UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

VALIDITY OF BIOIMPEDANCE FOR THE ASSESSMENT OF TOTAL BODY AND SEGMENTAL FAT-FREE MASS IN OLDER MEN AND WOMEN AND A COMPARISON OF METHODS USED TO CLASSIFY SARCOPENIA

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

JORDAN R. MOON
Norman, Oklahoma
2009

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/318520

VALIDITY OF BIOIMPEDANCE FOR THE ASSESSMENT OF TOTAL BODY AND SEGMENTAL FAT-FREE MASS IN OLDER MEN AND WOMEN AND A COMPARISON OF METHODS USED TO CLASSIFY SARCOPENIA

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY

Dr. Jeffrey R. Stout, Chair

Dr. Michael G. Bemben

Dr. Joel T. Cramer

Dr. Travis W. Beck

Dr. Trina L. Hope

© Copyright by JORDAN ROBERT MOON 2009
All Rights Reserved.

ACKNOWLEDGEMENTS

I would like to dedicate my dissertation to my friends, family, and mentors who have helped me every step of the way. Without your help I could never have accomplished this goal. I am forever indebted to my mentor Dr. Jeff Stout, who has believed in me and supported me for the last five years. I would not be where I am, nor who I am without you. I cannot thank you enough Dr. Stout; I hope someday to be the mentor, teacher, and friend that you are today. To my parents, thank you for your continuing support and dedication to my well-being. You taught me to work hard and always encouraged me; without your support this would not have been possible. I would also like to thank Dr. Joel Cramer, Dr. Mike Bemben, Dr. Travis Beck, and Dr. Trina Hope, for your mentorship and guidance throughout my doctoral efforts, and for your support as my doctoral committee members. Great thanks needs to be given to my friend Sarah Tobkin, you have made this journey not only possible, but exciting, without you my life would have been harder and my days longer. Thank you for your support, kindness, and dedication to my future.

“If we knew what it was we were doing, it would not be called research, would it?”

“Most people say that is it is the intellect which makes a great scientist.

They are wrong: it is character. “

- Albert Einstein

TABLE OF CONTENTS

ACKNOWLEDGEMENTS …… iv

ABSTRACT …… x

TABLES …… viii

FIGURES …… ix

CHAPTER

I. INTRODUCTION …… 1

Hypotheses …… 6

Definition of Terms …… 8

Abbreviations …… 8

Delimitations …… 10

Assumptions …… 10

Limitations …… 11

II. REVIEW OF LITERATURE …… 12

Basic Principles of Bioimpedance …… 13

Fat-Free Mass Equations …… 16

Total Body Muscle Mass Equations …… 18

Multi-Frequency BIA …… 20

Appendicular Lean Mass Equations …… 21

Sarcopenia Classification Methods …… 22

Conclusion …… 22

III. METHODS …… 24

Participants …… 24

Research Design …… 24

Variables …… 25

Bioimpedance Spectroscopy (BIS) …… 25

Bioelectrical Impedance Analysis (BIA) …… 27

Air-Displacement Plethysmography …… 28

Deuterium Oxide (D_2O) …… 29

Dual-Energy X-Ray Absorptiometry (DXA) …… 30

Four-Compartment Model (4C) …… 31

Statistical Analysis …… 32

II. RESULTS …… 33

Total Body Water …… 33

Bioimpedance Spectroscopy (BIS) Total Body Equations …… 34

Bioimpedance Spectroscopy (BIS) Segmental Equations …… 35

InBody720 (MFBIA) Segmental and Total Body Analysis …… 35

Bioimpedance Analysis (BIA) Fat-Free Mass Equations ....…… 37

Bioimpedance Analysis (BIA) Total Body Muscle Mass Equations … 37

Appendicular Lean Mass …… 38

Relative Skeletal Muscle Index (RSMI) …… 39

Accuracy of Relative Skeletal Muscle Index (RSMI) and Skeletal Muscle Index (SMI) Predictions …… 40

V. DISCUSSION …… 42

Total Body Water …… 42

Bioimpedance Spectroscopy (BIS) Total Body Equations 44
Bioimpedance Spectroscopy (BIS) Segmental Equations 47
InBody720 (MFBIA) Segmental and Total Body Analysis 49
Bioimpedance Analysis (BIA) Fat-Free Mass Equations 51
Bioimpedance Analysis (BIA) Total Body Muscle Mass Equations ... 54
Appendicular Lean Mass 55
Relative Skeletal Muscle Index (RSMI) 57
Accuracy of Relative Skeletal Muscle Index (RSMI) and Skeletal Muscle Index (SMI) Predictions 57
Conclusion 59
REFERENCES 61
APPENDIX 69
Prediction Equations 69
Tables 70
Figures 80
Figure Legends 88
Informed Consent 90
Health History Questionnaire 100

