

EVALUATION OF SELECTED FIELD AND
LABORATORY MEASURES OF
BODY COMPOSITION

by

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ABSTRACT

The four-compartment (4C) model is a laboratory method that is a valid method for the assessment of body composition. Three experiments were performed to 1) determine the validity of selected bioimpedance equations with the 4C model, 2) determine the impact of predicted vs. simultaneous residual lung volume (RLV) during underwater weighing (UWW) on the 4C model, and 3) develop a new body fat prediction equation. In the first study, subjects had body fat percentage (BF%) and fat-free mass (FFM) predicted from four bioelectrical impedance equations and compared to the 4C model. Three equations produced a significant mean difference, while another was non-significant. However, all four equations had a small standard error of the estimate (SEE) and fairly narrow limits of agreement. In a second study, RLV was measured simultaneously and predicted when determining UWW and 4C model BF%. The mean differences for UWW BF% was significant when comparing predicted vs. simultaneous RLV, but non-significant when the body density values derived via UWW were incorporated in the 4C model. The error was lower when using RLV prediction equations for determining BF% via the 4C model than UWW. In a third study the variables sex, body mass index (BMI), sum of combined handgrip strength, and vigorous physical activity was utilized in a regression equation to predict 4C model BF%. The new BF% equation, previous BMI-based BF% equations and skinfolds were compared to the 4C model. The new equation and BMI-based equations had significantly different BF% values and provided large 95% limits of agreement. Similarly, skinfolds had a significant mean difference, but the SEE was 3.7%, leading to the

recommendation of skinfolds over the new equation and BMI-based equations. In conclusion, BIA equations can be used in the field, but practitioners should consider the tendency of the equations to over-predict BF% and under-predict FFM. Furthermore, the prediction of RLV can be used for the UWW procedure when determining 4C model BF%, but should not be used for UWW BF% alone. Lastly, the new BF% equation and BMI-based equations did not compare favorably with 4C model and the use of skinfolds is recommended.

DEDICATION

Dedicated to my Wife, Son, Daughter, Mom, Scott, Jon-Michael and the rest of my family, friends, and loved ones who have provided me with the much needed support that has brought me to this point in my life. Thanks for all you have done. I am truly grateful that you have been supportive of me and I cannot express how appreciative I am of you.

LIST OF ABBREVIATIONS & SYMBOLS

2C	two-compartment
3C	three-compartment
4C	four-compartment
BD	body density
BIA	bioelectrical impedance analysis
BIS	bioimpedance spectroscopy
BF%	body fat percentage
BM	body mass
BMC	bone mineral content
BMI	body mass index
BV	body volume
CE	constant error
DXA	dual energy x-ray absorptiometry
ES	effect size
FFM	fat-free mass
FM	fat mass
HFBI	hand-to-foot bioelectrical impedance analysis
IPAQ	international physical activity questionnaire
LOA	limits of agreement

Mo	bone mineral
NHANES	national health and nutrition examination survey
PA	physical activity
R	resistance
RLV	residual lung volume
SEE	standard error of estimate
SF7	skinfold 7 site
TBW	total body water
USG	urine specific gravity
UWW	underwater weighing
Xc	reactance

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CONTENTS

ABSTRACT.....	ii
DEDICATION.....	iv
LIST OF ABBREVIATIONS & SYMBOLS.....	v
ACKNOWLEDGEMENTS.....	vii
LIST OF TABLES.....	xi
LIST OF FIGURES.....	xii
CHAPTER 1: INTRODUCTION.....	1
REFERENCES.....	5
CHAPTER 2: VALIDITY OF SELECTED BIOIMPEDANCE EQUATIONS FOR ESTIMATING BODY COMPOSITION IN MEN AND WOMEN: A FOUR-COMPARTMENT MODEL COMPARISON.....	8
ABSTRACT.....	8
INTRODUCTION.....	9
METHODS.....	12
RESULTS.....	16
DISCUSSION.....	19
PRACTICAL APPLICATIONS.....	23
REFERENCES.....	24
CHAPTER 3: IMPACT OF MEASURED VS. PREDICTED RESIDUAL LUNG VOLUME ON BODY FAT PERCENTAGE VIA UNDERWATER WEIGHING AND FOUR-COMPARTMENT MODEL.....	30

ABSTRACT.....	30
INTRODUCTION	31
METHODS	33
RESULTS	37
DISCUSSION.....	40
PRACTICAL APPLICATIONS.....	45
REFERENCES	45
CHAPTER 4: PREDICTING BODY FAT PERCENTAGE USING BMI, MUSCULAR PERFORMANCE, AND SUBJECTIVE PHYSICAL ACTIVITY LEVEL.....	52
ABSTRACT.....	52
INTRODUCTION	53
METHODS	55
RESULTS	61
DISCUSSION.....	62
PRACTICAL APPLICATIONS.....	68
REFERENCES	69
CHAPTER 5: CONCLUSION	78
REFERENCES	80
APPENDIX.....	81

LIST OF TABLES

Table 2.1. Subject characteristics (mean and standard deviation (SD) and range).....27

Table 2.2. Comparison of BF% values between the BIA equations and 4C model 28

Table 2.3. Comparison of FFM values between the BIA equations and 4C model29

Table 3.1. Subject characteristics (mean and standard deviation (SD) and range).....49

Table 3.2. Comparison of UWW BF% between simultaneously measured and predicted RLV50

Table 3.3. Comparison of 4C model BF% between simultaneously measured and predicted RLV51

Table 4.1. Correlation, Mean, and Standard Deviations for 2 independent samples73

Table 4.2. Comparison to the 4C model of methods for predicting BF%74

Table 4.3. Pearson correlation coefficient for predictor variables and body comp75

Table 4.4. Regression models for the prediction of 4C Model FFM76

LIST OF FIGURES

Figure 4.1. Bland and Altman plot comparing the prediction equations and 4C model BF% (n = 135). The middle solid line indicates the constant error or mean difference. The dashed lines represent the upper and lower limits of agreement (± 1.96 SD). The dashed-dotted regression lines represent the trend between the errors of each measurement77

CHAPTER 1

INTRODUCTION

Body composition is often assessed to help identify the success of weight loss interventions and for determining risk of obesity related diseases (1-3). Body fat percentage (BF%) is important to evaluate due to the negative health complications associated with obesity such as cardiovascular disease and type II diabetes, while fat-free mass (FFM) is important to know in assessing the effects of sarcopenia and osteoporosis or for determining success of training interventions (4).

The assessment of body composition is determined by indirect and doubly indirect methods (5). Examples of indirect methods are laboratory techniques such as underwater weighing (UWW) and dual energy x-ray absorptiometry (DXA) whereas field techniques such as bioelectrical impedance analysis (BIA) and skinfolds are considered doubly indirect methods. Doubly indirect methods are often fast and affordable methods that have been derived from a statistical relationship with an indirect method.

Multiple-compartment models have emerged as criterion measurements in body composition research over methods such as UWW and DXA due to the assumptions (i.e., hydration of FFM is 73%) of two-compartment (2C) models. For example, three-compartment (3C) models sub-divide the FFM into residual (bone mineral and muscle combined), and total body water (TBW), which accounts for the largest component of the body. Eliminating the hydration assumption of FFM in a 3C model is thought to add accuracy to this method (6).

However, four-compartment (4C) models are the leading reference method for body composition since they build upon the 3C model by including an additional measurement of bone mineral content (BMC) for estimation of body composition (7).

Bioimpedance spectroscopy, DXA, and UWW can be used in a 4C model to determine total body water (TBW), BMC, and body density (BD), respectively. Various factors can influence the measurements within a 4C model. Therefore, identifying these variables is important. For example, it has been shown that predicting residual lung volume (RLV) instead of measuring it influences BD measurements via UWW.

Due to the differences in BD reported when predicting and measuring RLV, the measurement of BF% is also influenced (8, 9). However, the influence of predicting RLV on BF% has only been examined for UWW BF%. Thus, the impact of predicting RLV on BD measurements that are being used to calculate 4C model BF% has yet to be investigated. Determining whether BD can be determined with predicted RLV instead of measuring it in a 4C model could be useful in research and laboratory settings when there are time constraints or limited equipment.

2C models are often used to assess body composition when multiple-compartment models are not available. 2C models divide the body into two compartments as its name suggests: fat mass (FM) and FFM. Though indirect methods such as UWW and DXA are often used as criterion measures, both have an underlying assumption that hydration of FFM is a constant 73%. This is problematic since large variations in the hydration of FFM (68-81%) have been reported (10). Consequently, multiple-compartment models of 3C or more, which include a measurement of TBW, are often considered superior to 2C models. However, the practicality of using these multiple-compartment models, UWW, or DXA in large population studies or in some

field and clinical settings is limited. As a result, doubly indirect methods (field methods) such as BIA and anthropometry have been developed as alternatives for practitioners.

Anthropometric methods of body composition include methods such as body mass index (BMI) and skinfold techniques (11). BMI is one of the most widely used methods in obesity research since it only requires the measurements of height and weight and it is useful in determining risk for developing cardiovascular disease, type II diabetes, and hypertension (4, 12, 13). However, BMI is not a measure of BF%. Therefore, regression equations have been developed to predict BF% with BMI as a prediction variable (14-16).

A disadvantage of using BMI as a predictor of BF% is that it does not distinguish between FM and FFM. Because of this limitation, using BMI to estimate BF% can result in a large standard error of the estimate, especially in athletic populations with a large muscle mass (17). These concerns raise the question of whether a regression equation that utilizes both BMI and muscular fitness variables (i.e., push-ups, sit-ups, and handgrip strength) can increase prediction equation accuracy when estimating BF%. In addition, physical activity (PA) has been found to have an inverse relationship with BF% regardless of age, sex, race, etc. (18-20). Therefore, it seems plausible that including a subjective measure of PA and muscular fitness variables with BMI could be a practical method for assessing body composition in various populations and settings.

BIA is often a surrogate of indirect methods (e.g., UWW and DXA). This method works by sending a painless electrical signal through the body to measure TBW. Single-frequency BIA devices utilize a low frequency (50 kHz) to estimate extracellular water. Conversely, higher frequencies (> 50 kHz) are capable of penetrating the cellular membrane and can be used to estimate intracellular water. Multi-frequency BIA devices employ both high and low

frequencies to estimate intra and extracellular water, respectively, which is thought to result in a more accurate measurement of TBW than single-frequency BIA devices (11, 21).

Similar to BMI, there are disadvantages practitioners need to be aware of when using BIA to estimate body composition. For example, BIA equations are often population specific. Furthermore, most published BIA equations were developed from a 2C model, which makes the assumption that FFM has a constant hydration of 73%. The agreement between published BIA equations derived from a 2C model and those developed from a 4C model is an area that is in need of investigation. Identifying the agreement between published BIA equations and a 4C model could be useful for practitioners when laboratory methods are not readily available.

The purpose of this dissertation was to evaluate selected field and laboratory measures of body composition. Specific purposes for each individual study are shown below.

Study 1. The purpose of this study was to compare BF% and FFM values derived from published BIA equations of Chumlea et al. (22) (BIA_{CH}), Deurenberg et al. (23) (BIA_{DE}), Kyle et al. (24) (BIA_{KYLE}), and Sun et al. (25) (BIA_{SUN}) to a 4C model criterion method.

We hypothesized that the BIA_{SUN} equation would have less error than BIA_{CH} , BIA_{DE} , and BIA_{KYLE} when compared to the 4C model.

Study 2. The purpose of this study was to compare UWW and 4C model BF% values when simultaneously measuring and predicting RLV to determine BD measurements via UWW. We hypothesized that the prediction of RLV when determining BD measurements would result in more error for UWW BF% than when used to calculate BF% with the 4C model.

Study 3. The purpose of this study was to develop a BF% regression model that utilized age, sex, BMI, a subjective measure of physical activity, and selected markers of muscular fitness as prediction variables. It was hypothesized that the muscular fitness and physical activity variables would add precision above that of only BMI when incorporated into a model that predicts BF%. Further, it was hypothesized that this model would result in smaller error compared to currently existing BMI-based BF% equations and skinfold prediction equations when compared against a reference measure of using the 4C model.

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CHAPTER 2

VALIDITY OF SELECTED BIOIMPEDANCE EQUATIONS FOR ESTIMATING BODY COMPOSITION IN MEN AND WOMEN: A FOUR-COMPARTMENT MODEL COMPARISON

ABSTRACT

The purpose of this study was to compare body fat percentage (BF%) and fat-free mass (FFM) values calculated from bioelectrical impedance analysis (BIA) equations (previously developed from a range of laboratory techniques), to values determined from the four-compartment (4C) model. Eighty-two adults (42 men and 40 women) volunteered to participate (age = 23 ± 5 years). BF% and FFM were estimated from previously developed BIA equations by Chumlea et al. (BIA_{CH}), Deurenberg et al. (BIA_{DE}), Kyle et al. (BIA_{KYLE}), and Sun et al. (BIA_{SUN}). Criterion BF% and FFM were calculated with the 4C model. The standard error of estimate (SEE) for group BF% and FFM ranged from 3.0-3.8% and 2.1-2.7kg, respectively. The constant error (CE) was significantly higher and lower for BF% and FFM ($p < 0.001$), respectively for three BIA equations (BIA_{CH} , CE = 3.1% and -2.2kg; BIA_{DE} , CE = 3.7% and -2.9kg; BIA_{KYLE} , CE = 2.3% and -1.9kg), but was non-significant for BF% ($p = 0.702$) and FFM ($p = 0.677$) for BIA_{SUN} (CE = -0.1% and 0.1kg). The 95% limits of agreement were narrowest for BIA_{CH} ($\pm 5.9\%$; ± 4.2 kg) and largest for BIA_{DE} ($\pm 7.4\%$; ± 6.2 kg). The significant CE yielded by BIA_{CH} , BIA_{DE} , and BIA_{KYLE} indicates that these equations tend to over-predict group BF% and under-estimate group FFM. However, all BIA equations produced low SEEs and fairly narrow limits of agreement. When the use of a 4C model is not available, practitioners might consider

using one of the selected BIA equations, but should consider the associated CE for each equation.

KEYWORDS: Percent Body Fat; Fat-Free Mass; Adiposity, Bioelectrical Impedance, Multi-Compartment

INTRODUCTION

Body composition refers to the relative proportions of the tissues that comprise the human body and is an important parameter of physical fitness with specific implications related to optimal physical functioning and health, especially considering the negative health consequences associated with excessive adiposity (1). Likewise, a sufficient level of fat-free-mass (FFM) is important for combating age-related sarcopenia and maintaining basal metabolic rate (2). When interpreted correctly, body composition metrics are also useful for tracking responses to exercise training programs, competitive athletic seasons, and long-term nutritional interventions (3). These points underscore that chosen methods of measuring body composition should be precise, yet practically convenient.

