EVALUATION OF METHODS FOR DETERMINING VARIOUS COMPONENTS OF BODY COMPOSITION

by

ZACKARY S. CICONE

MICHAEL R. ESCO, COMMITTEE CHAIR
MICHAEL V. FEDEWA
HAYLEY V. MACDONALD
YOUN-JENG CHOI
JONATHAN E. WINGO

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ABSTRACT

Doubly indirect methods of assessing body composition are commonly used in laboratory and practical settings. The purpose of this dissertation was to expand upon the methodological discrepancies associated with various techniques, and to provide improved equations to overcome these limitations. A series of three studies was conducted to 1) improve the estimation of underwater residual lung volume (RLV), 2) systematically review and quantify the error associated with single-frequency bioimpedance analysis (SFBIA) for the determination of total body water (TBW), and 3) develop a novel equation for predicting percent body fat (%BF) from skinfolds using a criterion multi-compartment model. The first study developed an equation for the prediction of underwater RLV in healthy adults using age and height as predictor variables. The new equation produced superior validity statistics upon cross-validation compared to four existing equations, indicating that it may be used by practitioners to accurately estimate underwater RLV during hydrostatic weighing. The second study systematically reviewed and meta-analyzed 264 effects from 51 original studies designed to compare SFBIA to criterion dilution methods for TBW estimation. Although a non-significant overall effect was identified, there was significant variability associated with SFBIA methodology (i.e., frequency and resistivity index) and sample sex (% female). Moderator analyses indicated that SFBIA procedures utilizing Ht^2/R at 100 kHz produced the most accurate estimate of TBW when compared to isotope dilution techniques. The third study developed a skinfold-based equation for the prediction of five-compartment model %BF in a sample of healthy adults. The new equation

outperformed selected existing equations when cross-validated, indicating its potential utility for practitioners concerned with obtaining accurate estimates of %BF in the general population.

DEDICATION

To Noni, who always pushed me to pursue education to the highest degree possible and would most definitely follow up her reading of this document with a “what’s next?”, rather than a “congratulations.” Additionally, I would not have been able to complete this monumental task without the support of my parents, who have been behind me throughout all the interests, hobbies, and obsessions I have taken on over the years. Thank you.

LIST OF ABBREVIATIONS AND SYMBOLS

%BF	Percent body fat
2C	Two-compartment
3C	Three-compartment
4C	Four-compartment
5C	Five-compartment
ADP	Air displacement plethysmography
BIA	Bioimpedance analysis
BIS	Bioimpedance spectroscopy
d	Cohen's *d* effect size
D_2O	Deuterium oxide
DXA	Dual energy X-ray absorptiometry
FFM	Fat-free mass
FM	Fat mass
ICC	Intraclass correlation coefficient
LOA	Limits of agreement
MFBIA	Multi-frequency bioimpedance analysis
M_o	Osseous mineral
M_s	Non-osseous mineral
R	Resistance
RLV	Residual lung volume

SEE	Standard error of the estimate
SFBIA	Single frequency bioimpedance analysis
SMD	Standardized mean difference
TBW	Total body water
TE	Total error
Xc	Reactance
Z	Impedance

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CHAPTER 1

INTRODUCTION

The measurement of body composition is of great importance to the health and medical sciences. Excess adiposity, commonly expressed as percent body fat (%BF), is associated with increased risk for various metabolic disorders (1-4), as well as some cancers (5). Additionally, high levels of body fat have been shown to impede physical performance in various sports (6-10). Further, changes in %BF are often used to monitor the efficacy of training and nutrition interventions in clinical, research, and athletic settings.

Numerous techniques have been developed for quantifying %BF in humans, and include direct, indirect, and doubly indirect methods (11). Direct methods, such as the cadaver studies discussed in Brozek et al. (12), require direct measurement of fat tissue and are impractical for *in vivo* studies. Accordingly, the preferred methods commonly utilized in laboratory and field settings are categorized as either indirect or doubly indirect. Laboratory techniques such as hydrodensitometry, air displacement plethysmography (ADP), and dual energy X-ray absorptiometry (DXA) are indirect, as they determine %BF by first measuring a specific property of the body (i.e., volume or density) and then estimating the ratio of fat to fat-free mass (FFM) based on that property (13-15). Doubly indirect methods such as skinfold thickness, anthropometric measurements, and bioimpedance analysis (BIA) include an additional degree of separation and are based on population-specific regression equations derived from indirect methods (16-18).

The latter category is particularly prevalent in the exercise sciences because they allow practitioners to estimate variables of interest when more sophisticated laboratory techniques are unavailable. However, the equations used for body composition assessment are numerous and varied, and many were developed using samples or testing conditions different from those in which they are applied today. For example, the criterion method of body density, hydrodensitometry, requires a measure of participant residual lung volume (RLV) to accurately determine body volume during water submersion (12). While it is preferable to measure RLV during the underwater weighing procedure (15), equipment limitations and participant restrictions often lead to the use of RLV prediction equations (19). Although these are simple to use and typically require few variables, the coefficients in the equations were derived from dry on-land measures of RLV (20-22) and thus may not accurately reflect underwater RLV (19). Thus, errors in the estimation of RLV may affect the subsequent calculations of body volume, density, and %BF.

In addition to errors born of methodological discrepancies between equation development and application, there are issues underlying the actual direct techniques on which many equations are based. Previously mentioned indirect methods including hydrodensitometry, ADP, and DXA are based on two-compartment (2C) models of body composition, which assume body mass is comprised of fat mass and fat-free mass (FFM), and that each compartment has a constant density (15). Further, 2C models assume that the hydration of FFM is constant, although this has been shown to vary greatly across individuals (23). Because common equations for estimating %BF from skinfolds are based on the 2C model (16, 17, 24), they are susceptible to errors incurred from individual variability in protein content, mineral content, and total body water (TBW) volume.

Multi-compartment models expand on the 2C approach by measuring additional individual compartments, thereby reducing the number of assumptions required of the model. The three-compartment (3C) model accounts for much of the unaccounted variability of the 2C model by including a measure of total body water (TBW), acknowledged to be the largest individual compartment (15), though it still assumes constant densities of protein and mineral. Estimates of TBW for the 3C models are often made using the single-frequency BIA (SFBIA) technique because it is considerably less expensive, easier to administer, and less time-consuming than the criterion chemical dilution methods. Unfortunately, there is a large amount of variability in SFBIA methodology, with discrepancies across studies regarding SFBIA device, electrode arrangement, current frequency, prediction equation, and reference method (25, 26). The degree to which these inter-study differences affect TBW determination has yet to be examined.

Limitations of the 3C model were addressed by Selinger (27), who took advantage of the newly developed single-photon absorptiometry technique to quantify mineral content and develop a four-compartment (4C) model, which was later refined as X-ray technology improved (28). Due to the disparate densities of osseous and non-osseous mineral (29), Wang et al. (30) further separated the mineral compartment to yield a five-compartment (5C) model. Despite the recognition that multi-compartment models produce a more comprehensive assessment of body composition than their predecessors (31-34), they are seldom used as the reference method against which doubly indirect methods are developed. For instance, the most ubiquitous skinfold equations in use today are those originally developed against 2C-derived hydrodensitometry (16, 17, 24). In light of the limitations of the 2C model already discussed, it is important that less

direct methods of %BF determination be derived from the most accurate criterion measures available.

Working to address the issues outlined above, the purpose of this dissertation was to critically examine existing techniques and develop new equations for determining various components of body composition. The specific aims of each study were as follows:

Study 1: to develop and cross-validate an equation for predicting underwater RLV from anthropometric variables in healthy men and women, and to compare the new equation to existing equations. We hypothesized that the new prediction equation would perform better than existing equations when cross-validated against measured underwater RLV, indicated by a large intraclass correlation coefficient, small mean bias and standard error, and narrow limits of agreement.

Study 2: to systematically review and meta-analyze the available literature regarding validation of SFBIA for estimating TBW in comparison with isotope dilution techniques, and to identify potential moderating variables associated with the error in TBW estimation. We hypothesized that there would be significant error in the accuracy of SFBIA attributable to between-study differences in device, electrode placement, and criterion method.

Study 3: to develop and cross-validate an equation for predicting 5C model %BF in healthy men and women using skinfolds and anthropometric variables, and to compare the new equation to existing equations. We hypothesized that the new model would outperform existing equations upon cross-validation against the criterion 5C model, indicated by a large intraclass correlation coefficient, small mean bias and standard error, and narrow limits of agreement.

REFERENCES

1. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, et al. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study. The Journal of Clinical Endocrinology and Metabolism. 2010;95(12):5419-26.

2. Smith SR, Lovejoy JC, Greenway F, Ryan D, de la Bretonne J, Volafova J, et al. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism-Clinical and Experimental. 2001;50(4):425-35.

3. Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nature Genetics. 2017;49(6):842.

4. Fox C, Massaro J, Hoffmann U, Pou K, Maurovich-Horvat P, Liu C. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116:39-48.

5. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nature Reviews Cancer. 2015;15(8):484.

6. Chaouachi A, Brughelli M, Chamari K, Levin GT, Abdelkrim NB, Laurencelle L, et al. Lower limb maximal dynamic strength and agility determinants in elite basketball players. The Journal of Strength and Conditioning Research. 2009;23(5):1570-7.

7. Esco M, Fedewa M, Cicone Z, Sinelnikov O, Sekulic D, Holmes C. Field-based performance tests are related to body fat percentage and fat-free mass, but not body mass index, in youth soccer players. Sports. 2018;6(4):105.

8. Green MR, Pivarnik JM, Carrier DP, Womack CJ. Relationship between physiological profiles and on-ice performance of a National Collegiate Athletic Association Division I hockey team. Journal of Strength and Conditioning Research. 2006;20(1):43.

9. Silvestre R, Kraemer WJ, West C, Judelson DA, Spiering BA, Vingren JL, et al. Body composition and physical performance during a National Collegiate Athletic Association Division I men's soccer season. Journal of Strength and Conditioning Research. 2006;20(4):962.

10. Tangalos C, Robertson SJ, Spittle M, Gastin PB. Predictors of individual player match performance in junior Australian football. International Journal of Sports Physiology and Performance. 2015;10(7):853-9.

11. Wang Z, Heshka S, Pierson Jr RN, Heymsfield SB. Systematic organization of body-composition methodology: an overview with emphasis on component-based methods. The American Journal of Clinical Nutrition. 1995;61(3):457-65.

12. Brožek J, Grande F, Anderson JT, Keys A. Densitometric analysis of body composition: revision of some quantitative assumptions. Annals of the New York Academy of Sciences. 1963;110(1):113-40.

13. Behnke AR, Feen B, Welham W. The specific gravity of healthy men: body weight÷ volume as an index of obesity. Journal of the American Medical Association. 1942;118(7):495-8.

14. Goldman R, Buskirk E. Body volume measurement by underwater weighing: description of a method. Techniques for Measuring Body Composition. 1961. p. 78-89.

15. Siri WE. Body composition from fluid spaces and density: analysis of methods. Techniques for Measuring Body Composition. 1961;61:223-44.

16. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. British Journal of Nutrition. 1978;40(3):497-504.

17. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Medicine and Science in Sports and Exercise. 1980;12(3):175-81.

18. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. The American Journal of Clinical Nutrition. 2003;77(2):331-40.

19. Nickerson BS, Esco MR, Bishop PA, Schumacker RE, Richardson MT, Fedewa MV, et al. Impact of measured vs. predicted residual lung volume on body fat percentage using underwater weighing and 4-compartment model. The Journal of Strength & Conditioning Research. 2017;31(9):2519-27.

20. Goldman H, Becklake MR. Respiratory function tests, normal values at median altitudes and the prediction of normal results. American Review of Tuberculosis and Pulmonary Diseases. 1959;79(4):457-67.

21. Grimby G, Soderholm B. Spirometric Studies in Normal Subjects: III. Static Lung Volumes and Maximum Voluntary Ventilation in Adults with a Note on Physical Fitness 1. Acta Medica Scandinavica. 1963;173(2):199-206.

22. Miller WC, Swensen T, Wallace JP. Derivation of prediction equations for RV in overweight men and women. Medicine and science in sports and exercise. 1998;30(2):322-7.

23. Moon J. Body composition in athletes and sports nutrition: an examination of the bioimpedance analysis technique. European Journal of Clinical Nutrition. 2013;67(S1):S54.

24. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. British Journal of Nutrition. 1974;32(1):77-97.

25. Khalil SF, Mohktar MS, Ibrahim F. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. Sensors (Basel, Switzerland). 2014;14(6):10895-928.

26. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, et al. Bioelectrical impedance analysis—part I: review of principles and methods. Clinical Nutrition. 2004;23(5):1226-43.

27. Selinger A. The body as a three component system [dissertation]. Urbana and Champaign, Illinois: University of Illinois at Urbana-Champaign; 1977.

28. Heymsfield SB, Lichtman S, Baumgartner RN, Wang J, Kamen Y, Aliprantis A, et al. Body composition of humans: comparison of two improved four-compartment models that differ in expense, technical complexity, and radiation exposure. The American Journal of Clinical Nutrition. 1990;52(1):52-8.

29. Heymsfield SB, Waki M, Kehayias J, Lichtman S, Dilmanian FA, Kamen Y, et al. Chemical and elemental analysis of humans in vivo using improved body composition models. American Journal of Physiology-Endocrinology And Metabolism. 1991;261(2):E190-E8.

30. Wang Z, Pi-Sunyer FX, Kotler DP, Wielopolski L, Withers RT, Pierson Jr RN, et al. Multicomponent methods: evaluation of new and traditional soft tissue mineral models by in vivo neutron activation analysis. The American Journal of Clinical Nutrition. 2002;76(5):968-74.

31. Moon JR, Tobkin SE, Roberts MD, Dalbo VJ, Kerksick CM, Bemben MG, et al. Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. Nutrition & Metabolism. 2008;5(1):7.

32. Nickerson BS, Fedewa MV, Cicone Z, Esco MR. The Relative Accuracy Of Skinfolds Compared To Four-Compartment Estimates Of Body Composition. Clinical Nutrition. 2019.

33. Nickerson BS, Tinsley GM, Esco MR. Validity of Field and Laboratory Three-Compartment Models in Healthy Adults. Medicine and Science in Sports and Exercise. 2019;51(5):1032-9.

34. Wang Z, Deurenberg P, Wang W, Pietrobelli A, Baumgartner RN, Heymsfield SB. Hydration of fat-free body mass: review and critique of a classic body-composition constant. The American Journal of Clinical Nutrition. 1999;69(5):833-41.

CHAPTER 2

DEVELOPMENT AND CROSS-VALIDATION OF AN EQUATION TO PREDICT UNDERWATER RESIDUAL LUNG VOLUME IN HEALTHY MEN AND WOMEN

ABSTRACT

Researchers commonly use regression equations to predict residual lung volume (RLV) during underwater weighing when its measurement is not practical. However, the equations currently available were derived from on-land measures of RLV and do not account for changes in lung capacity during water immersion, and thus may lead to inaccuracies in subsequent body composition assessment. The purpose of this study was to 1) develop a novel equation for the prediction of RLV during water immersion, and 2) cross-validate the new equation and compare it to existing RLV prediction equations. A sample of 175 healthy adults were recruited to complete RLV measurement while completely submerged in water. The sample was randomly divided into a development cohort (n = 131) and cross-validation cohort (n = 44). Regression analysis in the development cohort resulted in a significant ($p < 0.001$; $R^2 = 0.54$; SEE = 0.25) equation for predicting RLV from age and height: RLV = -3.419 + 0.026 × Height (cm) + 0.019 × Age (y). In the cross-validation cohort, repeated measures ANOVA revealed that the existing equations produced significantly different mean values from measured RLV (all $p < 0.001$), while the new equation did not. Further, Bland-Altman analysis indicated that the new equation produced the smallest mean bias and limits of agreement (0.07 ± 0.50 L) when compared to existing equations, as well as the lowest SEE. The results of this study suggest that the

new equation is more accurate than existing equations for predicting underwater RLV, and it should be used during hydrostatic weighing when RLV measurement is not possible.

INTRODUCTION

The long-standing gold standard for body composition assessment is hydrostatic weighing, a technique for determining body volume based on the displacement of water (1). A potential source of error in hydrostatic weighing is the volume of residual air within the lungs and gastrointestinal tract, which increases corporeal buoyancy and results in less water displacement, thus artificially decreasing body density. While the amount of air in the gastrointestinal tract is considered negligible (~0.1 liters) (1), the volume of residual air in the lungs is much larger (generally 1.0 to 2.0 liters) and should be measured and removed from the total body volume (2).

Residual lung volume (RLV) can be determined either by direct measurement or estimation based on variables including age and height (3). The most common direct measurement is the oxygen dilution method, which measures the change in concentration of an inert gas within the pulmonary-spirometer system during a period of rebreathing following maximal exhalation (4). This measurement can be done either on land or underwater during the hydrostatic weighing procedure, though controversy exists as to which is best (5). Simultaneous hydrostatic weighing and RLV measurement is often cited to be the gold standard for body composition assessment (3, 6). However, research suggests that water immersion may affect lung volumes, with several authors reporting significant changes in RLV during submersion compared to on-land measurements (7-11).

Various equations for predicting RLV have been described in the literature, including those by Miller et al. (12), Goldman and Becklake (13), Paoletti et al., and Grimby and

Soderholm (14). These particular models use variables including height, weight, and age to predict RLV. While the use of these equations for the densitometric analysis of body composition provide convenient alternatives to direct measurement of RLV, investigations into their validity found that they may introduce additional error when compared to chemical dilution techniques (3, 15, 16). Further, these equations are used to estimate underwater-RLV during hydrostatic weighing, despite being derived from regression analyses using on-land RLV (12, 13, 17, 18). Though the potential effect of water immersion on RLV may be of consequence, it is entirely discounted by existing estimation equations. Furthermore, it is unknown whether this effect contributes to inaccuracies in underwater RLV prediction (6, 8, 19).

Despite the limitations of current RLV prediction equations, estimation is cost-effective and limits participant burden. For example, RLV measurement equipment is expensive and requires routine maintenance and calibration. Additionally, underwater measurement requires a great deal of subject cooperation and comfortability. Data collection can be compromised, slowed or negated in the event a subject becomes panicked while underwater and floods the respiratory tubing. Developing a valid equation for predicting underwater RLV would enable researchers to expedite body composition measurements when testing large groups of subjects, and importantly, to accommodate subjects who may be uncomfortable or unable to perform RLV testing underwater. Therefore, the purpose of this study was to develop an equation for predicting underwater RLV in healthy adults. Further, the new model was cross-validated in a separate cohort against measured underwater RLV, as well as compared to existing equations from Miller et al.(12), Goldman and Becklake (13), Paoletti et al. (20), and Grimby and Soderholm (14). We hypothesized that the new equation would produce a smaller mean

difference, tighter limits of agreement, and a smaller standard error than the existing equations when compared to the criterion.

METHODS

Participants

The sample consisted of healthy, adult volunteers who had been recruited from the local community and University for a previous study (3). All participants provided written informed consent prior to participation. Participants with any cardiac, pulmonary, or metabolic conditions were excluded. Women who were pregnant, pregnant within the previous 12 months, or currently lactating were also excluded. The testing protocol, recruitment flyers, and consent forms were reviewed and approved by the University's institutional review board. The final sample consisted of 175 participants, which was randomly divided into a development cohort (75% of the total sample, n = 131, 49.6% female) and a cross-validation cohort (25% of the total sample, n = 44, 50.0% female) using the IBM SPSS software package (Version 25.0, IBM, Somers, NY, USA).

Power analyses were conducted using G*Power (version 3.1.9.4, Universität Kiel, Germany) to confirm that adequate statistical power was achieved during 1) regression analysis for model development and 2) cross-validation of the new model. For model development, the large effect size (f^2 = 1.188) from multiple linear regression using 131 participants and 2 predictor variables, with α level of 0.05, resulted in an achieved power (1 – β) level of 1.000. For cross-validation, the large effect size (f = 1.612) from the repeated measures ANOVA of 44 participants and 5 comparisons, with α level of 0.05, resulted in an achieved power (1 – β) level of 1.000.

Procedures

Testing occurred during a single session at the University's Exercise Physiology Laboratory. Participants were instructed to adhere to the following pre-testing guidelines: abstain from alcohol and exercise for 24 hours; abstain from food and caffeine for 8 to 12 hours; abstain from drinking water for ≥ 2 hours. Participants wore loose-fitting athletic attire for anthropometric measurements and changed into tight-fitting or compression-type clothing for the underwater measurements. Standing height was measured to the nearest 0.1 centimeter using a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany) and body mass was measured to the nearest 0.1 kilogram using a digital scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan).

Calibration and setup of the residual lung volume equipment

A nitrogen analyzer ("VacuMed"; Model 17750A, VacuMed, Ventura, CA, USA) was used to measure RLV. The VacuMed system pulls expired air into a high-voltage ionization chamber and uses a photodiode to measure the light emitted from the ionized gas, which is proportional to the nitrogen concentration (21). The change in nitrogen concentration during the rebreathing procedure is used to calculate RLV using the method described by Wilmore et al. (22). The VacuMed system was calibrated to the low (0%) and high (80%) ends of the expected nitrogen concentration range according to the manufacturer's instructions prior to each use.

The rebreathing procedure uses a five or six-liter anesthesia bag fitted to the bottom of a "T"-shaped three-way valve. An anti-microbial spirometer filter (Pulmogard #3421, VacuMed, Ventura, CA, USA) was attached to one end of the three-way valve to serve as a mouthpiece for the participant, with the opposite end left open to the room air. A two-way stopcock attached to the "T"-valve allows the subject to breathe either room air (stopcock closed) or air within the bag (stopcock open). Once assembled and ready for measurement, the bag will be evacuated, flushed

with 0.5 liters of oxygen, and then filled with 5 liters of 100% oxygen. Two bags will be filled per subject, and additional ones may be assembled if needed.

Underwater residual lung volume measurement

Underwater measurement was carried out in a 500-gallon heated water tank. Participants sat in a nylon seat harness which was suspended from an aluminum frame mounted around the tank. The three-way valve was attached to the side of the tank in order to be used without any subject handling. The mouthpiece was attached to a length of corrugated hose to allow the subject to breathe through the apparatus while completely submerged. The dead space from the tube was accounted for in the RLV calculation. Participants completed a maximal exhalation through the mouthpiece during submersion. After reaching maximal exhalation, the two-way valve was switched to allow rebreathing from the oxygen-filled bag. Participants were instructed to sit up during the rebreathing procedure and take deep breaths to adequately mix the lung air with the bag air until equilibration of pulmonary nitrogen. The procedure was repeated until two measures within 200 ml were obtained. The average of the two smallest measures was used as the criterion RLV.

Statistical analyses

Data were managed using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were completed using SPSS version 25 for Windows (IBM, Somers, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Hierarchical multiple regression analysis was performed in the development cohort to predict criterion RLV. Independent variables of interest for developing the model were participant age, height, sex, and weight, as they have been shown to correlate significantly with on-land RLV (12, 13, 17, 20). Standard assumptions for regression analysis were assessed prior

to analysis: 1) linearity between the predictors and the outcome variable, assessed by visual examination of scatterplots and by significant ($p < 0.05$) bivariate correlations; 2) independence of error terms, indicated by a Durbin-Watson statistic between 1.0 and 3.0 (23, 24); 3) equality of error variance, assessed by visual examination of scatterplots of the standardized predicted values and residuals for homoscedasticity (24); 4) normal distribution of errors, assessed by visual inspection of histograms and normal Q-Q plots of standardized residuals and by the Shapiro-Wilk test ($p > 0.05$ indicates no significant departure from normality) (24, 25); 5) multicollinearity of independent variables, defined as a variance inflation factor (VIF) > 10.0 (26).

Within the cross-validation sample, new model performance was compared to criterion RLV as well as RLV predicted from equations by Miller et al. (12), Goldman and Becklake (13), Paoletti et al. (20), and Grimby and Soderholm (14). Mean differences were tested for significance using repeated measures ANOVA with simple planned contrasts and quantified using Cohen's *d* effect size. Agreement was examined using the Bland-Altman method for calculating limits of agreement (27), Pearson's product-moment correlation coefficient, standard error of the estimate (SEE), and total error (TE). Data are presented as mean ± standard deviation ($M \pm SD$) unless otherwise specified. Statistical significance was accepted at $p < 0.05$.

RESULTS

Physical characteristics of the participants in the development and cross-validation cohorts are presented in Table 1. There were no significant differences in age, height, weight, BMI, or RLV between the development and cross-validation groups. On average men were taller, heavier, and had a larger RLV than women in both groups.

All assumptions for multiple regression were satisfied: a Durbin-Watson statistic of 1.849 suggests independence of error terms; homoscedasticity of standardized residuals versus predicted values suggests equality of error variance; normality of standardized residuals was observed graphically and statistically, $W(131) = 0.993$, $p = 0.731$. Additionally, the hypothesized predictor variables height ($r = 0.692$), weight ($r = 0.435$), age ($r = 0.293$), and sex ($r = 0.539$) were significantly (all $p < 0.001$) correlated with the outcome variable. Multicollinearity was not indicated in the model (VIF = 1.004).

Underwater RLV was regressed on height, weight, age, and sex using multiple linear regression. Sex and weight were not significant predictors of RLV when included in the model with height and age (all $p > 0.05$). The final model regressing RLV on age and height was statistically significant, $F(2, 128) = 75.373$, $p < 0.001$, and was defined by the following equation: RLV = -3.419 + (0.026 × height) + (0.019 × age), where RLV is in liters, height is in centimeters, and age is in years. Regression statistics are presented in Table 2.