LIST OF TABLES

Table 1. Descriptive Characteristics of Subject …… 70

Table 2. Total Body Water Validity Results …… 71

Table 3. Bioimpedance Spectroscopy (BIS) Total Body Equations

Validity Results …… 72

Table 4. Bioimpedance Spectroscopy (BIS) Segmental Equations

Validity Results …….. 73

Table 5. InBody720 (MFBIA) Segmental and Total Body Analysis

Validity Results ……. 74

Table 6. Bioimpedance Analysis (BIA) Fat-Free Mass Equations

Validity Results ……. 75

Table 7. Bioimpedance Analysis (BIA) Total Body Muscle Mass

Validity Results ……. 77

Table 8. Appendicular Lean Mass Validity Results …… 78

Table 9. Relative Skeletal Muscle Index (RSMI) Validity Results …… 79

LIST OF FIGURES

Figure 2. Correct Percentage for RSMI Equations for classifying sarcopenia … 80

Figure 2. Incorrect Percentage for RSMI Equations for classifying sarcopenia … 81

Figure 3. Total Accuracy of RSMI Prediction equations for classifying sarcopenia …… 82

Figure 4. Correct Percentage for SMI Equations for classifying sarcopenia … 83

Figure 5. Incorrect Percentage for SMI Equations for classifying sarcopenia … 84

Figure 6. Total Accuracy of SMI Prediction equations for classifying sarcopenia …….. 85

Figure 7. Number of Subjects Classified as Sarcopenic by Both Methods …… 86

Figure 8. Total Agreement Between Methods for Classifying as Sarcopenia …… 87

ABSTRACT

VALIDITY OF BIOIMPEDANCE FOR THE ASSESSMENT OF TOTAL BODY AND SEGMENTAL FAT-FREE MASS IN OLDER MEN AND WOMEN AND A COMPARISON OF METHODS USED TO CLASSIFY SARCOPENIA

Jordan R. Moon, Ph.D.

The University of Oklahoma, 2009

Supervising Professor: Jeffrey R. Stout, Ph.D.

The purpose of the current investigation was to evaluate the validity of several total body fat-free mass (FFM), muscle mass (TBMM), water (TBW), and appendicular lean mass (ALM) equations in older adults compared to a criterion four-compartment (4C) model and dual-energy X-ray absorptiometry (DXA). Additionally, this investigation examined two body composition-based methods for the classification of sarcopenia. Seventy-four healthy older men (n = 32) and women (n = 42) participated in the investigation (mean ± SD, age = 72 ± 6 years, height =167.5 ± 8.5 cm, mass = 69.49 ± 12.71 kg). Body composition was assessed using bioimpedance analysis (BIA/MFBIA) and spectroscopy (BIS) and compared to a 4C model and DXA. Additionally, relative skeletal muscle index (RSMI) and skeletal muscle index (SMI) were calculated using DXA and BIA, respectively. In both men and women, TBW methods produced low SEE values (< 1.57 kg) and high r values (> 0.92), but mean differences were observed (> -2.01 L) compared to deuterium oxide. A BIS-estimated TBW two-compartment model produced low SEE values (< 2.17 kg) and high r values (> 0.88), but mean differences were observed (> -3.71 kg) compared to the 4C model. BIS

TBMM equations resulted in low SEE values (< 2.28 kg) and high r values (> 0.79), but mean differences were observed (> -0.68 kg) in all but one equation for men and women combined, and one for the women alone compared to DXA muscle mass values. Equations for ALM estimated via BIS resulted in low SEE values (< 2.07 kg) and high r values (> 0.69), but mean differences were observed (> -1.20 kg) compared to DXA lean mass values. A MFBIA device produced low SEE values (< 2.14 kg) and high r values (> 0.77), but mean differences were observed (> -0.42 kg) with the exception of lean mass in the legs for men, compared to the 4C model and DXA. All BIA FFM equations produced low SEE values (< 2.29 kg) and high r values (> 0.83), but mean differences were observed (> 1.11 kg) with the exception of one equation for all groups and one equation for women only, compared to the 4C model. Total body muscle mass estimated via BIA resulted in low SEE values (< 2.14 kg) and high r values (> 0.81), but mean differences were observed (> -0.50 kg). Appendicular lean mass BIA and MFBIA produced low SEE values (< 1.68 kg) and high r values (> 0.83), but mean differences were observed (> -0.71 kg) with the exception of one equation for all groups and the MFBIA in men. Of the ALM equations, one produced valid results ($r > 0.75$, SEE < 0.48) for RSMI compared to DXA. This equation resulted in a total accuracy of 91% in all men and women compared to DXA RSMI for the classification of sarcopenia. Comparing a TBMM equation that has been used to classify sarcopenia to DXA muscle mass, there was a 69% agreement. The BIA-based sarcopenia classification method indicated forty-four subjects as sarcopenic, while the DXA-based method only classified sixteen as sarcopenic. Total agreement between sarcopenia classification methods was only 24%. Mean differences suggest corrections are needed for systematic deviations produced by nearly all equations. However, there are accurate BIA equations, and more complicated MFBIA and BIS equations were no better than these BIA equations. Therefore, BIA is an acceptable method to predict both FFM and ALM in older men and women and can be used

as an alternative to DXA or a 4C model. Poor agreement between sarcopenia classification methods indicates a need for a standardized procedure. Nonetheless, the accurate ALM BIA equation used to predict RSMI for use in sarcopenia classification produced an individual accuracy of 91%, suggesting that using an ALM equation to predict DXA ALM is more appropriate than generating a new BIA-based sarcopenia classification method.