Bioelectrical impedance (BIA) provides an attractive body composition field tool since it is noninvasive, requires limited training, and can be quickly administered. The principle underlying this procedure is that electrical conductivity is proportional to total body water (TBW). A small, painless electrical current is sent through the body. The current receives less resistance from hydrated tissues, such as skeletal muscle, compared to compartments of lower water content, such as adipose tissue. The current is sent from one pole to another and its direction depends on the specific BIA device. For example, hand-to-hand and foot-to-foot BIA transmits the signal through only the upper or lower portions of the body, respectively.

These types of BIA devices are limited in their ability to capture total body fat due to differences in distribution (i.e., android versus gynoid). However, with hand-to-foot BIA (HFBI A), the current is sent from the upper to lower portion of the body. Thus, HFBI A is more accurate compared to the other portable BIA devices because it is not as influenced by body fat distribution patterns.

Numerous equations are available that estimate BF% and FFM from the speed of the electrical current provided by HFBI A, along with other descriptive variables. Generally, the equations were validated from laboratory techniques of body composition hence making the BIA method a “double indirect” approach (4). However, each BIA equation may provide a different estimated value of BF% and FFM due to the range of laboratory techniques that were utilized as the criterion within the validation studies. For example, two BIA equations that are commonly utilized within field settings were formerly developed against dual energy x-ray absorptiometry (DXA) (5) and underwater weighing (UWW) (6). Unfortunately, neither DXA nor UWW account for TBW, which can vary among individuals (7, 8). The error associated with UWW and DXA could be incorporated into the BIA prediction equations. As a result, the prediction error of each equation might be compounded with error inherent in the criterion method (e.g., UWW). Consequently, these issues could result in BIA equations not having good agreement with multi-compartment models.

The four-compartment (4C) model incorporates a number of body tissues, including estimated TBW and bone mineral content (BMC). UWW with simultaneously determined residual lung volume has traditionally been considered the “gold standard” for body composition testing (9, 10). However, the ability to include various FFM compartments (i.e., TBW and

BMC) for body composition testing has led some researchers to suggest that the 4C model should be used in future validation studies when available (11-13).

Sun et al. (14) developed a BIA equation from a 4C model with a low-frequency (50 kHz) HFBIA device. The prediction equation was derived on a large number of participants (734 men and 1095 women) representing a broad range of age and body sizes from 5 different study sites (14). Other equations are available that have been validated against the Sun et al. (14) equation. For example Chumlea et al. (15) developed a BIA prediction equation, using the Sun et al. (14) equation as a criterion, from data on children and adults who took part in the third National Health and Nutrition Examination Survey (NHANES III). The Sun et al. (14) equation was used to develop a BIA equation from the NHANES III data because researchers had impedance measurements on NHANES III subjects, but no FFM or BF% values from laboratory methods such as DXA and UWW. As a result, the Sun et al. (14) BIA equation, which was derived from a 4C model, was chosen to be used as the criterion measurement.

Because of the number of available BIA equations, with each being validated against different criterion methods, research is needed to evaluate which equation(s) provide the highest degree of accuracy compared to the 4C model. Therefore, the purpose of this study was to compare BF% and FFM values derived from published BIA equations that were previously developed from a range of laboratory techniques, by using the 4C model as a comparison, in young-adult men and women. Due to the differences in fat distribution across sex, the relative accuracies of each BIA equation were determined within the entire group of participants, as well as within separate cohorts of men and women. It was hypothesized that the equation previously developed from the 4C model would provide the greatest degree of accuracy compared to the other methods.

METHODS

Experimental Approach to the Problem

Participants completed one visit to the laboratory for this study. Subjects had body composition measured with single-frequency HFBIA, bioimpedance spectroscopy (BIS), UWW, and DXA. The 4C model (16) was used to compute the criterion variables, BF% and FFM. The predicted BF% and FFM values were derived via the published equations of Chumlea et al. (15) (BIA_{CH}), Deurenberg et al. (6) (BIA_{DE}), Kyle et al. (5) (BIA_{KYLE}), and Sun et al. (14) (BIA_{SUN}).

Subjects

Forty-two men and forty women participated in this study and were fully informed of the methods and procedures prior to participation. Subject characteristics are depicted in Table 2.1. Recruitment occurred via flyers, word of mouth, and classroom recruitment. Inclusion criteria consisted of subjects who were 18 – 40 years of age that were free from orthopedic disorders and who had no known signs or symptoms of cardiovascular, pulmonary, or metabolic diseases. Before participation, subjects provided written informed consent and completed a medical history questionnaire to ensure inclusion criteria were met. Subjects were asked to avoid eating or drinking, except water, 3 h prior to participating in the study. In addition, subjects were asked to avoid exercise 12 h before testing. Institutional review board approval for subject participation was approved by the host university.

Procedures

Prior to any testing, subjects were required to be adequately hydrated. The assessment of hydration was determined by a refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan) via a urine specific gravity (USG) value of < 1.020 . When USG values were ≥ 1.020 , subjects were given thirty minutes to ingest water *ad libitum* and then retested. After hydration

confirmation, subjects provided a nude body mass (BM) measurement (to the nearest 0.1 kg) with a digital weighing scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Next, subjects were asked to dress into shorts and t-shirt, and their height was measured to the nearest 0.1 cm with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany).

Bioelectrical Impedance Analysis

HFBIAs for all subjects involved removing the right shoe and sock. Subjects laid down on a gurney with their arms 30° from their body and their legs not touching. All body jewelry on the electrode side was removed. The electrode sites were cleaned with alcohol. Prior to electrode placement, excess hair was removed with a razor. Once subjects were fully prepped, electrodes were placed on the right hand and foot. The measurement of resistance (R) and reactance (Xc) values were taken with a single frequency (50 kHz) HFBIAs device (Quantum IV, RJL systems, Clinton MI) and used to derive BF% and FFM. The equations are as follows: (R = Resistance, Xc = Reactance, Wt = Weight, Ht = Height)

BIA_{CH}

$$FFM_{Men} = -10.678 + (0.262 \times Wt) + (0.652 \times Ht^2/R) + (0.015 \times R)$$

$$FFM_{Women} = -9.529 + (0.168 \times Wt) + (0.696 \times Ht^2/R) + (0.016 \times R)$$

BIA_{DE}

$$FFM = -12.44 + (0.34 \times Ht^2/R) + (0.1534 \times Ht) + (0.273 \times Wt) - (0.127 \times age) + (4.56 \times sex);$$

men = 1, women = 0)

BIA_{KYLE}

$$\text{FFM} = -4.104 + (0.518 \times \text{Ht}^2/\text{R}) + (0.231 \times \text{Wt}) + (0.130 \times \text{Xc}) + (4.229 \times \text{sex; men} = 1, \text{ women} = 0)$$

BIA_{SUN}

$$\text{FFM Men} = -10.68 + (0.65 \times \text{Ht}^2/\text{R}) + (0.26 \times \text{Wt}) + (0.02 \times \text{R})$$

$$\text{FFM Women} = -9.53 + (0.69 \times \text{Ht}^2/\text{R}) + (0.17 \times \text{Wt}) + (0.02 \times \text{R})$$

The BF% from each method was calculated as follows:

$$\text{BF\%} = ([\text{BM} - \text{FFM}] / \text{BM}) \times 100$$

Bioimpedance Spectroscopy

BIS was used to determine TBW (ImpTM SFB7, ImpediMed Limited, Queensland, Australia). BIS has been shown to be valid when compared to a criterion measure (i.e., deuterium oxide) (12, 17-19). Furthermore, the TBW values derived from BIS have been used in multi-compartment equations for validation studies (20-22). Subjects removed their right shoe and sock. Subjects were asked to lie down on a gurney with the arms $\geq 30^\circ$ away from the body with legs separated. Excess hair at electrode sites was removed and sites were cleaned with alcohol pads prior to electrode placement. When subjects were fully prepped, TBW was measured on the right side of the body. All BIS measurements were conducted immediately following HF BIA measurements (< 5 min).

Dual Energy X-Ray Absorptiometry

Dual energy X-ray absorptiometry (DXA) was used to measure BMC. The total body BMC was converted to total body bone mineral (Mo). Prior to each whole-body scan, DXA was

calibrated according to the manufacturer's instructions via a standard calibration block (GE Lunar Prodigy, Software version 14.10.022, GE Lunar Corporation, Madison, WI). Subjects removed shoes, socks, and all jewelry. Subjects were instructed to lie supine on the scanning bed with hands by their side. During all body scans, subjects were asked to remain motionless. Scans lasted approximately 6 to 10 min. Mo was calculated as follows: Mo = total body BMC(kg) × 1.0436 (23).

Underwater Weighing

UWW was performed in an apparatus specifically designed for densitometry measurements (Vacu-Med, Ventura, CA). Prior to the UWW measurement, subjects changed into compression type clothing or a bathing suit. Subjects sat on a sling seat during testing. The subjects were instructed to perform a maximum expiration and submerge completely underwater. Simultaneous determination of lung volume was completed with the oxygen dilution technique via a nitrogen analyzer (Vacu-Med, Ventura, CA). Body volume (BV) derived from UWW was used to calculate the 4C model.

Four-Compartment Model Calculations

The 4C model calculation is described by Wang et al. (16). The equation for fat mass (FM) and BF% are as follows

$$FM \text{ (kg)} = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(BM)$$

$$BF\% = (FM/BM) \times 100$$

Statistical Analyses

All data were analyzed with SPSS version 23 (Somers, NY, USA). The differences in mean BF% and FFM among the BIA equations and the 4C model were evaluated using a one-way repeated measures analysis of variance. In the event of a significant omnibus test, paired

samples t-tests were conducted to determine individual differences. A Bonferroni-adjusted alpha level was applied to reduce the chances of obtaining a type I error when multiple pairwise tests were performed. Therefore, the adjusted alpha level for significance of the mean comparisons was determined as 0.0125. Effect size (ES) of the differences was determined using Cohen's *d*. The magnitude of the ES was determined by Hopkin's scale (24) as follows: 0-0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, >2.0 = very large. The constant error (CE) was determined as the differences between the 4C model and BIA equations (e.g., $CE = BIA_{CH} - 4C$ model). Regression procedures were used to determine the Pearson product moment correlation coefficients (*r*) and standard error of the estimate (SEE) for each BIA equation and the 4C model. The following thresholds were used to describe the *r* of BF% values: 0 to 0.30 small, 0.31 to 0.49 moderate, 0.50 to 0.69 large, 0.70 to 0.89 very large, and 0.90 to 1.00 near perfect (24). The method of Bland-Altman (25) was used to identify the 95% limits of agreement between each BIA equation and the 4C model. Due to the differences in body fat distribution and body composition between men and women, all aforementioned analyses were conducted on both sexes combined and on each sex separately.

RESULTS

Body Fat Percentage

Results comparing the BIA equations to the 4C model for BF% are depicted in Table 2.2. When BF% values from men and women were combined, BIA_{CH} , BIA_{DE} , and BIA_{KYLE} all provided significantly higher estimates of BF% (all $p < 0.001$). However, BIA_{SUN} was not significantly different compared to the 4C model ($p = 0.702$). The Cohen's *d* statistic showed a small ES for BIA_{CH} , BIA_{DE} , and BIA_{KYLE} and a trivial ES for BIA_{SUN} . The correlation coefficients were very large and near perfect for the BIA equations. The SEE values ranged

from 3.0% (BIA_{CH}) to 3.8% (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{DE} ($\pm 7.4\%$) and narrowest for BIA_{CH} ($\pm 5.9\%$). The trends, which were calculated as the correlation between the x- (i.e., the mean of actual and predicted values) and the y- (i.e., the difference between the predicted and actual values) axes of the Bland Altman plots, were not statistically significant for BIA_{CH} ($r = -0.06$; $p = 0.58$), BIA_{KYLE} ($r = -0.20$; $p = 0.07$), and BIA_{SUN} ($r = -0.16$; $p = 0.15$), but were statistically significant for BIA_{DE} ($r = -0.26$; $p < 0.05$).

When men were analyzed independently, BIA_{CH} , BIA_{DE} , and BIA_{KYLE} provided significantly lower estimates of BF% (all $p < 0.001$). However, BIA_{SUN} was not significantly different compared to the 4C model in men ($p = 0.29$). The Cohen's d statistic showed a trivial ES for BIA_{SUN} , small ES for BIA_{CH} and BIA_{KYLE} , and moderate ES for BIA_{DE} . The correlation coefficients were very large and near perfect for the BIA equations. The SEE values ranged from 2.7% (BIA_{CH}) to 4.0% (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{DE} ($\pm 8.0\%$) and narrowest for BIA_{CH} ($\pm 5.3\%$). The trends were not statistically significant for BIA_{CH} ($r = -0.28$; $p = 0.07$), BIA_{DE} ($r = -0.09$; $p = 0.58$), and BIA_{KYLE} ($r = -0.12$; $p = 0.46$), and BIA_{SUN} ($r = 0.19$; $p = 0.24$).

When women were analyzed independently, BIA_{CH} , BIA_{DE} , and BIA_{KYLE} provided significantly lower estimates of BF% (all $p < 0.0125$). However, BIA_{SUN} was not significantly different compared to the 4C model in women ($p = 0.116$). The Cohen's d statistic showed a trivial ES for BIA_{SUN} , small ES for BIA_{KYLE} , and moderate ES for BIA_{CH} and BIA_{DE} . The correlation coefficients were very large for the BIA equations. The SEE values ranged from 3.2% (BIA_{CH} , BIA_{KYLE} , and BIA_{SUN}) to 3.4% (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{SUN} ($\pm 6.8\%$) and narrowest for BIA_{KYLE} ($\pm 6.3\%$). The trends were not statistically

significant for BIA_{CH} ($r = -0.06$; $p = 0.96$), BIA_{DE} ($r = -0.29$; $p = 0.06$), and BIA_{KYLE} ($r = -0.07$; $p = 0.67$), and BIA_{SUN} ($r = 0.15$; $p = 0.36$).

Fat-Free Mass

Results comparing BIA equations and 4C model for FFM are depicted in Table 2.3. When men and women FFM values were combined, BIA_{CH}, BIA_{DE}, and BIA_{KYLE} provided significantly lower estimates of FFM (all $p < 0.001$). However, BIA_{SUN} was not significantly different compared to the 4C model ($p = 0.677$). The Cohen's d statistic showed a small ES for BIA_{DE}, and trivial ES for BIA_{CH}, BIA_{KYLE}, and BIA_{SUN}. The correlation coefficients were near perfect for all BIA equations. The SEE values ranged from 2.1kg (BIA_{CH} and BIA_{SUN}) to 2.7kg (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{DE} (± 6.4 kg) and narrowest for BIA_{CH} (± 4.2 kg). The trends were statistically significant for BIA_{CH} ($r = -0.26$; $p < 0.05$), BIA_{DE} ($r = -0.62$; $p < 0.001$), and BIA_{KYLE} ($r = -0.61$; $p < 0.001$), and BIA_{SUN} ($r = -0.39$; $p < 0.001$).