Cross-validation of RLV prediction equations against measured RLV was performed using repeated measures ANOVA with simple contrasts. Standardized residuals for all RLV values were normally distributed according to visual inspection of histograms, Q-Q plots, and Kolmogorov-Smirnov test of normality (all $p > 0.05$). Mauchly's test indicated a violation of sphericity, $\chi^2(14) = 190.640$, $p < 0.001$, therefore degrees of freedom were corrected using the Greenhouse-Geisser estimate ($\varepsilon = 0.353$). The corrected repeated measures ANOVA was statistically significant, $F(1.766, 75.924) = 111.616$, $p < 0.001$. Simple contrasts revealed that the previously published equations produced mean values that were different than measured RLV (all $p < 0.001$). The mean value from the newly developed equation was not different from measured RLV ($p = 0.08$).

Pearson correlations between predicted values and the criterion were significant for all equations (all $p < 0.001$) and ranged from 0.52 to 0.76. Cohen's *d* statistic ranged from small to large, with the new equation producing the smallest standardized mean difference. The SEE was less than 0.30 liters for all equations. Bland-Altman plots (Figure 1) show comparable limits of agreement (± 1.96 *SD* of the bias) for all equations, ranging from 0.50 to 0.63 liters. Additionally, proportional bias was indicated by significantly (all $p < 0.001$) large and negative trends between the mean and difference of RLV values (*r* values ranging from -0.51 to -0.72). Comparison statistics between predicted and measured RLV are presented in Table 3.

DISCUSSION

The purpose of this study was to develop and cross-validate a novel equation for the prediction of underwater RLV in healthy adults. In agreement with our hypothesis, the final regression equation explained over half the variability in underwater RLV using age and height as predictors. The new equation also provided acceptable individual estimates of RLV during the cross-validation procedure, as evidenced by the high *r* value, low effect size, low SEE and TE, small mean difference, and narrow limits of agreement. Finally, the new equation produced better overall agreement with underwater RLV than the selected equations; Miller et al. (12) and Grimby and Soderholm (14) produced underestimated mean values of RLV, while Goldman and Becklake (13) and Paoletti et al. (20) both overestimated RLV. These findings suggest that the equation described in this study may provide greater accuracy in the estimation of RLV during water immersion than previously derived equations.

The equations previously described are commonly used to estimate underwater RLV during hydrostatic weighing, despite being developed using on-land RLV (12-14, 20). While suitable for on-land measures, their application to underwater RLV is predicated on the

assumption that lung volumes remain unchanged while underwater. However, decreases in RLV ranging from 4.2% to 19.7% have been reported during immersion (6, 7, 9-11, 19, 28), and are due to compression of the thoracic wall and diaphragm during inspiratory muscle relaxation (7, 11, 19). Considering the impact RLV measurement can have on body volume and subsequent percent body fat estimations (3, 16), it is necessary to account for the effect of submersion when developing equations for the prediction of underwater RLV. Despite the potential impact of submersion on RLV, the equation developed in this study is the first to be derived from underwater RLV rather than on-land measures. Accordingly, the explained variance of our equation is larger, and the SEE is lower than those previously reported in similar samples (12-14, 17, 20, 29).

Although derived from a different criterion RLV, the significance of age and height as predictor variables in our regression model supports previous research. Goldman and Becklake (13) described sex-specific equations to predict RLV from age and height in a sample of 94 healthy men (R^2 = 0.41) and women (R^2 = 0.30) at altitude using age and height. During cross-validation in our study, the Goldman and Becklake equations overestimated underwater RLV by an average of 0.26 L and produced limits of agreement of ±0.61 L. This overestimation is in line with research by Nickerson et al. (3), who reported the Goldman and Becklake equation overestimated underwater RLV by an average of 0.23 L. The authors further demonstrated that the error in RLV associated with the Goldman and Becklake equations resulted in 2.0% and 1.5% underestimations of percent body fat from underwater weighing and 4-compartment models, respectively (3). The overestimations in RLV associated with these equations may in part be due to the methods used to determine RLV, which were different from those reported in the current study. Rather than use a dilution technique, Goldman and Becklake calculated RLV

as the difference between functional residual capacity and expiratory reserve volume, which were measured using a closed-circuit, constant-volume technique (30, 31).

Similarly, Grimby and Soderholm (14) developed sex-specific equations to predict RLV in a sample of 210 healthy men and women. The female-specific equation used age and height as the predictor variables and had a SEE of 0.32 L (14). Further, the coefficients and intercept of the female-specific equation were nearly identical to the male-specific equations of Goldman and Becklake (13) and the equation developed in the current study. Additionally, Grimby and Soderholm (14) found that weight was a significant predictor of RLV in the male cohort and included it in the final model (SEE = 0.38 L). Paoletti et al. (20) reported a similar finding in a sample of 279 healthy women aged 18 to 64 y and described an equation which included weight as a predictor with a similar SEE as Grimby and Soderholm's male-specific equation, though the R^2 value was less than 0.20. However, Paoletti et al.(20) did not find weight to be significant in the prediction of RLV in men. The inclusion of weight in these equations did not improve their accuracy in our cross-validation cohort. The equations from Paoletti et al.(20) overestimated underwater RLV by 0.36 L on average, while Grimby and Soderholm (14) underestimated by 0.17 L. The latter equation also produced the widest limits of agreement (±0.63 L) compared to the other equations.

Although the significance of weight as a predictor of RLV reported by the previous studies (14, 20) is in conflict with the present findings, the discrepancy may be in part due to the body composition of the participants. When comparing RLV prediction between normal weight and overweight men and women, Miller et al.(12) observed that in addition to age and height, weight was a significant predictor in the overweight groups only. The authors further reported that when data from the normal weight and overweight groups were combined, the mean percent

body fat for the sample increased to 30.6% and caused weight to become significant in the regression model (12). They went on to note that previous studies that had identified weight as significant for the prediction of RLV were carried out in samples of predominantly overweight men (29) and women (32), even if the sample was not explicitly classified as overweight. The final equations reported by Miller et al.(12) were weight-specific (overweight and normal weight), and produced R^2 values larger than previously published equations, though the SEE was comparable. Further, the normal weight equation compared better to our new model than the others, underestimating underwater RLV by 0.16 L. Because our cross-validation cohort was not overweight, the inclusion of weight in the prediction of RLV may have contributed to the errors associated with those equations (14, 20).

Another discrepancy between the current and previous studies was that of participant sex. While our analysis found that sex was not significant for predicting RLV, the equations from Goldman and Becklake (13), Paoletti et al. (20), and Grimby and Soderholm (14) are sex-specific. The derivation of sex-specific equations implies that RLV varies differently between men and women, hence the need for separate equations. However, none of these studies reported statistically significant findings when comparing men and women or including sex as a predictor variable in a regression analysis. Our findings are supported by Miller et al. (12), who examined sex as a potential predictor in groups of overweight and normal weight adults and found it was not significant. Additionally, the sex-specific equations did not provide better agreement than the new equation or that of Miller et al. (12). Based on results from our analysis as well as the investigation by Miller et al. (12), we speculate that sex differences in RLV are likely due to differences in height, as women are on average shorter than men and thus have proportionally smaller lung capacities.

This study is not without limitations. In terms of random sampling, we were limited to members of the local university and surrounding area, which led to a disproportionate number of white participants. This may limit the generalizability of the equation described here, as discrepancies in lung volumes between those of Caucasian and African American descent have been reported previously (33-35). Further, the age range of participants reported in the current study (18 to 37 years) is markedly smaller than the age ranges used to develop previous equations, which generally span 18 to 72 years (12-14, 20). This truncated age range may further limit applicability of the new equation. A final consideration is the method used to determine RLV during the development of each of the studies. Both the current study and Miller et al. (12) utilized the oxygen dilution technique described by Wilmore (4), while the other studies calculated RLV from functional residual capacity (13, 14, 20). This discrepancy between measurement techniques may have an impact on the validity of the equations and thus, create additional variability between equations.

CONCLUSION

The equation developed in this study was able to predict underwater RLV from age and height in a sample of healthy adults with greater explained variance and lower error than previously developed equations. When compared to measured RLV, the new equation outperformed previously published equations, resulting in a small mean difference and narrow limits of agreement. This study also suggests that sex-specific equations may not be necessary in the prediction of RLV, though additional work in this area is needed. Further, the finding to exclude weight as a predictor variable supports previous findings that weight may only become influential in groups with large amounts of adiposity; however, our sample was non-obese, and we did not explicitly test this hypothesis. Overall, our findings suggest that the new equation

presented here may be appropriate for the prediction of underwater RLV during hydrostatic weighing procedures when measurement is not feasible. Due to the sampling limitations previously discussed, we recommend limiting its use to healthy, normal weight adults under the age of 40 years, and employing caution when applying it to other demographics.

REFERENCES

1. Goldman R, Buskirk E. Body volume measurement by underwater weighing: description of a method. Techniques for Measuring Body Composition. 1961. p. 78-89.

2. Behnke AR, Feen B, Welham W. The specific gravity of healthy men: body weight÷ volume as an index of obesity. Journal of the American Medical Association. 1942;118(7):495-8.

3. Nickerson BS, Esco MR, Bishop PA, Schumacker RE, Richardson MT, Fedewa MV, et al. Impact of measured vs. predicted residual lung volume on body fat percentage using underwater weighing and 4-compartment model. The Journal of Strength and Conditioning Research. 2017;31(9):2519-27.

4. Wilmore JH. A simplified method for determination of residual lung volumes. Journal of Applied Physiology. 1969;27(1):96-100.

5. Brodie D, Vicki Moscrip MSC H, Hutcheon R. Body composition measurement: a review of hydrodensitometry, anthropometry, and impedance methods. Nutrition. 1998;14(3):296-310.

6. Brožek J, Henschel A, Keys A. Effect of submersion in water on the volume of residual air in man. Journal of Applied Physiology. 1949;2(5):240-6.

7. Agostoni E, Gurtner G, Torri G, Rahn H. Respiratory mechanics during submersion and negative-pressure breathing. Journal of Applied Physiology. 1966;21(1):251-8.

8. Girandola RN, Wiswell RA, Mohler JG, Romero GT, Barnes WS. Effects of water immersion on lung volumes: implications for body composition analysis. Journal of Applied Physiology. 1977;43(2):276-9.

9. Ostrove S, Vaccaro P. Effect of immersion on RV in young women: implications for measurement of body density. International Journal of Sports Medicine. 1982;3(04):220-3.

10. Sawka MN, Weber H, Knowlton RG. The effect of total body submersion on residual lung volume and body density measurements in man. Ergonomics. 1978;21(2):89-94.

11. Withers R, Hamdorf P. Effect of immersion on lung capacities and volumes: implications for the densitometric estimation of relative body fat. Journal of Sports Sciences. 1989;7(1):21-30.

12. Miller WC, Swensen T, Wallace JP. Derivation of prediction equations for RV in overweight men and women. Medicine and Science in Sports and Exercise. 1998;30(2):322-7.

13. Goldman H, Becklake MR. Respiratory function tests, normal values at median altitudes and the prediction of normal results. American Review of Tuberculosis and Pulmonary Diseases. 1959;79(4):457-67.

14. Grimby G, Soderholm B. Spirometric studies in normal subjects: III. Static lung volumes and maximum voluntary ventilation in adults with a note on physical fitness 1. Acta Medica Scandinavica. 1963;173(2):199-206.

15. Forsyth R, Plyley M, Shephard RJ. Residual volume as a tool in body fat prediction. Annals of Nutrition and Metabolism. 1988;32(2):62-7.

16. Morrow JJ, Jackson AS, Bradley PW, Hartung GH. Accuracy of measured and predicted residual lung volume on body density measurement. Medicine and Science in Sports and Exercise. 1986;18(6):647-52.

17. Boren HG, Kory RC, Syner JC. The Veterans Administration-Army cooperative study of pulmonary function: II. The lung volume and its subdivisions in normal men. The American Journal of Medicine. 1966;41(1):96-114.

18. Wilmore JH. The use of actual, predicted and constant residual volumes in the assessment of body composition by underwater weighing. Medicine and Science in Sports. 1969;1(2):87-90.

19. Craig Jr AB, Ware DE. Effect of immersion in water on vital capacity and residual volume of the lungs. Journal of Applied Physiology. 1967;23(4):423-5.

20. Paoletti P, Viegi G, Carrozzi L, Di Pede F, Modena P, Ballerin L, et al. Residual volume in a general population: effects of body size, age, cigarette smoking, and respiratory symptoms. Chest. 1992;102(4):1209-15.

21. Rumble J. CRC Handbook of Chemistry and Physics: CRC press; 2017.

22. Wilmore JH, Vodak PA, Parr RB, Girandola RN, Billing JE. Further simplification of a method for determination of residual lung volume. Medicine and Science in Sports and Exercise. 1980;12(3):216-8.

23. Durbin J, Watson GS. Testing for serial correlation in least squares regression. II. Biometrika. 1951;38(1/2):159-77.

24. Field A. Discovering statistics using IBM SPSS statistics: Sage; 2013.

25. Shapiro S, Wilk M. Approximations for the null distribution of the W statistic. Technometrics. 1968;10(4):861-6.

26. Wetherill GB. Regression Analysis with Application: Chapman & Hall, Ltd.; 1987.

27. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. 1986;327(8476):307-10.

28. Sloan A, Bredell G. A comparison of two simple methods for determining pulmonary residual volume. Human Biology. 1973:23-9.

29. Chinn K, Allen TH. Prediction of residual lung volume for purposes of determining total body tissue volume. Report. US Army Medical Research and Nutrition Laboratory. 1960 Oct;252:1-9.

30. Briscoe W, Becklake M, Rose T. Intrapulmonary mixing of helium in normal and emphysematous subjects. Clinical Science. 1951;10(1):37-51.

31. McMichael J. A rapid method of determining lung capacity. Clinical Science. 1939;4:167.

32. Hall A, Heywood C, Cotes J. Lung function in healthy British women. Thorax. 1979;34(3):359-65.

33. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general US population. American Journal of Respiratory and Critical Care Medicine. 1999;159(1):179-87.

34. Harik-Khan RI, Muller DC, Wise RA. Racial difference in lung function in African-American and White children: effect of anthropometric, socioeconomic, nutritional, and environmental factors. American Journal of Epidemiology. 2004;160(9):893-900.

35. Hsi BP, Hsu KH, Jenkins DE. Ventilatory functions of normal children and young adults: Mexican-American, white, and black. III. Sitting height as a predictor. The Journal of Pediatrics. 1983;102(6):860-5.

Table 1. Descriptive characteristics of participants

Variable	Total (n = 175)	Development (n = 131)	Cross-validation (n = 44)
Age (y)	22 ± 5	22 ± 5	21 ± 5
Height (cm)	171.6 ± 9.5	171.5 ± 9.6	171.7 ± 9.2
Weight (kg)	72.3 ± 15.8	72.8 ± 16.6	70.5 ± 12.9
BMI ($kg \cdot m^{-2}$)	24.4 ± 4.5	24.6 ± 4.8	23.8 ± 3.4
RLV (L)	1.47 ± 0.38	1.50 ± 0.37	1.38 ± 0.38
Caucasian/white (%)	82.3	79.4	90.9
Female (%)	49.7	49.6	50.0

BMI, body mass index; cm, centimeter; kg, kilogram; L, liter; m, meters; RLV, underwater residual lung volume as determined by oxygen dilution; y, years.

Table 2. Prediction of underwater RLV (n = 131)

Model	Model coefficients					F change (p value)	Adjusted R^2	SEE
	Constant	Height (cm)	Age (y)	Weight (kg)	Sex			
1	-3.116*	0.027*	---	---	---	< 0.001	0.475	0.270
2	-3.419*	0.026*	0.019*	---	---	< 0.001	0.534	0.254
3	-3.511*	0.027*	0.020*	-0.001	---	0.624	0.531	0.255
4	-3.030*	0.024*	0.021*	-0.002	0.102	0.123	0.536	0.254

Adjusted R^2, coefficient of determination adjusted for multiple predictors; cm, centimeters; F change, p-value of change in F-statistic; kg, kilograms; RLV, residual lung volume; SEE, standard error of the estimate; y, years.
* significant at $p < 0.001$.

Table 3. Comparison statistics between criterion and predicted RLV (n = 44)

						Limits of agreement			
	Mean ± *SD* (L)	*r*	*d*	SEE	TE	Bias ± 1.96 *SD*	Lower	Upper	Trend
Criterion RLV	1.38 ± 0.38								
Cicone et al.	1.45 ± 0.25	0.73**	0.22	0.17	0.26	0.07 ± 0.50	-0.43	0.57	-0.51***
Miller et al.	1.22 ± 0.21*	0.71**	-0.53	0.19	0.31	-0.16 ± 0.53	-0.69	0.37	-0.65***
Goldman and Becklake	1.64 ± 0.20*	0.56**	0.86	0.26	0.40	0.26 ± 0.61	-0.35	0.87	-0.63***
Paoletti et al.	1.74 ± 0.21*	0.76**	1.17	0.17	0.44	0.36 ± 0.50	-0.14	0.86	-0.67***
Grimby and Soderholm	1.21 ± 0.17*	0.52**	-0.58	0.28	0.36	-0.17 ± 0.63	-0.80	0.46	-0.72***

Bias, mean difference between predicted and criterion; d, Cohen's standardized mean difference; L, liters; r, Pearson's product-moment correlation coefficient; RLV, residual lung volume; SD, standard deviation; SEE, standard error of the estimate; TE, total error; trend, correlation (expressed as Pearson's r) between the bias and average.
*different from criterion ($p < 0.001$).
**significant correlation ($p < 0.001$).
***trend is significant ($p < 0.001$).

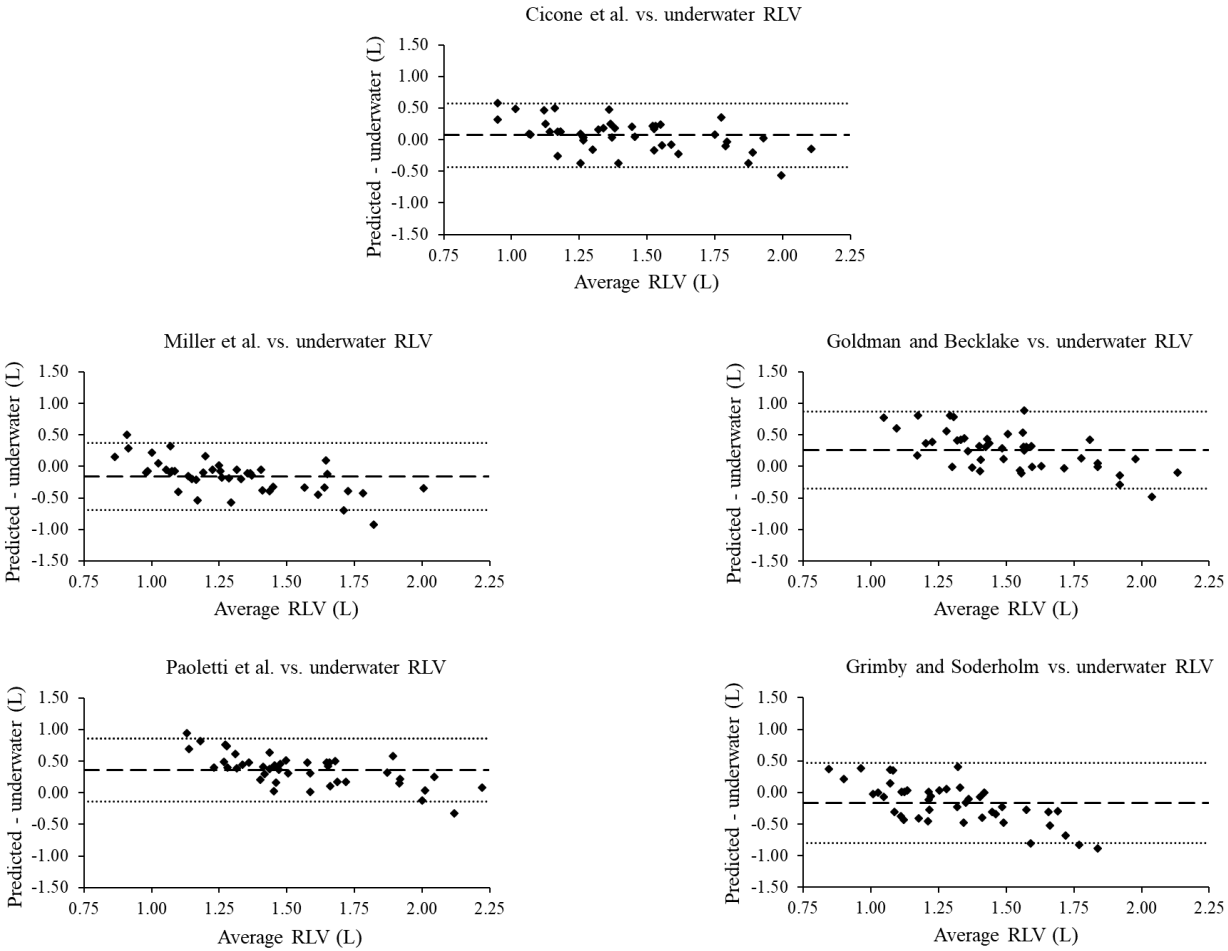

Figure 1. Bland-Altman plots of measured versus predicted RLV (n = 44). Dark center line represents mean bias. Light dotted outer lines represent limits of agreement (bias ± 1.96 *SD* of bias). RLV, residual lung volume.

CHAPTER 3

ESTIMATION OF TOTAL BODY WATER USING SINGLE FREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

ABSTRACT

Single frequency bioimpedance analysis (SFBIA) is a simple and non-invasive alternative to criterion chemical dilution techniques for assessing total body water (TBW). However, the impact of the various methodological considerations associated with SFBIA are unknown. The purpose of this study was to systematically review and meta-analyze the available literature regarding the validation of SFBIA for TBW determination. Electronic database searches were carried out to identify potentially relevant reports. Multi-level random-effects models were used to generate an overall standardized mean difference (SMD) effect size and identify potential moderators from the final sample of included study effects. The systematic search of five databases resulted in the identification of 264 effects from 51 original studies for quantitative analysis. The overall SMD indicated a small difference between SFBIA and criterion dilution methods (SMD = 0.02, $p = 0.823$) and a large degree of heterogeneity between effects (I^2 = 95.8%). Moderator analysis revealed that error in SFBIA estimation of TBW was primarily influenced the selection of frequency and index ($\beta = -1.10$, $p < 0.0001$) used to predict TBW, as well as the proportion of women in study samples ($\beta = -0.002$, $p < 0.0001$). Use of resistance index (Ht^2/R) produced less error than impedance index (Ht^2/Z) across all reported frequencies, with Ht^2/R at 100 kHz producing the smallest overall SMD. The results presented in this study suggest that SFBIA provides accurate estimates of TBW compared to criterion dilution

techniques. However, care should be taken to use appropriate SFBIA parameters to minimize error in the estimate.

INTRODUCTION

Accurate assessment of total body water (TBW) is critical for monitoring various aspects of physical health and performance. For instance, a decrease in body mass from water loss as little as 1-4% can cause decline in cognitive function (1), mood (2), exercise performance (3), and occupational work capacity (4). Thus, TBW is an important metric for athletic monitoring. Furthermore, it is an important consideration in body composition assessment. Multi-compartment body fat techniques that include TBW are more accurate than standard two-compartment models (e.g., hydrostatic weighing, skinfolds) that are based on assumed constant values for FFM hydration and density (5-11). Additionally, TBW assessment is an important consideration when monitoring patients with a metabolic condition that affects fluid status, such as hemodialysis (12, 13), peritoneal dialysis (14, 15), chronic kidney disease (16), cachexia (17), and lymphedema (18, 19). Last, tracking TBW is essential during nutritional interventions to prevent undesirable decreases in FFM with weight loss.

Despite the importance of TBW assessment, such measurement techniques have primarily been limited to laboratory settings. The “gold standard” biochemical methods include dilution of isotopic tracers such as deuterium oxide, tritium dilution, and oxygen-18 (20-22). Administration of the dilution methods requires considerable cooperation from the participants, including multiple urine or blood samples and time commitments of up to six hours (22). Furthermore, each method is relatively expensive and requires a skilled technician to initiate the isotope measurement, collect and store each specimen, and perform the chemical analysis (23).

These inconveniences often preclude the widespread utility of dilution techniques in clinical and practical settings.

A safe, non-invasive, and inexpensive alternative is bioelectrical impedance analysis (BIA) (24-26). This technique is based on the inverse relationship between TBW and electrical resistance (27). The most common BIA technology, single-frequency BIA (SFBIA), passes a current at 50 kHz through the body via surface electrodes attached to various contact points that serve as electrical poles. The rate of the current, along with descriptive characteristics, is used to estimate TBW using general or population-specific regression equations (27, 28). This technique is user independent and requires minimal time, making it suitable for assessing large groups and reporting of results quickly (i.e., within a few minutes). Though SFBIA may not be considered as sophisticated as dilution techniques or more advanced bioimpedance spectrometry methods, it remains prominent due to its low cost, ease of administration, and availability (27, 28).

There are important distinctions in SFBIA methods that are commercially available, namely the direction of the current and the locations and number of contact points. For instance, the direction of the current may pass from two poles (i.e., bipolar) that are placed either at both hands, at both feet, or from hand to foot. These differences may result in errors of up to 70 ohms in resistance values due to the discrepancies regarding electrode placement (25, 29). Additionally, the number of contact points affects the accuracy of SFBIA devices, as bipolar devices may be less accurate than tetrapolar devices that pass the current between both hands *and* both feet (25, 30, 31).

Independent of the SFBIA device, the chemical dilution method used as the criterion variable may also affect the outcome of TBW measurements. Although deuterium oxide is the most commonly reported chemical dilution method, others have been used with varying results.

For example, Schoeller et al. (22) reported differences in TBW as large as 3% between deuterium oxide and oxygen-18 dilution techniques. Further, there are a large number of BIA devices from different manufacturers that are used to validate the SFBIA procedure (28). It is not known if there are specific differences in estimation error across the various types of available devices. In sum, SFBIA may be a valid method for predicting TBW; however, there are several important device and methodological considerations—SFBIA model, electrode placement, frequency of current, criterion dilution technique—that could impact the validity and reliability of measures obtained via SFBIA. No study to date has investigated whether the aforementioned discrepancies associated with SFBIA influence TBW estimations, and if so, to what extent.