CHAPTER I

INTRODUCTION

By 2050, the number of Americans age 85 and older will be nearly 18 million (16). In 2011 the first of 76 million Baby Boomers will turn 65 years of age. By 2021, this group will reach 75 years of age, the age when healthcare costs start to escalate (64), making the ten-year period from 65 to 75 years of age crucial for reducing health-related costs and improving the quality of life of aging individuals. More importantly, in the fastest growing age group, the Medicare expenditures per enrollee for those 85 and older are much higher compared to younger groups (75). Specifically, average nursing home costs for individuals aged 85 and older are nearly nine times the costs for those aged 69 and 70 (75). While most older adults consider themselves to be in good health and live independent lives, the National Advisory Council on Aging found that 91% of Canadians had one or more chronic conditions, 40% lived with a disability, and a large number (10-25%) were considered frail (27). Frailty, and other chronic health conditions, can be attributed a decline across multiple physiological systems, resulting in a reduction of one's ability to complete tasks of everyday living (25). Recent investigations have determined that neuromuscular function is closely associated with these activities of daily living (ADL) (34, 58). Therefore, muscular and neuromuscular function contribute directly to maintenance of ADLs, as well as to frailty and other chronic health conditions.

Muscle fatigue can be defined as the fall in maximum force-generating capacity of the muscle (63) and the failure of the muscle to maintain the required

force (36). Some experts suggest that "low tolerance for muscular work" could be a better indicator of frailty than muscle weakness alone (68, 90). Nonetheless, muscle fatigue and frailty together can be associated with factors such as aging, disease, inflammation, physical inactivity, malnutrition, hormonal deficiencies, subjective fatigue, and neuromuscular function and structure (74). Moreover, strength decrements can lead to sarcopenia (muscle loss), which increases the possibility of accidental falls in older adults, leading to potential hip fractures and other injuries (10, 42). Along with a six-fold increase in government health care costs for the aged by 2040, hip fracture costs alone are projected to be six billion dollars in the year 2040, (65). More importantly, 20% of those with hip fractures will not be able to walk (47), and the average individual at 80 years old lacks the muscle capacity to rise unassisted from a chair (15). These muscular and injury-related limitations not only increase health-related costs but detrimentally affect quality of life, as well as the ability to perform ADLs (33). Therefore, due to the direct association between sarcopenia-related injuries and the subsequent effects on ADLs and quality of life, there exists a need for methods that identify the early onset of sarcopenia, as well as a guide to reduce the further development of sarcopenia at its earliest occurrence.

Currently, sarcopenia can be defined as a relative skeletal muscle mass index (RSMI = appendicular fat-free mass (kg) / height in m^2) two standard deviations below healthy standards (men < 7.26 kg/m^2, women < 5.45 kg/m^2) (6). However, the calculation of RSMI requires appendicular lean mass assessments from a dual-energy X-ray absorptiometry (DXA) scanner (6). These scanning devices expose individuals to radiation, require a trained technician, and are expensive to purchase

and maintain. Another investigation by Janssen et al. (33) suggested classifying sarcopenia using a bioelectrical impedance analysis (BIA) total body muscle mass (TBMM) equation (32) using a skeletal muscle index (SMI = TBMM / body mass x 100) with classifications based on between one and two standard deviations (Class I, men 31% - 37%, women 22% - 28%), and above two standard deviations (Class II, men < 31%, women < 22%) from a normal population. However, neither of these methods is considered standard, and the comparison of the two for the classification of sarcopenia is not known. Furthermore, the validity of the Janssen et al. (32) equation in an older population had not been established for predicting TBMM or for the classification of sarcopenia in Americans. The authors (33) simply state that the equation was valid compared to magnetic resonance imaging in a wide age range (18-86) and adiposity (BMI = 16-48 kg/m^2), and the internal cross validation produced an r value of 0.93. However, this equation has not been externally validated in an older population of Americans. Still, other past methods that have been used to identify sarcopenia have included body composition analysis, as well as physical functioning tests. Unfortunately, by the time physical function has decreased, sarcopenia may have contributed to a significant loss of muscle, thereby, increasing the risk for a fall or other related injuries/illnesses. Therefore, the ideal method to identify sarcopenia or muscle loss would be to directly assess muscle mass MM. However, typical body composition devices that can assess muscle mass (MM) or fat-free mass (FFM), such as X-ray computed tomography (CT), magnetic resonance imaging (MRI), DXA, air-displacement plethysmography (BODPOD), and underwater weighing scanners/apparatuses, are highly expensive and require

travel to a testing center. The limitations of these devices are specifically their lack of portability and the cost of not only one, but multiple, measurements.

