of multiple frequency BIA devices, research using this equipment is sparse; however, there are a few prior studies to support these results. A study from 1991, was conducted by Segal et al.,75 in men (age=37±2 years, weight=94.1±4.2 kg; Mean±SEM) for a multiple frequency, four-terminal portable impedance analyzer (Tri-Frequency TVI-10, Daninger Medical Technology, Columbus, OH). Using multiple regression and double cross-validation, researchers computed the regression coefficients for total body water (Rxx=0.947, SEE=2.64), and for extracellular water (Rxx=0.930, SEE=1.94). A more recent study from 2010, was conducted by Park et al.,68 in men and women at two testing centers, center x (age=53.7±9.0 years, height=159.0±7.4 cm, weight=61.4±9.2 kg; Mean±SD) and center y (age=53.5±6.9 years, height=161.5±8.3 cm, weight=64.7±9.9 kg; Mean±SD) for two multiple frequency bioelectrical impedance analyzers (Inbody 330, Biospace, Seoul, Korea; Zeus 9.9, Jawon Medical, Kyoungsan, Korea). Using mean in difference, inter-rater reliability coefficients
(λ), and intra-class coefficients (ICC) were computed between devices at the two testing centers. These researchers found for body fat percentage at center x, low mean in difference (1.37±1.21) and excellent reliability (λ=0.952). At center y, there was also low mean in difference (1.95±1.67) and excellent reliability (λ=0.943)\textsuperscript{68}. Additionally, researchers found excellent ICC=0.93 (0.92-0.94). Further research needs to be conducted on the reliability of multiple frequency BIA devices amongst various sample populations.

*Reliability for Single and Multiple Frequency BIA.* Uniquely to this study, single and multiple frequency BIA devices within day reliability was tested using Bland-Altman plots for bias, and Chronbach’s α for internal consistency. For day 1, there was a strong, statistically significant internal consistency (r=.946, p<.0001) with a 3.771% mean range with a tendency to overestimate the values. For day 2, there was a strong, statistically significant internal consistency (r=.919, p<.0001) with a 3.391% mean range with a tendency to overestimate the values. The purpose of the analyses was to demonstrate that these two single and multiple BIA devices can be interchangeable for within day testing.

There were several limitations to this study. Due to the high temperatures and humidity coupled with extremely limited parking and distance between the labs, hydration could have been altered between techniques in a very short amount of time. Additionally, diet and physical activity were not controlled for during the two week timespan of testing. Even though menstruation was part of the exclusion criteria for this study, research by Gleichauf found that single frequency BIA to be reliable during female menstruation\textsuperscript{29}. Lastly, the comparative method to the BIA devices was the Bod Pod, which is not a gold standard technique. However, there is copious amounts of research
that indicate very strong correlations between the current gold standard of DXA and the previous one of HW with the Bod Pod\textsuperscript{3,9,21,23,26,45,46,54,58,59}. The results could have direct clinical application with regards to body composition testing availability. The single frequency BIA model Tanita BC 418 MA has a price tag of $5,500\textsuperscript{81}, while the multiple frequency BIA model InBody 520 costs $7,250\textsuperscript{38}. If these two models are highly correlated, then the less expensive, single frequency BIA model would be a viable method for various healthcare and fitness centers to provide more readily available, reliable, and valid body composition testing.

**Conclusion**

The findings of this study indicate that single and multiple frequency BIA devices are both reliable and valid techniques to measure body composition as compared to the Bod Pod in healthy, young adults. Additionally, multiple frequency BIA exhibited no greater accuracy than the single frequency BIA, despite the claims and much greater cost. Further research on a much larger scale is needed to determine the true comparability of these techniques, especially between various sub-populations.
FIGURE LEGEND

Figure 3.1. Study Consort Chart

Figure 4.1. Body Fat Percentages (Mean±SD) for Single-Frequency BIA, Multi-Frequency BIA and Bod Pod

Figure 4.2. Bland-Altman Plot of Single Frequency BIA Testing Session 1 and Bod Pod
Figure 4.3. Bland-Altman Plot of Single Frequency BIA Testing Session 2 and Bod Pod
Figure 4.4. Bland-Altman Plot of Multiple Frequency BIA Testing Session 1 and Bod Pod
Figure 4.5. Bland-Altman Plot of Multiple Frequency BIA Testing Session 2 and Bod Pod
Figure 4.6. Bland-Altman Plot of Single Frequency BIA between Testing Sessions
Figure 4.7. Bland-Altman Plot of Multiple Frequency BIA between Testing Sessions
Figure 4.8. Bland-Altman Plot between BIAs for Testing Session 1
Figure 4.9. Bland-Altman Plot between BIAs for Testing Session 2
Figure 3.1

- Assessed for eligibility (n=50)
  - Refused consent (n = 0)
  - Excluded (n=15)
  - Dropout (n=15)
  - Experimental (n=20)

Randomized

- Single and Multiple Frequency BIAs (n=20)
- Bod Pod (n=20)
- Single and Multiple Frequency BIAs (n=20)