Therefore, a comprehensive review and synthesis of the SFBIA literature is needed to systematically explore these device and methodological considerations. Aggregate-level meta-analysis techniques will allow for the exploration of potential moderating factors at the study level (i.e., sample characteristics, bioimpedance procedures, criterion dilution techniques), and will enable the provision of recommendations to be used by researchers and clinicians alike. The purpose of this study was to 1) systematically review and meta-analyze the available literature regarding the accuracy of SFBIA for the estimation of TBW, 2) identify potential moderators that explain error in the estimation of TBW, and 3) identify best practices for using SFBIA for the estimation of TBW in clinical and research settings.

METHODS

Procedures

Search strategy

This study fully satisfies the criteria implied by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (32, 33) and the Assessment of

Multiple Systematic Reviews (AMSTAR) Methodological Quality Tool (34, 35). The electronic database search included PubMed, CINAHL Plus with Full Text, Web of Science, and Scopus. Additionally, the ProQuest database was searched for theses and dissertations related to the validity of SFBIA. Databases were searched from inception until December 2019 using key terms related to bioelectrical impedance, validity, total body water, and isotope tracer. Hand-searching and cross-referencing was also performed using the reference lists of included studies to supplement electronic database searches. Included studies were required to meet the following *a priori* criteria: i) peer-reviewed, ii) full-text article available in English, iii) involved human participants, iv) compared TBW values from SFBIA to chemical dilution, v) reported sufficient information so that the standardized mean difference (SMD) effect size and its components could be calculated, vi) reported the specific type of bioimpedance analysis procedures used.

Assessment of study quality

Methodological study quality (SQ) was assessed using the scoring system for BIA validation studies proposed by Gonzalez et al. (36). The scoring system allows up to 400 points and consists of weighted questions regarding study design, procedures, statistical analysis, and technique standardization. Assessment of SQ was carried out in duplicate by two independent coders (ZSC and second coder, CJH). Discrepancies between SQ results were resolved by discussion between the two coders. Total SQ (expressed as a percentage of items satisfied of the total points possible) was included in the moderator analysis to identify the impact of SQ on error in TBW estimation. The SFBIA SQ survey utilized for this study is provided in Supplemental Digital Content (SDC) 1.

Study outcomes, effect size calculation, and heterogeneity

The within-subject standardized mean difference (SMD) effect size was used to quantify the accuracy of SFBIA compared to isotope dilution techniques for determining TBW (38). The SMD was defined as the mean difference between SFBIA and criterion TBW divided by the standard deviation (SD) of the mean difference, adjusted for small sample bias (37, 38). Multiple effects from a single study were disaggregated and analyzed separately (39). A multi-level random-effects model with maximum likelihood estimation was used to generate an overall SMD and 95% confidence intervals. The multi-level model was used to account for effects nested within studies (40, 41). A positive effect indicates that SFBIA overestimated TBW and the magnitude of the effect size were qualified as small (≤ 0.20), medium (0.50), and large (≥ 0.80) (42). Effect size heterogeneity was identified using Cochran's *Q* statistic (43) and quantified with the I^2 statistic (44, 45).

Moderator analysis

In the presence of significant heterogeneity, pre-selected study-level moderators were evaluated to identify potential sources of variability across effect sizes. Potential study-level moderators included SFBIA device and procedure information (model, electrode arrangement, side of the body, frequency of current, index), isotope tracer used for chemical dilution technique (deuterium oxide, oxygen-18, tritium), sampling medium used for dilution technique (blood, urine, saliva, respiration), sample characteristics (sex [% female], age, height, weight, body mass index [BMI], and health status), and total SQ (expressed as a percentage of items satisfied). Health status of participants was dichotomized into "healthy" and "unhealthy", with effects from studies using participants suffering from chronic illness (e.g., COPD, HIV/AIDS, cancer, cerebral palsy) collapsed into the "unhealthy" category. Similarly, frequency was split into 3

categories: 50 kHz, 100 kHz, and "other". The latter category was comprised of uncommon frequencies including 5, 400, and 1000 kHz. Additionally, the interaction between frequency and index was examined. See SDC 2a and 2b for more information regarding study-level moderators. Individual moderators were examined using a multi-level random-effects model with maximum likelihood estimation. Significant and trending ($p < 0.10$) moderators were incorporated into a multi-level multiple-moderator model to identify significant relationships between variables, and to determine the proportion of unique variance attributable to study-level moderators. An adjusted pseudo-R^2 (46) was calculated to estimate the amount of heterogeneity accounted for by the final model. The number of moderators included in the model was limited to 26 (one per every ten effects) to avoid overfitting (47).

Potential bias

Potential reporting biases were identified by visual examination of a funnel plot for asymmetries (48) and confirmed using rank correlation (49) and regression tests (50). Outlying individual effects (i.e., studentized residual $\geq |3.0|$) were identified using procedures described by Viechtbauer and Cheung (51). Because results from meta-analyses may be unduly impacted by extreme values (40, 52, 53), a sensitivity analysis was performed in order to gauge the effect of potential outliers on the overall SMD.

Statistical analysis

Data were managed using Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Examination of effect size distribution, meta-regression, model comparison statistics, and plot construction were completing using the 'metafor' package (54) in R (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria). Aggregate-level descriptive characteristics are presented as mean ± SD unless otherwise stated. SMD effect size estimates

are reported as mean (95% confidence intervals). Standardized β coefficients, 95% confidence intervals, and *p* values are reported for moderators included in the multi-level regression models. Statistical significance for analyses was accepted at $p < 0.05$.

RESULTS

Study selection

Our systematic search of 5 databases yielded 2,050 potentially relevant records for review. Systematic screening of the records ultimately resulted in 51 original studies being identified for inclusion (55-105). Extraction of multiple effects from studies with > 1 comparison yielded a total of 264 effects. A detailed description of the search, screening, and identification of relevant records is shown in Figure 1.

Study and participant characteristics

On average, studies included 82 ± 197 participants and yielded 5 ± 5 (range 1 to 23) effects. Aggregate-level data from 21,556 predominantly healthy (i.e., free from metabolic, pulmonary, and cardiovascular disease) (68.2%) participants (54.5% female, 29.0 ± 18.8 years, 22.7 ± 4.5 $kg \cdot m^{-2}$) across 51 studies were included in the final analysis. Race/ethnicity of participants was infrequently reported (38.4% of total effects), so it was excluded from the quantitative analysis. A majority of studies reported mean bias (97.0%) and SD of the bias (73.1%), while others reported mean values, limits of agreement, *t*-statistics, or SEM values. Reported mean TBW values were 30.5 ± 12.0 L (predicted from SFBIA) and 30.5 ± 10.9 L (determined by chemical dilution). Average mean bias between predicted and criterion TBW was 0.1 ± 2.5 L (Table 1).

The most utilized SFBIA devices for the prediction of TBW were the RJL Model BIA 101 (28.0%), the Xitron Technologies Models 4000 and 4000B (23.5%), and the Bodystat

Quadscan 4000 (20.5%). A total of 12 different manufacturers and 21 SFBIA models were represented across our sample of studies. SFBIA procedures applying 50 kHz (90.5%) to the right side of the body (46.2%) utilizing a hand-to-foot (92.4%) electrode arrangement were most commonly reported. The predominant criterion technique for determination of TBW was D_2O dilution (95.1%), and most studies reported using salivary analysis for sampling (53.4%). See SDC 3 for procedural details of the included studies.

Assessment of study quality

The studies included in our analysis achieved a moderate (71.6 ± 9.7%) quality, with scores ranging from 40.4 to 85.5%. Items least likely to be satisfied included those related to sample bias (SQ8, 13.7%), adherence to pre-testing exercise guidelines (SQ11, 21.6%), and SFBIA test-retest reliability (SQ16, 33.3%). Regarding SFBIA and dilution technique standardization, only six of the 21 total items were reported in 50% or more of the studies. Overall and itemized SQ scores are provided in SDC 4.

Accuracy of SFBIA for estimating TBW

The cumulative results from 264 effects extracted from 51 original studies published between 1986 and 2019 revealed that SFBIA accurately estimated TBW when compared to isotope dilution techniques (SMD = 0.02, 95% *CI*: -0.17 to 0.18) (Figure 2). However, the mean SMD lacked homogeneity (Cochran's Q_{17} = 9240.3, $p < 0.001$, I^2 = 95.8%, 95% *CI*: 93.8 to 97.3%), indicating a large degree of between-study variability. Moderator analyses were conducted to identify potential sources of error in effect size.

Moderator analysis

Pre-planned subgroup comparisons were used to examine categorical moderators and revealed a significant ($p < 0.001$) interaction between frequency and index (Table 2). Impedance

index (i.e., Ht^2/Z) produced significant moderate to large errors in TBW estimation at 50 kHz (k = 57, SMD = -0.82, $p < 0.001$), 100 kHz (k = 12, SMD = 0.68, $p < 0.001$), and other reported frequencies (k = 1, SMD = 1.82, $p < 0.001$). Resistance index (i.e., Ht^2/R) exhibited small error at 50 kHz (k = 174, SMD = 0.40, $p < 0.001$) only. The effects of frequency and index are displayed in Figure 3. Prediction equations that included anthropometric measures were more accurate (k = 194, SMD = 0.12, $p = 0.19$) than those that only used resistivity index (k = 61, SMD = -0.37, $p < 0.001$). When equations were categorized by sex-specificity, neither group produced significant error in TBW estimation (all $p > 0.05$). Regarding criterion methodology, SFBIA significantly underestimated TBW determined via oxygen-18 dilution (k = 8, SMD = -0.60, $p = 0.007$) while comparisons with D_2O and tritium dilution produced small ($p > 0.10$) error. See Table 3 for a detailed summary of subgroup comparisons. Univariate meta-regressions were used to examine the potential moderating effects of continuous variables (Table 4). Results indicated that error in TBW estimation increased proportionally with sample height ($\beta = 0.011$, $p < 0.001$) and weight ($\beta = 0.012$, $p < 0.001$), while an inverse relationship was observed with increasing proportion of females in the sample ($\beta = -0.002$, $p < 0.001$). When significant subgroup and univariate moderators were combined into a single, multi-level multiple-moderator model, frequency and resistivity index were shown to exert the most influence on error in TBW estimation ($\beta = -1.10$, $p < 0.001$). Proportion of females in the sample also had a small effect ($\beta = -0.002$, $p < 0.001$). The full model accounted for approximately 63.3% of the between-study variability in effect size ($p < 0.001$).

Assessment of publication bias

Examination of the funnel plot (Figure 4) revealed a wide spread of study-level effects. Nine potential outliers with residual magnitudes ≥ 3.0 were identified from the 264 effects

(3.4%). The outliers were from 6 studies with very homogeneous samples of participants, including ill and healthy children (58, 59, 73), African women (66, 84), and elderly adults (98). All but one (98) study used Ht^2/Z to predict TBW, one study used 100 kHz (58), and the rest used a hand-to-foot configuration at 50 kHz. Despite the range of effect sizes and the potential outliers, an approximately symmetrical distribution of effects was observed and confirmed using rank correlation (Kendall's $\tau = -0.060$, $p = 0.15$) and regression ($z = -1.88$, $p = 0.06$) tests. A sensitivity analysis in which the 9 outlying effects were removed produced a similarly small overall effect size (SMD = 0.01, 95% *CI*: -0.17 to 0.18, $p = 0.95$) with a large degree of heterogeneity ($I^2 = 96.5$). Additionally, the distribution of effect sizes remained symmetrical in the reduced funnel plot (Figure 5) according to visual inspection as well as Begg's and Egger's tests (all $p > 0.05$).

DISCUSSION

The primary aim of this systematic review and meta-analysis was to examine the accuracy of SFBIA-derived estimations of TBW compared to criterion isotope dilution techniques, and to identify sources of variability in the observed error. The cumulative results from 264 effects extracted from 51 original studies indicate that SFBIA provides accurate estimates of TBW when compared to isotope dilution techniques. However, there was a large degree of heterogeneity in the degree of estimation error associated with SFBIA procedures (i.e., frequency and resistivity index), prediction equations, and participant characteristics. Other factors related to TBW assessment methodology including device, electrode arrangement, body side of the measurement, isotope tracer, and sampling medium did not modulate the overall effect size. Further, methodological SQ, publication year, participant health status, age, weight, and BMI did not impact the accuracy of SFBIA.

The primary factors associated with error in TBW estimation using SFBIA were frequency and resistivity index. Regarding frequency, 50 kHz was the most commonly used with SFBIA techniques (90.5% of the total effects). The use of 50 kHz is predicated on the assumption that electrical currents move through both intra- and extracellular fluid at lower frequencies, thereby providing an estimate of TBW (28). This assertion has been challenged, however, primarily by the allegation that 50 kHz is too low to completely penetrate cell membranes and thus does not adequately capture intracellular fluid volume (106). Although the use of 100 kHz is sometimes used to combat this issue (107-110), the same criticisms are often applied (20, 108, 111). Despite these limitations, the results of our aggregate-level analysis indicated that overall SMD was not independently affected by measures at either 50 or 100 kHz. Other reported frequencies were associated with significant error, however. These included SFBIA at 5 kHz, which is typically used to capture extracellular fluid volumes (100, 110, 112); 400 kHz, the rationale for which was not explicitly provided (100); and 1000 kHz, which has been shown to perform better than 50 kHz in HIV-positive patients (86). These frequencies were grouped into a single category due to lack of statistical power (only 8 effects reported in total).

Both resistivity indexes significantly modulated the overall SMD. Estimations of TBW using Ht^2/R produced overestimations compared to dilution techniques, while Ht^2/Z generally underestimated TBW. This finding is particularly interesting because it suggests that resistance and impedance values may not be interchangeable as commonly reported (25, 26, 113). Determination of fluid volumes via BIA is based upon the relationship between impedance and resistance, defined as $Z^2 = R^2 + Xc^2$, where Z is impedance, R is resistance, and Xc is reactance. Because the magnitude of reactance is very small at low frequencies (i.e., 50 kHz), it is generally considered negligible in practice (114, 115). Theoretically, this means that impedance and

resistance values are equivalent at low frequencies and thus can be used interchangeably. Although this rationale has been used to justify substitution of one value for another when using previously developed equations (59, 92, 116), there is a lack of empirical evidence demonstrating that Ht^2/R and Ht^2/Z can be swapped without consequence. Considering the findings of the present meta-analysis, we suggest that resistivity indexes be considered separate entities, and that authors use the original variables reported when using SFBIA prediction equations.

Examination of the interaction between frequency and resistivity indexes revealed that Ht^2/R outperformed Ht^2/Z across all reported frequencies. Notably, Ht^2/Z produced moderate effects at 50 and 100 kHz, while Ht^2/R produced small to moderate effects. Overall, the least error was observed when Ht^2/R was used in combination with 100 kHz. While improved accuracy of 100 kHz over 50 kHz when using Ht^2/R has been reported (110, 117), other authors have suggested that the use of 100 kHz offers no practical improvement over 50 kHz (108). Further, the previously discussed assertions that both frequencies are too low for fully encapsulating intracellular fluid volume seem to imply that the use of either is inadequate for assessment of TBW. Based on the results presented here, there appears to be increased accuracy when using Ht^2/R rather than Ht^2/Z, and 100 kHz rather than 50 kHz. From a practical standpoint, attention should be given to equations that utilize these particular parameters, although there are other considerations to be made regarding equation selection.

Across our sample of effects, more than 70 equations were used to predict TBW from SFBIA. Our analysis demonstrated that univariate equations (i.e., resistivity index is the sole predictor of TBW) contributed small but significant error to the overall SMD, while those that included other variables did not. This was not unexpected, as researchers frequently report that additional measures, especially weight, improve validity statistics when developing SFBIA

equations (63, 76, 98, 99, 108, 118-123). Theoretically, variables such as weight, age, and sex may help control for differences in body build, adiposity, and distribution of body water between the extra- and intracellular compartments (108). However, the inclusion of other variables for prediction of TBW, specifically weight, is not without its critics. For example, it has been suggested that a measure of body volume rather than weight be used in obese subjects, because the former more closely approximates the biophysical model on which BIA is based (63, 107). Additionally, rapid weight loss during caloric restriction may produce spurious results regarding fat-free mass change during nutritional interventions, while a measure of body volume reflects changes in adiposity without being directly related to fat-free mass (63). When entered into the multiple-moderator model, the effect of equation type was inconsequential, suggesting that other aspects of SFBIA methodology (i.e., frequency and index) were likely contributing to differences between equations.

The selection of isotope tracer used for criterion TBW determination was a source of error independent of other factors, though this effect was attenuated when combined with other factors. Of the 3 tracers reported in the reviewed studies, the use of oxygen-18 was associated with significant underestimation of TBW. This supports earlier work by Scholler et al. (22), who reported that oxygen-18 produced 3.0% lower TBW than D_2O. The authors suggested that oxygen-18 may yield more accurate estimates of TBW than deuterated or tritiated water because less exchange with nonaqueous body compartments (i.e., proteins and carbohydrates) occurs (22), a phenomenon to which the former isotopes are especially prone (124-126). Additionally, oxygen-18 is non-toxic, nonradioactive, and can be measured via expired CO_2 (22), making it a more appropriate tracer than D_2O or tritium for use with special populations including pregnant women, young children (75), older adults (98), and chronically ill persons (91). Despite the

proposed benefits, only 3.1% of the effects reviewed in the present study reported using oxygen-18 for SFBIA validation, while the vast majority (95.3%) used D_2O.

Sample characteristics including sex ($\beta = -0.002$), height ($\beta = 0.004$), and weight ($\beta = 0.005$) produced similarly small effects on TBW error in the multiple-moderator model, while only sex (expressed as the percentage of female participants within the sample) remained influential. Although this result implies error between SFBIA and criterion TBW values decrease as the proportion of women in a sample increases, the magnitude of the coefficient suggests that the effect is trivial. Additionally, subgroup comparison of equations categorized by sex-specificity showed that the inclusion of sex did not moderate the overall SMD, which indicates that differences between men and women may be accounted for by anthropometric variables. Differences in estimation error between men and women have been demonstrated in studies comparing BIS to criterion dilution techniques, with several authors reporting smaller error in women than men when comparing BIS to criterion methods (23, 127, 128).

There were a number of unexpected results that emerged from this meta-analysis. For instance, subgroup comparisons indicated that error in TBW estimation was similar across electrode arrangement (i.e., hand to foot versus foot to foot), body side used for measurement, and SFBIA device. Regarding criterion TBW measures, the sampling medium did not contribute to error in overall SMD, although differences between methods has been reported (129). Participant characteristics including health status, age, and BMI were also inconsequential. The lack of effect of health status is especially interesting, as researchers often note that the presence of disease may alter hydration status and thus impact TBW estimates (74, 130, 131). It is possible that the sum of positive and negative errors associated with different conditions produced a “washout” effect when categorized together and compared against effects with

healthy participants. Due to the variability in disease conditions represented in the selected studies, however, it was not possible to fractionate the subgroup into smaller categories.

This systematic review and meta-analysis is not without limitations. The search strategy was broad and inclusive, so as to capture as much of the desired literature as possible. However, variations in search terms or use of another database may have produced different results. Additionally, the initial records returned from the electronic search (n = 2,050) were screened for relevance using the built-in filters associated with each database before being imported into a citation manager for title/abstract review. It is entirely possible that different screening procedures may have resulted in different records. There were also methodological limitations imposed by the available data. For instance, the pre-identified moderators were often either highly homogeneous (i.e., > 90% of the total effects used the same frequency, isotope tracer, and electrode arrangement) or highly heterogeneous (i.e., > 70 different equations, > 20 different models) across the effects, making certain subgroup comparisons statistically unviable and thus limiting our ability to explore potential interactions. In the latter case, the decision was made to collapse certain factor levels in order to allow for meaningful statistical comparisons, which may potentially obscure large effects or create a "washout" effect as previously discussed. Despite these limitations, we feel confident that the results presented here accurately reflect the current body of literature.

CONCLUSION

Although another meta-analysis evaluating the accuracy of SFBIA, MFBIA, and BIS techniques has been published (132), the scope of the paper was extremely limited (n = 16 studies) and the authors did not report homogeneity of effect size, conduct moderator analyses, or assess potential publication bias. To our knowledge, this is the first comprehensive systematic

review and meta-analysis evaluating the accuracy of the SFBIA technique for estimation of TBW. Our search strategy allowed for the inclusion of wide ranges of age, body composition, health condition, race and ethnicity, and measurement techniques. Additionally, the literature search was not restricted by SFBIA or criterion methodology. The multi-level approach to meta-analysis permitted the calculation of an overall weighted mean effect size while accounting for nesting of effects within studies and yielded a small SMD across all 255 effects. Moderator analyses showed that error in SFBIA estimation of TBW was primarily affected by frequency, resistivity index, and equation. Specifically, the use of Ht^2/R at 100 kHz produced the least error in TBW estimation, while Ht^2/Z resulted in statistically significant error across all frequencies. Additionally, equations that included a measure of weight or some other anthropometric variable generally outperformed equations based solely on resistivity index. Overall, these findings indicate that SFBIA is a valid method for estimating TBW. However, care should be taken to ensure the parameters discussed in this report are utilized in order to minimize the potential error associated with TBW estimation.

REFERENCES

1. Ganio MS, Armstrong LE, Casa DJ, McDermott BP, Lee EC, Yamamoto LM, et al. Mild dehydration impairs cognitive performance and mood of men. British Journal of Nutrition. 2011;106(10):1535-43.

2. Armstrong LE, Ganio MS, Casa DJ, Lee EC, McDermott BP, Klau JF, et al. Mild dehydration affects mood in healthy young women. The Journal of Nutrition. 2011;142(2):382-8.

3. Armstrong LE, Costill DL, Fink WJ. Influence of diuretic-induced dehydration on competitive running performance. Medicine and Science in Sports and Exercise. 1985;17(4):456-61.

4. Montazer S, Farshad AA, Monazzam MR, Eyvazlou M, Yaraghi AAS, Mirkazemi R. Assessment of construction workers' hydration status using urine specific gravity. International Journal of Occupational Medicine and Environmental Health. 2013;26(5):762-9.

5. Siri WE. Body composition from fluid spaces and density: analysis of methods. Techniques for Measuring Body Composition. 1961;61:223-44.

6. Wang Z, Deurenberg P, Guo SS, Pietrobelli A, Wang J, Pierson Jr R, et al. Six-compartment body composition model: inter-method comparisons of total body fat measurement. International Journal of Obesity. 1998;22(4):329.

7. Withers RT, Laforgia J, Heymsfield S. Critical appraisal of the estimation of body composition via two-, three-, and four-compartment models. American Journal of Human Biology: The Official Journal of the Human Biology Association. 1999;11(2):175-85.

8. Withers RT, Laforgia J, Heymsfield S, Wang Z-M, Pillans R. Two, three and four-compartment chemical models of body composition analysis. Anthropometrica. UNSW, Sydney, Australia1996. p. 199-231.

9. Withers RT, LaForgia J, Pillans R, Shipp N, Chatterton B, Schultz C, et al. Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women. Journal of Applied Physiology. 1998;85(1):238-45.

10. Kerr A, Slater G, Byrne N, Chaseling J. Validation of bioelectrical impedance spectroscopy to measure total body water in resistance-trained males. International Journal of Sport Nutrition and Exercise Metabolism. 2015;25(5):494-503.

11. Moon JR, Eckerson JM, Tobkin SE, Smith AE, Lockwood CM, Walter AA, et al. Estimating body fat in NCAA Division I female athletes: a five-compartment model validation of laboratory methods. European Journal of Applied Physiology and Occupational Physiology. 2009;105(1):119-30.

12. Machek P, Jirka T, Moissl U, Chamney P, Wabel P. Guided optimization of fluid status in haemodialysis patients. Nephrology Dialysis Transplantation. 2009;25(2):538-44.

13. Wizemann V, Wabel P, Chamney P, Zaluska W, Moissl U, Rode C, et al. The mortality risk of overhydration in haemodialysis patients. Nephrology Dialysis Transplantation. 2009;24(5):1574-9.

14. Devolder I, Verleysen A, Vijt D, Vanholder R, Van Biesen W. Body composition, hydration, and related parameters in hemodialysis versus peritoneal dialysis patients. Peritoneal Dialysis International. 2010;30(2):208-14.

15. Van Biesen W, Williams JD, Covic AC, Fan S, Claes K, Lichodziejewska-Niemierko M, et al. Fluid status in peritoneal dialysis patients: the European Body Composition Monitoring (EuroBCM) study cohort. PloS one. 2011;6(2):e17148.

16. Hung S-C, Kuo K-L, Peng C-H, Wu C-H, Lien Y-C, Wang Y-C, et al. Volume overload correlates with cardiovascular risk factors in patients with chronic kidney disease. Kidney International. 2014;85(3):703-9.

17. Simons J, Schols A, Westerterp KR, Ten Velde G, Wouters E. The use of bioelectrical impedance analysis to predict total body water in patients with cancer cachexia. The American Journal of Clinical Nutrition. 1995;61(4):741-5.

18. Cornish B, Chapman M, Hirst C, Mirolo B, Bunce I, Ward L, et al. Early diagnosis of lymphedema using multiple frequency bioimpedance. Lymphology. 2001;34(1):2-11.

19. York S, Ward L, Czerniec S, Lee M, Refshauge K, Kilbreath S. Single frequency versus bioimpedance spectroscopy for the assessment of lymphedema. Breast Cancer Research and Treatment. 2009;117(1):177.

20. Moon J. Body composition in athletes and sports nutrition: an examination of the bioimpedance analysis technique. European Journal of Clinical Nutrition. 2013;67(S1):S54.

21. Schloerb PR, Friis-Hansen BJ, Edelman IS, Solomon A, Moore FD. The measurement of total body water in the human subject by deuterium oxide dilution: With a consideration of the dynamics of deuterium distribution. The Journal of Clinical Investigation. 1950;29(10):1296-310.