Imaging devices such as CT, MRI, and DXA are now considered the gold standard for estimating segmental MM and volume. Analysis of a series of CT and MRI images taken along the human body can provide very accurate measurements of body composition. Unfortunately, the high cost and radiation dose associated with CT makes this technique impractical for routine or regular body composition measurements. Although MRI imaging does not produce radiation, it still remains a costly procedure and is unlikely to become a routine screening tool for body composition. Currently, the gold standard for total body FFM and fat mass (FM) assessment is the four-compartment model, which includes a measurement of bone, body water, body volume/density, and body fat. As with the previous models, the high cost and technical skill required to utilize this model reduces its practicality. Nonetheless, these methods can accurately identify MM and accurately track changes in body composition. In fact, the four-compartment model has been suggested for use when tracking changes due to its accuracy in several populations (49, 50, 53, 87). More importantly, due to individual variations in FFM hydration/density and changes in the extracellular water-to-intracellular water ratio, a multiple-compartment model that includes a total body water estimation is required to accurately predict or track changes in both fat and FFM in older men and women (21, 70, 82). Still, a four-compartment (4C) model involves several measurements and techniques. Specifically, criterion total body water methods, such as isotope dilution, require long equilibration periods and expensive equipment and analysis.

However, several field techniques have been developed which claim to rapidly and affordably assess MM, FFM, and total body water (TBW).

Field techniques include bioelectrical impedance analysis (BIA) and bioimpedance spectroscopy (BIS). Bioimpedance has become a popular technique because in its simplest form it only requires someone to remove his or her shoes to get an estimate of FM and FFM. The principle underlying BIA/S is the fact that the electrical impedance of FFM and adipose tissue is different. State of the art BIA/S systems use multiple electrodes and multiple frequencies to determine body composition including water fraction. These multi-electrode systems could potentially provide an approximate distribution of both total body and segmental MM. However, these devices have not been validated in older adults and, to date, there has not been an investigation using the BIA/S segmental electrode placements suggested by Kaysen et al. (35) in any population other than hemodialysis patients. In addition, there is a lack of evidence to support the use of BIS to predict total body water for the potential use in a multiple-compartment model or for predicting fat-free and FM using the two-compartment (2C) model of Pace et al. (55). However, bioimpedance spectroscopy has been proven to be valid in younger healthy populations of men and women and could potentially be used to predict TBW, FFM, MM, and FM in older adults (35, 44, 50, 52, 53). In addition, a recent publication suggests “a clinical definition of sarcopenia ought to use methods of assessment that are valid, reliable, specific to skeletal muscle, predictive of future health events, non-invasive, practical, low cost and widely accessible” (56).

Therefore, if BIA or BIS techniques are found to be valid in an older population, these devices could potentially be used for sarcopenia screening in nursing homes, hospitals, fitness centers, or in any commercial or clinical environment. Furthermore, this type of device would allow for facilities and individuals the opportunity to monitor changes in MM, FFM, and FM and potentially increase their quality of life allowing for a healthier, longer, and less medically expensive life. Additionally, the cost of government funded healthcare due to sarcopenia-related illnesses and injuries could dramatically be reduced.

Hypotheses

1. It is hypothesized that both BIA and BIS devices will result in valid total body water estimations compared to deuterium oxide.

2. It is hypothesized that the two-compartment model using total body water estimated via BIS will result in larger errors compared to a four-compartment model than muscle mass predictions using BIS compared to dual-energy X-ray absorptiometry muscle mass.

3. It is hypothesized that the BIS muscle mass equations of Tengvall et al. (73) will produce more accurate results than the BIS muscle mass equation of Kaysen et al. (35) developed in hemodialysis patients.

4. It is hypothesized that the segmental BIS equations of Kaysen et al. (35) developed in hemodialysis patients would result in good agreement with dual-energy X-ray absorptiometry but produce mean differences and subsequent large total error values.

5. It is hypothesized that the InBody720 MFBIA would result in good agreement with dual-energy X-ray absorptiometry but produce mean differences and subsequent large total error values.

6. It is hypothesized that all BIA fat-free mass equations would produce good agreement with the four-compartment model but may produce mean differences and subsequent large total error values.

7. It is hypothesized that the BIA total body muscle mass equation of Janssen et al. (32) developed using magnetic resonance imaging would be less accurate than the equation of Tengvall et al. (73) developed using dual-energy X-ray absorptiometry.

8. It is hypothesized that the most recent BIA appendicular lean mass equation of Macdonald et al. (44) would produce more accurate results than the older equation of Kyle et al. (40).