When data for men were analyzed separately from women, BIA_{CH}, BIA_{DE}, and BIA_{KYLE} provided significantly lower estimates of FFM (all $p < 0.001$). However, BIA_{SUN} was not significantly different compared to the 4C model in men ($p = 0.151$). The Cohen's d statistic showed a trivial ES for BIA_{SUN} and a small ES for all other BIA equations. The correlation coefficients were near perfect for all BIA equations. The SEE values ranged from 2.2kg (BIA_{CH} and BIA_{KYLE}) to 2.9kg (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{DE} (± 7.2 kg) and narrowest for BIA_{CH} (± 4.4 kg). The trends were statistically significant for BIA_{DE} ($r = -0.76$; $p < 0.001$), and BIA_{KYLE} ($r = -0.78$; $p < 0.001$), and BIA_{SUN} ($r = -0.33$; $p < 0.05$), but not statistically significant for BIA_{CH} ($r = -0.26$; $p = 0.10$).

When women were analyzed separately, BIA_{CH}, BIA_{DE}, and BIA_{KYLE} provided significantly lower estimates of FFM (all $p < 0.0125$). However, BIA_{SUN} was not significantly

different compared to the 4C model in women ($p = 0.298$). The Cohen's d statistic showed a trivial ES for BIA_{SUN} and a small ES for all other BIA equations. The correlation coefficients were near perfect for all BIA equations. The SEE values ranged from 1.6kg (BIA_{SUN}) to 2.0kg (BIA_{DE}) and the 95% limits of agreement were largest for BIA_{DE} (± 4.2 kg) and narrowest for BIA_{KYLE} and BIA_{SUN} (± 4.0 kg). The trends were statistically significant for BIA_{CH} ($r = -0.62$; $p < 001$), BIA_{DE} ($r = -0.57$; $p < 001$), and BIA_{KYLE} ($r = -0.73$; $p < 001$), and BIA_{SUN} ($r = -0.75$; $p < 001$).

DISCUSSION

This investigation compared BF% and FFM values derived from four published equations with HF BIA to the 4C model. The primary findings of the current study were that the BIA equations had very large to near perfect correlations with the 4C model for group BF% and FFM, along with small SEEs (3.0-3.8% and 2.1-2.7kg, respectively) and fairly narrow 95% limits of agreement for BF% and FFM (± 5.9 -7.4% and ± 4.2 -6.2kg respectively). BIA_{CH} , BIA_{DE} , and BIA_{KYLE} provided significantly higher group BF% ($CE > 2.2\%$) and lower group FFM ($CE > -1.8$ kg) values compared to the 4C model, while BIA_{SUN} had non-significant CEs of -0.1% and -0.1kg respectively. The significant CE suggests that BIA_{CH} , BIA_{DE} , and BIA_{KYLE} might consistently overestimate BF% and underestimate FFM for individuals within the age-range of the current study. However, despite the significant CE, all four BIA equations had small SEEs and narrow 95% limits of agreement when used to predict BF% and FFM. Specifically, BIA_{CH} tended to have the lowest SEE and narrowest 95% limits of agreement of BF% and FFM compared to the other three BIA equations. For all four equations, the correlation between the x- (i.e., the mean of actual and predicted values) and the y- (i.e., the difference between the predicted and actual values) axes of the Bland Altman plots were significant and negative

concerning group FFM, but not for BIA_{CH} , BIA_{KYLE} , and BIA_{SUN} when estimating group BF%. This finding demonstrates that each equation could over-predict FFM in individuals with lower actual measured values while under-predicting those with higher actual FFM and remained consistent when stratified by sex. For example, BIA_{CH} , BIA_{DE} , and BIA_{KYLE} each yielded significantly higher BF% values in men ($> 2.8\%$) and women ($> 1.5\%$) and significantly lower FFM estimates (> 2.3 and 1.6kg , respectively). Additionally, there was a non-significant CE for BIA_{SUN} when estimating BF% and FFM in men (0.6% and -0.5kg , respectively) and women (-0.9% and 0.3kg , respectively). The significant correlations, small SEEs, and fairly narrow 95% limits of agreement remained for each equation when stratified by sex. BIA_{SUN} appears to have acceptable agreement with the 4C model when men and women were examined separately. Although BIA_{CH} , BIA_{DE} , and BIA_{KYLE} each had a significant CE, these equations could potentially be used if adjusting for the CE (e.g., decrease BIA_{CH} by 3.0% when determining group BF%). Given the potential limitations within the current sample, further research is needed to replicate these results in different age and racial and ethnic groups.

Results of previous research have shown similar findings to that of the current study (26-29). Stout et al. (28) found that 5 different HFBIA equations significantly over-estimated BF% (CE = $1.3 - 6.1\%$), had small SEEs ($3.4 - 3.6\%$) and very strong to near perfect correlations ($r = 0.69 - 0.88$) in women when compared to UWW. Stout et al. (26) also reported HFBIA significantly over-estimated BF% compared to UWW in a group of college-aged men (CE = 2.7% ; SEE 4.2% , and $r = 0.74$). Similarly, Eckerson et al. (29) reported that 7 different HFBIA equations under-estimated FFM compared to UWW (CE $2.24 - 8.06\text{kg}$; $r > 0.89$; SEEs $1.77 - 5.12\text{kg}$) in a group of lean men. The current and previous finding suggests that HFBIA may consistently over-estimate group BF% and under-estimate group FFM.

The findings of the current study are most likely related to the different criterion methods that were previously used to develop each BIA equation. BIA_{DE} , BIA_{KYLE} , and BIA_{SUN} were developed from UWW, DXA, and the 4C model, respectively (5, 6, 14) while the BIA_{CH} was specifically developed from the BIA_{SUN} equation (15). Therefore, because BIA_{SUN} was developed from a 4C model, it may come to no surprise that it had the lowest CE of all the BIA equations in the current study. Furthermore, 2C laboratory body composition models, such as DXA and UWW, assume that the body is comprised of fat and fat-free components with densities of $0.9 \text{ g}\cdot\text{cm}^{-3}$ and $1.1 \text{ g}\cdot\text{cm}^{-3}$, respectively (10) and that the relative amounts of three major components of FFM (water, mineral, and protein) are constant in all individuals (9). However, these assumptions likely vary from person-to-person due to factors such as age, health status, gender, etc. As a result, most of the error associated with many 2C models lies in the validity of outlined assumptions, which are based on the analyses of just three male cadavers (9). It may come with little surprise that Clasey et al. (8) reported UWW and DXA to significantly over-estimate BF% (CE 3.4 and 4.5%, respectively) compared to a 4C model. They concluded that the differences may be due to the hydration of subjects' FFM (76.3%), which was different from the assumed constant made by 2C models (8). Therefore, BIA methods that were developed from a 2C model may carry additional inherent error, which should be taken into consideration when practitioners utilize them.

Another potential explanation for the findings could be due to the differences in subject body composition characteristics between the current study and validation studies used to derive each BIA equation. It appears that the current sample was larger (i.e., had higher BM and FFM) and leaner (i.e., had lower BF%) compared to the samples studied in the former studies (5, 6, 14, 15). As such, the current sample is considerably leaner than the general population (15).

Therefore, the results of the current study may align more closely to previous research among individuals with lower BF% and greater FFM. The over-estimation of BF% and under-estimation of FFM is a common finding regarding the agreement between BIA and criterion measures in athletic subjects (30-34). For example, Loenneke et al. (30) reported a segmental BIA device to significantly over-estimate BF% compared to DXA in collegiate baseball players (CE = 2.0%; SEE 3.5%; $r = 0.88$). In a group of collegiate football players, Oppliger et al. (31) found three different BIA devices to significantly over-estimate BF% and under-estimate FFM compared to UWW, despite strong correlations between the BIA methods and UWW ($r = 0.90 - 0.92$ for FFM; $r = 0.71 - 0.81$ for BF%). Interestingly, the mode selection of BIA has also been found to influence testing results of athletes. In a group of collegiate wrestlers, Dixon et al. (34) reported that leg-to-leg BIA significantly overestimated BF% (CE 3.0%) when the standard mode was selected, but was significantly underestimated BF% (CE 2.0%) with the athletic mode selected when compared to UWW, despite strong correlations and small SEEs. These findings further support the notion that BIA equations derived from a general population without the use of a multiple-compartment model might have a tendency to over-predict BF% and under-predict FFM when determining group body composition in physically active men and women. Consequently, BIA equations may consistently over-estimate BF% and under-estimate FFM in heavier and leaner more athletic individuals.

Limitations of the current study could be the use of BIS to estimate TBW rather than the “gold standard” deuterium oxide. Nonetheless, the BIS device utilized in this study has been used in 4C models (20-22, 35) and has also been cross-validated to the deuterium oxide technique in healthy men and women (17). In addition, this study examined apparently healthy

men and women between the ages of 18 to 35 years. Therefore, our results are limited for being generalized to clinical populations and older or younger subjects.

In conclusion, the current study found that BIA_{CH} , BIA_{DE} , and BIA_{KYLE} all had a significant CE, strong correlations, small SEEs, and tight limits of agreement when compared to the 4C model. Although there was a significant CE for BIA_{CH} , BIA_{DE} , BIA_{KYLE} , these equations could potentially be useful when correcting for the CE, especially BIA_{CH} , which tended to have the lowest SEE and narrowest limits of agreement for all BIA equations. BIA_{SUN} was the only equation that provided a non-significant mean difference for BF% and FFM, in addition to near perfect correlations, small SEE and narrow limits of agreement. Therefore, BIA_{SUN} can also be used as a surrogate for the 4C model of body composition.

PRACTICAL APPLICATIONS

This study utilized a single-frequency HF BIA analyzer to calculate impedance measurements (i.e., resistance and reactance) of men and women ages 18-35 years old. BIA_{CH} , BIA_{DE} , and BIA_{KYLE} provided significantly different CEs when compared to the 4C model while the CE for BIA_{SUN} was non-significant. However, all equations had strong to near perfect correlations, small SEEs, and fairly narrow limits of agreement. Therefore, when the use of a 4C model is not practical, practitioners utilizing a single-frequency HF BIA analyzer to calculate impedance can use the selected BIA equations evaluated in the current study, but should take the CE into consideration when doing so (e.g., BIA_{CH} tendency to over-predict group BF% on average 3%). However, practitioners should be aware that all four BIA equations have a tendency to over-predict FFM for individuals with low actual FFM and under-predict it for those with high actual FFM.

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Table 2.1. Subject characteristics (mean and standard deviation (SD) and range)

	All (n = 82)			Men (n = 42)			Women (n = 40)		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Age (years)	23 \pm 5	18	35	23 \pm 5	18	35	22 \pm 4	18	35
Height (cm)	171.7 \pm 9.7	154.2	194.6	178.2 \pm 8.1	160.0	194.6	164.8 \pm 5.6	154.2	176.5
Body Mass (kg)	71.3 \pm 15.8	45.9	130.9	81.8 \pm 13.8	62.2	130.9	60.3 \pm 8.6	45.9	80.7

Table 2.2. Comparison of BF% values between the BIA equations and 4C model

Value	Mean \pm SD	p	Cohen's <i>d</i>	r	SEE	Limits of Agreement			
						CE \pm 1.96 SD	Upper	Lower	Trend
All (n = 82)									
4C BF%	21.8 \pm 7.8								
BIA _{CH}	24.9 \pm 7.6	<0.001	-0.40	0.93	3.0	3.1 \pm 5.9	9.0	-2.8	-0.06
BIA _{DE}	25.5 \pm 6.9	<0.001	-0.50	0.87	3.8	3.7 \pm 7.4	11.1	-3.8	-0.26*
BIA _{KYLE}	24.1 \pm 7.2	<0.001	-0.31	0.90	3.4	2.3 \pm 6.6	8.8	-4.3	-0.20
BIA _{SUN}	21.7 \pm 7.3	0.702	0.01	0.91	3.3	-0.1 \pm 6.4	6.2	-6.5	-0.16
Men (n = 42)									
4C BF%	16.7 \pm 6.2								
BIA _{CH}	19.7 \pm 5.5	<0.001	-0.51	0.90	2.7	3.0 \pm 5.3	8.2	-2.3	-0.28
BIA _{DE}	21.2 \pm 5.9	<0.001	-0.74	0.77	4.0	4.4 \pm 8.0	12.4	-3.6	-0.09
BIA _{KYLE}	19.6 \pm 5.8	<0.001	-0.48	0.84	3.4	2.9 \pm 6.6	9.5	-3.8	-0.12
BIA _{SUN}	17.3 \pm 5.7	0.209	-0.10	0.89	2.9	0.6 \pm 5.7	6.2	-5.1	-0.19
Women (n = 40)									
4C BF%	27.2 \pm 5.3								
BIA _{CH}	30.4 \pm 5.3	<0.001	-0.60	0.81	3.2	3.2 \pm 6.5	9.7	-3.3	0.08
BIA _{DE}	30.1 \pm 4.4	<0.001	-0.60	0.78	3.4	2.9 \pm 6.5	9.4	-3.6	-0.29
BIA _{KYLE}	28.8 \pm 5.1	0.002	-0.31	0.81	3.2	1.6 \pm 6.3	7.9	-4.7	-0.07
BIA _{SUN}	26.3 \pm 5.8	0.116	0.16	0.81	3.2	-0.9 \pm 6.8	5.9	-7.7	0.15

4C = four-compartment model; BF% = body fat percentage; BIA_{CH} = BIA-BF% prediction model from Chumlea et al. (2002); BIA_{DE} = BIA-BF% prediction model from Deurenberg et al. (1991); BIA_{KYLE} = BIA-BF% prediction model from Kyle et al. (2001); BIA_{SUN} = BIA-BF% prediction model from Sun et al. (2003); SEE = standard error of estimate; CE = constant error

*Significant trend (p < 0.05).