22. Schoeller D, Van Santen E, Peterson D, Dietz W, Jaspan J, Klein P. Total body water measurement in humans with 18O and 2H labeled water. The American Journal of Clinical Nutrition. 1980;33(12):2686-93.

23. Moon JR, Tobkin SE, Roberts MD, Dalbo VJ, Kerksick CM, Bemben MG, et al. Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. Nutrition and Metabolism. 2008;5(1):7.

24. Chumlea WC, Guo SS. Bioelectrical impedance and body composition: present status and future directions. Nutrition Reviews. 1994;52(4):123-31.

25. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. Journal of Applied Physiology. 1986;60(4):1327-32.

26. van Marken Lichtenbelt WD, Westerterp KR, Wouters L, Luijendijk SC. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. The American Journal of Clinical Nutrition. 1994;60(2):159-66.

27. Khalil SF, Mohktar MS, Ibrahim F. The theory and fundamentals of bioimpedance analysis in clinical status monitoring and diagnosis of diseases. Sensors. 2014;14(6):10895-928.

28. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, et al. Bioelectrical impedance analysis—part I: review of principles and methods. Clinical Nutrition. 2004;23(5):1226-43.

29. Lukaski HC. Comparison of proximal and distal placements of electrodes to assess human body composition by bioelectrical impedance. In Human Body Composition 1993 (pp. 39-43). Springer, Boston, MA.

30. Bosy-Westphal A, Later W, Hitze B, Sato T, Kossel E, Glüer C-C, et al. Accuracy of bioelectrical impedance consumer devices for measurement of body composition in comparison to whole body magnetic resonance imaging and dual X-ray absorptiometry. Obesity Facts. 2008;1(6):319-24.

31. Esco MR, Olson MS, Williford HN, Lizana SN, Russell AR. The accuracy of hand-to-hand bioelectrical impedance analysis in predicting body composition in college-age female athletes. The Journal of Strength and Conditioning Research. 2011;25(4):1040-5.

32. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Journal of Clinical Epidemiology. 2009;62:1006-12.

33. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Reviews. 2015;4(1):1.

34. Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, et al. Development of AMSTAR: a measurement tool to assess the methodological quality of systematic reviews. BMC Medical Research Methodology. 2007;7(1):10.

35. Shea BJ, Hamel C, Wells GA, Bouter LM, Kristjansson E, Grimshaw J, et al. AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. Journal of Clinical Epidemiology. 2009;62(10):1013-20.

36. Gonzalez C, Evans J, Smye S, Holland P. Total body water measurement using bioelectrical impedance analysis, isotope dilution and total body potassium: a scoring system to facilitate intercomparison. European Journal of Clinical Nutrition. 2002;56(4):326.

37. Gibbons RD, Hedeker DR, Davis JM. Estimation of effect size from a series of experiments involving paired comparisons. Journal of Educational Statistics. 1993;18(3):271-9.

38. Hedges LV. Distribution theory for Glass's estimator of effect size and related estimators. Journal of Educational Statistics. 1981;6(2):107-28.

39. Lipsey MW, Wilson DB. Practical Meta-Analysis: Sage Publications, Inc; 2001.

40. Pastor DA, Lazowski RA. On the multilevel nature of meta-analysis: a tutorial, comparison of software programs, and discussion of analytic choices. Multivariate Behavioral Research. 2018;53(1):74-89.

41. Olkin I, Gleser L. Stochastically dependent effect sizes. The Handbook of Research Synthesis and Meta-Analysis. 2009:357-76.

42. Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.

43. Cochran WG. The combination of estimates from different experiments. Biometrics. 1954;10(1):101-29.

44. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in Medicine. 2002;21(11):1539-58.

45. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I^2 index? Psychological Methods. 2006;11(2):193.

46. McFadden D. Quantitative methods for analyzing travel behavior of individuals: some recent developments: Institute of Transportation Studies, University of California Berkeley, CA; 1977.

47. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions 5.1. 0. The Cochrane Collaboration. 2011:33-49.

48. Sterne J, Egger M, Moher D. Addressing Reporting Biases. In: Higgins J, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions: The Cochrane Collaboration; 2008.

49. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088-101.

50. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical research ed). 1997;315(7109):629-34.

51. Viechtbauer W, Cheung MWL. Outlier and influence diagnostics for meta-analysis. Research Synthesis Methods. 2010;1(2):112-25.

52. Hedges L, Olkin I. Statistical Models for Meta-Analysis: New York: Academic Press; 1985.

53. Huffcutt AI, Arthur W. Development of a new outlier statistic for meta-analytic data. Journal of Applied Psychology. 1995;80(2):327.

54. Viechtbauer W. Conduction meta-analysis in R with the metafor package. Journal of Statistical Software. 2010;36(3):1-48.

55. Aglago KE, El Menchawy I, El Kari K, El Hamdouchi A, Barkat A, Benguedour R, et al. Development and validation of bioelectrical impedance analysis equations for predicting total body water and fat-free mass in North-African adults. European Journal of Clinical Nutrition. 2013;67(10):1081-6.

56. Al-Bachir M, Othman I. Body Fat Assessment of Young Syrian Adults Using Bioelectrical Impedance Analysis and Deuterium Dilution. Malaysian Journal of Nutrition. 2016;22(2):173-82.

57. Bauer J, Capra S, Davies PSW. Estimation of total body water from foot-to-foot bioelectrical impedance analysis in patients with cancer cachexia -- agreement between three prediction methods and deuterium oxide dilution. Journal of Human Nutrition and Dietetics. 2005;18(4):295-300.

58. Beertema W, van Hezewijk M, Kester A, Forget PP, van Kreel B. Measurement of total body water in children using bioelectrical impedance: a comparison of several prediction equations. Journal of Pediatric Gastroenterology and Nutrition. 2000;31(4):428-32.

59. Bell KL, Boyd RN, Walker JL, Stevenson RD, Davies PSW. The use of bioelectrical impedance analysis to estimate total body water in young children with cerebral palsy. Clinical Nutrition. 2013;32(4):579-84.

60. Bell NA, McClure PD, Hill RJ, Davies PS. Assessment of foot-to-foot bioelectrical impedance analysis for the prediction of total body water. European Journal of Clinical Nutrition. 1998;52(11):856-9.

61. Ben Jemaa H, Mankai A, Khlifi S, Minaoui R, Ghozzi D, Zediri M, et al. Development and validation of impedance-based equations for the prediction of total body water and fat-free mass in children aged 8-11 years. Clinical Nutrition. 2019;38(1):227-33.

62. Danford LC, Schoeller DA, Kushner RF. Comparison of two bioelectrical impedance analysis models for total body water measurement in children. Annals of Human Biology. 1992;19(6):603-7.

63. De Lorenzo A, Sorge R, Candeloro N, Di Campli C, Sesti G, Lauro R. New insights into body composition assessment in obese women. Canadian Journal of Physiology and Pharmacology. 1999;77(1):17-21.

64. Diouf A, Diongue O, Nde M, Idohou-Dossou N, Thiam M, Wade S. Validity of bioelectrical impedance analysis in predicting total body water and adiposity among Senegalese school-aged children. PLoS One. 2018;13(10):e0204486.

65. Diouf A, Gartner A, Dossou NI, Sanon DA, Bluck L, Wright A, et al. Validity of impedance-based predictions of total body water as measured by 2H dilution in African HIV/AIDS outpatients. The British Journal of Nutrition. 2009;101(9):1369-77.

66. Dioum A, Gartner A, Cisse AS, Delpeuch F, Maire B, Wade S, et al. Validity of impedance-based equations for the prediction of total body water as measured by deuterium dilution in African women. American Journal of Clinical Nutrition. 2005;81(3):597-604.

67. El Harchaoui I, El Hamdouchi A, Baddou I, El Menchawy I, Benjeddou K, Saeid N, et al. Development and validation of bioelectrical impedance analysis equations for prediction total body water and fat-free mass using D2O technique in Moroccan children aged between 8 and 11 years old. European Journal of Clinical Nutrition. 2018;72(12):1663-72.

68. Fjeld CR, Freundt-Thurne J, Schoeller DA. Total body water measured by 18 O dilution and bioelectrical impedance in well and malnourished children. Pediatric Research. 1990;27(1):98.

69. Gregory J, Greene S, Scrimgeour C, Rennie M. Body water measurement in growth disorders: a comparison of bioelectrical impedance and skinfold thickness techniques with isotope dilution. Archives of Disease in Childhood. 1991;66(2):220-2.

70. Haas V, Schutz T, Engeli S, Schroder C, Westerterp K, Boschmann M. Comparing single-frequency bioelectrical impedance analysis against deuterium dilution to assess total body water. European Journal of Clinical Nutrition. 2012;66(9):994-7.

71. Haroun D, Taylor SJ, Viner RM, Hayward RS, Darch TS, Eaton S, Cole TJ, Wells JC. Validation of bioelectrical impedance analysis in adolescents across different ethnic groups. Obesity. 2010 Jun;18(6):1252-9.

72. Hastuti J, Kagawa M, Byrne NM, Hills AP. Proposal of new body composition prediction equations from bioelectrical impedance for Indonesian men. European Journal of Clinical Nutrition. 2016;70(11):1271-7.

73. Horlick M, Arpadi SM, Bethel J, Wang J, Moye J, Cuff P, et al. Bioelectrical impedance analysis models for prediction of total body water and fat-free mass in healthy and HIV-infected children and adolescents. American Journal of Clinical Nutrition. 2002;76(5):991-9.

74. Isenring E, Bauer J, Capra S, Davies PSW. Evaluation of foot-to-foot bioelectrical impedance analysis for the prediction of total body water in oncology outpatients receiving radiotherapy. European Journal of Clinical Nutrition. 2004;58(1):46-51.

75. Kushner R, Schoeller DA, Fjeld CR, Danford L. Is the impedance index (ht2/R) significant in predicting total body water? The American Journal of Clinical Nutrition. 1992;56(5):835-9.

76. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. The American Journal of Clinical Nutrition. 1986;44(3):417-24.

77. Liu A, Byrne NM, Ma G, Nasreddine L, Trinidad TP, Kijboonchoo K, et al. Validation of bioelectrical impedance analysis for total body water assessment against the deuterium dilution technique in Asian children. European Journal of Clinical Nutrition. 2011;65(12):1321-7.

78. Lukaski HC, Bolonchuk WW. Estimation of body fluid volumes using tetrapolar bioelectrical impedance measurements. Aviation, Space, and Environmental Medicine. 1988;59(12):1163-9.

79. Masuda T, Komiya S. A prediction equation for total body water from bioelectrical impedance in Japanese children. Journal of Physiological Anthropology and Applied Human Science. 2004;23(2):35-9.

80. Matias CN, Judice PB, Santos DA, Magalhaes JP, Minderico CS, Fields DA, et al. Suitability of Bioelectrical Based Methods to Assess Water Compartments in Recreational and Elite Athletes. Journal of the American College of Nutrition. 2016;35(5):413-21.

81. Matias CN, Santos DA, Júdice PB, Magalhães JP, Minderico CS, Fields DA, et al. Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models. Clinical Nutrition. 2016;35(2):468-74.

82. Medoua GN, Abomo ACN, Essa'a VJ, Sobgui CM, Messomo MTG, Rikong HA. Validity of impedance-based equations for the prediction of total body water as measured by deuterium dilution in Cameroonian HIV-infected patients treated with antiretroviral treatment. Clinical Nutrition. 2008;27(6):881-8.

83. Medoua GN, Essa'a VJ, Tankou CT, Ndzana ACA, Dimodi HT, Ntsama PM. Validity of anthropometry- and impedance-based equations for the prediction of total body water as measured by deuterium dilution in Cameroonian haemodialysis patients. Clinical Nutrition ESPEN. 2015;10(5):E167-E73.

84. Medoua GN, Nana ES, Essa'a VJ, Ntsama PM, Matchawe C, Rikong HA, et al. Body composition of Cameroonian lactating women determined by anthropometry, bioelectrical impedance, and deuterium dilution. Nutrition. 2011;27(4):414-9.

85. Patel RV, Matthie JR, Withers PO, Peterson EL, Zarowitz BJ. Estimation of total body and extracellular water using single- and multiple-frequency bioimpedance. The Annals of Pharmacotherapy. 1994;28(5):565-9.

86. Paton NI, Elia M, Jennings G, Ward LC, Griffin GE. Bioelectrical impedance analysis in human immunodeficiency virus-infected patients: Comparison of single frequency with multifrequency, spectroscopy, and other novel approaches. Nutrition. 1998;14(9):658-66.

87. Puiman PJ, Francis P, Buntain H, Wainwright C, Masters B, Davies PS. Total body water in children with cystic fibrosis using bioelectrical impedance. Journal of Cystic Fibrosis. 2004 Dec 1;3(4):243-7.

88. Rallison LR, Kushner RF, Penn D, Schoeller DA. Errors in estimating peritoneal fluid by bioelectrical impedance analysis and total body electrical conductivity. Journal of the American College of Nutrition. 1993;12(1):66-72.

89. Maria Mendes Resende C, Simon Camelo Junior J, Neves Campanelli Marcal Vieira M, da Silva Castro Perdona G, Ferriolli E, Pfrimer K, Giaretta Mathias M, Pontes Monteiro J. Body composition in obese adolescents: deuterium oxide dilution method, bioelectrical impedance and predictive equations. Current Nutrition and Food Science. 2013 Feb 1;9(1):73-81.

90. Richards ML, Bell SC, Edmiston KA, Davies PS. Assessment of bioelectrical impedance analysis for the prediction of total body water in cystic fibrosis. Asia Pacific Journal of Clinical Nutrition. 2003;12(2):161-5.

91. Ritz P. Bioelectrical impedance analysis estimation of water compartments in elderly diseased patients: the source study. The journals of gerontology Series A, Biological Sciences and Medical Sciences. 2001;56(6):M344-8.

92. Scalfi L, Bedogni G, Marra M, DiBiase G, Caldara A, Severi S, et al. The prediction of total body water from bioelectrical impedance in patients with anorexia nervosa. British Journal of Nutrition. 1997;78(3):357-65.

93. Sen B, Mahalanabis D, Kurpad AV, Shaikh S, Bose K. Total body water and fat-free mass: evaluation of equations based on bioelectrical impedance analysis in infants and young children in India. British Journal of Nutrition. 2010 Jul;104(2):256-64.

94. Seoane F, Abtahi S, Abtahi F, Ellegard L, Johannsson G, Bosaeus I, et al. Slightly superior performance of bioimpedance spectroscopy over single frequency regression equations for assessment of total body water. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. 2015;2015:3707-10.

95. Shaikh S, Mahalanabis D, Kurpad AV, Khaled MA. Validation of an anthropometric equation and bioelectrical impedance analysis (BIA) technique to measure body composition of children in India using D2O dilution method. Nutrition Research. 2002;22(6):685-94.

96. Sluys T, Van der Ende M, Swart G, Van den Berg J, Wilson J. Body composition in patients with acquired immunodeficiency syndrome: a validation study of bioelectric impedance analysis. Journal of Parenteral and Enteral Nutrition. 1993;17(5):404-6.

97. Strain GW, Wang J, Gagner M, Pomp A, Inabnet WB, Heymsfield SB. Bioimpedance for severe obesity: Comparing research methods for total body water and resting energy expenditure. Obesity. 2008;16(8):1953-6.

98. Vache C, Rousset P, Gachon P, Gachon AM, Morio B, Boulier A, et al. Bioelectrical impedance analysis measurements of total body water and extracellular water in healthy elderly subjects. International Journal of Obesity. 1998;22(6):537-43.

99. Van Loan M, Mayclin P. Bioelectrical impedance analysis: is it a reliable estimator of lean body mass and total body water? Human Biology. 1987:299-309.

100. van Marken Lichtenbelt WD, Westerterp KR, Wouters L, Luijendijk SC. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. Am J Clinical Nutrition. 1994;60(2):159-66.

101. Visser M, Deurenberg P, Van Staveren WA. Multi-frequency bioelectrical impedance for assessing total body water and extracellular water in elderly subjects. European Journal of Clinical Nutrition. 1995;49(4):256-66.

102. Wickramasinghe V, Lamabadusuriya S, Cleghorn GJ, Davies P. Assessment of body composition in Sri Lankan children: validation of a bioelectrical impedance prediction equation. European Journal of Clinical Nutrition. 2008;62(10):1170.

103. Widen EM, Strain G, King WC, Yu W, Lin S, Goodpaster B, et al. Validity of bioelectrical impedance analysis for measuring changes in body water and percent fat after bariatric surgery. Obesity Surgery. 2014;24(6):847-54.

104. Woodrow G, Oldroyd B, Turney JH, Davies PSW, Day JME, Smith MA. Measurement of total body water by bioelectrical impedance in chronic renal failure. European Journal of Clinical Nutrition. 1996;50(10):676-81.

105. Zillikens MC, van den Berg JW, Wilson JH, Rietveld T, Swart GR. The validity of bioelectrical impedance analysis in estimating total body water in patients with cirrhosis. Journal of Hepatology. 1992;16(1-2):59-65.

106. Matthie JR. Bioimpedance measurements of human body composition: critical analysis and outlook. Expert Review of Medical Devices. 2008;5(2):239-61.

107. De Lorenzo A, Sasso G, Andreoli A, Sorge R, Candeloro N, Cairella M. Improved prediction formula for total body water assessment in obese women. International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity. 1995;19(8):535-8.

108. Deurenberg P, Tagliabue A, Schouten FJ. Multi-frequency impedance for the prediction of extracellular water and total body water. British Journal of Nutrition. 1995;73(3):349-58.

109. Vache C, Rousset P, Gachon P, Gachon A, Morio B, Boulier A, et al. Bioelectrical impedance analysis measurements of total body water and extracellular water in healthy elderly subjects. International Journal of Obesity. 1998;22(6):537.

110. Segal KR, Burastero S, Chun A, Coronel P, Pierson Jr RN, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. The American Journal of Clinical Nutrition. 1991;54(1):26-9.

111. Thomasset M. Bioelectric properties of tissue. Impedance measurement in clinical medicine. Significance of curves obtained. Lyon Medical. 1962;94:107.

112. Jenin P, Lenoir J, Roullet C, Thomasset A, Ducrot H. Determination of body fluid compartments by electrical impedance measurements. Aviation, Space, and Environmental Medicine. 1975;46(2):152-5.

113. National Institutes of Health (US). Office of Medical Applications of Research. Bioelectrical impedance analysis in body composition measurement: National Institutes of Health Technology Assessment Conference Statement, December 12-14, 1994. NIH Office of Medical Applications of Research; 1994.

114. Baumgartner RN, Chumlea WC, Roche AF. Bioelectric impedance phase angle and body composition. The American Journal of Clinical Nutrition. 1988;48(1):16-23.

115. Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. American Journal of Clinical Nutrition. 1985;41(4):810-7.

116. Borghil A, Bedogni G, Rocchi E, Severi S, Farina F, Battistini N. Multi-frequency bioelectric impedance measurements for predicting body water compartments in patients with non-ascitic liver cirrhosis. British Journal of Nutrition. 1996;76(3):325-32.

117. Khaled MA, Kabir I, Goran MI, Mahalanabis D. Bioelectrical impedance measurements at various frequencies to estimate human body compositions. Indian Journal of Experimental Biology. 1997 Feb;35(2):159-61.

118. Cornish BH, Ward LC, Thomas BJ, Jebb SA, Elia M. Evaluation of multiple frequency bioelectrical impedance and Cole-Cole analysis for the assessment of body water volumes in healthy humans. European Journal of Clinical Nutrition. 1996;50(3):159-64.

119. Heitmann B. Prediction of body water and fat in adult Danes from measurement of electrical impedance. A validation study. International Journal of Obesity. 1990;14(9):789-802.

120. Kotler DP, Burastero S, Wang J, Pierson RN. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: Effects of race, sex, and disease. American Journal of Clinical Nutrition. 1996;64(3):489-97.

121. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. The American Journal of Clinical Nutrition. 2003;77(2):331-40.

122. Cox-Reijven PL, Soeters PB. Validation of bio-impedance spectroscopy: Effects of degree of obesity and ways of calculating volumes from measured resistance values. International Journal of Obesity. 2000;24(3):271-80.

123. Visser M, Deurenberg P, van Staveren WA. Multi-frequency bioelectrical impedance for assessing total body water and extracellular water in elderly subjects. European Journal of Clinical Nutrition. 1995;49(4):256-66.

124. Wagner DR, Heyward VH. Techniques of body composition assessment: a review of laboratory and field methods. Research Quarterly for Exercise and Sport. 1999;70(2):135-49.

125. Pinson EA. Water exchanges and barriers as studied by the use of hydrogen isotopes. Physiological Reviews. 1952;32(2):123-34.

126. Culebras JM, Moore FD. Total body water and the exchangeable hydrogen. I. Theoretical calculation of nonaqueous exchangeable hydrogen in man. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1977;232(1):R54-R9.

127. Moon JR, Stout JR, Smith AE, Tobkin SE, Lockwood CM, Kendall KL, et al. Reproducibility and validity of bioimpedance spectroscopy for tracking changes in total body water: implications for repeated measurements. British Journal of Nutrition. 2010;104(9):1384-94.

128. Moon JR, Smith AE, Tobkin SE, Lockwood CM, Kendall KL, Graef JL, et al. Total body water changes after an exercise intervention tracked using bioimpedance spectroscopy: a deuterium oxide comparison. Clinical Nutrition. 2009;28(5):516-25.

129. Wong WW, Cochran WJ, Klish WJ, Smith E, Lee LS, Klein PD. In vivo isotope-fractionation factors and the measurement of deuterium-and oxygen-18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. The American Journal of Clinical Nutrition. 1988;47(1):1-6.

130. Simons JP, Schols AM, Westerterp KR, ten Velde GP, Wouters EF. The use of bioelectrical impedance analysis to predict total body water in patients with cancer cachexia. The American Journal of Clinical Nutrition. 1995;61(4):741-5.

131. Koulmann N, Jimenez C, Regal D, Bolliet P, Launay JC, Savourey G, et al. Use of bioelectrical impedance analysis to estimate body fluid compartments after acute variations of the body hydration level. Medicine and Science in Sports and Exercise. 2000;32(4):857-64.

132. Martinoli R, Mohamed E, Maiolo C, Cianci R, Denoth F, Salvadori S, et al. Total body water estimation using bioelectrical impedance: a meta-analysis of the data available in the literature. Acta Diabetologica. 2003;40(1):s203-s6.

Table 1. Summary statistics of the aggregate sample (n = 21,556) from 51 studies

Variable	Mean ± *SD*	Range
Sample size	82 ± 197	6 to 1,170
Number of effects	5 ± 5	1 to 23
Age (y)	29 ± 19	0.0 to 81.8
Height (cm)	156.4 ± 26.4	41.0 to 185.0
Weight (kg)	56.5 ± 20.3	1.6 to 128.2
BMI ($kg \cdot m^{-2}$)	22.7 ± 4.5	9.5 to 50.2
Sex (% female)	54.5 ± 38.6	0.0 to 100.0
SFBIA TBW (L)	30.5 ± 12.0	4.9 to 49.5
Criterion TBW (L)	30.5 ± 10.9	1.3 to 51.2
Bias (SFBIA – Criterion) (L)	0.1 ± 2.5	-14.3 to 8.6

SFBIA, single frequency bioimpedance analysis; TBW, total body water.

Table 2. Effect of frequency and resistivity index on the accuracy of SFBIA

Index	Frequency (kHz)	SMD (95% *CI*)	*p* value
Ht^2/Z	50	-0.821 (-1.035 to -0.607)	< 0.001
Ht^2/Z	100	0.676 (0.407 to 0.946)	< 0.001
Ht^2/Z	Other	1.818 (1.167 to 2.470)	< 0.001
Ht^2/R	50	0.397 (0.185 to 0.609)	< 0.001
Ht^2/R	100	0.102 (-0.146 to 0.350)	0.42
Ht^2/R	Other	0.237 (-0.213 to 0.687)	0.30

CI, confidence interval; Ht, height; kHz, kilohertz; *R*, resistance; SE, standard error; SFBIA, single-frequency bioelectrical impedance analysis; SMD, standardized mean difference; TBW, total body water; *Z*, impedance.

Table 3. Summary of subgroup analyses

Moderator	*k* (%)	SMD (95% *CI*)	*p* value
SFBIA device			
Model BIA 101	74 (28.0)	0.185 (-0.090 to 0.460)	0.19
Quadscan 4000	54 (20.5)	0.167 (-0.347 to 0.681)	0.53
Xitron 4000/B	62 (23.5)	-0.174 (-0.630 to 0.282)	0.45
Other	74 (28.0)	-0.042 (-0.276 to 0.191)	0.72
Frequency (kHz)			
50	239 (90.5)	0.002 (-0.176 to 0.180)	0.98
100	20 (7.6)	0.185 (-0.018 to 0.388)	0.07
Other	5 (1.9)	0.547 (0.171 to 0.922)	0.004
Resistance index			
Ht^2/Z	78 (29.5)	-0.716 (-0.929 to -0.504)	< 0.001
Ht^2/R	186 (70.5)	0.433 (0.222 to 0.644)	< 0.001
Electrode arrangement			
Hand-to-foot	244 (92.4)	0.030 (-0.157 to 0.217)	0.75
Foot-to-foot	20 (7.6)	-0.071 (-0.635 to 0.494)	0.81
Side of the body			
Right	122 (46.2)	-0.161 (-0.402 to 0.079)	0.19
Left	41 (15.5)	0.150 (-0.219 to 0.520)	0.43
Other	101 (38.3)	0.218 (-0.034 to 0.470)	0.09
Equation variables			
Index only	65 (24.6)	-0.365 (-0.550 to -0.180)	< 0.001
Anthropometric	199 (75.4)	0.122 (-0.061 to 0.304)	0.19
Equation type (biological sex)			
Not sex-specific	166 (62.9)	0.090 (-0.086 to 0.267)	0.32
Sex-specific	98 (37.1)	-0.108 (-0.286 to 0.071)	0.24
Criterion TBW isotope tracer			
Deuterium oxide	251 (95.1)	0.062 (-0.124 to 0.248)	0.51
Tritium	4 (1.5)	-0.313 (-1.583 to 0.956)	0.63
Oxygen-18	9 (3.4)	-0.595 (-1.027 to -0.164)	0.01
Criterion TBW fluid sample			
Blood	49 (18.6)	-0.114 (-0.503 to 0.276)	0.57
Urine	72 (27.3)	-0.171 (-0.436 to 0.095)	0.21
Saliva	141 (53.4)	0.244 (-0.007 to 0.496)	0.06
Respiratory	2 (0.8)	0.224 (-1.029 to 1.476)	0.73
Health status of participants			
Healthy	187 (70.8)	-0.014 (-0.197 to 0.170)	0.88
Unhealthy	77 (29.2)	0.077 (-0.119 to 0.273)	0.44

%, proportion of total effects; *CI*, confidence interval; Ht, height; kHz, kilohertz; *k*, number of effects; *R*, resistance; SFBIA, single-frequency bioelectrical impedance analysis; SMD, standardized mean difference effect size; TBW, total body water; *Z*, impedance.