9. It is hypothesized that the BIA appendicular lean mass equations would produce more accurate relative skeletal muscle index values compared to the InBody720 MFBIA and the combination of two segmental equations.

10. It is hypothesized that there will be little agreement between sarcopenia classification methods due to the differences in the methods used for assessing lean mass.

Definition of Terms

Appendicular lean mass – The sum of lean mass from both the left and right arms and the right and left leg.

Appendicular muscle mass – The sum of muscle mass from both the left and right arms and the right and left leg.

Relative skeletal muscle index – Calculated using appendicular lean mass divided by height in meters squared.

Skeletal muscle index – Calculated using total body muscle mass divided by body mass multiplied by 100.

Abbreviations

HT – Height (cm)

BM – Body mass (kg)

R – Resistance

Xc – Reactance

Ri – Intracellular resistance

Re – extracellular resistance

ICW – intracellular water

ECW – Extracellular water

BIA – Bioelectrical impedance analysis

BIS – Bioimpedance spectroscopy

MFBIA – Multiple frequency bioelectrical impedance analysis

DXA – Dual-energy X-ray absorptiometry

TBW – Total body water (L)

D_2O – Deuterium oxide

r – Pearson product moment correlation coefficient

SEE – Standard error of estimated

TE – Total error

CE – Constant error/mean difference

LOA – Limits of agreement

FFM – Fat-free mass

FM – Fat mass

TBMM – Total body muscle mass

MM – Muscle mass

ALM – Appendicular lean mass

AMM – Appendicular muscle mass

RSMI – Relative skeletal muscle index

SMI – Skeletal muscle index

2C – Two-compartment

4C – Four-compartment

CT - X-ray computed tomography

MRI - magnetic resonance imaging

Delimitations

At least sixty men and women over the age of sixty-five will be recruited for this investigation. All subjects will complete a general health history questionnaire and a written informed consent prior to all testing sessions. In order to be eligible for participation, subjects must be healthy and implant- and pacemaker-free. Additionally, all subjects must be ambulatory.

Assumptions

Theoretical Assumptions

1. The health history document will be completed accurately.
2. Subjects will be fasting for a minimum of twelve hours with *ad libitum* water consumption.
3. Equipment will perform properly.
4. Proper hydration is accurately reflected in urine specific gravity.

Statistical Assumptions

1. Normality – The sample population is evenly distributed.
2. Independent observations – Each condition is independent of each other.
3. Equal variances – The variances between variables are equal.

Limitations

1. Subjects will be recruited from Norman, Oklahoma and surrounding areas and may not represent all men and women sixty-five and over. Additionally, all subjects will be volunteers, so the sample is not a true random selection from the population.
2. Despite the fact that isotope dilution techniques are criterion for predicting total body water, there is evidence that indicated the choice of sample and isotope may influence the predictions. Therefore, methods using this technique may not directly compare to other dilution methods.
3. The use of air-displacement rather than hydrostatic weighing could also be a limitation. While data suggest both methods are valid, hydrostatic weighing is considered the gold standard for estimating body volume.
4. Because subjects are healthy, there is no way of knowing a-priori if any of them are sarcopenic. Therefore, there may not be enough sarcopenic subjects to make accurate comparisons of methods.

CHAPTER II

REVIEW OF LITERATURE

Over the past several decades, body composition methods have been advancing for use in all populations. Improvements include such methods as bioelectrical impedance analysis and bioimpedance spectroscopy, as well as advancements in four-compartment (4C) models and dual-energy X-ray absorptiometry (DXA) technology. One population for which body composition assessments are particularly important is older men and women. Specifically, muscle mass (fat-free mass) has been shown to decrease with age (22). This loss of muscle mass has been termed sarcopenia. Sarcopenia has been associated with a decrease in quality of life due to the reduced ability to perform typical activities of daily living (33). Therefore, there is a need for an accurate method to estimate the early onset of sarcopenia. More importantly, due to the rising number of older adults, the method used to estimate muscle or lean mass should be easy to use, cost effective, and portable, allowing for assessments in clinics and offices. Unfortunately, the most accurate methods for predicting muscle are expensive, time consuming, and not portable. Such techniques include magnetic resonance imaging (MRI), DXA, and multiple compartment models which use a combination of non-portable methods. While some bioimpedance methods are portable and other are not, all bioimpedance methods are fast, non-invasive, and simple to perform, suggesting bioimpedance may be a useful alternative for predicting muscle mass to more complicated methods. However, literature does not agree regarding which bioimpedance methods and

equations are valid in older men and women. More importantly, there are multiple methods using muscle mass predictions for the classification of sarcopenia. This review will focus on bioimpedance methods and equations used in the past to predict muscle mass (MM), fat-free mass (FFM), and lean mass in older adults. Additionally, this review will discuss two currently acceptable muscle mass-based sarcopenia classification methods.