Table 2.3. Comparison of FFM values between the BIA equations and 4C model

Value	Mean \pm SD	p	Cohen's <i>d</i>	r	SEE	Limits of Agreement			
						CE \pm 1.96 SD	Upper	Lower	Trend
All (n = 82)									
4C FFM	56.0 \pm 14.4								
BIA _{CH}	53.7 \pm 13.9	<0.001	0.16	0.99	2.1	-2.2 \pm 4.2	2.0	-6.4	-0.26*
BIA _{DE}	53.1 \pm 12.5	<0.001	0.22	0.98	2.7	-2.9 \pm 6.2	3.3	-9.1	-0.62*
BIA _{KYLE}	54.1 \pm 12.8	<0.001	0.14	0.99	2.2	-1.9 \pm 5.2	3.3	-7.0	-0.61*
BIA _{SUN}	55.9 \pm 13.6	0.677	0.01	0.99	2.1	-0.1 \pm 4.4	4.3	-4.5	-0.39*
Men (n = 42)									
4C FFM	67.7 \pm 9.7								
BIA _{CH}	65.3 \pm 9.1	<0.001	0.26	0.97	2.2	-2.4 \pm 4.4	1.9	-6.8	-0.26
BIA _{DE}	63.8 \pm 7.0	<0.001	0.46	0.96	2.9	-3.9 \pm 7.2	3.3	-11.1	-0.76*
BIA _{KYLE}	65.1 \pm 7.4	<0.001	0.30	0.98	2.2	-2.6 \pm 5.8	3.2	-8.4	-0.78*
BIA _{SUN}	67.2 \pm 8.9	0.151	0.05	0.97	2.3	-0.5 \pm 4.6	4.1	-5.1	-0.33*
Women (n = 40)									
4C FFM	43.6 \pm 5.4								
BIA _{CH}	41.6 \pm 4.1	<0.001	0.42	0.94	1.9	-2.1 \pm 4.1	2.0	-6.1	-0.62*
BIA _{DE}	41.8 \pm 4.2	<0.001	0.37	0.93	2.0	-1.8 \pm 4.2	2.3	-6.0	-0.57*
BIA _{KYLE}	42.5 \pm 3.9	0.001	0.23	0.95	1.7	-1.1 \pm 4.0	2.9	-5.1	-0.73*
BIA _{SUN}	44.0 \pm 3.9	0.298	-0.08	0.95	1.6	0.3 \pm 4.0	4.4	-3.7	-0.75*

4C = four-compartment model; FFM = fat-free mass; BIA_{CH} = BIA-FFM prediction model from Chumlea et al. (2002); BIA_{DE} = BIA-FFM prediction model from Deurenberg et al. (1991); BIA_{KYLE} = BIA-FFM prediction model from Kyle et al. (2001); BIA_{SUN} = BIA-FFM prediction model from Sun et al. (2003); SEE = standard error of estimate; CE = constant error
 *Significant trend (p < 0.05).

CHAPTER 3

IMPACT OF MEASURED VS. PREDICTED RESIDUAL LUNG VOLUME ON BODY FAT PERCENTAGE VIA UNDERWATER WEIGHING AND FOUR-COMPARTMENT MODEL

ABSTRACT

The purpose of this study was to compare underwater weighing (UWW) and four-compartment (4C) model body fat percentage (BF%) for predicted vs. simultaneously measured residual lung volume (RLV). Forty-seven women and thirty-three men (age=22±5 years) had their UWW and 4C model BF% determined using Boren et al. (RLV_{BOREN}), Goldman and Becklake (RLV_{GB}), and Miller et al. (RLV_{MILLER}) RLV prediction equations. Criterion UWW BF% included body density (BD) values with simultaneous RLV. Criterion 4C model BF% included BD via UWW with simultaneous RLV, total body water via bioimpedance spectroscopy, and bone mineral content via dual energy x-ray absorptiometry. The standard error of estimate (SEE) for UWW and 4C model BF% determined via RLV prediction equations varied from 2.0-2.6% and 1.3-1.5%, respectively. The constant error (CE) was significantly different ($p<0.016$) for UWW BF% when using RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} (CE=0.7, -2.0, 1.0%, respectively). However, the CEs for RLV_{BOREN} and RLV_{MILLER} were not significant ($p=0.73$ and $p=0.11$, respectively) in the 4C model (CE=0.1 and 0.2%, respectively) whereas the CE for RLV_{GB} was significant than the criterion ($p<0.001$; CE= -1.5%). The 95% limits of agreement were less than ±5.2% for UWW BF% and less than ±3.1% for the 4C model when using the RLV equations. Results suggest RLV equations produced low SEEs and narrow limits

of agreement for both UWW and the 4C model BF% when compared to measurements obtained with simultaneous RLV. When simultaneous RLV is not feasible, practitioners can use RLV prediction equations to determine BD measurements that will be incorporated into the 4C model, but should use caution when utilized to determine UWW BF%.

KEYWORDS: multi-compartment model; hydrostatic weighing; percent fat; body density

INTRODUCTION

Body composition is an important health-related characteristic that can be useful for determining risk of chronic diseases and monitoring progress of interventions designed to decrease body fat percentage (BF%) and increase fat-free mass (FFM) (1, 2). Traditional indirect methods for assessing body composition, such as underwater weighing (UWW), are based on a two-compartment (2C) model, in which the body is assumed to be comprised of fat and fat-free components with densities of $0.9 \text{ g}\cdot\text{cm}^{-3}$ and $1.1 \text{ g}\cdot\text{cm}^{-3}$, respectively (3).

UWW with simultaneous residual volume measurement has traditionally been considered the “gold standard” for body composition measurements (4). However, multiple-compartment models have emerged and are also recommended for use in body composition research (5-7). This is because 2C models estimate BF% by determining fat mass (FM) and FFM (i.e., body density), whereas multiple-compartment models determine fat and FFM, but also include components within the latter tissue. For example, Wang et al. (8) developed a four-compartment (4C) model that can be used to estimate BF% from body density (FM and FFM), total body water (TBW) and bone mineral content (BMC). Components within the FFM such as TBW and BMC vary from person-to-person, despite the assumed constants integrated in a 2C model for estimating body composition, which is a likely reason why the 4C model has emerged as another criterion method.

The assessment of BF% via the 4C model can be measured by UWW for determination of body density (BD), dual energy X-ray absorptiometry (DXA) for determination of BMC, and deuterium oxide or bioimpedance spectroscopy (BIS) for determination of TBW. Concerning the assessment of BD via UWW, the influence of residual lung volume (RLV) is of particular interest due to the significant effect it can have on results. For instance, RLV can be measured simultaneously during UWW measurements, measured on dry land, or in water (head above water), predicted, or assumed as a constant (9-11). UWW with simultaneously measured RLV is recommended for determining BD vs. dry land RLV measurements (3). The RLV measured on dry land may not reflect the actual RLV achieved during the UWW procedure. However, measuring simultaneous RLV can be problematic for individuals who might not be comfortable underwater or who otherwise experience difficulty fully expiring air in the lungs during testing. In these cases, dry land determination of RLV could be utilized. Lastly, if the measurement of RLV is not possible, then it will have to be predicted. However, predicting RLV instead of simultaneously measuring it can introduce additional measurement error. RLV prediction equations have been developed, which estimate RLV from height and age (10, 12, 13). For example, Boren et al. (13), Goldman and Becklake (10), and Miller et al. (12) have developed RLV prediction equations that have been used to predict RLV which are often used for calculating BD for an UWW assessment (14-17).

The use of a RLV prediction equation is attractive because it provides a simpler and less expensive approach. However, the prediction of RLV can result in significantly different BD values compared to results obtained with measured RLV (14, 16, 17). The reason for the differences might be due to subject compliance during testing (i.e., some subjects may be more comfortable than others in water), the prediction equation being utilized, and the method being

used to measure RLV (18). Consequently, the differences in BD determined when measuring vs. predicting RLV have resulted in significantly different mean UWW BF% by as much as 5.0% (15, 16). Thus, the prediction of RLV should be used with caution when determining UWW BF%.

The effect of predicted vs. actual RLV when utilizing UWW for the determination of BD values to be used within a 4C model has yet to be investigated. The overall BF% prediction error associated with predicting RLV may be lessened due to the inclusion of other measures, such as TBW and BMC. If this is the case, then predicting RLV within a 4C model may be useful for future studies utilizing laboratories that conduct body composition research with limited time and resources, and for subjects who might not be comfortable with the requirements of simultaneous RLV. Therefore, the purpose of this study was to compare UWW and 4C model BF% values when simultaneously measuring vs. predicting RLV. Because of previous research findings (15, 17, 18), it was hypothesized that RLV prediction would decrease the accuracy of UWW BF% when compared to simultaneous measurements. However, since BD is one of three measurements used for calculating 4C model BF%, it is less likely that the inherent error associated with predicting RLV when determining BD would have as large of an impact on the overall measure of BF% via the 4C model. Therefore, it was also hypothesized that the prediction of RLV would produce less error in the 4C model than UWW BF%.

METHODS

Experimental Approach to the Problem

A group of men and women (n=80) performed 6 to 10 trials of the UWW procedure, with the mean of the 3 highest underwater weights recorded. BD was derived via simultaneous RLV and the associated underwater weight. BD was also determined when using the RLV prediction

equations and mean of the 3 highest underwater weights. BF% from UWW and the 4C method were compared between the BD values derived from measured vs. predicted RLV measures. Due to the differences in fat percentages between sexes, the relative accuracies of each RLV equation was determined within the entire group of participants, as well as stratified by sex.

Subjects

Eighty participants (47 women and 33 men) participated and were fully informed of the methods and procedures prior to participation in the study. Descriptive statistics are shown in Table 3.1. Subjects were asked to avoid eating or drinking, except water, 3 h prior to participating in the study. Before participation, subjects provided written informed consent and completed a medical history questionnaire. University institutional review board approval for subject participation was provided by the host university.

Procedures

Hydration was assessed prior to all testing via urine specific gravity (USG) using a handheld optical refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan). In order to be considered hydrated, subjects had to provide a USG value of < 1.020 (19, 20). When USG values were ≥ 1.020 , subjects were given thirty minutes to ingest water *ad libitum* and then retested. After hydration confirmation, subjects provided a nude (BM) measurement (to the nearest 0.1 kg) with a digital weighing scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Height was measured (to the nearest 0.1 cm) with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Body composition tests were taken after hydration, weight, and height measurements.

Underwater Weighing

UWW tests were measured to the nearest 0.025 kg in an apparatus specifically designed for densitometry measurements (Vacu-Med, Ventura, CA). Subjects sat in a sling seat during testing and completed 6 to 10 UWW trials. The mean of the 3 trials in which the subject had the highest mass were used to determine BV.

Residual Lung Volume

Simultaneous RLV was assessed with a nitrogen analyzer (Vacu-Med, Ventura, CA) during all UWW tests. The 3 trials in which the highest UWW measurements were recorded as previously described were also used to determine BD when predicting RLV. The equations used to predict RLV were as follows:

Boren et al. (10) (RLV_{BOREN}):

$$(0.019 \times \text{height [cm]}) + (0.0115 \times \text{age [years]}) - 2.24$$

Goldman and Becklake (10) (RLV_{GB})

$$\text{Men: } (0.017 \times \text{age [years]}) + (0.06858 \times \text{height [in.]}) - 3.477$$

$$\text{Women: } (0.032 \times \text{height [in.]}) + (0.009 \times \text{age [years]}) - 3.9$$

Miller et al. (12) (RLV_{MILLER})

$$(0.0275 \times \text{age [years]}) + (0.0189 \times \text{height [cm]}) - 2.6139$$

Dual Energy X-Ray Absorptiometry

BMC was measured with DXA (GE Lunar Prodigy, Software version 14.10.022, GE Lunar Corporation, Madison, WI) and converted to total body bone mineral (Mo) using the following equation: $Mo = BMC \times 1.0436$ (21). Calibration of the DXA was performed each day before use according to the manufacturer's instructions. Prior to positioning on the scanning bed, subjects were asked to remove shoes and all metal material (e.g., jewelry). During DXA

scans, subjects laid supine and motionless with the arms by the side. When participants were in proper position, a certified technician began the DXA scan.

Total Body Water

BIS was used to measure TBW according to manufacturer guidelines (ImpTM SFB7, ImpediMed Limited, Queensland, Australia). BIS has been shown to be valid when compared to criterion measures (i.e., deuterium oxide) (22-25) and has previously been used in multi-compartment equations for validation studies (7, 26, 27). For BIS measurements, subjects removed their right shoe and sock and then lied down on a gurney with the arms $\geq 30^\circ$ away from the body and legs separated. Prior to electrode placement, excess hair was removed (when applicable) and sites were cleaned with alcohol pads. When subjects were fully prepped, TBW measurements were taken.

Four-Compartment Model Calculation

Criterion (simultaneous RLV) and predicted (RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER}) 4C model BF% values were calculated using the Wang et al. equation (8). The 4C model equation included measurements of BM, body volume (BV), TBW, and Mo. The equation for fat mass (FM) and BF% were as follows:

$$FM \text{ (kg)} = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(BM)$$

$$BF\% = (FM/BM) \times 100$$

Statistical Analyses

All data were analyzed with SPSS version 23 (Somers, NY, USA). The difference in mean BF% for UWW and the 4C model when predicting vs. simultaneously measuring RLV for BD (e.g., UWW BF% with predicted RLV – UWW BF% with simultaneous RLV) were compared using a one-way repeated measures analysis of variance. In the event of a significant

omnibus test, paired samples t-tests were conducted to determine individual differences. A Bonferroni-adjusted alpha level was applied to reduce the chances of obtaining a type I error when multiple pairwise tests were performed. Therefore, the adjusted alpha level for significance of the mean comparisons was determined as $p < 0.016$. Effect size (ES) of the differences was determined using Cohen's d . The magnitude of the ES was determined by Hopkin's scale (28) as follows: 0-0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, >2.0 = very large. The constant error (CE) was determined as the differences between the UWW and 4C model BF% when simultaneously measured RLV, vs. predicted RLV for body density measurements. Regression procedures were used to determine the Pearson product moment correlation coefficients (r) and standard error of the estimate (SEE). The following qualitative thresholds were used to describe the r values: 0 to 0.30 small, 0.31 to 0.49 moderate, 0.50 to 0.69 large, 0.70 to 0.89 very large, and 0.90 to 1.00 near perfect (28). The method of Bland-Altman (29) was used to identify the 95% limits of agreement.

RESULTS

UWW Body Fat Percentage

Results comparing UWW BF% with simultaneous vs. predicted RLV are depicted in Table 3.2. When UWW BF% was combined for men and women, RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} each provided significantly different BF% values than BF% determined using simultaneous measurement of RLV (all $p < 0.016$). The Cohen's d statistic showed a trivial ES for RLV_{BOREN} and RLV_{MILLER} and a small ES for RLV_{GB} . The correlation coefficients were near perfect for UWW BF% when measuring vs. predicting RLV. The SEE values were 2.1, 2.6, and 2.0% and the 95% limits of agreement were ± 4.1 , ± 5.1 , and $\pm 3.9\%$ for RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} , respectively. The trends, which were determined as the correlation between the x-

(i.e., the mean of actual and predicted values) and the y- (i.e., the difference between the predicted and actual values) axes of the Bland Altman plots, were not statistically significant for RLV_{BOREN} ($r = -0.23$; $p = 0.84$), RLV_{GB} ($r = -0.08$; $p = 0.49$), and RLV_{MILLER} ($r = -0.05$; $p = 0.68$).