Table 4. Summary of univariate multi-level meta-regression

Moderator	β (95% *CI*)	*p* value
Publication year	0.002 (-0.017 to 0.021)	0.83
Study quality (%)	-0.001 (-0.019 to 0.017)	0.90
Age	0.003 (-0.004 to 0.011)	0.40
Sex (% female)	-0.002 (-0.003 to -0.002)	< 0.001
Height	0.011 (0.008 to 0.015)	< 0.001
Weight	0.013 (0.008 to 0.017)	< 0.001
Body mass index	-0.005 (-0.032 to 0.021)	0.69

β, standardized beta; *CI*, confidence interval.

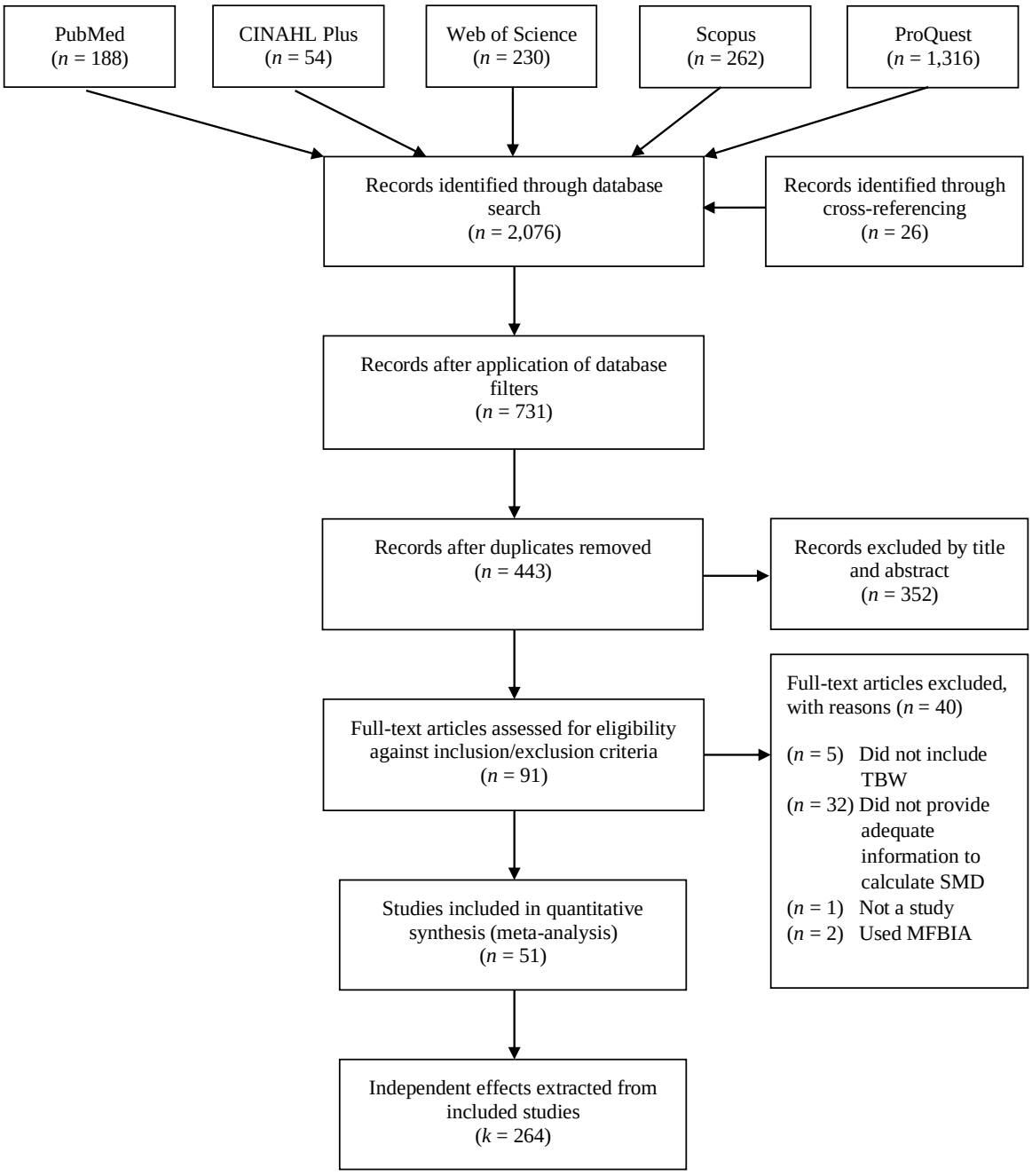

Figure 1. PRISMA flowchart of study selection. *k*, number of effects; MFBIA, multi-frequency bioimpedance analysis; *n*, number of studies; SMD, standardized mean difference effect size; TBW, total body water.

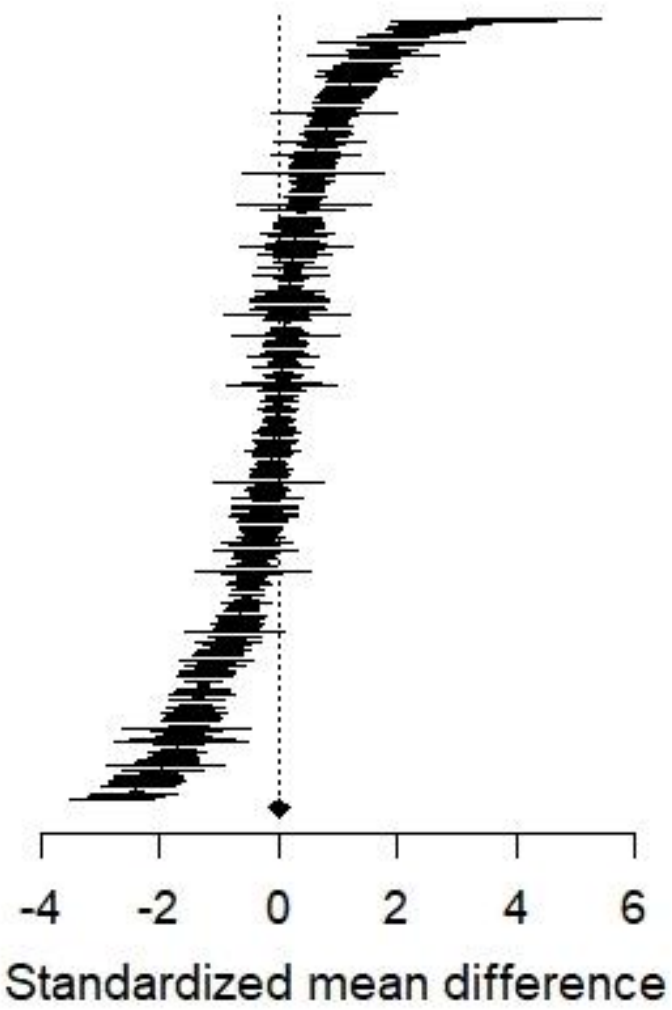

Figure 2. Forest plot of individual effect sizes (k = 264). Effect sizes are displayed in ascending order. Values to the left of the zero indicate an underestimation of total body water by single frequency bioelectrical impedance analysis compared to criterion isotope dilution; values to the right indicate overestimation. The mean effect size (0.02, 95% *CI*: -0.16 to 0.20, p = 0.82) is indicated by the polygon at the base of the vertical dotted line.

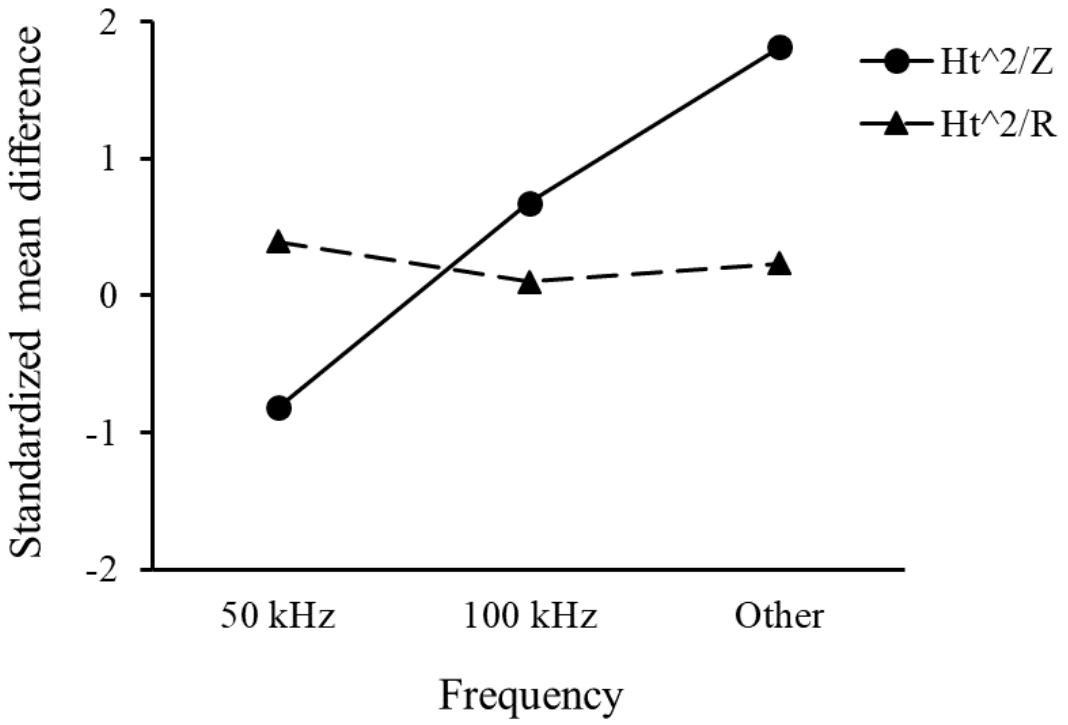

Figure 3. The effect of frequency and index on the accuracy of SFBIA. R, resistance; SFBIA, single-frequency bioimpedance analysis; kHz, kilohertz; Z, impedance.

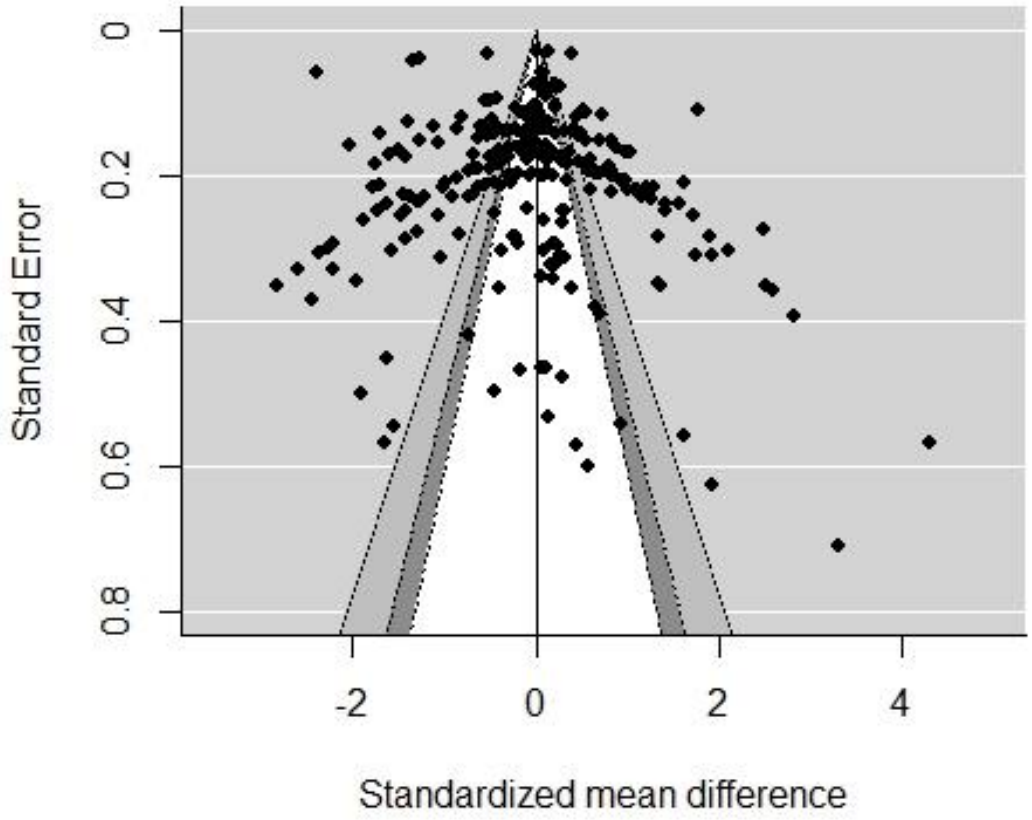

Figure 4. Funnel plot of SMD versus standard error (k = 264). SMD, standardized mean difference. Inner, middle, and outer dashed lines represent 90, 95, and 99% confidence intervals, respectively.

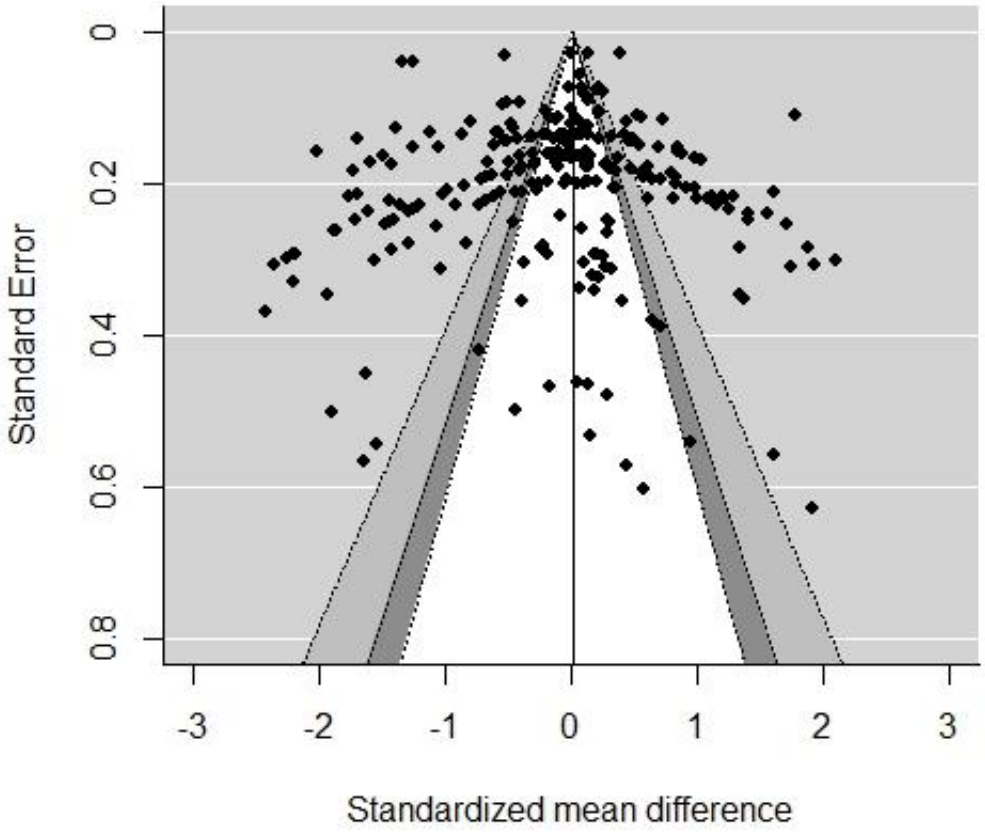

Figure 5. Reduced funnel plot with outliers removed (k = 255). Inner, middle, and outer dashed lines represent 90, 95, and 99% confidence intervals, respectively.

SDC 1. Study quality for BIA validation research

A. STUDY DESIGN	ANSWER	SCORE
1. What type of study design was used? - Case report - Uncontrolled experiment - Cohort or case control study - Not clear		**5** **30** **40** **0**
2. Were the inclusion criteria clarified?	Yes No NC	**30**
3. Were the exclusion criteria clarified?	Yes No NC	**30**

B. SUBJECTS	ANSWER	SCORE
4. Was the source of the study group described?	Yes No NC	**20**
5. Was there a description of the demographic characteristics of the subjects? (ethnicity, gender, age)	Yes No NC	**20**
6. Was there a description of the clinical characteristics of the subjects? (normal or type of disease and severity)	Yes No NC	**20**
7. Was the study group free of patients with co-morbid conditions?	Yes No NC	**20**
8. Was the sample selected in a way that avoids bias?	Yes No NC	**20**

C. PROCEDURES	ANSWER	SCORE
9. Was the weight measured to the nearest 0.1 Kg (1-5 g in children less than 3 years)?	Yes No NC	**0.7**
10. Did the subjects fast overnight or for 8 h before the test? (3 hr before in children < years)	Yes No NC	**5**
11. Did the subjects avoid strenuous exercise for 8 h before the test?	Yes No NC	**3.7**
12. Was the diagnostic BIA performed in a Standardized manner? [***See questionnaire, page 2***]	**Insert subtotal here →**	
13. Was the Gold Standard (GS) performed in a Standardized manner? [***See questionnaire, page 3***]	**Insert subtotal here →**	
14. Were BIA and GS test done at the same time and in similar conditions?	Yes No NC	**1**
15. Was the GS performed on all subjects?	Yes No NC	**0.5**
16. Was a test of BIA reproducibility carried out?	Yes No NC	**2.8**
17. Was non-random exclusion of patients avoided?	Yes No NC	**0.3**
18. Was the hydration status likely to have remained the same during the whole measurement period?	Yes No NC	**6**

D. STATISTICAL ANALYSIS	ANSWER	SCORE
19. Was/were the algorithm(s) to calculate the water volumes given?	Yes No NC	**15**
20. Were all results included in the analysis?	Yes No NC	**15**
21. What was the primary statistical analysis used?	Non parametric Correlation/regression Bland/Altman analysis	**30** **50** **70**

If there is reference to another study this should be evaluated with the same questionnaire

Question	Answer			Score
1. Was the bladder emptied before the test?	Yes	No	NC	**1.8**
2. Was the menstrual cycle considered or not applicable?	Yes	No	NA	**2.1**
3. Was a non-conductive surface used for the test	Yes	No	NC	**1.1**
4. Was the skin cleaned with alcohol?	Yes	No	NC	**1.0**
5. Was the environment or skin temperature noted?	Yes	No	NC	**13.1**
6. Were metallic objects removed from the patients?	Yes	No	NC	**1.1**
7. Was the patient position and time in that position recorded?	Yes	No	NC	**5.9**
8. Was the height measured to the nearest 0.5 cm? (0.1 cm in children under 3 years)	Yes	No	NC	**0.6**
9. Was the abduction of limbs described?	Yes	No	NC	**4.8**
10. Was the electrode position described? (In children under 3 yr. the minimal distance between the sense3 and voltage electrodes should be 3cm)	Yes	No	NC	**3.7**
11. Were the brand or type and size of electrodes stated?	Yes	No	NC	**0.7**
12. Did the study state the side of the body on which measurements were taken?	Yes	No	NC	**4.1**
SUBTOTAL (40 points possible)				

Enter subtotal into C-12 Score column in page 1 table

If there is reference to another study this should be evaluated with the same questionnaire

Question	Answer			Score
1. Were drinks avoided for several hrs before the test?	Yes	No	NC	**4.4**
2. Were baseline physiologic samples taken before the dose of tracer?	Yes	No	NC	**4.4**
3. Did each patient take the complete dose? (The container used for the dose should be washed with water and this should be administered to the subject)	Yes	No	NC	**4.4**
4. Was the reported period of equilibration adequate? TBW: 3-4 h ECW: 3-4 h If ECW expanded TBW: 4-5 h If ECW expanded ECW: 5-6 h	Yes	No	NC	**4.4**
5. Were the samples stored in airtight tubes?	Yes	No	NC	**4.4**
6. Was the measurement procedure the same for all subjects?	Yes	No	NC	**4.4**
7. Was eating or drinking avoided during the equilibration period?	Yes	No	NC	**4.4**
8. If the back-extrapolation method was used were foods avoided for 1 hr after the dose or not applicable?	Yes	No	NA	**4.4**
9. If urine was sampled for TBW was the 1st specimen discarded and then two samples collected after the dose or it was not applicable?	Yes	No	NA	**4.4**
SUBTOTAL (40 points possible)				

NC: Not clear
NA: Not applicable

Enter subtotal into the C-13 Score column in page 1 table

SDC 2a. Description of categorical moderators

Moderator	Levels	Description
SFBIA device	Model BIA 101 Quadscan 4000 Xitron 4000/B Other	Model of SFBIA device used to measure TBW and impedance values.
Frequency (kHz)	50 100 Other (5, 400, and 1000)	Current frequency used to measure bioelectrical impedance.
Index	Ht^2/Z Ht^2/R	Resistivity index. BIA parameter used to predict TBW. Z = impedance, R = resistance.
Electrode arrangement	Hand-to-foot Foot-to-foot	Refers to placement of electrodes on the limbs.
Side of the body	Right Left Other	Side of the body electrodes are placed for measurement. NA for foot-to-foot SFBIA.
Equation type	Index only Anthropometric variables	Variables in regression equation to predict TBW.
Equation type – sex	Not sex-specific Sex-specific	Identifies if regression equation used is sex-specific or accounts for sex.
Criterion TBW isotope tracer	Deuterium oxide Tritium Oxygen-18	Isotope tracer used to determine criterion TBW.
Criterion TBW fluid sample	Blood Urine Saliva Respiratory	Fluid medium in which criterion TBW was measured.
Health status of participants	Healthy Unhealthy	Identifies whether sample was comprised of healthy or unhealthy participants. “Unhealthy” category included effects derived from samples of participants suffering from chronic disease including COPD, HIV/AIDS, and cancer.

SDC 2b. Description of continuous moderators

Moderator	Description
Publication year	Study year of publication.
Study quality (%)	Total study quality expressed as a percentage.
Age	Mean sample age (year).
Sex (% female)	Proportion of females in sample.
Height	Mean sample height (cm).
Weight	Mean sample weight (kg).
Body mass index	Mean sample body mass index ($kg \cdot m^{-2}$).