Basic Principles of Bioimpedance

Bioimpedance methods are classified by the number of frequencies used for analysis. Single frequency devices use "bioelectrical impedance analysis" (BIA), while multiple frequency devices use "bioimpedance spectroscopy" (BIS) for predicting body composition and fluid volumes. The term spectroscopy is used because BIS methods utilize a "spectra" of frequencies. However, the number of frequencies needed before a BIA device can be considered a BIS device is unknown. Typically, BIS devises utilize Cole modeling (12) and mixture theories (26) rather than regression equations to predict body composition variables (45). Therefore, BIA devices that use multiple frequencies are typically called "multi-frequency bioelectrical impedance analyzers" (MFBIA). However, it has been reported that BIS using the Cole model (12) is the "best model" for predicting body composition via bioimpedance (45) yet the main principles behind how these devices can be used to predict body composition are the same.

By sending electrical currents through the body, bioimpedance devices can calculate impedance, otherwise known as the resistivity (R) and reactance (Xc) of the

current. This is possible because cell membranes in the human body behave as capacitors, and impedance to electrical flow is dependent on the frequency of the electrical current (12, 23). At low frequencies (< 50 kHz) the electrical current cannot penetrate cell membranes and, therefore, can be used to predict extracellular water. Higher frequencies (> 50 kHz) can penetrate cell membranes and be used estimate intracellular volumes. This basic principle is the foundation for BIA, MFBIA, and BIS devices to estimate body composition. However, there is a fundamental assumption made by all bioimpedance devices that the human body is composed of uniform cylinders. While this is not the case, total body bioimpedance can still accurately predict body composition compartments. This is possible because the body's fluid is evenly distributed and body segmental lengths are proportional to segmental circumferences (17). BIA devices use a single 50 kHz current to calculate the body's impedance (R and Xc). These values are then used in regression equations to predict various body composition compartments. Surprisingly, the use of 50 kHz was not intended for predicting body composition, but for tracking changes in dialysis patients (54). It has been reported that the BIA technique using 50 kHz is "scientifically unsound" (45). Still, 50 kHz remains the standard for BIA devices. Typically, body composition equations predict FFM because there is a relative constant relationship between total body water (TBW) and FFM (0.68 – 0.74) (84). Since the electrolytes in the body's water are the best conductors of electrical current, bioimpedance most accurately predicts fluid volumes. However, TBW contains both intracellular water (ICW) and extracellular water (ECW), and a 50 kHz frequency may not account for all of the ICW because it may not penetrate cell

membranes. In fact, it has been reported that a frequency of 100 kHz cannot completely penetrate through a cell (76). Because muscles contain a large portion of ICW, bioimpedance methods that utilize higher frequencies are preferred for predicting FFM (45, 61).

Advanced MFBIA devices utilize several frequencies to predict body composition compartments. MFBIA devices typically utilize frequencies ranging from 5 to 500 kHz, allowing for a more accurate estimate of intracellular and extracellular volume compared to single frequency devices. However, MFBIA equations are limited by the same assumptions as single frequency devices and are also considered inferior to BIS because they do not utilize modeling techniques (45). Nonetheless, arguments exist for both BIA and BIS techniques (39, 45, 59, 60). Still, BIS is the most comprehensive bioimpedance method, and data support its accuracy for predicting fluid volumes and other body composition variables (1, 14, 51, 52, 79). Bioimpedance spectroscopy is considered superior to BIA and MFBIA because the calculation of fluid volumes is not based on equations but on Cole modeling (12) and mixture theories (26). However, BIS is subject to the same assumptions as BIA and MFBIA. Nonetheless, BIS can calculate resistivity at both an infinite frequency and at a frequency of zero. Using these resistance values, intracellular (Ri) and extracellular resistance (Re) can be calculated, and subsequent volumes can be calculated. However, BIS still uses a constant FFM hydration (0.73) to predict FFM. Recently, due to the complexity of the method, BIS has been used to develop prediction equations for TBMM. Overall, the appropriateness of BIA, MFBIA, and

BIS for the prediction of total body muscle mass (TBMM), FFM, appendicular lean mass (ALM), or TBMM for use in an older population remains unclear.

Fat-Free Mass Equations

One of the oldest two-compartment models was developed for predicting body composition using only TBW (55). Similar to the assumptions of BIA and BIS, this method assumes a constant FFM hydration status of 0.732. It has been reported that TBW can vary with age (66). A common assumption is that TBW decreases with age causing a dehydration of FFM. While some studies support this finding (43, 72), others suggest the opposite (28, 30, 66, 93). Nonetheless, if hydration remains around 0.732, the Pace and Rathbun 2C (55) model using TBW should be accurate. A study by Wang et al. (87) found that the 2C model of Pace and Rathbun (55) produced slight underestimations (1.04 kg) with an SEE of (0.95 kg) for fat mass compared to a six-compartment model. Still, the accuracy of the 2C model of Pace and Rathbun (55) is dependent on the ability of BIS to predict TBW. Several studies have indicated accurate TBW and FFM predictions in younger populations (1, 14, 51, 52, 79). However, there is limited, but promising, research using BIS in the elderly (73).