When data for men were analyzed independently, each RLV prediction equation provided significantly different UWW BF% values compared to UWW BF% with simultaneous RLV measurement (all $p < 0.016$). The Cohen's d statistic showed a trivial ES for RLV_{BOREN} , and a small ES for RLV_{GB} and RLV_{MILLER} . The correlation coefficients were near perfect for UWW BF% when measuring vs. predicting RLV. The SEE values were 1.5, 1.4, and 1.3% while the 95% limits of agreement were ± 2.8 , ± 2.9 , and $\pm 2.6\%$ for RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} , respectively. The trends were not statistically significant for RLV_{BOREN} ($r = -0.03$; $p = 0.86$), RLV_{GB} ($r = -0.10$; $p = 0.59$), and RLV_{MILLER} ($r = -0.01$; $p = 0.95$).

When data for women were analyzed independently, RLV_{GB} and RLV_{MILLER} provided significantly different UWW BF% values compared to UWW BF% determined with simultaneous RLV measurement in women (both $p < 0.016$). However, RLV_{BOREN} was not significantly different ($p = 0.07$). The Cohen's d statistic showed a trivial ES for RLV_{BOREN} and RLV_{MILLER} and small ES for RLV_{GB} . The correlation coefficients were very strong for UWW BF% when measuring vs. predicting RLV. The SEE values were 2.4, 3.0, and 2.4% while the 95% limits of agreement were ± 4.8 , ± 6.1 , and $\pm 4.6\%$ for RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} , respectively. The trends were not statistically significant for RLV_{BOREN} ($r = -0.03$; $p = 0.84$), RLV_{GB} ($r = -0.07$; $p = 0.62$), and RLV_{MILLER} ($r = -0.10$; $p = 0.52$).

4C Model Body Fat Percentage

Results comparing 4C model BF% determined with simultaneously measured vs. predicted RLV are depicted in Table 3.3. When 4C model BF% data from men and women were analyzed together, RLV_{BOREN} and RLV_{MILLER} resulted in mean values that were not significantly different from the criterion measurement ($p = 0.729$ and 0.112 , respectively), while BF% determined with RLV_{GB} was significantly lower than the criterion mean ($p < 0.001$). The Cohen's d statistic showed a trivial ES for RLV_{BOREN} and RLV_{MILLER} and was small for RLV_{GB} . The correlation coefficients were near perfect for the 4C model BF% when measuring vs. predicting RLV. The SEE values were 1.3, 1.5, and 1.3% while the 95% limits of agreement were ± 2.6 , ± 3.0 , and $\pm 2.6\%$ for RLV_{BOREN} , RLV_{GB} , and RLV_{MILLER} , respectively when compared to 4C model BF% determined with simultaneous RLV. The trends were statistically significant for RLV_{BOREN} ($r = -0.26$; $p < 0.05$), RLV_{GB} ($r = -0.39$; $p < 0.001$), and RLV_{MILLER} ($r = -0.26$; $p < 0.05$).

Analyses of data on men revealed RLV_{GB} and RLV_{MILLER} both provided significantly different 4C model BF% values compared to the criterion (both $p < 0.016$). However, BF% determined with RLV_{BOREN} was non-significant compared to the criterion mean ($p = 0.10$). The Cohen's d statistic showed a trivial ES for all three 4C model BF% values when using the RLV equations. The correlation coefficients were near perfect for the 4C model BF% when measuring vs. predicting RLV. The SEE values were 0.8% for all RLV equations whereas the 95% limits of agreements were less than $\pm 1.8\%$ for all RLV equations when compared to 4C model BF% determined with simultaneous RLV. The trends were not statistically significant for RLV_{BOREN} ($r = -0.26$; $p = 0.14$), RLV_{GB} , ($r = -0.18$; $p = 0.33$), and RLV_{MILLER} ($r = -0.24$; $p = 0.18$).

In women, RLV_{GB} provided a significantly different 4C model BF% value than the criterion in women ($p < 0.001$). However, RLV_{BOREN} and RLV_{MILLER} were each not significantly different from the criterion mean ($p = 0.71$ and 0.62 , respectively). The Cohen's d statistic showed a trivial ES for RLV_{BOREN} and RLV_{MILLER} and small ES for RLV_{GB} . The correlation coefficients were near perfect for the 4C model BF% when measuring vs. predicting RLV. The SEE values were less than 1.8% while the 95% limits of agreement were less than ± 3.4 for all RLV equations compared to 4C model BF% determined with simultaneous RLV. The trends were not statistically significant for RLV_{BOREN} ($r = -0.25$; $p = 0.09$), RLV_{GB} ($r = -0.22$; $p = 0.14$), and RLV_{MILLER} ($r = -0.28$; $p = 0.06$).

DISCUSSION

This study compared UWW and 4C model BF% values determined with simultaneously measured vs. predicted RLV when determining BD measurements via UWW. One of the major findings of the current study was that there was less error for 4C model BF% when using predicted RLV to determine BD than there was for UWW BF%. When comparing UWW and 4C model BF% determined with measured vs. predicted RLV, there were small to trivial ESs, very large to near perfect correlations, small SEE (2.0 to 2.6% for UWW and 1.3 to 1.5% for 4C model), and narrow limits of agreement (± 3.9 to 4.1% for UWW and ± 2.6 to 3.0% for 4C model). However, RLV_{BOREN} and RLV_{MILLER} resulted in a significantly higher group UWW BF% (CE > 0.7%), while RLV_{GB} resulted in a significantly lower value (CE = 2.0%). When utilized with the 4C model, RLV_{BOREN} and RLV_{MILLER} resulted in non-significant mean differences (0.1 and 0.2%, respectively) for group BF%, while RLV_{GB} resulted in significantly lower mean BF% (CE = 1.5%; $p < 0.001$). The trends were negative, but not statistically significant ($p > 0.05$) for all 3 RLV equations when determining UWW BF%. Moreover, the

trends in the 4C model were negative, but statistically significant ($p < 0.05$). However, the r values of the trend were non-significant when assessed in men and women separately, despite yielding similar r values to group findings. With a sufficiently large sample, as there was when examining men and women together as a group, a statistical test may demonstrate significance with little practical relevance (28, 30). Thus, the significant, yet small r value when examining the relationship between the x- and y-axes of the Bland-Altman plot for group 4C model BF% is likely due to an increased power and of no practical significance.

When examined in men and women separately, RLV_{BOREN} in women was the only equation not significantly different than UWW BF% determined with simultaneous RLV. Furthermore, in the 4C model, RLV_{BOREN} in both men and women, and RLV_{MILLER} in women, were the only equations that yielded BF% values that were not significantly different from BF% determined with simultaneously measured BF%. However, the significant mean difference for RLV_{GB} and RLV_{MILLER} were trivial for men, while RLV_{GB} yielded a small ES for women when used in the 4C model. This finding demonstrates why interpreting mean differences alone should be done with caution. The p value can be used to determine whether an effect exists, but it does not reveal the size of the effect (30). For example, RLV_{MILLER} resulted in a significant mean difference of only 0.4% when used to calculate 4C model BF% in men, which is of little practical significance. As a result, the interpretation of whether an RLV prediction equation is accurate when used to calculate UWW or 4C model BF% should not be based solely on the p value. Instead, other statistical procedures such as the ES, SEE, and limits of agreement should all be taken into consideration collectively. In the current study, when using RLV prediction equations, the small to trivial ES, near perfect correlations, small SEE, and narrow limits of agreement were similar for each sex when compared to group 4C model BF% results determined

when using simultaneous RLV. Thus, the use of RLV prediction equations in the 4C model introduces a trivial amount of error in the estimate of BD. As such, predicted RLV can be used as an alternative to simultaneous RLV in young adults having BF% assessed via the 4C model.

The current study supports previous research investigating the use of RLV equations to predict UWW BF%. For example, Morrow et al. (15) reported a significant over-prediction of UWW BF% (3.5%) when using RLV_{BOREN} in a group of men, but a significant under-prediction (-1.3%) in a group of male athletes. Similarly, Withers et al. (16) reported RLV_{GB} to under-predict a group of female athletes UWW BF% by 5.7%, which is a larger CE than what was observed for the non-athlete females of the current study (2.5%) and by Forsyth et al. (3.1%) (18). Withers et al. (16) suggested that the reason for the large differences in UWW BF% when predicting vs. measuring RLV in athletes could be because athletes have better developed respiratory musculature, which would facilitate a larger expiratory reserve volume, and hence a smaller RLV.

In addition, all of the equations utilized in the current study were derived on dry land. This is problematic since Ostrove et al. (31) found that RLV was approximately 200 mL higher on dry land compared to values obtained with subjects immersed in water up to the neck. The reason for this difference is likely due to the hydrostatic pressure, which assists the specific skeletal muscles responsible for exhalation (32) and allows for a more complete emptying of the lungs and compresses the RLV. In addition, Forsyth et al. (18) reported mean RLV measures in a group of men and women to be 1.24 ± 0.1 , 1.52 ± 0.2 , and $1.76 \pm 0.3L$, when using helium equilibration, oxygen dilution, and the nitrogen elimination method, respectively, which were significantly different from each other. It was further reported that a difference of 0.2L for RLV caused UWW BF% to change by 1.5% (18). Consequently, the method (i.e., on dry land) and

technique (e.g., oxygen dilution) used to derive the RLV equations evaluated in the current study could have each contributed to the differences between the prediction equations and simultaneous measurements of RLV observed in the current study.

A final issue in RLV prediction that cannot be ignored is subject cooperation. Familiarity with the UWW technique, comfort during testing, or willingness to participate could all influence the results derived via UWW. The CE for men when using RLV_{BOREN} in the current study was (0.7%), which is much lower than that reported by Latin et al. (17) (4.4%). The reason for the larger CE could be because subjects in the current study were much younger (age = 22 ± 5 vs. 62 ± 4). Latin et al. (17) stated that an older population such as those in their study might be less comfortable with the RLV measured in conjunction with UWW, which could have impacted the results of testing. Therefore, subject comfort during testing could contribute in some aspect to the differences in BF% when measuring vs. predicting RLV.

The virtue of using laboratory methods such as the 4C model over field techniques is also worth discussing. In a study by Moon et al. (33) the three-site skinfold technique (Jackson and Pollock) produced a SEE of 2.8% when compared to a three-compartment (3C) model, while the 95% limits of agreement ranged from 8.1% above the CE (-2.6%) to -2.8% below the CE in a group of college women. Similarly, in a group of collegiate female athletes, Moon et al. (27) found the SEE to range from 2.26-2.56% for skinfolds and the 95% limits of agreement to be > 4.90% when comparing BF% derived from Jackson and Pollock skinfolds to the 4C model (34). In the current study, the SEE and 95% limits of agreement were lower for men when predicting RLV to determine UWW BF% compared to findings based on skinfolds in previous research, but were similar for women.

The use of skinfolds might be more practical than UWW with predicted RLV for women. However, when BD is determined via predicted RLV and incorporated into the 4C model, the SEE and 95% limits of agreement are small (SEE 0.8% for men and 1.6-1.7% for women). These findings suggest that the prediction of RLV to determine UWW BF% when predicting RLV should be done with caution, especially in women, and that skinfolds might be a better alternative. However, the prediction of RLV when determining BD values that will be incorporated into the 4C model will yield very low SEE and narrow 95% limits of agreement and is recommended over skinfolds and UWW BF% determined with predicted RLV.

A limitation of the current study could be the age of the subjects. Although the age range was 18-35 years, a majority of subjects (n=64) were 18-22 years old. Since age is a significant predictor of RLV (10, 12, 13), generalizing the results to individuals who fall outside this age range should be done with caution. Another limitation could be the population tested. Most subjects were Caucasian undergraduate students. Therefore, whether the results would be similar for other populations cannot currently be determined.

In conclusion, the RLV prediction equations produced significantly different UWW BF% when compared to simultaneous measurement of RLV in men and women. However, the SEEs for UWW BF% were 1.3-1.5% and 2.4-3.0%, while the 95% limits of agreement were $\pm 2.6-2.9\%$ and $\pm 4.6-6.1\%$ for men and women, respectively. These values were even lower when BD derived via UWW with predicted RLV was included in the 4C model. The results of the current study found that using predicted RLV in a 4C model yielded smaller error than values obtained for UWW BF%. Therefore, the prediction of RLV in the 4C model via the three RLV equations examined in the current study could be a suitable alternative when simultaneous RLV is not feasible.

PRACTICAL APPLICATIONS

Practitioners and researchers should be aware of the error when using RLV for determining UWW and 4C model BF%. Predicted RLV is easy to determine since age and height are the only variables needed for calculation. Because predicting RLV is quicker and more practical than simultaneous RLV measurements, the former method is more desirable than the latter for body composition testing. The results of the current study found that when UWW BF% determined using predicted RLV was compared to UWW determined with simultaneously measured RLV, the SEE ranged from 1.3-1.5% for men and 2.4-3.0% for women. This error was reduced even more when RLV prediction equations were used for determining BD values that were incorporated into the 4C model (SEE < 0.9% for men and < 1.8% for women). Therefore, the results of this investigation support the use of RLV prediction equations during UWW when BD is used for calculating 4C model BF%, but not for predicting BF% via UWW.

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Table 3.1. Subject characteristics (mean and standard deviation (SD) and range)

	All (n = 80)			Men (n = 33)			Women (n = 47)		
	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max	Mean \pm SD	Min	Max
Age (years)	22 \pm 5	18	35	22 \pm 5	18	35	22 \pm 5	18	35
Height (cm)	170.5 \pm 10.0	151.3	194.6	179.1 \pm 8.2	160.0	194.6	164.8 \pm 5.8	151.3	176.5
Body Mass (kg)	68.4 \pm 14.3	42.9	108.1	80.7 \pm 11.3	62.2	108.1	59.8 \pm 8.9	42.9	80.7

Table 3.2. Comparison of UWW BF% between simultaneously measured and predicted RLV

Value	Mean \pm SD	p	Cohen's <i>d</i>	r	SEE	Limits of Agreement			
						CE \pm 1.96 SD	Upper	Lower	Trend
All (n = 80)									
UWW BF%									
Simultaneous RLV	21.8 \pm 7.1								
RLV _{BOREN}	22.5 \pm 7.1	0.005	-0.09	0.96	2.1	0.7 \pm 4.1	4.8	-3.4	-0.23
RLV _{GB}	19.8 \pm 6.9	<0.001	0.28	0.93	2.6	-2.0 \pm 5.1	3.1	-7.1	-0.08
RLV _{MILLER}	22.8 \pm 7.1	<0.001	-0.14	0.96	2.0	1.0 \pm 3.9	4.9	-2.9	-0.05
Men (n = 33)									
UWW BF%									
Simultaneous RLV	16.5 \pm 5.1								
RLV _{BOREN}	17.2 \pm 5.1	0.012	-0.14	0.96	1.5	0.7 \pm 2.8	3.5	-2.2	-0.03
RLV _{GB}	15.3 \pm 5.2	<0.001	0.23	0.96	1.4	-1.3 \pm 2.9	1.6	-4.1	0.10
RLV _{MILLER}	17.5 \pm 5.1	<0.001	-0.20	0.97	1.3	1.0 \pm 2.6	3.5	-1.6	-0.01
Women (n = 47)									
UWW BF%									
Simultaneous RLV	25.5 \pm 6.0								
RLV _{BOREN}	26.2 \pm 5.9	0.071	-0.12	0.91	2.4	0.7 \pm 4.8	5.5	-4.2	-0.03
RLV _{GB}	23.0 \pm 6.2	<0.001	0.41	0.87	3.0	-2.5 \pm 6.1	3.6	-8.6	0.07
RLV _{MILLER}	26.5 \pm 5.8	<0.001	-0.17	0.92	2.4	1.0 \pm 4.6	5.7	-3.6	-0.10

UWW = underwater weighing; BF% = body fat percentage; RLV = residual lung volume; RLV_{BOREN} = RLV prediction model from Boren et al. (1966); RLV_{GB} = RLV prediction model from Goldman and Becklake (1959); RLV_{MILLER} = RLV prediction model from Miller et al. (1998);

SEE = standard error of estimate; CE = constant error

*Significant trend (p < 0.05).