SDC 3. Summaries of the 51 studies included in the quantitative analysis

Author	Year	*k*	Sample description	Country	*n*	% female	Electrode arrangement	SFBIA device	Isotope
Aglago et al.	2013	10	Healthy North-African adults	Morocco	250	77.6	Hand to foot	Bodystat Quadscan 4000	D_2O
Al-Bachir and Othman	2016	1	Young Syrian adults	Syria	215	0.0	Hand to foot	Bodystat Quadscan 4000	D_2O
Bauer et al.	2005	3	Adults with cancer cachexia	Australia	7	28.6	Foot to foot	Tanita Model 300GS	D_2O
Beertema et al.	2000	4	Children with various diseases	Netherlands	38	39.5	Hand to foot	Xitron Hydra ECF/ICF Model 4200	D_2O
Bell et al.	2013	6	Young children with cerebral palsy	Australia	55	30.9	Foot to foot	Bodystat 1500MDD	D_2O
Bell et al.	1998	1	Apparently healthy adults	Australia	57	49.1	Foot to foot	Tanita Model TBF 305	D_2O
Ben Jemaa et al.	2019	7	Healthy Tunisian children	Tunisia	134	45.5	Foot to foot	Tanita Model TBF 401A	D_2O
Danford et al.	1992	7	Healthy children	United States	37	51.4	Hand to foot	RJL Model BIA-101	D_2O
De Lorenzo et al.	1999	2	Obese adult women	Italy	55	100.0	Hand to foot	Xitron Model 4000 B	D_2O
Diouf et al.	2018	1	Healthy Senegalese children	Senegal	151	51.0	Hand to foot	Xitron Model 4000 B	D_2O
Diouf et al.	2009	10	HIV-infected African adults	Senegal	34	58.8	Hand to foot	Xitron Model 4000 B	D_2O

Dioum et al.	2005	23	Healthy African women	Senegal	36	100.0	Hand to foot	Xitron Model 4000 B	D_2O
El Harchaoui et al.	2018	10	Healthy Moroccan children	Morocco	247	50.6	Hand to foot	Bodystat Quadscan 4000	D_2O
Fjeld et al.	1990	2	Healthy and protein-energy malnourished children	Peru	65	NR	Hand to foot	RJL Model BIA-101	D_2O
Gregory et al.	1991	2	Children with growth disorders	England	34	17.6	Hand to foot	Holtain bioimpedance analyzer	D_2O
Haas et al.	2012	2	Healthy adults	Germany	52	50.0	Hand to foot	BIACorpus RX 4000	D_2O
Haroun et al.	2010	8	Healthy adolescents of various ethnicities	England	382	45.8	Foot to foot	Tanita TBF-300	D_2O
Hastuti et al.	2016	1	Healthy Indonesian men	Indonesia	292	0.0	Hand to foot	ImpediMed Imp DF50	D_2O
Horlick et al.	2002	8	Healthy and HIV-positive children and adolescents	United States	1291	48.6	Hand to foot	RJL Model BIA-101	D_2O
Isenring et al.	2004	1	Adults receiving radiotherapy	Australia	27	14.8	Foot to foot	Tanita Models TBF 300GS and 410	D_2O
Kushner et al.	1992	8	Healthy neonates, children, and adults	United States	59	27.1	Hand to foot	RJL Model BIA-101	18-O, D_2O
Kushner and Schoeller	1986	4	Adults with various health conditions	United States	18	66.7	Hand to foot	RJL Model BIA-101	D_2O
Liu et al.	2011	1	Healthy Asian children	China, Lebanon, Malaysia, Philippines, Thailand	948	48.1	Hand to foot	ImpediMed Imp DF50	D_2O

Lukaski and Bolonchuk	1988	2	Healthy adults	United States	110	53.6	Hand to foot	RJL Model BIA-101	D_2O
Masuda and Komiya	2004	2	Healthy Japanese children	Japan	70	44.3	Hand to foot	Toyo Physical TP-95K	D_2O
Matias et al.	2016	21	Elite and recreational adult athletes	Portugal	212	26.9	Hand to foot	RJL Model BIA-101	D_2O
Matias et al.	2016	7	Adult athletes	Portugal	208	33.7	Hand to foot	RJL Model BIA-101	D_2O
Medoua et al.	2008	12	Cameroonian HIV-positive adults	Cameroon	56	66.1	Hand to foot	Bodystat Quadscan 4000	D_2O
Medoua et al.	2015	9	Cameroonian dialysis patients	Cameroon	40	30.0	Hand to foot	Bodystat Quadscan 4000	D_2O
Medoua et al.	2011	12	Cameroonian lactating women	Cameroon	44	100.0	Hand to foot	Bodystat Quadscan 4000	D_2O
Patel et al.	1994	2	Healthy adults	United States	15	46.7	Hand to foot	RJL Model BIA-101	D_2O
Paton et al.	1998	1	HIV-positive men	England	33	0.0	Hand to foot	Uniquest SEAC SFB3	D_2O
Puiman et al.	2004	1	Children with cystic fibrosis	Australia	56	55.4	Hand to foot	Bodystat 1500 analyser	D_2O
Rallison et al.	1993	2	Healthy adults and adults undergoing CAPD	United States	17	41.2	Hand to foot	RJL Model BIA-101	D_2O
Resende et al.	2013	1	Obese adolescents	Brazil	55	47.3	Hand to foot	Bodystat 1500	D_2O

Richards et al.	2003	2	Healthy adults and adults with cystic fibrosis	Australia	78	59.0	Hand to foot	Bodystat 1500	D_2O
Ritz	2001	2	Older adults with various diseases	France	169	64.5	Hand to foot	Spengler Analycor-3	D_2O
Scalfi et al.	1997	12	Healthy women and women with anorexia	Italy	46	100.0	Hand to foot	Dietosystem Human-IM Scan	D_2O
Sen et al.	2010	3	Healthy young Indian children	India	86	48.8	Hand to foot	Xitron Model 4000 B	D_2O
Seoane et al.	2015	4	Adults on growth hormone replacement therapy	Sweden	94	40.4	Hand to foot	Fresenius Body Scout Spectrometer	Tritium
Shaikh et al.	2002	1	Poor, young Indian children	India	10	30.0	Hand to foot	Xitron Model 4000 B	D_2O
Sluys et al.	1993	1	Adults with AIDS	Netherlands	11	9.1	Hand to foot	RJL Model BIA-101	D_2O
Strain et al.	2008	1	Obese adults	Netherlands	42	65.0	Foot to foot	Tanita 310	D_2O
Vache et al.	1998	3	Healthy older adults	France	58	53.4	Hand to foot	Eugedia ANALYCOR-3	18-O
Van Loan and Mayclin	1987	2	Healthy adults	United States	188	34.6	Hand to foot	RJL Model BIA-101	D_2O
van Marken Lichtenbelt et al.	1994	8	Healthy adults	Netherlands	29	51.7	Hand to foot	Xitron Model 4000	D_2O
Visser et al.	1995	14	Healthy older adults	Netherlands	117	69.2	Hand to foot	Xitron Model 4000	D_2O

Wickramasinghe et al.	2008	6	Healthy Sri Lankan children	Sri Lanka	94	43.6	Hand to foot	Bodystat	D_2O
Widen et al.	2014	1	Bariatric surgery candidates	United States	50	72.0	Hand to foot	Tanita TBF-310	D_2O
Woodrow et al.	1996	4	Healthy adults and adults with renal failure	England	96	51.0	Hand to foot	RJL Model BIA-101 and Holtain BIA	D_2O
Zillikens et al.	1992	6	Adults with cirrhosis	Netherlands	27	44.4	Hand to foot	RJL Model BIA-101	D_2O

D_2O, deuterium oxide; *k*, number of individual effects extracted from the study; *n*, study sample size; SFBIA, single frequency bioelectrical impedance analysis.

SDC 4. Itemized and total study quality scores for included studies

			Design			Subjects				
Author	Year	SQ	1	2	3	4	5	6	7	8
Aglago et al.	2013	312.3	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Al-Bachir and Othman	2016	287.8	30.0	0.0	30.0	20.0	20.0	20.0	20.0	0.0
Bauer et al.	2005	227.6	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Beertema et al.	2000	245.9	30.0	0.0	0.0	20.0	20.0	20.0	20.0	0.0
Bell (KL) et al.	2013	308.5	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Bell (NA) et al.	1998	161.4	30.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0
Ben Jemaa et al.	2019	297.8	30.0	0.0	30.0	20.0	20.0	20.0	20.0	20.0
Danford et al.	1992	220.1	30.0	0.0	0.0	0.0	20.0	20.0	20.0	20.0
De Lorenzo et al.	1999	231.6	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Diouf et al.	2018	296.3	30.0	30.0	0.0	20.0	20.0	20.0	20.0	20.0
Diouf et al.	2009	318.3	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Dioum et al.	2005	342.0	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
El Harchaoui et al.	2018	318.4	30.0	30.0	0.0	20.0	20.0	20.0	20.0	20.0
Fjeld et al.	1990	305.4	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Gregory et al.	1991	260.7	30.0	30.0	0.0	20.0	20.0	20.0	0.0	0.0
Haas et al.	2012	259.6	30.0	0.0	0.0	20.0	0.0	20.0	20.0	0.0
Haroun et al.	2010	234.5	30.0	30.0	0.0	20.0	20.0	0.0	0.0	0.0
Hastuti et al.	2016	312.3	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Horlick et al.	2002	293.2	30.0	30.0	30.0	20.0	0.0	20.0	20.0	20.0
Isenring et al.	2004	263.5	30.0	30.0	30.0	0.0	20.0	20.0	20.0	0.0
Kushner et al.	1992	224.4	30.0	0.0	0.0	20.0	20.0	0.0	0.0	20.0
Kushner and Schoeller	1986	195.5	30.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0
Liu et al.	2011	300.9	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Lukaski and Bolonchuk	1988	231.5	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Masuda and Komiya	2004	247.0	30.0	0.0	0.0	20.0	20.0	20.0	20.0	0.0
Matias et al.	2016	267.7	30.0	30.0	0.0	0.0	20.0	20.0	20.0	0.0
Matias et al.	2016	267.7	30.0	30.0	0.0	0.0	20.0	20.0	20.0	0.0
Medoua et al.	2008	310.4	30.0	30.0	0.0	20.0	20.0	20.0	20.0	0.0
Medoua et al.	2015	292.0	30.0	30.0	0.0	20.0	20.0	20.0	20.0	0.0
Medoua et al.	2011	326.1	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Patel et al.	1994	334.2	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Paton et al.	1998	272.6	30.0	30.0	0.0	0.0	20.0	20.0	20.0	0.0
Puiman et al.	2004	253.9	30.0	30.0	0.0	20.0	20.0	20.0	20.0	0.0
Rallison et al.	1993	261.5	30.0	0.0	0.0	20.0	20.0	20.0	20.0	0.0
Resende et al.	2013	323.1	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Richards et al.	2003	238.9	30.0	0.0	0.0	20.0	20.0	20.0	20.0	0.0
Ritz	2001	319.1	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Scalfi et al.	1997	286.1	30.0	30.0	30.0	20.0	20.0	20.0	0.0	0.0
Sen et al.	2010	292.8	30.0	30.0	0.0	20.0	20.0	20.0	20.0	0.0
Seoane et al.	2015	216.3	30.0	0.0	0.0	20.0	20.0	20.0	0.0	0.0
Shaikh et al.	2002	245.9	30.0	0.0	0.0	20.0	20.0	20.0	0.0	0.0
Sluys et al.	1993	220.7	30.0	0.0	0.0	20.0	20.0	20.0	20.0	0.0
Strain et al.	2008	283.5	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Vache et al.	1998	237.3	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Van Loan and Mayclin	1987	283.1	30.0	30.0	30.0	0.0	20.0	20.0	20.0	0.0
van Marken Lichtenbelt et al.	1994	237.3	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Visser et al.	1995	320.0	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0

Wickramasinghe et al.	2008	314.4	30.0	30.0	30.0	20.0	20.0	20.0	20.0	20.0
Widen et al.	2014	286.3	30.0	30.0	30.0	20.0	20.0	20.0	20.0	0.0
Woodrow et al.	1996	210.4	30.0	0.0	0.0	0.0	20.0	20.0	20.0	0.0
Zillikens et al.	1992	278.3	30.0	30.0	0.0	20.0	20.0	20.0	20.0	0.0

SDC 4. Itemized and total study quality scores for included studies

			Procedures									
Author	Year	SQ	9	10	11	12	13	14	15	16	17	18
Aglago et al.	2013	312.3	0.7	0.0	0.0	16.2	17.6	1.0	0.5	0.0	0.3	6.0
Al-Bachir and Othman	2016	287.8	0.7	5.0	0.0	20.1	26.4	1.0	0.5	2.8	0.3	6.0
Bauer et al.	2005	227.6	0.7	5.0	0.0	6.5	17.6	1.0	0.5	0.0	0.3	6.0
Beertema et al.	2000	245.9	0.7	0.0	0.0	16.0	26.4	1.0	0.5	0.0	0.3	6.0
Bell (KL) et al.	2013	308.5	0.7	0.0	0.0	14.3	13.2	1.0	0.5	2.8	0.0	6.0
Bell (NA) et al.	1998	161.4	0.7	0.0	0.0	4.7	13.2	1.0	0.5	0.0	0.3	6.0
Ben Jemaa et al.	2019	297.8	0.7	0.0	0.0	16.1	13.2	1.0	0.5	0.0	0.3	6.0
Danford et al.	1992	220.1	0.7	0.0	0.0	8.4	13.2	1.0	0.5	0.0	0.3	6.0
De Lorenzo et al.	1999	231.6	0.7	5.0	0.0	6.1	22.0	1.0	0.5	0.0	0.3	6.0
Diouf et al.	2018	296.3	0.7	0.0	0.0	10.2	17.6	1.0	0.5	0.0	0.3	6.0
Diouf et al.	2009	318.3	0.7	0.0	0.0	15.0	22.0	1.0	0.5	2.8	0.3	6.0
Dioum et al.	2005	342.0	0.7	5.0	3.7	37.2	17.6	1.0	0.5	0.0	0.3	6.0
El Harchaoui et al.	2018	318.4	0.7	5.0	0.0	14.1	30.8	1.0	0.5	0.0	0.3	6.0
Fjeld et al.	1990	305.4	0.7	0.0	0.0	15.3	17.6	1.0	0.5	0.0	0.3	0.0
Gregory et al.	1991	260.7	0.7	5.0	0.0	8.4	22.0	1.0	0.5	2.8	0.3	0.0
Haas et al.	2012	259.6	0.7	5.0	3.7	22.6	30.8	0.0	0.5	0.0	0.3	6.0
Haroun et al.	2010	234.5	0.7	0.0	0.0	8.4	17.6	1.0	0.5	0.0	0.3	6.0
Hastuti et al.	2016	312.3	0.7	5.0	3.7	20.7	4.4	1.0	0.5	0.0	0.3	6.0
Horlick et al.	2002	293.2	0.7	0.0	0.0	9.1	8.8	1.0	0.5	2.8	0.3	0.0
Isenring et al.	2004	263.5	0.7	0.0	0.0	8.4	17.6	1.0	0.5	0.0	0.3	0.0
Kushner et al.	1992	224.4	0.7	5.0	0.0	6.5	17.6	1.0	0.5	2.8	0.3	0.0
Kushner and Schoeller	1986	195.5	0.7	5.0	0.0	13.2	22.0	1.0	0.5	2.8	0.3	0.0
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Masuda and Komiya	2004	247.0	0.7	5.0	0.0	11.9	17.6	1.0	0.5	0.0	0.3	0.0
Matias et al.	2016	267.7	0.7	5.0	3.7	16.1	17.6	1.0	0.5	2.8	0.3	0.0
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Medoua et al.	2015	292.0	0.7	0.0	0.0	21.5	22.0	1.0	0.5	0.0	0.3	6.0
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Rallison et al.	1993	261.5	0.7	5.0	0.0	13.2	22.0	1.0	0.5	2.8	0.3	6.0
Resende et al.	2013	323.1	0.7	5.0	0.0	13.2	26.4	1.0	0.5	0.0	0.3	6.0
Richards et al.	2003	238.9	0.7	0.0	0.0	13.2	13.2	1.0	0.5	0.0	0.3	0.0
Ritz	2001	319.1	0.7	5.0	0.0	16.8	22.0	1.0	0.5	2.8	0.3	0.0
Scalfi et al.	1997	286.1	0.7	5.0	0.0	8.2	17.6	1.0	0.5	2.8	0.3	0.0
Sen et al.	2010	292.8	0.7	0.0	3.7	14.2	26.4	1.0	0.5	0.0	0.3	6.0
Seoane et al.	2015	216.3	0.7	5.0	0.0	10.0	8.8	1.0	0.5	0.0	0.3	0.0
Shaikh et al.	2002	245.9	0.7	5.0	0.0	16.0	26.4	1.0	0.5	0.0	0.3	6.0
Sluys et al.	1993	220.7	0.7	5.0	0.0	0.6	17.6	1.0	0.5	0.0	0.3	0.0
Strain et al.	2008	283.5	0.0	5.0	0.0	8.5	13.2	1.0	0.5	0.0	0.3	0.0
Vache et al.	1998	237.3	0.7	5.0	0.0	15.0	22.0	1.0	0.5	2.8	0.3	0.0
Van Loan and Mayclin	1987	283.1	0.7	0.0	0.0	10.2	17.6	1.0	0.5	2.8	0.3	0.0
van Marken Lichtenbelt et al.	1994	237.3	0.7	5.0	0.0	15.0	22.0	1.0	0.5	2.8	0.3	0.0
Visser et al.	1995	320.0	0.7	5.0	0.0	16.1	17.6	1.0	0.5	2.8	0.3	6.0

Wickramasinghe et al.	2008	314.4	0.7	0.0	3.7	0.6	17.6	1.0	0.5	0.0	0.3	0.0
Widen et al.	2014	286.3	0.7	0.0	0.0	0.6	13.2	1.0	0.5	0.0	0.3	0.0
Woodrow et al.	1996	210.4	0.7	0.0	0.0	4.7	13.2	1.0	0.5	0.0	0.3	0.0
Zillikens et al.	1992	278.3	0.7	5.0	0.0	13.2	17.6	1.0	0.5	0.0	0.3	0.0

SDC 4. Itemized and total study quality scores for included studies

			Statistics		
Author	Year	SQ	19	20	21
Aglago et al.	2013	312.3	15.0	15.0	70.0
Al-Bachir and Othman	2016	287.8	0.0	15.0	70.0
Bauer et al.	2005	227.6	15.0	15.0	70.0
Beertema et al.	2000	245.9	15.0	0.0	70.0
Bell (KL) et al.	2013	308.5	15.0	15.0	70.0
Bell (NA) et al.	1998	161.4	0.0	15.0	70.0
Ben Jemaa et al.	2019	297.8	15.0	15.0	70.0
Danford et al.	1992	220.1	15.0	15.0	50.0
De Lorenzo et al.	1999	231.6	15.0	15.0	70.0
Diouf et al.	2018	296.3	15.0	15.0	70.0
Diouf et al.	2009	318.3	15.0	15.0	70.0
Dioum et al.	2005	342.0	15.0	15.0	70.0
El Harchaoui et al.	2018	318.4	15.0	15.0	70.0
Fjeld et al.	1990	305.4	15.0	15.0	70.0
Gregory et al.	1991	260.7	15.0	15.0	70.0
Haas et al.	2012	259.6	15.0	15.0	70.0
Haroun et al.	2010	234.5	15.0	15.0	70.0
Hastuti et al.	2016	312.3	15.0	15.0	70.0
Horlick et al.	2002	293.2	15.0	15.0	70.0
Isenring et al.	2004	263.5	0.0	15.0	70.0
Kushner et al.	1992	224.4	15.0	15.0	70.0
Kushner and Schoeller	1986	195.5	15.0	15.0	70.0
Liu et al.	2011	300.9	15.0	15.0	70.0
Lukaski and Bolonchuk	1988	231.5	15.0	15.0	70.0
Masuda and Komiya	2004	247.0	15.0	15.0	70.0
Matias et al.	2016	267.7	15.0	15.0	70.0
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Medoua et al.	2008	310.4	15.0	15.0	70.0
Medoua et al.	2015	292.0	15.0	15.0	70.0
Medoua et al.	2011	326.1	15.0	15.0	70.0
Patel et al.	1994	334.2	15.0	15.0	70.0
Paton et al.	1998	272.6	15.0	15.0	70.0
Puiman et al.	2004	253.9	0.0	15.0	70.0
Rallison et al.	1993	261.5	15.0	15.0	70.0
Resende et al.	2013	323.1	15.0	15.0	70.0
Richards et al.	2003	238.9	15.0	15.0	70.0
Ritz	2001	319.1	15.0	15.0	70.0
Scalfi et al.	1997	286.1	15.0	15.0	70.0
Sen et al.	2010	292.8	15.0	15.0	70.0
Seoane et al.	2015	216.3	15.0	15.0	70.0
Shaikh et al.	2002	245.9	15.0	15.0	70.0
Sluys et al.	1993	220.7	0.0	15.0	70.0
Strain et al.	2008	283.5	0.0	15.0	70.0
Vache et al.	1998	237.3	15.0	15.0	70.0
Van Loan and Mayclin	1987	283.1	15.0	15.0	70.0
van Marken Lichtenbelt et al.	1994	237.3	15.0	15.0	70.0
Visser et al.	1995	320.0	15.0	15.0	70.0

Wickramasinghe et al.	2008	314.4	15.0	15.0	70.0
Widen et al.	2014	286.3	15.0	15.0	70.0
Woodrow et al.	1996	210.4	15.0	15.0	70.0
Zillikens et al.	1992	278.3	15.0	15.0	70.0

SDC 4. Itemized and total study quality scores for included studies

			SFBIA standardization procedures											
Author	Year	SQ	1	2	3	4	5	6	7	8	9	10	11	12
Aglago et al.	2013	312.3	1.8	2.1	1.1	1.0	0.0	1.1	0.0	0.6	4.8	3.7	0.0	0.0
Al-Bachir and Othman	2016	287.8	0.0	0.0	0.0	1.0	0.0	0.0	5.9	0.6	4.8	3.7	0.0	4.1
Bauer et al.	2005	227.6	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Beertema et al.	2000	245.9	1.8	0.0	0.0	1.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Bell (KL) et al.	2013	308.5	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.6	4.8	3.7	0.0	4.1
Bell (NA) et al.	1998	161.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Ben Jemaa et al.	2019	297.8	1.8	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.0	4.1
Danford et al.	1992	220.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	4.1
De Lorenzo et al.	1999	231.6	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	0.0
Diouf et al.	2018	296.3	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	4.1
Diouf et al.	2009	318.3	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.6	4.8	3.7	0.0	0.0
Dioum et al.	2005	342.0	1.8	0.0	1.1	1.0	13.1	1.1	5.9	0.6	4.8	3.7	0.0	4.1
El Harchaoui et al.	2018	318.4	1.8	0.0	1.1	1.0	0.0	1.1	0.0	0.6	4.8	3.7	0.0	0.0
Fjeld et al.	1990	305.4	0.0	0.0	0.0	1.0	0.0	1.1	0.0	0.6	4.8	3.7	0.0	4.1
Gregory et al.	1991	260.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	4.1
Haas et al.	2012	259.6	1.8	0.0	0.0	1.0	0.0	0.0	5.9	0.6	4.8	3.7	0.7	4.1
Haroun et al.	2010	234.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	4.1
Hastuti et al.	2016	312.3	1.8	0.0	1.1	0.0	13.1	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Horlick et al.	2002	293.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.7	4.1
Isenring et al.	2004	263.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	4.1
Kushner et al.	1992	224.4	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Kushner and Schoeller	1986	195.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Liu et al.	2011	300.9	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Lukaski and Bolonchuk	1988	231.5	1.8	0.0	1.1	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.7	4.1
Masuda and Komiya	2004	247.0	1.8	0.0	0.0	1.0	0.0	0.0	0.0	0.6	0.0	3.7	0.7	4.1
Matias et al.	2016	267.7	1.8	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.0	4.1
Matias et al.	2016	267.7	1.8	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.0	4.1
Medoua et al.	2008	310.4	1.8	0.0	1.1	0.0	13.1	1.1	5.9	0.6	4.8	3.7	0.0	4.1
Medoua et al.	2015	292.0	0.0	0.0	0.0	0.0	13.1	0.0	0.0	0.6	0.0	3.7	0.0	4.1
Medoua et al.	2011	326.1	0.0	0.0	1.1	0.0	13.1	1.1	5.9	0.6	4.8	3.7	0.0	4.1
Patel et al.	1994	334.2	1.8	0.0	1.1	1.0	0.0	0.0	0.0	0.6	4.8	3.7	0.7	4.1
Paton et al.	1998	272.6	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.7	4.1
Puiman et al.	2004	253.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Rallison et al.	1993	261.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Resende et al.	2013	323.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Richards et al.	2003	238.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Ritz	2001	319.1	1.8	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.7	4.1
Scalfi et al.	1997	286.1	1.8	2.1	0.0	0.0	0.0	0.0	0.0	0.6	0.0	3.7	0.0	0.0
Sen et al.	2010	292.8	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Seoane et al.	2015	216.3	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0	4.1
Shaikh et al.	2002	245.9	1.8	0.0	0.0	1.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1
Sluys et al.	1993	220.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
Strain et al.	2008	283.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	0.7	4.1
Vache et al.	1998	237.3	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.7	4.1
Van Loan and Mayclin	1987	283.1	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	0.0
van Marken Lichtenbelt et al.	1994	237.3	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.7	4.1
Visser et al.	1995	320.0	0.0	0.0	1.1	0.0	0.0	0.0	5.9	0.6	0.0	3.7	0.7	4.1

Wickramasinghe et al.	2008	314.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
Widen et al.	2014	286.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
Woodrow et al.	1996	210.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	4.1
Zillikens et al.	1992	278.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	4.8	3.7	0.0	4.1

SDC 4. Itemized and total study quality scores for included studies

			Dilution technique standardization								
Author	Year	SQ	1	2	3	4	5	6	7	8	9
Aglago et al.	2013	312.3	0.0	4.4	4.4	4.4	0.0	4.4	0.0	0.0	0.0
Al-Bachir and Othman	2016	287.8	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Bauer et al.	2005	227.6	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Beertema et al.	2000	245.9	4.4	4.4	4.4	4.4	4.4	4.4	0.0	0.0	0.0
Bell (KL) et al.	2013	308.5	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Bell (NA) et al.	1998	161.4	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Ben Jemaa et al.	2019	297.8	0.0	4.4	4.4	0.0	0.0	4.4	0.0	0.0	0.0
Danford et al.	1992	220.1	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
De Lorenzo et al.	1999	231.6	4.4	4.4	4.4	4.4	0.0	4.4	0.0	0.0	0.0
Diouf et al.	2018	296.3	0.0	4.4	4.4	4.4	0.0	4.4	0.0	0.0	0.0
Diouf et al.	2009	318.3	0.0	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Dioum et al.	2005	342.0	4.4	4.4	4.4	0.0	0.0	4.4	0.0	0.0	0.0
El Harchaoui et al.	2018	318.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	0.0	0.0
Fjeld et al.	1990	305.4	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	4.4
Gregory et al.	1991	260.7	4.4	4.4	0.0	4.4	4.4	4.4	0.0	0.0	0.0
Haas et al.	2012	259.6	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	4.4
Haroun et al.	2010	234.5	0.0	4.4	0.0	4.4	4.4	4.4	0.0	0.0	0.0
Hastuti et al.	2016	312.3	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Horlick et al.	2002	293.2	0.0	4.4	0.0	0.0	0.0	4.4	0.0	0.0	0.0
Isenring et al.	2004	263.5	0.0	4.4	0.0	4.4	4.4	4.4	0.0	0.0	0.0
Kushner et al.	1992	224.4	4.4	4.4	0.0	4.4	0.0	0.0	0.0	0.0	4.4
Kushner and Schoeller	1986	195.5	4.4	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
Liu et al.	2011	300.9	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Lukaski and Bolonchuk	1988	231.5	4.4	4.4	4.4	4.4	0.0	4.4	0.0	0.0	0.0
Masuda and Komiya	2004	247.0	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Matias et al.	2016	267.7	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Matias et al.	2016	267.7	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Medoua et al.	2008	310.4	4.4	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
Medoua et al.	2015	292.0	4.4	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
Medoua et al.	2011	326.1	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Patel et al.	1994	334.2	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Paton et al.	1998	272.6	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Puiman et al.	2004	253.9	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Rallison et al.	1993	261.5	4.4	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
Resende et al.	2013	323.1	4.4	4.4	0.0	4.4	4.4	4.4	4.4	0.0	0.0
Richards et al.	2003	238.9	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Ritz	2001	319.1	4.4	4.4	0.0	4.4	4.4	4.4	0.0	0.0	0.0
Scalfi et al.	1997	286.1	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Sen et al.	2010	292.8	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Seoane et al.	2015	216.3	4.4	0.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0
Shaikh et al.	2002	245.9	4.4	4.4	4.4	4.4	0.0	4.4	4.4	0.0	0.0
Sluys et al.	1993	220.7	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Strain et al.	2008	283.5	4.4	0.0	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Vache et al.	1998	237.3	4.4	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
Van Loan and Mayclin	1987	283.1	0.0	4.4	0.0	4.4	0.0	4.4	4.4	0.0	0.0
van Marken Lichtenbelt et al.	1994	237.3	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	4.4
Visser et al.	1995	320.0	4.4	0.0	0.0	4.4	0.0	4.4	4.4	0.0	0.0

Wickramasinghe et al.	2008	314.4	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Widen et al.	2014	286.3	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Woodrow et al.	1996	210.4	0.0	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0
Zillikens et al.	1992	278.3	4.4	4.4	0.0	4.4	0.0	4.4	0.0	0.0	0.0

CHAPTER 4

GENERALIZED EQUATIONS FOR PREDICTING PERCENT BODY FAT FROM ANTHROPOMETRIC MEASURES USING A CRITERION FIVE-COMPARTMENT MODEL

ABSTRACT

Skinfold-based prediction equations are commonly used to estimate percent body fat (%BF) when laboratory methods are impractical or not available. However, most equations are derived from two-compartment models, which assume constant hydration of fat-free mass. The purpose of this study was to develop and cross-validate a skinfold-based equation for the prediction of %BF, using a five-compartment (5C) model as the criterion measure. A sample of 357 healthy adults completed body composition measurements which allowed for calculation of 5C %BF (i.e., hydrostatic weighing, dual-energy X-ray absorptiometry, and bioimpedance spectroscopy) as well as skinfolds and waist circumference measurements. The sample was randomly divided into a development cohort (n = 279) and a cross-validation cohort (n = 78). Regression analysis in the development group produced a significant equation ($p < 0.001$, adjusted $R^2 = 0.775$, SEE = 4.01) using sum of 3 skinfolds (midaxillary, subscapular, and thigh), sex, age, waist circumference, body mass index (BMI), and the interaction term sex*age. The new equation cross-validated well against the criterion 5C %BF when compared to other skinfold-based equations, producing a large intraclass correlation coefficient (ICC = 0.90), small bias and limits of agreement (0.42 ± 8.60%), and small measures of error (SEE = 2.48). The new equation improved on previous skinfold-based equations developed using other criterion methods and provided acceptable individual estimates of %BF in our sample of healthy adults.