There have been several attempts to predict FFM using BIA in an older population (5, 18-20, 41, 62, 88). As early as 1990, BIA equations have been developed for older adults (18, 19). Deurenberg et al. (19) developed two equations compared to a 2C model (71) using hydrostatic weighing and found good agreement ($r > 0.91$, SEE < 2.85 kg). In the same year, using the same 2C model, Deurenberg et

al. (18) developed another equation with similar results to their earlier investigation ($r > 0.81$, SEE < 3.22 kg). Later, Kyle et al. (41) evaluated this equation and found a high r value (0.96) and low SEE (2.4 kg) compared to DXA, yet Kyle et al. (41) found a mean difference of 2.9 kg. This mean difference is most likely related to the criterion method used, 2C model vs. DXA. One year later Baumgartner et al. (5) developed a new BIA equation for predicting FFM in older adults. Like the results from the Deurenberg et al. (18, 19) studies, the Baumgartner et al. (5) equation found good agreement comparing a BIA FFM equation to a 4C model ($r = 0.91$, SEE = 2.51 kg). Several years later, Kyle et al. (41) cross-validated the Baumgartner et al. (5) equation with similar findings to the original investigation ($r = 0.94$, SEE = 2.8 kg). However, Kyle et al. (41) found a mean difference of -2.9 kg, which is most likely due to the criterion method used, 4C model vs. DXA. Similar to the Baumgartner et al. (5) study, Williams et al. (88) discovered a good relationship between a 4C model and a BIA FFM prediction equation ($r > 0.86$, SEE < 1.6 kg). Another finding by Williams et al. (88) was that the Siri et al. (71) 2C model was no different than a 4C model, suggesting the hydration of FFM and the density of FFM in older adults are no different than a reference cadaver. Years later, Roubenoff et al. (62) found good agreement with a new BIA prediction equation compared to DXA ($r > 0.84$, SEE, < 3.5 kg). Subsequently, Kyle et al. (41) cross-validated the Roubenoff et al. (62) equation with similar results in men and women 22 to 94 years of age compared to DXA FFM ($r = 0.98$, SEE = 1.8 kg). However, Kyle et al. (41) found a mean difference of 2.6 kg compared to DXA. This mean difference could be related to the DXA models used. Kyle et al. (41) used a Hologic QDR-4500, while the

Roubenoff et al. (62) study used a GE Lunar DPX-L DXA model. Studies have shown that different DXA models may provide different results (77, 78). Kyle et al. (41) also developed a new BIA equation in men and women 22 to 94 years of age. Compared to DXA, Kyle et al. (41) found good agreement ($r = 0.97$, SEE = 1.7 kg) using the new BIA FFM equation. Another, more recent investigation developed a BIA FFM equation using a 4C model that included total body potassium and TBW (20). Dey et al. (20) also found good agreement between methods (r 0.95, SEE = 2.64 kg, LOA ± 5.21 kg). However, this equation was developed in a Swedish population of older men and women and has not been evaluated in Americans. Overall, the current BIA FFM equations appear to have good agreement with whatever criterion method used for development. However, there are mean differences between equations when compared to different criterion methods. Furthermore, the only investigation that has cross-validated other FFM equations in older adults used DXA as a criterion, and to date no investigation has utilized the most recent 4C model of Wang et al. (85), with updated soft tissue mineral constants, for comparing BIA FFM equations in older adults.

Total Body Muscle Mass Equations

In the year 2000, Jansen et al. (32) developed a TBMM BIA equation using MRI in men and women 18 to 86 years of age. Results indicated good agreement when two separate equations were cross-validated in different laboratories ($r > 0.81$, SEE < 2.8 kg). When the subjects from both laboratories were pooled, the final regression equation also produced a good relationship ($r = 0.94$, SEE = 2.6 and 2.7

kg). Recently, the final Janssen et al. (32) equation was evaluated in Swedish and Taiwanese populations (11, 73). In Taiwanese older adults, compared to MRI, the Janssen et al. (32) BIA TBMM equation produced better agreement than the original investigation ($r = 0.98$, an SEE of 1.56 kg) in Caucasian Americans. However, a mean difference of -0.44 kg was observed. Additionally, a significant mean difference (men -4.05 kg, women -1.02 kg, $p < 0.03$) was observed in Swedish older adults when the Janssen et al. equation was compared to a DXA MM equation (37). Mean differences between investigations could be related to the conflicting populations and criterion methods. In hemodialysis patients (33-73 yr), Kaysen et al. (35) developed BIS MM equations using MRI as the criterion. Muscle mass equations were produced that predict TBMM and segmental MM. Using intracellular water as the main predictor variable, Kaysen et al. (35) was able to predict both segmental (SEE arms = 0.63 kg, legs = 2.03 kg) and total ($r > 0.87$, SEE < 3.29 kg) body MM with good agreement compared to MRI. To date, the equations of Kaysen et al. (35) have not been evaluated in any population. Tengvall et al. (73) used both BIS and BIA to predict TBMM estimated by a DXA MM equation (37). Using standard BIA variables (resistance and reactance) a TBMM prediction equation resulted in a high correlation ($r = 0.96$, SEE = 1.59 kg) with DXA predicted MM. Bioimpedance spectroscopy MM equations produced similar results to the BIA equation with ($r = 0.96$, SEE = 1.60 kg) or without ($r = 0.96$, SEE = 1.64 kg) body mass as a predictor. Unlike the BIS equation of Janssen et al. (32), both BIS equations used raw intracellular resistance (Ri) and extracellular resistance (Re) rather than converting Ri to ICW. However, when the Janssen et al. (32) equation