Table 3.3. Comparison of 4C model BF% between simultaneously measured and predicted RLV

Value	Mean \pm SD	p	Cohen's <i>d</i>	r	SEE	Limits of Agreement			
						CE \pm 1.96 SD	Upper	Lower	Trend
All (n = 80)									
4C model BF%									
Simultaneous RLV	22.1 \pm 7.4								
RLV _{BOREN}	22.1 \pm 7.0	0.729	0.00	0.98	1.3	0.1 \pm 2.6	2.7	-2.6	-0.26*
RLV _{GB}	20.6 \pm 6.8	<0.001	0.21	0.98	1.5	-1.5 \pm 3.0	1.5	-4.4	-0.39*
RLV _{MILLER}	22.3 \pm 7.0	0.112	-0.03	0.98	1.3	0.2 \pm 2.6	2.8	-2.3	-0.26*
Men (n = 33)									
4C model BF%									
Simultaneous RLV	16.1 \pm 5.0								
RLV _{BOREN}	16.4 \pm 4.8	0.100	-0.06	0.99	0.8	0.2 \pm 1.7	1.9	-1.4	-0.26
RLV _{GB}	15.3 \pm 4.9	<0.001	0.16	0.99	0.8	-0.8 \pm 1.6	0.8	-2.5	-0.18
RLV _{MILLER}	16.5 \pm 4.8	0.005	-0.08	0.99	0.8	0.4 \pm 1.6	2.0	-1.1	-0.24
Women (n = 47)									
4C model BF%									
Simultaneous RLV	26.3 \pm 5.7								
RLV _{BOREN}	26.2 \pm 5.3	0.713	0.02	0.96	1.6	-0.1 \pm 3.1	3.1	-3.2	-0.25
RLV _{GB}	24.3 \pm 5.4	<0.001	0.36	0.96	1.7	-2.0 \pm 3.3	1.4	-5.3	-0.22
RLV _{MILLER}	26.4 \pm 5.3	0.621	-0.02	0.96	1.6	0.1 \pm 3.1	3.2	-3.0	-0.28

4C = four-compartment; BF% = body fat percentage; RLV = residual lung volume; RLV_{BOREN} = RLV prediction model from Boren et al. (1966); RLV_{GB} = RLV prediction model from Goldman and Becklake (1959); RLV_{MILLER} = RLV prediction model from Miller et al. (1998);

SEE = standard error of estimate; CE = constant error

*Significant trend (p < 0.05).

CHAPTER 4

PREDICTING BODY FAT PERCENTAGE USING BMI, MUSCULAR PERFORMANCE, AND SUBJECTIVE PHYSICAL ACTIVITY LEVEL

ABSTRACT

The purpose of this study was to determine the accuracy of body fat percentage (BF%) predicted using age, sex, body mass index (BMI), physical activity (PA), handgrip strength, push-ups and sit-ups. The relationship between independent variables and criterion BF% was determined via an all-possible subset regression in 135 participants. Criterion BF% was determined via the four-compartment (4C) model. Sample 1 had 32 females and 35 males. Sample 2 had 33 females and 35 males. A common regression equation was developed from both samples. The common regression equation found that sex, BMI, handgrip strength, and PA were significantly associated with fat-free mass (FFM). The regression equation was as follows: $FFM = (10.147 * \text{sex}) + (0.0229 * \text{Vigorous PA}) + (0.231 * \text{combined sum of handgrip}) + (1.110 * \text{BMI})$ ($R^2=0.89$). Predicted BF% = $((\text{body mass} - \text{FFM}) / \text{BM}) * 100$. Predicted BF% from the new equation, BMI-based equations of Jackson et al. (BMI_{JA}), Deurenberg et al. (BMI_{DE}) Gallagher et al. (BMI_{GA}), and 7-site skinfolds from Brozek et al. (SF7_{BR}) were compared to the 4C model. The mean BF% values were significantly higher when using the new BMI-based equation ($\text{CE}=7.9\%$) and earlier BMI-based equations ($\text{CE} 1.7\text{-}2.3\%$), whereas SF7_{BR} was significantly lower ($\text{CE}=4.7\%$). The SEE ranged from 5.5-6.5% for the new equation and earlier equations and was 3.7% for SF7_{BR} . The 95% limits of agreement were $\pm 12.4, 13.6, 11.6, 12.7,$ and 7.2%

for the new equation, BMI_{JA} , BMI_{DE} , BMI_{GA} , and $SF7_{BR}$, respectively. These findings indicate that $SF7_{BR}$ provide a more accurate field based measure of BF% when compared to BMI-based equations.

KEYWORDS: Multi-Compartment Model; Body Mass Index; Skinfold; Muscular Fitness; Percent Body Fat; Prediction

INTRODUCTION

Body mass index (BMI) is one of the most commonly used methods for estimating fatness in obesity research due to its simple calculation. It is the primary method utilized to establish the standards for body weight classification (1, 2). For example, the World Health Organization and National Institutes of Health have established fatness categories based upon BMI. The consequence of a high BMI is an elevated risk for developing chronic diseases such as hypertension, high cholesterol, diabetes and coronary heart disease (1, 2). On the other hand, a low BMI may be associated with body image and eating disorders, sarcopenia, and low bone mineral density (3, 4).

A disadvantage of BMI is that it is not a valid measure of body fat percentage (BF%). However, there are several regression equations available to predict BF% based on BMI (5-8). Whereas some equations use BMI alone as the prediction variable (7), others also include variables such as sex, race, and age (5, 6, 8). Previous research has shown that these equations have large measurement errors and hence should not be utilized to predict BF% (9, 10). The errors may be related to the fact that neither BMI nor the BMI-based BF% equations account for fat and fat-free mass (FFM), which can vary dramatically between individuals despite similar levels of BMI (e.g., a very muscular individual may have a greater FFM and lower fat mass

compared to a sedentary person of the same BMI). As a result, accounting for muscular fitness might increase the accuracy of estimating BF% when incorporated into a BMI-based equation.

Sit-ups, push-ups, and handgrip tests are commonly utilized in field settings to assess muscular fitness, yet may also relate to body composition. Vaara et al. (11) found that muscular endurance tests (push-ups and sit-ups) were indicative of body fat content as determined by bioelectrical impedance. Handgrip strength has been found to be positively associated with lean body mass (11, 12). Hypothetically, these relatively simple tests could be useful as performance-based prediction variables of body composition.

In addition, individuals who display higher levels of overall physical activity (PA) tend to have lower BF% compared to less active individuals (13). For example, Zanovec et al. (14) found that PA had an inverse relationship with BF%. Since subjective measures of PA are useful when objective measures (e.g., accelerometry) are not available, a simple activity questionnaire when used with other anthropometric measures may help improve the accuracy of estimating body composition.

The aforementioned raises the possibility that easily obtainable measures of muscular fitness and PA may increase the accuracy of predicting BF% when employed in a model that utilizes BMI. This offers the possibility of more accurate BF% prediction by including field-based measures of muscular fitness. To the authors knowledge no prediction equation has been developed with these variables using the 4C model as a criterion method. Therefore, the purpose of this study was to develop a BF% regression model that utilized age, sex, BMI, a subjective measure of PA, and selected markers of muscular fitness as prediction variables. It was hypothesized that the muscular fitness and PA variables would add increased precision to the use of BMI alone when incorporated into a model that predicts BF%. Moreover, it was hypothesized

that this model would result in smaller error compared to currently existing BF% prediction equations when compared against a reference measure of BF% using the four-compartment (4C) model.

METHODS

Experimental Approach to the Problem

BF% was determined using a 4C model that employed bioimpedance spectroscopy (BIS) for total body water (TBW), underwater weighing (UWW) for body density, and dual energy X-ray absorptiometry (DXA) for bone mineral content (BMC). The 4C model was used to compute the criterion variable, BF%. The relationship between the independent variables (age, sex, BMI, handgrip strength, push-ups, sit-ups, and PA) and criterion BF% was determined via an all-possible subset regression. The new BF% equation, earlier BMI-based equations and a skinfold were then compared to the 4C model BF%.

Subjects

Participants completed one visit to the exercise physiology laboratory. The study consisted of 70 men (age = 34 ± 6 y; ht = 178.3 ± 7.4 cm; wt = 82.0 ± 12.8 kg) and 65 women (age = 22 ± 4 y; ht = 164.3 ± 6.0 cm; wt = 60.9 ± 9.3 kg) between 18 and 37 years old. All participants were fully informed of the methods and procedures prior to participation. Subjects were recruited with flyers, word of mouth, and classroom recruitment. Before participation, subjects provided written informed consent, completed an International Physical Activity Questionnaire (IPAQ), and medical history questionnaire. Subjects were asked to avoid eating or drinking, except water, 3 h prior to participating in the study. Before participation, subjects provided written informed consent and completed a medical history questionnaire. University institutional review board approval for subject participation was provided by the host university.

Procedures

Prior to any testing, subjects had to be adequately hydrated. Adequate hydration was based on a urine specific gravity (USG) value of < 1.020 (15). When USG values were ≥ 1.020 , subjects were given thirty minutes to ingest water *ad libitum* and then retested. After hydration confirmation, subjects provided a nude body mass (BM) measurement (to the nearest 0.1 kg) with a digital weighing scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Next, subjects were asked to dress in comfortable athletic attire (shorts and t-shirt). Height was then measured (to the nearest 0.1 cm) with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). BMI was calculated as weight (kg) divided by height (m^2). Following height and weight measurements, subjects had body composition measured with skinfolds and the 4C model, which included DXA, BIS, and UWW. Once body composition measurements were completed, subjects had their muscle strength and endurance assessed by handgrip strength, and maximal number of sit-ups and push-ups.

Dual Energy X-Ray Absorptiometry

BMC was estimated using a DXA device (GE Lunar Prodigy, Software version 14.10.022, GE Lunar Corporation, Madison, WI). BMC was converted to total body bone mineral (Mo) using the following equation: $Mo = BMC \times 1.0436$ (16). The DXA machine was calibrated each day before use according to manufacturer's instructions using a standard calibration block. Prior to positioning on the scanning bed, subjects were asked to remove shoes and anything metal (e.g., jewelry). During DXA scans, subjects were asked to lie supine and motionless with the arms by the side. Velcro straps were placed around the ankles and knees to help minimize movement during each scan. When participants were in proper position, a certified technician began the scan, which lasted approximately 6 to 10 min.

Total Body Water

BIS was used to measure TBW according to manufacturer guidelines (ImpTM SFB7, ImpediMed Limited, Queensland, Australia). BIS has been shown to be valid when compared to criterion measures (i.e., deuterium oxide) for the estimation of TBW (17-20) and has been used in multi-compartment equations for validation studies (21-23). Prior to BIS measurements, subjects removed their right shoe and sock. Subjects then lied down on a gurney with the arms $\geq 30^\circ$ away from the body with legs separated. Excess hair on electrode sites was removed and sites were cleaned with alcohol pads prior to electrode placement. When subjects were fully prepped, TBW were measured.

Underwater Weighing

UWW was performed in an apparatus specifically designed for taking densitometry measurements (Vacu-Med, Ventura, CA). Prior to the UWW measurement, subjects were asked to change into compression type clothing or a bathing suit. Subjects sat on a sling seat during testing. The subjects were instructed to perform a maximum expiration and submerge underwater. Simultaneous determination of lung volume was completed with the oxygen dilution technique. Body volume (BV) derived from UWW was used to calculate BF% via the 4C model.

Four-Compartment Model Calculation

The 4C model calculation has been described by Wang et al. (24), which includes the measurements of BV, BM, TBW, and Mo. The calculations of fat mass (FM) and BF% are listed below:

$$FM \text{ (kg)} = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(BM)$$

$$\text{BF}\% = (\text{FM}/\text{BM}) \times 100$$

Sit-Ups

For sit-ups, subjects were asked to wear a t-shirt and shorts and assume a supine position on a mat with the knees flexed to 90°. The feet were flat on the floor and secured by a co-examiner. Subjects were then asked to place their hands across the chest with each hand on the opposite shoulder. A metronome was set at 50 beats·min⁻¹ (25 sit-ups per min). Once in a proper position, subjects completed as many sit-ups as possible until failure. Subjects flexed their trunk and raised their lower back off the mat until the elbows came in contact with the thighs, which was counted as a full sit-up. The test was terminated when the subject was unable to maintain the required cadence or the proper technique for two consecutive repetitions. The maximum number of sit-ups performed correctly were counted and recorded by an examiner.

Push-Ups

Subjects placed their hands shoulder width apart on the ground and maintained a horizontal spinal position when performing push-ups. Women kept their knees flexed approximately 90° and feet plantar flexed to perform a modified push-up using the knees as a pivot point. Men performed standard push-ups with the toes as a pivotal point. A metronome was set at 50 beats·min⁻¹ (25 push-ups per min). All subjects bent their elbows until their chin touched the ground or the examiner's fist positioned underneath the chest (25). Once in the "down" position, subjects pushed-up and extended the elbows back to the starting position. A complete push-up was counted when subjects performed this motion correctly. The test was terminated if the subject was unable to maintain the required cadence or the proper technique for two consecutive repetitions. The maximum number of push-ups performed correctly were counted and recorded by an examiner.

Handgrip Strength

All handgrip tests were completed while standing. The dynamometer was adjusted such that the second joint of the finger was bent 90°. The dynamometer was reset to zero by the examiner prior to each handgrip test. Subjects were instructed to hold the dynamometer with the elbow flexed at 90°. The subject was then instructed to squeeze the dynamometer as hard as possible while avoiding the Valsalva maneuver. The handgrip strength was recorded in kg. The same procedure was repeated with the opposite hand. This procedure was repeated two additional times for each hand. The highest value of the three readings for each hand was added together and recorded as the combined sum (25).