INTRODUCTION

The assessment of body composition, specifically percent body fat (%BF), is of great importance to the health and medical sciences. Excess adiposity is associated with increased risk for metabolic disorders such as type 2 diabetes, hypertension, dyslipidemia, and non-alcoholic fatty liver disease (1-4). Additionally, %BF has been linked to several cancers, including esophageal, pancreatic, colorectal, postmenopausal breast, endometrial, renal, and gallbladder cancers (5). High levels of body fat have also been shown to impede performance in sports including soccer (6, 7), hockey (8), basketball (9) and Australian rules football (10). Further, changes in %BF are often used to monitor the efficacy of training and nutrition interventions in clinical, research, and athletic settings.

Anthropometric methods are often used to determine body composition when more advanced laboratory methods are unavailable (11, 12). Most prevalent is the use of skinfold-based regression equations, which allow for the estimation of body density based on the thickness of the skinfold measurements. These equations are often used in conjunction with two-compartment (2C) models, which calculate %BF from body density (13, 14). However, 2C models of body composition assume that the density of fat mass (FM, 0.9007 $g \cdot cm^{-3}$) and fat-free mass (FFM, 1.1000 $g \cdot cm^{-3}$) are constant. Although the composition of FM is relatively consistent across sex, age, and nutrition status (15), large inter-individual variation in the make-up of FFM has been observed in healthy and athletic populations (16, 17). This variation is due to the unique composition of the tissue, which is comprised of water, protein, and mineral (13).

In an effort to account for the individual variability of FFM, multi-compartment models that estimate body water, protein, and mineral content have been developed. The inclusion of these subdivisions of FFM allow multi-compartment models to better explain FFM variability

and thus more precisely quantify %BF. Models have been developed that include estimates of three (13), four (18, 19), and five (18) compartments. Although the former 2 include measures of total body water (TBW) and bone mineral content in addition to body density, the five-compartment (5C) distinguishes between osseous and non-osseous mineral content. Because the density of non-osseous mineral is greater than fat, water, protein, and bone mineral (20), variation in this particular tissue may contribute substantially to measurement errors when estimating body density (18).

Considering the widespread use of skinfold-based measurement techniques, accurate equations based on the most precise measure of %BF should be available for use in the general population. A skinfold equation based on a 5C model would improve upon previous equations by accounting for more variability in FFM, thus yielding more accurate estimates of %BF. Accordingly, the purpose of this study was to develop an equation for the prediction of %BF from skinfolds and anthropometric measures in healthy men and women, using the 5C %BF as the criterion ($\%BF_{5C}$). Additionally, we sought to compare the new equation to existing equations from Jackson and Pollock (21, 22), Evans et al. (23), and Peterson et al. (24). We hypothesized that the new equation would outperform the existing equations when cross-validated against the 5C model, indicated by a smaller mean difference, narrower limits of agreement, and lower standard error.

METHODS

Participants

The sample consisted of healthy adult volunteers which had been recruited for 2 separate body composition studies. All participants provided written informed consent prior to participation in the study. Participants with any cardiac, pulmonary, or metabolic conditions

were excluded, as were women who were pregnant, pregnant within the previous 12 months, or currently lactating. The testing protocol, recruitment flyers, and consent forms were reviewed and approved by the University's institutional review board. The final sample consisted of 357 participants, which was randomly divided into a development cohort (78% of the total sample, n = 279, 55.4% female) and a cross-validation cohort (22% of the total sample, n = 78, 41.0% female) using the IBM SPSS software package (Version 25.0, IBM, Somers, NY, USA).

Power analyses were conducted using G*Power (version 3.1.9.4, Universität Kiel, Germany) to confirm that adequate statistical power was achieved during regression and cross-validation procedures. For model development, the large effect size (f^2 = 3.27) from multiple linear regression using 279 participants and 6 predictor variables, with α level of 0.05, resulted in an achieved power (1 – β) of 1.000. For cross-validation, the large effect size (f = 0.70) from the repeated measures ANOVA of 78 participants and 5 comparisons, with α level of 0.05, resulted in an achieved power (1 – β) of 1.000.

Testing protocol

Testing occurred during a single visit to the University's Exercise Physiology Laboratory. Participants were instructed to adhere to the following pre-testing guidelines: abstain from alcohol and exercise for 24 hours; abstain from food and caffeine for 8 to 12 hours; abstain from drinking water for ≥2 hours. Hydration was assessed via urine specific gravity (USG) using a refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan) to ensure adequate hydration prior to testing; USG values <1.030 were required for participation (25). Participants exceeding this threshold were asked to reschedule their testing time for another day. Shoes, jewelry, and any metallic objects were removed prior to body composition assessments.

Anthropometrics and skinfolds

Standing height was measured to the nearest 0.1 centimeter using a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Body mass (BM) was measured to the nearest 0.1 kg using a digital scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Skinfold thicknesses were measured using a calibrated Lange caliper (Beta Technology Incorporated, Cambridge, Massachusetts, USA) at 7 sites (chest, midaxillary, subscapular, triceps, suprailiac, abdominal, and thigh) as described by Jackson and Pollock (26). Skinfolds were taken in duplicate on the right side of the body, with a third measurement taken if measures varied more than 2 mm (27). The average of the 2 measures was calculated for each site and recorded to the nearest 0.5 mm. Waist circumference (just above the umbilicus) was measured in duplicate using a spring-loaded measuring tape, with a third measurement taken if the second was not within 5 mm of the first (27). The average of the 2 circumferences was calculated and recorded to the nearest 0.5 cm.

Body composition measurement

Body volume

Body volume was determined using hydrostatic weighing and residual lung volume as described previously (16, 24, 28). Hydrostatic weighing was performed by placing participants on a nylon seat harness in a submersion tank with water heated to approximately body temperature. The harness was suspended from a 15-kg scale (Chatillon, Largo, FL, USA). Five to ten trials were performed, and the mean of the 3 heaviest values were used to calculate body volume. All underwater weights were recorded to the nearest 0.025 kg.

Pulmonary nitrogen concentrations were measured using closed-circuit spirometry. Raw nitrogen concentrations following a maximal exhalation and after a rebreathing period were determined using either a metabolic cart (ParvoMedics TrueOne 2400, Sandy, UT, USA) or a

nitrogen analyzer (VacuMed, Ventura, CA, USA). Residual lung volume was calculated using the technique described by Wilmore (29). A minimum of 2 trials were performed, and the average of 2 trials within 0.2 liters was used in the calculation of body volume. Agreement analysis in a sample of 34 participants demonstrated that the use of either system for the determination of residual lung volume did not impact within-subject body volume (ICC = 1.00, $p < 0.001$; bias ± 95% limits of agreement, 0.36 ± 0.52 L).

Body volume was calculated using the following equation described by Goldman and Buskirk (30):

$$Body\ volume = \left[\left[\frac{(M_{body} - M_{UW})}{(D_{water})}\right] - (V_{residual} + V_{GI})\right]$$

where body volume is in liters, M_{UW} and M_{body} are underwater weight and body weight, respectively, in kilograms, D_{water} is the density of water, $V_{residual}$ is residual lung volume in liters, and V_{GI} is the volume of air assumed to be in the gastrointestinal tract (approximately 0.1 liters) (31).

Total body water

TBW was determined using whole-body bioimpedance spectroscopy (BIS; Imp™ SFB7, ImpediMed Ltd., Brisbane, Australia). Participants were instructed to lie supine on a padded gurney with arms angled approximately 30 degrees to the torso with legs shoulder-width apart. Prior to electrode placement, excess hair was removed from the electrode sites. Dual-tab electrodes were placed on the back of the right wrist and top of the right ankle after being cleaned with alcohol (32). TBW was determined using the right-side whole-body configuration outlined by the manufacturer. The average of the 2 measurements to the nearest 0.1 liter were used in the body composition calculations.

Total body bone mineral and total body soft tissue mineral

Bone mineral content (BMC) was measured using dual-energy X-ray absorptiometry (DXA). The DXA was calibrated according to manufacturer guidelines prior to each use using a standardized calibration block (GE Lunar Prodigy; Software version 14.10.022; GE Lunar Corporation, Madison, WI, USA). Participants were positioned supine on the DXA platform with arms held at the sides and feet secured with Velcro straps around the ankles to prevent movement. BMC provided by the DXA was converted to total body bone mineral (M_O) and total body soft tissue mineral (M_S) using the following equations (18, 28):

$$M_O = BMC \times 1.0436$$

$$M_S = [0.882 \times (12.9 * TBW) + 37.9]/1000$$

where BMC, M_O, and M_S are measured in kilograms and TBW is in liters.

Calculation of percent body fat

The criterion 5C model %BF ($\%BF_{5C}$) was determined by calculating fat mass using the 5C model described by Wang et al. (18) and then dividing by total body mass. Additionally, several existing skinfold-based equations were used to predict %BF for comparison within the cross-validation cohort. Body density equations by Jackson and Pollock (21, 22), converted to %BF using the Siri equation (13), were selected due to their common use in research and practice. Equations by Evans et al. (23) and Peterson et al. (24) were also selected, since they were developed more recently using multi-compartment models as the criterion and included both men and women. Outcomes from these equations are referred to as $\%BF_{JP}$, $\%BF_{Evans}$, and $\%BF_{Peterson}$. Equations are described in detail in Table 1.

Selection of predictor variables for inclusion in the model

The potential independent variables in this model were the sum of 7 skinfolds, sum of 3 skinfolds, age, sex, body mass index (BMI), and waist circumference. Skinfolds were selected as the primary independent variable due to the well-documented association with body density and subcutaneous body fat (21-23, 33-36). Additionally, three-site skinfold tests are considerably quicker to perform and have been shown to produce comparable results to seven-site tests (21-23, 36). Age, sex, BMI, and waist circumferences were also selected as these have been shown to significantly relate to variation in %BF (37-40). Additionally, interaction terms between sex and age (sex*age) and sex and waist circumference (sex*waist) were examined using one-way ANOVA and interaction plots, as differences in fat distribution have been shown to change with age and vary between sexes (34, 41-43).

Statistical analysis

Data were managed using Microsoft Excel for Windows (Microsoft Corporation, Redmond, WA, USA). Statistical analyses were completed using SPSS for Windows (Version 25.0, IBM, Somers, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Significance for all statistical tests was accepted at $p < 0.05$. Data are presented as mean ± standard deviation ($M \pm SD$) unless otherwise specified.

Model development

Standard assumptions for regression analysis were checked prior to building the model: 1) Linearity between the predictors and the outcome variable, assessed by visual examination of scatterplots for a linear relationship and by significant ($p < 0.05$) bivariate correlations; 2) Independence of error terms, indicated by a Durbin-Watson statistic between 1.0 and 3.0 (44, 45); 3) Equality of error variance, assessed by visual examination of scatterplots of the

standardized predicted values and residuals for homoscedasticity (44); 4) Normal distribution of errors, assessed by visual inspection of histograms and normal Q-Q plots of standardized residuals and by the Shapiro-Wilk test ($p > 0.05$ indicates no significant departure from normality) (44, 46). Multicollinearity, defined as a variance inflation factor (VIF) > 10.0 (47), was also assessed during the model-building process.

The base model regressed $\%BF_{5C}$ on the sum of skinfolds. Bivariate correlational analyses were used to determine which skinfold variable, i.e., individual skinfold measurements, seven-site skinfold sum, three-site skinfold terms reported in Jackson and Pollock (21, 22), or novel three-site skinfold terms (developed in the current study), exhibited the strongest association with the outcome variable of interest, $\%BF_{5C}$, based on Pearson's product-moment correlation coefficient (r). Novel three-site skinfold terms were generated from combinations of individual skinfold sites, selected based on the magnitude of the correlation coefficients. Linear regression analysis to predict $\%BF_{5C}$ from seven-site and the various three-site skinfold terms were compared using explained variance. The model with the largest R^2 effect size served as the base model.

Participant characteristics including age, sex, BMI, and waist circumference, as well as the interaction terms, were added to the model using a hierarchical variable selection procedure (48-50) based on previous prediction models. Age and sex were added first, as they have been consistently shown to impact body composition and are included in most existing models (21, 22, 24, 34, 36, 38). Waist circumference and BMI were added next, as they have been used to improve the predictive ability of equations using only skinfolds (24, 36). The last variables added to the model were the interaction terms, added one at a time in order of the highest correlation

coefficient with $\%BF_{5C}$. Model fit was assessed using adjusted R^2 change. Non-significant variables and those that introduced multicollinearity (VIF > 10.0) were removed from the model.

Cross-validation of the new model

Within the cross-validation cohort, new model ($\%BF_{New}$) performance, as well as $\%BF_{JP}$, $\%BF_{Evans}$, and $\%BF_{Peterson}$ were compared to the criterion $\%BF_{5C}$. Mean differences between predicted and criterion %BF were assessed using repeated measures ANOVA with simple planned contrasts and quantified using Cohen's *d* effect size. Agreement between predicted and criterion %BF was examined using the Bland-Altman method for calculating limits of agreement (51), the intraclass correlation coefficient (52, 53), and the standard error of the estimate.

RESULTS

Descriptive statistics are provided for our total sample, the development cohort, and the cross-validation cohort in Table 2. There were no differences in age, BMI, or $\%BF_{5C}$ between groups. Participants in the cross-validation group were taller and heavier than in the developmental group, likely due to a greater proportion of men. Skewness and kurtosis values were < |2.0| for all body composition metrics of interest (all skinfolds, waist circumference, BMI, $\%BF_{5C}$), indicating a normal distribution of variables.

Model development

All assumptions for multiple linear regression were satisfied. A Durbin-Watson statistic of 1.420 suggests independence of error terms; homoscedasticity of standardized residuals versus predicted values suggests equality of error variance; normality of standardized residuals was observed graphically and statistically ($W(279) = 0.990$, $p = 0.06$). Multicollinearity was not indicated in the model (VIF < 10 for all variables). Additionally, all hypothesized predictor variables were significantly (all $p < 0.001$) correlated with the outcome variable. The strongest

combination of skinfolds observed in our sample was the sum of the thigh, triceps, and midaxillary sites (SS_{new}). Individually, these 3 sites had the largest associations with $\%BF_{5C}$ (r values between 0.69 and 0.79). When summed, they produced a larger correlation coefficient with $\%BF_{5C}$ than the other sum of skinfolds terms so it was included in the development of the new model. Bivariate correlations of potential predictor variables are presented in Table 3.

The interaction term sex*age was significant ($p < 0.05$), with older men displaying larger $\%BF_{5C}$ values than younger men (mean difference, 5.5% BF), while older and younger women displayed similar values (mean difference, 0.1% BF). The term sex*waist did not impact the model ($p = 0.16$), indicating that the association between waist circumference and $\%BF_{5C}$ was consistent between men and women. Only the term sex*age was included in the model.

Initial regression analysis showed that SS_{new} explained 68.7% of the variance in $\%BF_{5C}$ in the development group, which was greater than the other skinfold terms (R^2 values between 0.59 and 0.65). The addition of sex and age ($p < 0.001$) increased explained variance to 74.5%, while waist and BMI brought it to 76.6%. The interaction term sex*age further increased the explained variance of the model to 77.5%. The complete model resulted in $F(6, 278) = 160.645$, $p < 0.001$, adjusted $R^2 = 0.775$, SEE = 4.0, and is presented in Table 4 with regression statistics from the model building process. The final equation, along with simplified equations for men and women separately, are presented in Table 5.

Cross-validation and equation comparison

Standardized residuals for all %BF values were normally distributed according to visual inspection of histograms, Q-Q plots, and the Shapiro-Wilk test of normality (all $p > 0.05$). Mauchly's test indicated a violation of sphericity, $\chi^2(9) = 154.642$, $p < 0.001$, therefore degrees of freedom were corrected using the Greenhouse-Geisser estimate ($\varepsilon = 0.536$). There was an

omnibus effect based on the corrected repeated measures ANOVA, $F(2.144, 165.058) = 38.370$, $p < 0.001$. Simple contrasts revealed that $\%BF_{Peterson}$ overestimated $\%BF_{5C}$ by 4.2% BF ($p < 0.001$). Mean values from $\%BF_{New}$, $\%BF_{JP}$, and $\%BF_{Evans}$ were not different from $\%BF_{5C}$ (all $p > 0.05$).

Intraclass correlation coefficients were comparably strong for all equations, ranging from 0.87 to 0.90 (all $p < 0.001$). Cohen's *d* effect size ranged from small to large, with $\%BF_{Evans}$ producing the smallest standardized mean difference ($d = -0.01$) and $\%BF_{Peterson}$ producing the largest ($d = 0.62$). $\%BF_{New}$ produced the smallest SEE (2.5% BF) and TE (4.4), while the largest SEE (3.6% BF) and TE (6.4) values were produced by $\%BF_{JP}$ and $\%BF_{Peterson}$, respectively. Limits of agreement (± 1.96 *SD* of the bias; Figure 1) ranged from ±8.6 ($\%BF_{New}$) to ±11.4% BF ($\%BF_{JP}$). Additionally, $\%BF_{JP}$ demonstrated proportional bias as indicated by a positive trend ($r = 0.29$, $p < 0.001$) between the bias and average %BF values. Cross-validation and comparison statistics are presented in Table 6.

DISCUSSION

The purpose of this study was to develop and cross-validate a skinfold-based prediction equation for %BF based on a criterion 5C model in a sample of healthy adults. The new equation was based on the sum of 3 skinfolds (midaxillary, subscapular, and thigh), sex, waist circumference, age, and BMI, and explained a significant amount of the variability observed in $\%BF_{5C}$. All equations were strongly associated with $\%BF_{5C}$ during cross-validation (ICCs ≥ 0.87). When compared to the criterion, $\%BF_{Evans}$ produced the smallest mean bias, while $\%BF_{Peterson}$ produced the largest (4.2% BF). The largest estimates of mean and individual error were produced by $\%BF_{JP,}$ which also displayed a tendency to overestimate %BF at higher levels of adiposity. Overall, $\%BF_{New}$ produced the smallest estimates of error and the narrowest limits

of agreement when compared to the criterion. Notably, the new equation presented in this study produced a lower SEE than reported by other authors, who have shown classic 2C-based skinfold equations (21, 22, 33, 34) to produce SEE values ranging from 3.5 to 5.0% BF (23, 54). These findings suggest that $\%BF_{New}$ may provide greater accuracy in individual estimates of 5C model %BF than other equations.

The present study builds upon previous research aimed at developing anthropometric-based equations for the prediction of %BF. The equations of Jackson and Pollock (21, 22) were developed to predict body density using a 2C model (hydrodensitometry) as the criterion method. While this technique allows for the determination of body volume, the assumption of constant FFM density is a significant source of error in the estimation of %BF (13, 28). For example, Withers et al. (19) reported that $\%BF_{JP}$ significantly underestimated 4C model %BF in a sample of men and women, independent of training status. Similar findings have been reported in samples of college-age adults, with $\%BF_{JP}$ underestimating criterion %BF from 4C (55) and 3C (56) models by 4.8 %BF and 5.0 %BF, respectively. Peterson et al. (24) also found $\%BF_{JP}$ to underestimate 4C %BF in healthy women by 6.6% BF. These results are similar to the present study, though the mean difference observed in our sample was lower than that reported by others. Additionally, the SEE and TE of $\%BF_{JP}$ produced during cross-validation in our cohort was similar to those reported by Esco et al. (56) when comparing $\%BF_{JP}$ to a criterion 3C model. The discrepancies observed in the present study and others suggest that equations developed from hydrodensitometry may not adequately account for inter-individual variability in FFM, thus leading to error when compared to multi-compartment methods.

As well as improving upon the 2C-based equations of Jackson and Pollock, $\%BF_{New}$ produced less overall and individual error than other equations developed from a multi-

compartment criterion. The discrepancy in validity statistics between $\%BF_{New}$ and these other equations may be due to the fact that the 5C model used in the present study accounts for soft tissue mineral, which is more dense than fat, protein, water, and bone mineral and thus may significantly alter estimates of %BF when compared to 4C models (18, 20). Although the inclusion of soft tissue mineral is based on a physiological sound rationale, practical research comparing 5C and 4C model estimates of %BF is limited. In one investigation, Moon et al. (16) demonstrated near-identical results between %BF values from the 4C and 5C models proposed by Wang et al. (18). However, this study was limited to a small sample of 29 female collegiate athletes with similar physical characteristics and may not be representative of the actual differences between the 2 models. Future research should compare %BF from 4C and 5C models in larger, more heterogeneous samples of participants.

Differences in cross-validation performance are also likely due to methodological differences between the present study and those in which the other equations were developed. For example, $\%BF_{Peterson}$ was developed in a sample of 681 healthy, white adults aged 18 to 55 years using a 4C model as the criterion (24). The sex-specific equation reported by the authors explained 61.2% and 69.9% of the variance observed in 4C %BF in men and women, respectively, and produced a small mean difference (< 0.2% BF) during cross-validation (24). Although their sample was similar to ours in terms of age, height, and weight, $\%BF_{Peterson}$ produced the largest mean difference (4.2% BF) and TE (6.37) when cross-validated in our sample. Nickerson et al. (55) observed a similarly large mean difference, reporting that $\%BF_{Peterson}$ underestimated criterion 4C %BF by 3.1% BF. This error may be attributed to the criterion 4C model used in the development of $\%BF_{Peterson}$, which was originally developed in a sample of 10 male soldiers ranging in age from 19 to 24 years (57). This specificity in the

criterion 4C model of Peterson et al. likely limits its applicability to a more general population, such as that in our own sample. The criterion model used in the present study was derived from a sample of 156 healthy adults ranging in age from 25 to 74 years and included BMI values ranging from 21.7 to 50.1 $kg \cdot m^{-2}$ (18).

Compared to $\%BF_{Peterson}$, Evans et al. (23) used a more general 4C model (58) to develop their equation in a sample of 132 collegiate athletes. Further, the authors expanded on the anthropometric approach to predicting multi-compartment %BF by including sex and race as independent variables. The authors cross-validated the equation and found that $\%BF_{Evans}$ resulted in a strong correlation ($r = 0.85$) between criterion and predicted %BF, with a nonsignificant slope and intercept when compared to the line of identity (23). The equation was also cross-validated in Moon et al. (59), who found $\%BF_{Evans}$ to accurately predict 4C %BF with minimal error. Similarly, $\%BF_{Evans}$ produced a small mean difference when compared to our criterion 5C. However, the limits of agreement were wider than $\%BF_{New}$ and $\%BF_{Peterson}$ and SEE and TE were larger than $\%BF_{New}$. The error associated with $\%BF_{Evans}$ observed in our sample is likely due to differences in sample demographics. Their sample consisted of college athletes who were considerably younger (20.8 ± 2.3 years) and leaner (14.6 ± 6.5% BF) than our own sample, which would limit the ability of their equation to accurately predict higher %BF. Especially given that our regression analysis found an interaction between sex and age, $\%BF_{Evans}$ may be particularly prone to error when predicting %BF in older men.

Although not the explicit purpose of the present study, the interaction effect observed between sex and age clearly showed that %BF increased with age in the men, while there were no differences observed in the women. These findings are similar to those noted by van Der Ploeg et al. (36), who noted that the sum of skinfolds was associated with a higher %BF in older

men than younger men. These observations suggest that body composition status changes as age increases and may be the result of alterations in adipose tissue storage, an increase in skinfold compressibility, or a decrease in skin thickness (36). There could also be hormonal changes associated with age in men that impact body composition more than women, as longitudinal studies have shown that men tend to exhibit unfavorable changes in body composition (i.e., decreases in fat-free mass and increases in fat mass) than women over time (60, 61). This presents unique challenges for the measurement of subcutaneous body fat in older adults and is a topic that should be explored thoroughly in the future.