SPSS analysis (n=20)
Figure 4.1
Figure 4.2

PLETHYSMOGRAPHY VERSUS SINGLE FREQUENCY
BIOELECTRICAL IMPEDANCE FOR DAY 1

Plethysmography versus Single Frequency Day 1

$r = .875, p < .0001$

Bland-Altman Graph

Bias = -5.100
Std Dev = 4.4160
Limits of Agreement = -9.1693, 8.1453
Bias CI
95% CI = -2.5568 to 1.5656
Lower Limit of Agreement CI
95% CI = -12.7643 to -6.5743
Upper Limit of Agreement CI
95% CI = -4.5543 to 11.7443
Figure 4.3

PLETHYSMOGRAPHY VERSUS SINGLE FREQUENCY BEOELECTRICAL IMPEDANCE FOR DAY 2

Plethysmography versus Single Frequency Day 2

\[ r = 0.854, \ p < 0.0001 \]

Bland-Altman Graph

Bias = -4.350
Std Dev = 6.2142
Limits of Agreement = -8.3611, 3.7611

Bias CI
95% CI = -2.5745 to 1.7045
Lower Limit of Agreement CI
95% CI = -13.0699 to -6.6554
Upper Limit of Agreement CI
95% CI = 4.705 to 12.1909
Figure 4.4

**PLETHYSMOGRAPHY VERSUS MULTIPLE FREQUENCY BIOELECTRICAL IMPEDANCE FOR DAY 1**

Multiple Frequency Day 1 versus Plethysmography

$r = .828$, $p < .0001$

**Bland-Altman Graph**

Bias = 1.3750
Std Dev = 4.7616
Limits of Agreement = -7.9577, 10.7677
Bias CI 95% CI = -0.862 to 3.612
Lower Limit of Agreement CI 95% CI = -11.8322 to -4.0831
Upper Limit of Agreement CI 95% CI = 0.033 to 14.5022
Figure 4.5

**PLETHYSMOGRAPHY VERSUS MULTIPLE FREQUENCY BIOELECTRICAL IMPEDANCE FOR DAY 2**

Multiple Frequency Day 2 versus Plethysmography

![Graph showing correlation between Multiple Frequency Day 2 and Plethysmography](image)

$r = 0.825$, $p < 0.0001$

**Bland-Altman Graph**

![Bland-Altman graph showing bias and limits of agreement](image)

- Bias = 1.2600
- Std Dev = 4.7088
- Limits of Agreement = -8.1457, 10.6557
- Bias CI 95% CI = -0.9945 to 3.5145
- Lower Limit of Agreement CI 95% CI = -12.0089 to -4.2408
- Upper Limit of Agreement CI 95% CI = 0.7093 to 14.5700
Figure 4.6

SINGLE FREQUENCY BIOELECTRICAL IMPEDANCE

**Time 1 versus Time 2**

![Graph showing a scatter plot with a linear trend line. The correlation coefficient is r = 0.984, p < 0.0001.]

**Bland-Altman Graph**

![Graph showing the Bland-Altman plot with the mean difference, bias, and limits of agreement. The bias is -0.0750, with a 95% CI of -0.8661 to 0.7161. The limits of agreement are -3.3755 to 3.2215.]

**Summary**

- **Correlation Coefficient**: r = 0.984, p < 0.0001
- **Bias**: -0.0750, 95% CI: -0.8661 to 0.7161
- **Limits of Agreement**: -3.3755 to 3.2215
**Figure 4.7**

**Multiple Frequency Bioelectrical Impedance**

- **Time 1 versus Time 2**
  - Correlation coefficient: $r = 0.995$, $p < 0.0001$

**Bland-Altman Graph**

- Bias: 0.1150
- Standard Deviation: 0.7706
- Limits of Agreement: -1.4129 to 1.6429
- Bias CI: 95%
  - Lower Limit: -0.2512
  - Upper Limit: 0.4812
- 95% CI: -2.0472 to 0.7762
- Upper Limit of Agreement CI: -1.009 to 2.2772
Figure 4.8

**SINGLE VERSUS MULTIPLE FREQUENCY**
**BIOELECTRICAL IMPEDANCE FOR DAY 1**

Multiple versus Single Frequency

\[ r = .946, p < .0001 \]

**Bland-Altman Graph**

- Bias: 1.8850
- Std Dev: 3.0061
- Limits of Agreement: -4.1633, 7.9533
- Bias CI: 95% CI = 0.4305 to 3.3395
- Lower Limit of Agreement CI: 95% CI = -0.7026 to -1.664
- Upper Limit of Agreement CI: 95% CI = 5.434 to 10.4720
Figure 4.9

SINGLE VERSUS MULTIPLE FREQUENCY BIOELECTRICAL IMPEDANCE FOR DAY 2

Multiple versus Single Frequency

Single Frequency

Multiple Frequency

$r = .919, p < .0001$

Bland-Altman Graph

Mean = 1.6950
SD = 3.3400
Limits of Agreement = -4.8531, 8.2431
Bias CI
15% CI = 0.1255 to 3.2645
Lower Limit of Agreement CI
15% CI = -7.5717 to -2.1348
Upper Limit of Agreement CI
15% CI = 5.5240 to 10.8017
Table 4.1: Participant Characteristics (n=20) of Males (n=13) and Females (n=7).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.100</td>
<td>3.782</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.133</td>
<td>10.916</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.927</td>
<td>16.427</td>
</tr>
</tbody>
</table>

*SD=standard deviation
Table 4.2: Bland-Altman analyses for validity between bioelectric impedance and Bod Pod data.