International Physical Activity Questionnaire

The short version IPAQ was used as a subjective measure of total PA, as well as to provide an estimate of light, moderate, and vigorous PA. The four domains of the questionnaire included leisure-time physical activity, work-related physical activity, transport-related physical activity, and domestic and gardening (yard) activities. PA was calculated as a continuous score (MET-minutes/week) using the formula: Walking MET-minutes/week = 3.3 * walking minutes * walking days; Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate days; Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days; Total PA MET-minutes/week = sum of Walking + Moderate + Vigorous MET-minutes/week scores (26).

Skinfolds

Skinfold thickness measurements were conducted on the right side of the body with a calibrated Lange caliper. Measurements were taken based on the recommendations of Jackson

and Pollock (27) at the sites for chest, mid-axilla, triceps, abdomen, suprailium, subscapular, and thigh in order to determine body density. BF% was calculated from BD using the formula of Brozek et al. (SF7_{BR}) (28).

BMI-Based BF% Equations

Jackson et al. (7) (BMI_{JA}):

$$\text{BF\% (men)} = (3.76 \times \text{BMI}) - (0.04 \times \text{BMI}^2) - 47.80$$

$$\text{BF\% (women)} = (4.35 \times \text{BMI}) - (0.05 \times \text{BMI}^2) - 46.24$$

Deurenberg et al. (5) (BMI_{DE}):

$$\text{BF\%} = (1.20 \times \text{BMI}) + (0.23 \times \text{age}) - (10.8 \times \text{sex}) - 5.4; \text{sex} = 1 \text{ for men and } 0 \text{ for women}$$

Gallagher et al. (6) (BMI_{GA}):

$$\text{BF\%} = 76.0 - (1097.8 \times [1/\text{BMI}]) - (20.6 \times \text{sex}) + (0.053 \times \text{age}) + (95.0 \times \text{Asian} \times [1/\text{BMI}]) - (0.044 \times \text{Asian} \times \text{age}) + (154 \times \text{sex} \times [1/\text{BMI}]) + (0.034 \times \text{sex} \times \text{age}); \text{sex} = 1 \text{ for male and } 0 \text{ for female; Asian} = 1 \text{ for Asians and } 0 \text{ for the other races}$$

Statistical Analyses

An all-possible subset regression procedure was used to select the best set of independent predictor variables that have the highest R² value. The all-possible subset regression analysis considers all possible combinations of independent variables in explaining the variance of the dependent variable (4C BF%). The R² model fit value was used to select the best set of independent variables. Only regression equations in which all of the regression coefficients were significantly different from zero were considered, based on an alpha level of 0.05.

Multicollinearity may have been present amongst the set of eight independent predictor variables. Therefore, the variance inflation factor was reported in the final multiple regression solution. The variance inflation factor measures how much the variance of the estimated

regression coefficients are inflated as compared to when the predictor variables are not linearly related (29). It describes how much multicollinearity (correlation between predictors) exists in a regression analysis.

BF% means and standard deviations derived from the new BF% equation, previous BMI-based BF% equations, and skinfolds were compared to the criterion 4C model. Data were analyzed with SPSS version 23.0 (Somers, NY, USA) by a One-Way ANOVA repeated measures. In the event of a significant omnibus test, paired samples t-tests were conducted to determine individual differences. A Bonferroni-adjusted alpha level was applied to reduce the chances of obtaining a type I error when multiple pairwise tests were performed. Therefore, the adjusted alpha level for significance of the mean comparisons was determined as $p < 0.01$ ($0.05/5$; each of the 5 prediction equations vs. the 4C model BF%). The effect size (ES) of the mean differences was determined using Cohen's d . The magnitude of the ES was determined by Hopkin's scale (30) as follows: 0-0.2 = trivial, 0.2-0.6 = small, 0.6-1.2 = moderate, 1.2-2.0 = large, >2.0 = very large. The constant error (CE) was determined as the difference between the prediction equations and 4C model BF%. Pearson product moment correlation coefficients (r) were determined and interpreted according to the following thresholds: 0 to 0.30 small, 0.31 to 0.49 moderate, 0.50 to 0.69 large, 0.70 to 0.89 very large, and 0.90 to 1.00 near perfect (30). The r and standard error of the estimate (SEE) were determined with regression procedures. The method of Bland-Altman (31) was used to identify the 95% limits of agreement between BF% determined from the 4C model and all prediction equations.

RESULTS

Development of the Equation

Sex, vigorous PA, combined sum of handgrip and BMI were selected from the all-possible subset regression procedure due to statistical significance and used in the development of the new prediction equation. SPSS was used to *randomly split* the samples into two files (sample 1 and sample 2). Sample 1 had 32 females and 35 males; Sample 2 had 33 females and 35 males.

Statistical files were saved as local files (Microsoft Excel). SPSS was used to compute the descriptive statistics for the two samples. The variable names, sample size, correlation matrix, means, and standard deviations (Table 4.1) were then used in a statistical program (LISREL SIMPLIS). This program computes a chi-square statistic to determine whether there are sample differences in the multiple regression equation. The 4 predictor variables of FFM were all statistically significant for the common regression equation of both samples ($Z > 1.96$). The R-squared was .89. The model fit indicated a non-significant chi-square value ($\chi^2 = 2.14$, $df = 5$, $p = .83$), which implies that there was no difference in the regression equation for the two samples of data. The cross-validation index = .41, which also supports no difference in the multiple regression coefficients for the two samples.

The derived prediction equation was:

$$\text{FFM} = (10.147 * \text{Sex}) + (0.0229 * \text{Vigorous IPAQ}) + (0.231 * \text{combined sum of handgrip}) + (1.110 * \text{BMI}).$$

After the prediction of FFM for all subjects was completed using the new equation, each value was converted to BF% by the following:

$$\text{BF\%} = ((\text{BM} - \text{FFM}) / \text{BM}) \times 100$$

Comparison of Prediction Equations

Results comparing the new BF% equation, BMI-based BF% equations, and skinfolds to the 4C model derived BF% are depicted in Table 4.2. The new BF% equation, BMI_{JA}, BMI_{DE}, and BMI_{DE} each provided significantly higher estimates of BF%, whereas SF7_{BR} was significantly lower than the 4C model (all $p < 0.001$). The Cohen's d statistic showed a small ES for BMI_{JA}, BMI_{DE}, and BMI_{DE} and a moderate ES for the new BF% equation and SF7_{BR}. The correlation coefficients relating the prediction methods to the 4C model were large for BMI_{JA}, BMI_{DE}, and BMI_{DE} and very large for the new BF% equation and SF7_{BR}. The SEE values ranged from 3.7% (SF7_{BR}) to 6.5% (BMI_{JA}) and the 95% limits of agreement were widest for BMI_{JA} ($\pm 13.6\%$) and narrowest for SF7_{BR} ($\pm 7.2\%$). The Bland-Altman plots for all five prediction equations and the criterion 4C model BF% are presented in Figure 4.1. The correlation between the x- (i.e., the mean of actual and predicted values) and the y- (i.e., the difference between the predicted and actual values) axes of the Bland Altman plots were not statistically significant for the new equation ($r = 0.16$; $p = 0.7$), BMI_{JA} ($r = -0.15$; $p = 0.09$), and BMI_{GA} ($r = -0.05$; $p = 0.58$), but statistically significant for BMI_{DE} ($r = -0.30$; $p < 0.001$) and SF7_{BR} ($r = -0.20$; $p = 0.02$).

DISCUSSION

The purpose of this study was to develop a BF% regression model that utilizes age, sex, BMI, a subjective measure of PA, and selected markers of muscular fitness as prediction variables. The derived equation excluded the variables age and maximal sit-ups and push-ups. Compared to the 4C model, the new BF% equation and BMI-based BF% equations all significantly over-predicted BF% (CE 1.7-7.9%) whereas SF7_{BR} significantly under-estimated (CE = 4.7%) 4C model BF%. Although there were strong correlations for all the prediction equations, the SEE (SEE = 5.5% for new BF% equation; 5.8-6.5% for BMI-based BF%

equations; 3.7% for SF7_{BR}) were large for the new BF% equation and BMI-based BF% equations, but much smaller for SF7_{BR}.

The relatively small SEE for SF7_{BRis} when compared to the 4C model is consistent with the SEE previously reported by the American College of Sports Medicine when comparing skinfolds to UWW (25). In addition, the 95% limits of agreement were very large for the new BF% equation ($\pm 12.4\%$), BMI_{JA} ($\pm 13.6\%$), BMI_{DE} (11.6%), and BMI_{GA} ($\pm 12.7\%$), but narrower for SF7_{BR} ($\pm 7.2\%$).

The correlation between the x- (i.e., the mean of predicted and actual values) and the y- (i.e., the difference between the predicted and actual values) axes of the Bland Altman plots were small ($r = -0.30$ to 0.16) for all prediction equations, despite the significant negative trend for BMI_{DE} ($r = -0.30$) and SF7_{BR} ($r = -0.20$). The significant trend for BMI_{DE} and SF7_{BR} was likely due to the large sample size ($n=135$) of the study, which increased statistical power (30, 32). Thus, the significant trend for BMI_{DE} and SF7_{BR} likely has little practical meaning. Although all three BMI-based BF% equations produced a smaller CE ($< 2.4\%$) than SF7_{BR} (CE -4.7%) and the new BF% equation (7.9%), each BMI-based equation yielded very large individual differences between predicted vs. measured BF%. Similarly, the new BF% equation produced large errors in individual estimates of BF% that were comparable to the examined BMI-based equations.

Conversely, the CE was much larger for group values with the new BF% equation when compared to the BMI-based equations. This finding suggests the new BF% equation should not be used for group BF% estimates. SF7_{BR} also produced unacceptable group values (i.e., CE -4.7%). However, the SEE was much lower (3.7%) than the new BF% equation and BMI-based equation (SEE $> 5.7\%$). In addition, the 95% limits of agreement were smaller for SF7_{BR} ($\pm 7.2\%$) when compared to the new BF% equation and BMI-based equations ($> \pm 11.5\%$). As a

result, SF7_{BR} provides the lowest SEE and narrowest 95% limits of agreement of the selected equations and could potentially be used for individual estimates of BF%. Nevertheless, practitioners should be aware of the tendency of the skinfold technique for under-predicting BF%.

Previous research has found BMI-based equations to produce non-significant mean difference values when compared to UWW and DXA for BF% (10, 33-35). However, findings have also revealed there to be large individual errors in estimates of BF% when using these equations. For example, Loenneke et al. (33) reported BF% predicted from BMI yielded similar mean BF% values in a group of men and women (ages 18–33 y) when compared to DXA, but reported there to be a large SEE (6.2%), which is a similar statistical finding to that of the current study. Likewise, Esco et al. (10) found that BMI_{DE} and BMI_{JA} produced non-significantly different mean values when compared to DXA in a group of collegiate female athletes, but had large SEEs (4.53 and 4.75%, respectively) and 95% limits of agreement (± 9.40 and 9.50%, respectively). In the current study, the BMI-based equations generated a lower CE than the new BF% equation and SF7_{BR}. However, the SEEs and 95% limits of agreement were large when using the BMI-based equations, which is similar to the aforementioned studies (10, 33). Therefore, BMI-based equations and the newly derived BF% equation of the current study should not be used for individual estimates of BF%.

Contrary to our hypothesis, the new BF% equation, which included the variables sex, sum of handgrip strength, BMI, and vigorous PA, did not reduce the error of BMI-based equations. One noticeable difference between the current study and the studies used to derive BMI-based equations are the study samples. All of the studies that developed BMI-based equations included samples with a wider age range than that of the current study, which

consisted primarily of young adults (79% ages 18–25 y). As a result, age was not a significant predictor for the new BF% prediction equation. Another factor that is worth considering is the ethnicity of subjects tested. The new BF% equation was developed primarily on Caucasian men and women whereas BMI_{GA} derived their equation from a sample of African Americans (n=254), Asians (n=955), and Caucasians (n=417). Thus, in the current study a more heterogeneous group might have resulted in the inclusion of easily obtainable predictor variables such as age and ethnicity, which might have helped improve the accuracy of the new BF% equation.

The new BF% equation included the sum of combined handgrip, while excluding sit-ups and push-ups. Vaara et al. (11) found handgrip strength and FFM to have a moderate correlation ($r = 0.44$) as determined by bioelectrical impedance analysis. However, there was no relationship ($r = -0.06$) when comparing handgrip strength and BF% (11). Furthermore, the findings of Vaara et al. (11) demonstrated that sit-ups and push-ups had a moderate correlation ($r = -0.45$ and -0.47 , respectively) with BF%, but were not related to FFM ($r = -0.06$ and -0.07 , respectively). The equation in the current study was derived to predict FFM, which was then converted to BF% for statistical analyses. As a result, it seems plausible that designing the new equation to predict FFM could have been the reason push-ups and sit-ups were excluded and why handgrip strength was included as a significant predictor. However, since there is a lower correlation between handgrip strength and BF% than there is for handgrip strength and FFM (11), the conversion of FFM to BF% could cause there to be less precision when using handgrip strength to predict BF%. This position is further supported by the Pearson correlation coefficients between the predictor variables and 4C model FFM and BF% (Table 4.3). For example, the correlation for handgrip strength was higher for 4C model FFM than BF%, whereas sit-ups and push-ups had a higher correlation with 4C model BF% than FFM.

Another potential reason for the error in the new equation could be due to the inclusion of vigorous PA in the common regression equation. For instance, when the variables sex, vigorous PA, combined sum of handgrip, and BMI were used to develop separate regression equations for each of the two samples (Sample 1 and Sample 2), vigorous PA did not statically significantly contribute (Table 4.4). Conversely, vigorous PA was a significant contributor for the common regression equation. This finding has been referred to as Simpson's paradox (36), which states that when groups are combined together (as Sample 1 and Sample 2 were when developing the common regression equation), and data are analyzed in an aggregate form, the correlation may reverse itself due to lurking variables that have not been considered. However, it is possible that a lurking variable was not present and the reason why the correlation reversed itself when samples were combined. Consequently, the statistical significance of vigorous PA for the common regression equation is likely due to the fact that there was a larger sample size (n=135) when using the common regression, which increased statistical power.

Limitations of the current study included the use of a homogeneous sample to derive the new BF% equation. BF% tends vary between ethnicities and older and younger populations (6, 37). Therefore, the development of an equation from a heterogeneous sample could potentially reduce the errors that occurred with the newly derived BF% equation. Another limitation was the use of self-reported PA as a predictor variable. It has been suggested that self-reported PA is not an accurate method for the estimation of PA (38). Therefore, the use of an objective measure of PA, such as accelerometry, might improve the ability to predict BF% compared to subjective measures of PA such as the IPAQ questionnaire.

In conclusion, all of the selected equations evaluated in the current study yielded significantly different mean values when compared to the 4C model. Furthermore, the SEE and

95% limits of agreement were large for the new BF% equation and BMI-based equations, but were much smaller for SF7_{BR}. These findings suggest the selected equations in the current study should be used with caution when examining group values. The use of the new BF% equation and BMI-based equations are also not recommended for individual estimates of BF% due to the large SEE and 95% limits of agreement. However, SF7_{BR} produced an acceptable SEE (25) and smaller limits of agreement than the other selected equations. Therefore, SF7_{BR} appears to be a better field predictor for individual estimates of BF% when compared to the new equation and BMI-based equations. Therefore, SF7_{BR} is recommended over the new BF% equation and BMI-based equations when practitioners are seeking to use a field method for predicting BF%.