There are several limitations that should be considered before implementation of the equation described in this study. Most notably, the sample used in this study was generated by combining participants from an older project with the current study. This may lead to measurement error between the 2 groups of participants, as different investigators were involved in the 2 projects. Further, the use of BIS rather than chemical dilution may result in errors in estimation of TBW. Although the BIS device used in this study has been validated against the deuterium oxide dilution technique in healthy adults, the authors reported limits of agreement of approximately ± 6 L (62). Finally, our sample was predominantly college-age and Caucasian, which may limit generalizability of the equation. Although our age range is comparable to those reported by other researchers (24, 36), the majority of our participants were under the age of 26 and so our results may be skewed towards that age group, which is particularly important to consider in light of the interaction effect between sex and age described earlier.

CONCLUSION

This study provides researchers and practitioners with a simple equation for predicting 5C model %BF using simple skinfold and anthropometric measures. The new equation cross-

validated well against the criterion, providing a small mean difference, smaller estimates of error, and narrower limits of agreement when compared to existing equations. Additionally, our analysis revealed a significant interaction between sex and age, which suggests that body composition changes may occur differently in men than women as age increases. The equation developed in this study may be used for the estimation of %BF when laboratory methods are not available or practical and may provide better estimates than existing equations. However, due to the sampling limitations discussed previously, caution should be employed when using this equation in samples other than healthy, white adults.

REFERENCES

1. Fox C, Massaro J, Hoffmann U, Pou K, Maurovich-Horvat P, Liu C. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116:39-48.

2. Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nature Genetics. 2017;49(6):842.

3. Smith SR, Lovejoy JC, Greenway F, Ryan D, de la Bretonne J, Volafova J, et al. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. Metabolism-Clinical and Experimental. 2001;50(4):425-35.

4. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, et al. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study. The Journal of Clinical Endocrinology and Metabolism. 2010;95(12):5419-26.

5. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nature Reviews Cancer. 2015;15(8):484.

6. Esco M, Fedewa M, Cicone Z, Sinelnikov O, Sekulic D, Holmes C. Field-based performance tests are related to body fat percentage and fat-free mass, but not body mass index, in youth soccer players. Sports. 2018;6(4):105.

7. Silvestre R, Kraemer WJ, West C, Judelson DA, Spiering BA, Vingren JL, et al. Body composition and physical performance during a National Collegiate Athletic Association Division I men's soccer season. The Journal of Strength and Conditioning Research. 2006;20(4):962.

8. Green MR, Pivarnik JM, Carrier DP, Womack CJ. Relationship between physiological profiles and on-ice performance of a National Collegiate Athletic Association Division I hockey team. Journal of Strength and Conditioning Research. 2006;20(1):43.

9. Chaouachi A, Brughelli M, Chamari K, Levin GT, Abdelkrim NB, Laurencelle L, et al. Lower limb maximal dynamic strength and agility determinants in elite basketball players. The Journal of Strength and Conditioning Research. 2009;23(5):1570-7.

10. Tangalos C, Robertson SJ, Spittle M, Gastin PB. Predictors of individual player match performance in junior Australian football. International Journal of Sports Physiology and Performance. 2015;10(7):853-9.

11. Wang J, Thornton J, Kolesnik S, Pierson R. Anthropometry in body composition: an overview. Annals of the New York Academy of Sciences. 2000;904(1):317-26.

12. Heyward VH. Practical body composition assessment for children, adults, and older adults. International Journal of Sport Nutrition. 1998;8(3):285-307.

13. Siri WE. Body composition from fluid spaces and density: analysis of methods. Techniques for Measuring Body Composition. 1961;61:223-44.

14. Brožek J, Grande F, Anderson JT, Keys A. Densitometric analysis of body composition: revision of some quantitative assumptions. Annals of the New York Academy of Sciences. 1963;110(1):113-40.

15. Fidanza F, Keys A, Anderson JT. Density of body fat in man and other mammals. Journal of Applied Physiology. 1953;6(4):252-6.

16. Moon JR, Eckerson JM, Tobkin SE, Smith AE, Lockwood CM, Walter AA, et al. Estimating body fat in NCAA Division I female athletes: a five-compartment model validation of laboratory methods. European Journal of Applied Physiology and Occupational Physiology. 2009;105(1):119-30.

17. Withers RT, Laforgia J, Heymsfield SB, Wang ZM, Pillans RK. Two, three and four-compartment chemical models of body composition analysis. Anthropometrica, eds Norton KI, Olds TS (UNSW, Sydney, Australia). 1996:199-231.

18. Wang Z, Pi-Sunyer FX, Kotler DP, Wielopolski L, Withers RT, Pierson Jr RN, et al. Multicomponent methods: evaluation of new and traditional soft tissue mineral models by in vivo neutron activation analysis. The American Journal of Clinical Nutrition. 2002;76(5):968-74.

19. Withers RT, LaForgia J, Pillans R, Shipp N, Chatterton B, Schultz C, et al. Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women. Journal of Applied Physiology. 1998;85(1):238-45.

20. Heymsfield SB, Waki M, Kehayias J, Lichtman S, Dilmanian FA, Kamen Y, et al. Chemical and elemental analysis of humans in vivo using improved body composition models. American Journal of Physiology-Endocrinology And Metabolism. 1991;261(2):E190-E8.

21. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. British Journal of Nutrition. 1978;40(3):497-504.

22. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Medicine and Science in Sports and Exercise. 1980;12(3):175-81.

23. Evans EM, Rowe DA, Misic MM, Prior BM, Arngrímsson SA. Skinfold prediction equation for athletes developed using a four-component model. Medicine and Science in Sports and Exercise. 2005;37(11):2006-11.

24. Peterson MJ, Czerwinski SA, Siervogel RM. Development and validation of skinfold-thickness prediction equations with a 4-compartment model. The American Journal of Clinical Nutrition. 2003;77(5):1186-91.

25. Armstrong LE. Hydration assessment techniques. Nutrition Reviews. 2005 Jun 1;63(suppl_1):S40-54.

26. Jackson AS, Pollock ML. Practical assessment of body composition. The Physician and Sportsmedicine. 1985 May 1;13(5):76-90.

27. ACSM's Guidelines for Exercise Testing and Prescription: Wolters Kluwer; 2018.

28. Wang Z, Deurenberg P, Guo SS, Pietrobelli A, Wang J, Pierson Jr R, et al. Six-compartment body composition model: inter-method comparisons of total body fat measurement. International Journal of Obesity. 1998;22(4):329.

29. Wilmore JH. A simplified method for determination of residual lung volumes. Journal of Applied Physiology. 1969;27(1):96-100.

30. Goldman RF, Buskirk ER. Body volume measurement by underwater weighing: description of a method. Techniques for Measuring Body Composition. 1961;3:78-89.

31. Brožek J, Henschel A, Keys A. Effect of submersion in water on the volume of residual air in man. Journal of Applied Physiology. 1949;2(5):240-6.

32. Moon JR, Stout JR, Smith AE, Tobkin SE, Lockwood CM, Kendall KL, et al. Reproducibility and validity of bioimpedance spectroscopy for tracking changes in total body water: implications for repeated measurements. British Journal of Nutrition. 2010;104(9):1384-94.

33. Durnin J, Rahaman MM. The assessment of the amount of fat in the human body from measurements of skinfold thickness. British Journal of Nutrition. 1967;21(3):681-9.

34. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. British Journal of Nutrition. 1974;32(1):77-97.

35. Lohman TG. Skinfolds and body density and their relation to body fatness: a review. Human Biology. 1981;53(2):181.

36. van der Ploeg G, Gunn S, Withers R, Modra A. Use of anthropometric variables to predict relative body fat determined by a four-compartment body composition model. European Journal of Clinical Nutrition. 2003;57(8):1009.

37. Davidson LE, Wang J, Thornton JC, Kaleem Z, Silva-Palacios F, Pierson RN, et al. Predicting fat percent by skinfolds in racial groups: Durnin and Womersley revisited. Medicine and Science in Sports and Exercise. 2011;43(3):542.

38. Jackson A, Stanforth P, Gagnon J, Rankinen T, Leon A, Rao D, et al. The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. International Journal of Obesity. 2002;26(6):789.

39. Wang J, Thornton JC, Russell M, Burastero S, Heymsfield S, Pierson Jr RN. Asians have lower body mass index (BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. The American Journal of Clinical Nutrition. 1994;60(1):23-8.

40. Wellens RI, Roche AF, Khamis HJ, Jackson AS, Pollock ML, Siervogel RM. Relationships between the body mass index and body composition. Obesity Research. 1996;4(1):35-44.

41. Enzi G, Gasparo M, Biondetti PR, Fiore D, Semisa M, Zurlo F. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. The American Journal of Clinical Nutrition. 1986;44(6):739-46.

42. Nedungadi TP, Clegg DJ. Sexual dimorphism in body fat distribution and risk for cardiovascular diseases. Journal of Cardiovascular Translational Research. 2009;2(3):321-7.

43. Taylor RW, Grant AM, Williams SM, Goulding A. Sex differences in regional body fat distribution from pre-to postpuberty. Obesity. 2010;18(7):1410-6.

44. Field A. Discovering Statistics Using IBM SPSS Statistics: Sage; 2013.

45. Durbin J, Watson GS. Testing for serial correlation in least squares regression. II. Biometrika. 1951;38(1/2):159-77.

46. Shapiro S, Wilk M. Approximations for the null distribution of the W statistic. Technometrics. 1968;10(4):861-6.

47. Wetherill GB. Regression Analysis with Application: Chapman & Hall, Ltd.; 1987.

48. Lomax RG. An Introduction to Statistical Concepts for Education and Behavioral Sciences: Psychology Press; 2001.

49. Henderson DA, Denison DR. Stepwise regression in social and psychological research. Psychological Reports. 1989;64(1):251-7.

50. Streiner DL. Regression in the service of the superego: The do's and don'ts of stepwise multiple regression. The Canadian Journal of Psychiatry. 1994;39(4):191-6.

51. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. The Lancet. 1986;327(8476):307-10.

52. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychological Bulletin. 1979;86(2):420.

53. Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. The Journal of Strength and Conditioning Research. 2005;19(1):231.

54. Williams DP, Going SB, Lohman TG, Hewitt MJ, Haber AE. Estimation of body fat from skinfold thicknesses in middle-aged and older men and women: A multiple component approach. American Journal of Human Biology. 1992;4(5):595-605.

55. Nickerson BS, Fedewa MV, Cicone Z, Esco MR. The relative accuracy of skinfolds compared to four-compartment estimates of body composition. Clinical Nutrition. 2019.

56. Esco MR, Nickerson BS, Fedewa MV, Moon JR, Snarr RL. A novel method of utilizing skinfolds and bioimpedance for determining body fat percentage via a field-based three-compartment model. European Journal of Clinical Nutrition. 2018;72(10):1431.

57. Friedl K, DeLuca JP, Marchitelli L, Vogel JA. Reliability of body-fat estimations from a four-compartment model by using density, body water, and bone mineral measurements. The American Journal of Clinical Nutrition. 1992;55(4):764-70.

58. Lohman TG. Applicability of body composition techniques and constants for children and youths. Exercise and Sport Sciences Reviews. 1986;14:325-57.

59. Moon JR, Tobkin SE, Smith AE, Lockwood CM, Walter AA, Cramer JT, et al. Anthropometric estimations of percent body fat in NCAA Division I female athletes: a 4-compartment model validation. The Journal of Strength and Conditioning Research. 2009;23(4):1068-76.

60. Hughes VA, Frontera WR, Roubenoff R, Evans WJ, Singh MAF. Longitudinal changes in body composition in older men and women: role of body weight change and physical activity. The American Journal of Clinical Nutrition. 2002;76(2):473-81.

61. Michalakis K, Goulis D, Vazaiou A, Mintziori G, Polymeris A, Abrahamian-Michalakis A. Obesity in the ageing man. Metabolism: Clinical and Experimental. 2013;62(10):1341-9.

62. Moon JR, Tobkin SE, Roberts MD, Dalbo VJ, Kerksick CM, Bemben MG, et al. Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. Nutrition and Metabolism. 2008;5(1):7.

Table 1. Equations for the prediction of %BF

Wang 5C (criterion)	$FM = 2.748 \times BV - 0.715 \times TBW + 1.129 \times M_O + 1.222 \times M_S - 2.051 \times BM$
	$\%BF_{5C} = (FM/BM) \times 100$
Jackson and Pollock	
Men	$BD = 1.1093800 - 0.0008267 \times SS_1 + 0.0000016 \times SS_1^2 - 0.0002574 \times Age$
Women	$BD = 1.0994921 - 0.0009929 \times SS_2 + 0.0000023 \times SS_2^2 - 0.0001392 \times Age$
Siri et al.	$\%BF_{JP} = (4.95/BD - 4.50) \times 100$
Evans et al.	$\%BF_{Evans} = 8.997 + 0.24658 \times SS_3 - 6.343 \times Sex - 1.998 \times Race$
Peterson et al.	
Men	$\%BF_{Peterson} = 20.94878 + 0.1166 \times Age - 0.11666 \times Ht + 0.42696 \times SS_4 - 0.00159 \times SS_4^2$
Women	$\%BF_{Peterson} = 22.18945 + 0.06368 \times Age + 0.60404 \times BMI - 0.14520 \times Ht + 0.30919 \times SS_4 - 0.00099562 \times SS_4^2$

%BF, percent body fat; BD, body density in $g \cdot cm^{-3}$; BM, body mass in kg; BMI, body mass index in $kg \cdot m^{-2}$; BV, body volume in L; FM, fat mass in kg; Ht, height in cm; SS_x, sum of skinfolds in mm; TBW, total body water in L; M_o, osseous bone mineral content in kg; M_S, soft tissue bone mineral in kg.
Equation variables:
SS_1 = chest, abdomen, thigh
SS_2 = triceps, suprailiac, thigh
SS_3 = triceps, abdomen, thigh
SS_4 = triceps, subscapular, suprailiac, thigh
Gender coded as 0 = female, 1 = male
Race coded as 0 = white, 1 = black

Table 2. Descriptive characteristics of participants

Variable	Total (n = 357)	Development (n = 279)	Cross-validation (n = 78)
Age (y)	25.8 ± 10.7	26.0 ± 11.0	25.2 ± 9.5
Height (cm)	171.6 ± 9.3	171.0 ± 9.2	173.9 ± 9.4
Weight (kg)	74.8 ± 17.3	73.7 ± 17.9	78.6 ± 14.7
BMI ($kg \cdot m^{-2}$)	25.3 ± 4.9	25.1 ± 5.2	25.9 ± 3.7
Body fat (%)	22.6 ± 8.3	22.9 ± 8.4	21.5 ± 7.7
Caucasian/white (%)	84.4	83.2	88.5
Female (%)	52.2	55.4	41.0

BMI, body mass index; cm, centimeter; kg, kilogram; m, meters; y, years. Body fat determined by the 5-compartment model.

Table 3. Bivariate correlations between predictor variables and 5C %BF (n = 279)

Predictor variable	r
Skinfolds (mm)	
Abdominal	0.65
Triceps	0.73
Chest	0.43
Midaxillary	0.69
Subscapular	0.62
Suprailiac	0.67
Thigh	0.79
Sum of skinfolds (mm)	
SS_{new}	0.83
Evans et al. sum of 3	0.81
Peterson et al. sum of 4	0.80
Jackson and Pollock sum of 3	0.79
Sum of 7	0.77
Age (y)	0.14
Sex	-0.60
BMI ($kg \cdot m^{-2}$)	0.35
Waist circumference (cm)	0.37

5C %BF, five-compartment model percent body fat; BMI, body mass index; r, Pearson's correlation coefficient.

Table 4. Prediction of 5C %BF from anthropometric measures (n = 279)

Model	Intercept	Model coefficients						*F* change (*p* value)	Adjusted R^2	SEE (%)
		SS_{new} (mm)	Sex	Age (y)	BMI ($kg \cdot m^{-2}$)	Waist circumference (cm)	Sex*Age			
1	0.7810*	0.249*	--	--	--	--	--	< 0.001	0.687	4.73
2	12.433*	0.208*	-4.736*	--	--	--	--	< 0.001	0.745	4.26
3	12.428*	0.207*	-4.738*	0.000	--	--	--	0.99	0.745	4.27
4	10.536*	0.181*	-5.820*	0.001	0.157	--	--	0.06	0.747	4.25
5	3.934*	0.145*	-7.807*	-0.058*	-0.243*	0.255*	--	< 0.001	0.766	4.09
6	6.083*	0.143*	-12.058*	-0.150*	-0.233*	0.256*	0.162*	0.001	0.775	4.01

5C %BF, five-compartment model percent body fat; adjusted R^2, coefficient of determination adjusted for multiple predictors; BMI, body mass index; cm, centimeters; kg, kilograms; mm, millimeters; SS_{new}, sum of skinfolds (thigh, midaxillary, triceps); SEE, standard error of the estimate; y, years.
Sex is coded as 0=female, 1=male.
* $p < 0.05$.

Table 5. Final equations for predicting 5C %BF in healthy men and women

Full model

$$\%BF_{New} = 6.083 + (0.143 \times SS_{new}) - (12.058 \times Sex) - (0.150 \times Age) - (0.233 \times BMI) + (0.256 \times WC) + (0.162 \times Sex*Age)$$

Simplified models

Men

$$\%BF_{New} = -5.975 + (0.143 \times SS_{new}) + (0.012 \times Age) - (0.233 \times BMI) + (0.256 \times WC)$$

Women

$$\%BF_{New} = 6.083 + (0.143 \times SS_{new}) - (0.150 \times Age) - (0.233 \times BMI) + (0.256 \times WC)$$

5C %BF, five-compartment model percent body fat; Age, in y; BMI, body mass index in $kg \cdot m^{-2}$; SS_{new}, sum of thigh, midaxillary, and triceps skinfolds in mm; Sex, coded as 0=female, 1=male; WC, waist circumference in cm.

Table 6. Comparison statistics between criterion and predicted %BF (n = 78)

	Mean ± *SD* (%fat)	ICC	*d*	SEE	TE	Limits of agreement			
						Bias ± 1.96 *SD*	Lower	Upper	Trend
Criterion 5C	21.5 ± 7.7								
Cicone et al.	21.8 ± 6.9	0.90**	0.06	2.48	4.38	0.42 ± 8.60	-8.18	9.03	-0.21
Jackson and Pollock	20.5 ± 9.3	0.87**	-0.11	3.62	5.86	-1.00 ± 11.40	-12.40	10.40	0.29***
Evans et al.	21.5 ± 8.6	0.87**	-0.01	3.43	5.45	-0.05 ± 10.75	-10.80	10.69	0.18
Peterson et al.	25.7 ± 6.8*	0.87**	0.62	3.02	6.37	4.17 ± 9.50	-5.33	13.67	-0.21

Abbreviations: %BF, percent body fat; bias, mean difference between predicted and criterion body fat percentage; *d*, Cohen's standardized effect size; ICC, intraclass correlation coefficient; *SD*, standard deviation; SEE, standard error of the estimate; TE, total error; trend, correlation (expressed as Pearson's *r*) between the bias and average.

*$p < 0.001$ compared to criterion.

**$p < 0.001$.

***$p < 0.001$.

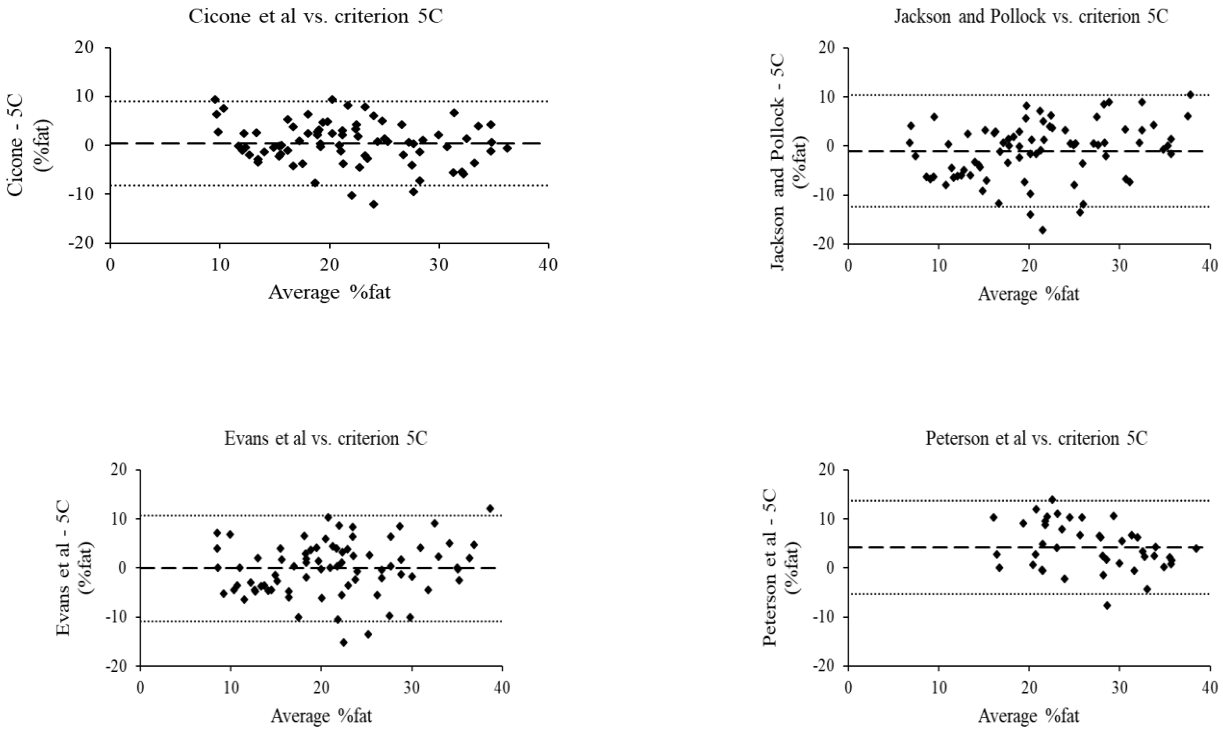

Figure 1. Bland-Altman plots of measured versus predicted %BF (n = 78). %BF, percent body fat. Dark center line represents mean bias. Light dotted outer lines represent limits of agreement (bias ± 1.96 *SD* of bias).

CHAPTER 5

CONCLUSION

Body composition measurement is a critical component in the toolbox of every exercise physiologist. Various methods of determining percent body fat (%BF) have been developed, and include indirect (i.e., underwater weighing) and doubly indirect (i.e., bioimpedance analysis, skinfold equations) techniques. While these assessment methods are commonly used in laboratory and practical settings, there are methodological discrepancies associated with them that have yet to be addressed. The purpose of this dissertation was to critically examine existing techniques and develop new equations for determining various components of body composition.

The first study sought to develop a novel equation for the prediction of underwater RLV in healthy adults. Regression analysis in a sample of 175 men and women produced a significant ($p < 0.001$) equation for predicting RLV from age and height. The new equation accurately predicted underwater RLV upon cross-validation, while existing equations misestimated ($p < 0.001$). Additionally, the new equation produced the smallest mean bias and limits of agreement (0.07 ± 0.50 L) and the smallest error (SEE = 0.17 L). The results of this study suggest that the new equation may be used by practitioners to accurately estimate underwater RLV during hydrostatic weighing procedures when RLV measurement is not possible.

Study 2 aimed to systematically review and meta-analyze the literature related to the accuracy of SFBIA for TBW determination. The standardized mean difference (SMD = 0.02, p =

0.82) of 264 effects extracted from 51 original studies indicated that SFBIA accurately estimated TBW compared to isotope dilution techniques. Moderator analyses revealed that there was statistically significant error in SFBIA estimation associated with frequency and resistivity index ($p < 0.0001$), as well as sample sex (% female, $p < 0.0001$). SFBIA procedures utilizing Ht^2/R produced less error than Ht^2/Z across all frequencies. Overall, the combination of Ht^2/R at 100 kHz produced the least error in TBW estimation. The results presented in this study suggest that SFBIA provides accurate estimates of TBW compared to criterion dilution techniques, although care should be taken to use appropriate SFBIA parameters to minimize error in the estimate.

The purpose of study 3 was to develop a novel equation to predict 5C model %BF from skinfolds and anthropometric measurements in healthy adults. Regression analysis in a sample of 279 men and women produced a significant equation ($p < 0.001$) using sum of three skinfolds (midaxillary, subscapular, and thigh), sex, age, waist circumference, and body mass index (BMI). Compared to existing equations, the new equation produced the strongest association (ICC = 0.90), narrowest limits of agreement (± 8.60%), and smallest error (SEE = 2.48%) when cross-validated against the criterion 5C model. These findings suggest that the new equation may provide more accurate estimates of %BF than other skinfold-based equations and can be used by practitioners when assessing body composition in healthy adults.

Overall, this dissertation expands upon limitations of common body composition assessment techniques. Accurate equations which produced stronger indices of agreement and validity than select existing equations are presented for use with the general population of healthy adults. Additionally, SFBIA was shown to provide accurate estimates of TBW when appropriate testing parameters are used.

APPENDIX

THE UNIVERSITY OF ALABAMA | Office of the Vice President for Research & Economic Development
Office for Research Compliance

February 25, 2020

Michael Esco, Ph.D.
Assistant Professor
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 15-019-ME-R4
"Development of a Novel Body Fat Prediction Equation: A Four-Compartment Model Approach"

Dr. Esco:

The University of Alabama Institutional Review Board has granted approval for your continuing review application. Your continuing review application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on December 4, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved informed consent form to obtain consent from your participants.

Good luck with your research,

[redacted]

J. Grier Stewart, MD, FACP
Medical IRB Chair

Jessup Building | Box 870127 | Tuscaloosa, AL 35487-0127
205-348-8461 | Fax 205-348-7189 | Toll Free 1-877-820-3066