<table>
<thead>
<tr>
<th>Test</th>
<th>Bias</th>
<th>Std Dev</th>
<th>Bias 95% CI</th>
<th>Chronbach's α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single 1-Bod Pod</td>
<td>-0.5100</td>
<td>4.4180</td>
<td>-2.5856 to 1.5656</td>
<td>0.875</td>
</tr>
<tr>
<td>Single 2-Bod Pod</td>
<td>-0.4350</td>
<td>4.5542</td>
<td>-2.5745 to 1.7045</td>
<td>0.854</td>
</tr>
<tr>
<td>Multi 1-Bod Pod</td>
<td>1.3750</td>
<td>4.7616</td>
<td>-.862 to 3.612</td>
<td>0.828</td>
</tr>
<tr>
<td>Multi 2-Bod Pod</td>
<td>1.2600</td>
<td>4.7988</td>
<td>.9945 to 3.5145</td>
<td>0.932</td>
</tr>
</tbody>
</table>

Std Dev = standard deviation, CI = Confidence Interval
Table 4.3: Bland-Altman analyses for reliability between bioelectric impedance measurements across days.

<table>
<thead>
<tr>
<th>Test</th>
<th>Bias</th>
<th>Std Dev</th>
<th>Bias 95% CI</th>
<th>Chronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single 1-Single 2</td>
<td>-0.0750</td>
<td>1.6839</td>
<td>-0.8661 to 0.761</td>
<td>0.984</td>
</tr>
<tr>
<td>Multi 1-Multi 2</td>
<td>0.1150</td>
<td>0.7795</td>
<td>-0.2512 to 0.4812</td>
<td>0.995</td>
</tr>
<tr>
<td>Multi 1-Single 1</td>
<td>1.8850</td>
<td>3.0961</td>
<td>0.4305 to 3.3395</td>
<td>0.946</td>
</tr>
<tr>
<td>Multi 2-Single 2</td>
<td>1.6950</td>
<td>3.3409</td>
<td>0.1255 to 3.2645</td>
<td>0.919</td>
</tr>
</tbody>
</table>

Std Dev= standard deviation, CI= Confidence Interval
Definition of Terms

**BD:** body density. A quantitative technique for calculating body composition by using gravity.\(^{13}\)

**BF%:** body fat percentage. The percentage of total mass that is body fat includes essential body fat and storage body fat.\(^{14}\)

**BIA:** Bioelectrical Impedance Analysis. A harmless amount of electrical current which is sent through the body to determine body fat percentage using total body water.\(^{10}\)

**Bod Pod:** A method for determining body fat percentage using air displacement.\(^{11}\)

**Body composition:** the relative proportions of protein, fat, water, and mineral components in the body. It varies among individuals as a result of differences in body density and degree of obesity.\(^{12}\)

**Capacitance:** “The property of an electric nonconductor that permits the storage of energy as a result of the separation of charge that occurs when opposite surfaces of the nonconductor are maintained at a difference of potential”.\(^{17}\)

**Conductance:** “The readiness with which a conductor transmits an electric current expressed as the reciprocal of electrical resistance”.\(^{19}\)

**Current:** “A flow of electric charge; the rate of such flow”.\(^{20}\)

**DXA:** Dual X-ray absorptiometry. A technique for scanning bone which measures bone mineral density and can be used to determine body fat percentage.\(^{24}\)

**Fat mass:** Portion of the body that is comprised of only fat.\(^{25}\)

**Fat-free mass:** Portion of the body including all its components except fat mass.\(^{44}\)

**Frequency:** “The number of complete alternations per second of an alternating current”.\(^{28}\)
**HW:** Hydrostatic weighing. A method for determining body fat percentage using water displacement.  

**Impedance:** “The apparent opposition in an electrical circuit to the flow of an alternating current that is analogous to the actual electrical resistance to a direct current and that is the ratio of effective electromotive force to the effective current”.  

**Reliability:** “The extent to which a test measurement or device produces the same results with different investigators, observers, or administration of the test over time. If repeated use of the same measurement tool on the same sample produces the same consistent results, the measurement is considered reliable”.  

**Resistance:** “the opposition offered by a body or substance to the passage through it of a steady electric current”.  

**Validity:** “The extent to which a test measurement or other device measures what it is intended to measure. A data collection tool should accurately reflect the concept that it is intended to measure”.
REFERENCES