PRACTICAL APPLICATIONS

The current study sought to improve upon BMI-based BF% equations by developing a prediction equation that included BMI, sex, measures of muscular fitness, and PA. It was hypothesized that the inclusion of muscular fitness and PA variables in a BMI-based BF% equation would improve the accuracy of estimating BF% compared to traditional BMI-based equations, which excluded these variables. Results of the current study indicated that the new BF% equation, which included the variables sex, BMI, vigorous PA, and sum of combined handgrip had an unacceptably high SEE (5.5%) and 95% limits of agreement ($\pm 12.4\%$). The SEE and limits of agreement were also large for three BMI-based BF% equations (SEE > 5.7%; 95% limits of agreement > 11.6%) when compared to the 4C model. However, the SEE was acceptable for SF7_{BR} (3.7%), which also had narrower limits of agreement ($\pm 7.2\%$). Therefore, when using a field predictor of adiposity, the use of the SF7_{BR} is recommended over the new BF% equation and BMI-based equations.

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Table 4.1. Correlation, Mean, and Standard Deviations for 2 independent samples

Sample 1 (n = 67)

Variable	Sex	PA	HG	BMI	4C FFM
Sex	1.0				
PA	.105	1.0			
HG	.809	.130	1.0		
BMI	.528	.141	.544	1.0	
4C FFM	.832	.195	.868	.734	1.0
Means	.52	42.0	77.4	24.1	56.9
SD	.50	49.8	24.2	4.3	14.5

Sample 2 (n = 68)

Variable	Sex	PA	HG	BMI	4C FFM
Sex	1.0				
PA	.355	1.0			
HG	.793	.239	1.0		
BMI	.259	.019	.316	1.0	
4C FFM	.834	.325	.838	.570	1.0
Means	.51	37.1	77.1	24.4	56.3
SD	.50	36.5	26.1	4.2	13.8

PA = Physical Activity; HG = sum of combined handgrip strength; BMI = body mass index; 4C FFM = four-compartment model fat-free mass

Table 4.2. Comparison to the 4C model of methods for predicting BF% (n = 135)

Value	Mean \pm SD	p	Cohen's <i>d</i>	r	SEE	Limits of Agreement			Trend
						CE \pm 1.96 SD	Upper	Lower	
4C BF%	21.5 \pm 7.8								
New BF% Equation	29.4 \pm 8.7	<0.001	-0.96	0.71	5.5	7.9 \pm 12.4	20.3	-4.5	0.16
BMI _{JA}	23.9 \pm 6.9	<0.001	-0.33	0.56	6.5	2.3 \pm 13.6	16.0	-11.3	-0.15
BMI _{DE}	23.3 \pm 6.2	<0.001	-0.26	0.66	5.8	1.7 \pm 11.6	13.3	-9.8	-0.30*
BMI _{GA}	23.7 \pm 7.5	<0.001	-0.29	0.64	6.0	2.2 \pm 12.7	14.4	-10.5	-0.05
SF7 _{BR}	16.8 \pm 7.1	<0.001	0.63	0.88	3.7	-4.7 \pm 7.2	2.4	-11.9	-0.20*

4C = four-compartment model; BF% = body fat percentage; BMI_{JA} = BMI-based BF% prediction equation from Jackson et al. (2002); BMI_{DE} = BMI-based BF% prediction equation from Deurenberg et al. (1991); BMI_{GA} = BMI-based BF% prediction equation from Gallagher et al. (2000); SF7_{BR} = skinfold BF% prediction equation from Brozek et al. (1963); SEE = standard error of estimate; CE = constant error; *Significant trend (p < 0.05).

Table 4.3. Pearson correlation coefficient for predictor variables and body comp (n =135)

	<u>4C Model FFM</u>		<u>4C Model BF%</u>	
	r	p	r	p
Vigorous PA	0.25	<0.05	-0.22	<0.05
Moderate PA	0.11	0.22	-0.14	0.09
Walking PA	-0.03	0.72	0.09	0.30
Total PA	0.18	<0.05	-0.14	0.09
Handgrip Strength	0.85	<0.001	-0.63	<0.001
Push-Ups	0.23	<0.05	-0.49	<0.001
Sit-Ups	0.01	0.87	-0.31	<0.001
BMI	0.65	<0.001	0.13	0.13
Age	0.33	<0.001	-0.17	0.84

BMI = body mass index, 4C = four-compartment; FFM = fat-free mass;
BF% = body fat percentage; PA = physical activity

Table 4.4. Regression models for the prediction of 4C Model FFM

Common Regression Equation for Samples 1 and 2 (n = 135)

$$4C \text{ Model FFM} = (10.147 * \text{Sex}) + (0.0229 * \text{Vigorous IPAQ}) + (0.231 * \text{Sum of Handgrip}) + (1.110 * \text{BMI}), \text{ Errorvar.} = 23.656, R^2 = 0.887$$

Variable	Sex	Vigorous IPAQ	Sum of Handgrip	BMI	Errorvar.
Standerr	(1.430)	(0.0100)	(0.0288)	(0.112)	(2.934)
Z-values	7.096	2.293	8.016	9.951	8.062
P-values	0.000	0.022	0.000	0.000	0.000

Sample 1 Regression Equation (n = 67)

$$4C \text{ Model FFM} = (8.442 * \text{Sex}) + (0.0176 * \text{Vigorous IPAQ}) + (0.268 * \text{Sum of Handgrip}) + (1.1126 * \text{BMI}), \text{ Errorvar.} = 24.879, R^2 = 0.882$$

Variable	Sex	Vigorous IPAQ	Sum of Handgrip	BMI	Errorvar.
Standerr	(2.160)	(0.0128)	(0.0455)	(0.179)	(4.433)
Z-values	3.908	1.374	5.878	6.274	5.612
P-values	0.000	0.169	0.000	0.000	0.000

Sample 2 Regression Equation (n = 68)

$$4C \text{ Model FFM} = (11.360 * \text{Sex}) + (0.0304 * \text{Vigorous IPAQ}) + (0.203 * \text{Sum of Handgrip}) + (1.112 * \text{BMI}), \text{ Errorvar.} = 21.791, R^2 = 0.886$$

Variable	Sex	Vigorous IPAQ	Sum of Handgrip	BMI	Errorvar.
Standerr	(1.984)	(0.0172)	(0.0374)	(0.146)	(3.852)
Z-values	5.725	1.767	5.432	7.632	5.657
P-values	0.000	0.077	0.000	0.000	0.000

4C = Four-Compartment Model; FFM = Fat-Free Mass (kg); Sex, Male = 1, Female = 0; IPAQ = International Physical Activity Questionnaire; BMI = Body Mass Index; Standerr = standard error.

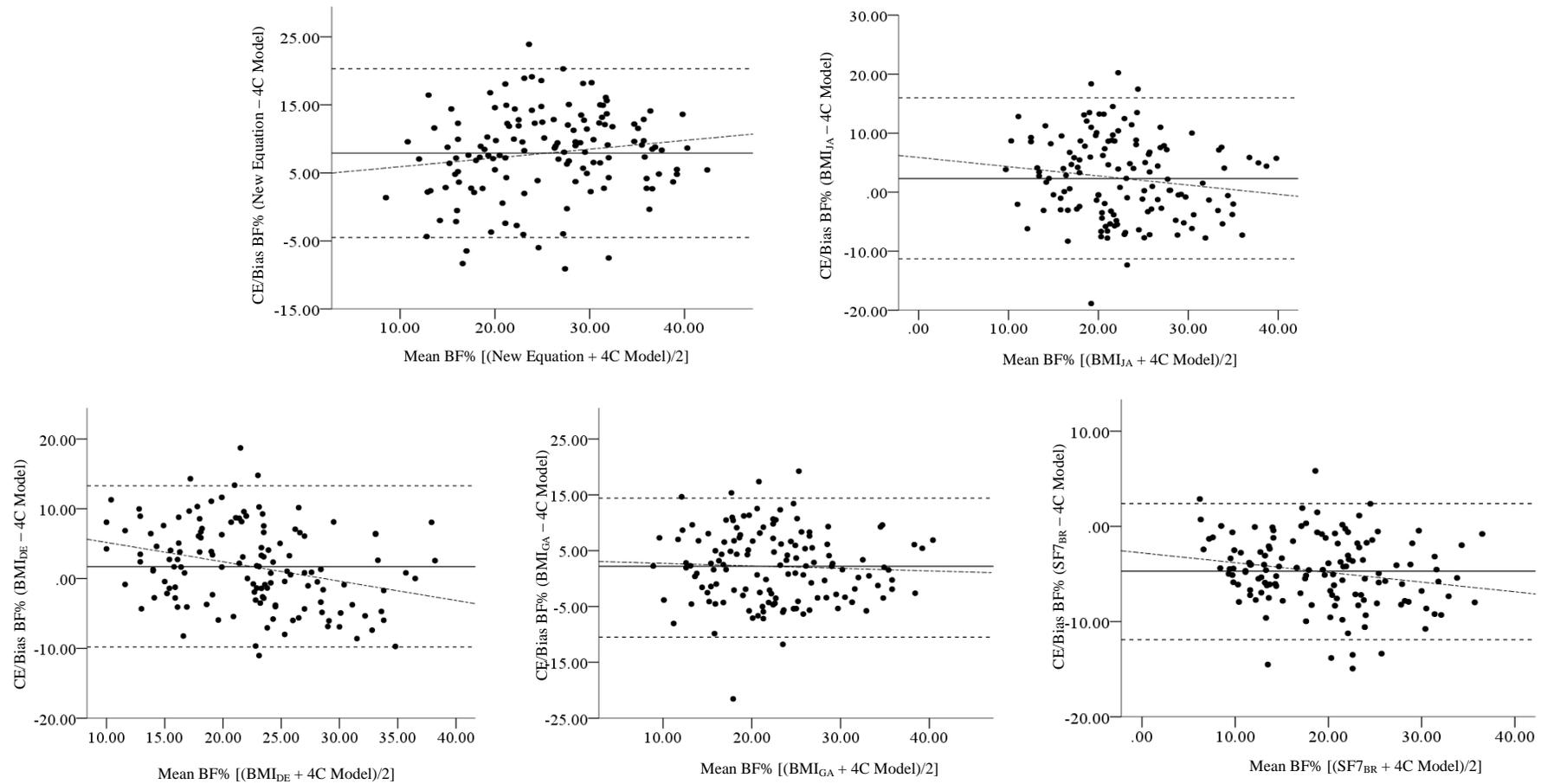


Figure 4.1. Bland and Altman plot comparing the prediction equations and 4C model BF% (n = 135). The middle solid line indicates the constant error or mean difference. The dashed lines represent the upper and lower limits of agreement (± 1.96 SD). The dashed-dotted regression lines represent the trend between the errors of each measurement.

CHAPTER 5

CONCLUSION

Body composition research on bioelectrical impedance analysis (BIA) has primarily focused on the agreement between BIA and two-compartment model methods such as underwater weighing (UWW) and dual energy X-ray absorptiometry. Similarly, the influence of predicting residual lung volume (RLV) has only been investigated for UWW body fat percentage (BF%). However, the four-compartment (4C) model has emerged as a criterion method in the determination of body composition (1-3). The agreement of BIA equations with the 4C model as well as the impact of predicting RLV when determining body density (BD) for the measurement of this criterion method was previously unknown.

The large error when using BMI-based BF% equations has been well documented (4-6), but the ability to improve upon BMI based equations by including muscular performance and physical activity (PA) variables was previously unknown. The current series of studies addressed gaps in the literature by 1) providing insight on the accuracy of BIA and predicting RLV and 2) developed a new equation to predict 4C model BF%, which included the variables BMI, PA, and handgrip strength.

The first study compared the 4C model to BF% and fat-free mass (FFM) values derived from published BIA equations that were previously developed from a range of laboratory techniques. One BIA equation (BIA_{SUN}) was found to yield non-significantly different mean BF% and FFM values compared to the 4C model, whilst the other three equations (BIA_{CH} , BIA_{DE} , and BIA_{KYLE}) were found to be significantly different from 4C values. However, the

standard error of estimate for BF% and FFM was small (SEE < 3.9% and 2.8kg, respectively) and the 95% limits of agreement were narrow (< $\pm 7.5\%$ and $\pm 6.3\text{kg}$, respectively) for all four of the BIA equations. These findings suggest practitioners could potentially use the selected BIA equations as an alternative to the 4C model, but the tendency of BIA_{CH}, BIA_{DE}, and BIA_{KYLE} to over-predict BF% and under-predict FFM should be appreciated.

The second study determined the impact that predicting vs. measuring RLV had on BD values that would be used for estimating UWW and 4C model BF%. For UWW BF%, predicting RLV resulted in significantly different mean BF% when compared to results obtained with simultaneous measurement of RLV (-2.0 to 1.0%). However, the prediction of RLV when determining BD values that were entered in the 4C model had little effect (-1.5 to 0.2%) on BF% when compared to simultaneous measurement of RLV. Therefore, when determining 4C model BF%, the prediction of RLV when using UWW is sufficient for obtaining BF%.

Finally, the third study sought to develop a regression equation that would improve upon previously developed BMI-based BF% equations by including variables of muscular fitness and PA. The newly derived BF% equation and previously developed BMI-based BF% equations were found to yield large standard error of estimates (SEE 5.5 to 6.5%) and 95% limits of agreement (± 11.6 to $\pm 13.6\%$) compared to the 4C model. Conversely, the 7-site skinfold technique had the smallest standard error of estimate (3.7%) and is recommended over the new BF% equation and BMI-based equations.

The collective findings of this dissertation support the use of BIA equations and the prediction of RLV when determining 4C model BF%. Furthermore, it supports the use of the skinfold technique over the new BF% equation and BMI-based equations. However, future research is needed to determine if other muscular fitness variables, PA assessment methods (e.g.,

accelerometers), and anthropometric measurements (e.g., height, weight, circumference, etc.) can better predict BF%.

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APPENDIX

Office for Research
Institutional Review Board for the
Protection of Human Subjects

THE UNIVERSITY OF
ALABAMA
R E S E A R C H

November 19, 2015

Brett Nickerson
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 15-019-ME
"Development of a Novel Body Fat Prediction Equation: A Four-
Compartment Model Approach"

Mr. Nickerson:

The University of Alabama Medical IRB has granted initial approval of the above application for a one-year period. Please be advised that your protocol will expire one year from the date of approval, 11/12/15

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent/assent forms to obtain consent from your participants.

Good luck with your research.

Sincerely,



J. Grier Stewart, MD, FACP
Medical IRB Chair



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