was cross-validated in Taiwanese older adults (r = 0.98, an SEE of 1.56 kg), the results were similar to those of the Tengvall et al. (73). Therefore, all TBMM equations (BIA and BIS) produced similar predictions compared to DXA MM or MRI. Still, the equations of Tengvall et al. (73) have not been validated in another lab and the various findings for the Janssen et al. (32) equation warrants further investigation.

Multi-frequency BIA (InBody720)

To date, there has not been an investigation comparing the InBody720 eight-polar MFBIA to anything other than DXA in an older population. Recently, Gibson et al. (24) compared the percent fat estimates from the InBody720 to a 4C model in men and women 18 to 82 years of age. Results from this investigation indicate that this method is not valid. Percent fat total error and SEE values were too large to be of practical use (> 4.84 kg). Significant ($p < 0.05$) mean differences were found in the women only. Similarly, in the same year, Volgyi et al. (83) found significant differences in FFM ($p < 0.05$) for both men (3.2 kg) and women (3.4 kg) compared to DXA. Based on the published research utilizing the InBody720 MFBIA, future investigations are needed before this device and method are used in any population. Nonetheless, a comparison of FFM values from the InBody720 MFBIA and a 4C model is needed.

Appendicular Lean Mass Equations

Currently, there are only two ALM equations, both utilizing BIA. In 2003, Kyle et al. (40) developed an ALM equation in healthy men and women 22 to 94 years of age. Results indicated good agreement between the BIA equation and DXA ALM ($r = 0.95$, SEE = 1.12 kg, CE = 0.1 kg, LOA ± 1.1 kg). Three years later, Macdonald et al. (44) cross-validated the equation of Kyle et al. (40) (mean ± SD, 65.1 ± 12.0) and found a significant ($p < 0.001$) overestimation of 2.3 kg compared to DXA. Additionally, compared to the original study by Kyle et al. (40), Macdonald et al. (44) found a lower r value (0.89) and larger SEE value (2.49 kg) compared to DXA. Similarly, Tengvall et al. (73) found a significant ($p < 0.03$) CE of -1.23 kg in men and -0.64 kg in women. Therefore, Macdonald et al. (44) developed another BIA ALM equation using DXA as the criterion. Similar to the equation of Kyle et al. (40), the Macdonald et al. (44) equation produced a high r value (0.96) and a low SEE value (1.57 kg). One reason for the dissonant findings could be related to the DXA models used. Specifically, both investigations utilized a Hologic DXA but used different models and software, and research supports variable findings with different software and DXA models (77, 78, 91). To date, the use of BIA ALM prediction equations for use in the classification of sarcopenia has not been investigated. Additionally, these ALM equations have not been compared to DXA in another lab or to different DXA models such as the GE Lunar used by Baumgartner et al. (6) for the classification of sarcopenia.

Sarcopenia Classification Methods

Two ALM-based methods are currently being used for the classification of sarcopenia. One method uses a relative skeletal muscle index (RSMI) calculated using DXA ALM (6). The other method uses a skeletal muscle index (SMI) calculated using the Janssen et al. (32) TBMM equation (33). However, both methods classify sarcopenia based on deviations from a young healthy population. The Baumgartner et al. (6) method considers individuals with RSMI values less than two standard deviation below a young healthy population as sarcopenic, while the Janssen et al. (33) method classifies sarcopenia into two classes: Class I is defined as one to two standard deviations below a young healthy population, and Class II is defined as over two standard deviations below a young healthy population. One significant difference between methods is the body composition technique used to predict ALM. Considering cost, ease of use, and availability, the Janssen et al. (33) method is superior to the Baumgartner et al. (6) method. However, there has never been a study comparing these methods. Therefore, depending on the method used to classify sarcopenia, individuals may or may not be considered sarcopenic. Without a standardized classification method, treatments for the sarcopenic cannot be suggested with confidence.

Conclusion

Currently, research supports the use of BIA equations for predicting FFM in older adults. Additionally, BIA TBMM equations appear to be valid in older adults. However, there are several new equations that have not been validated or compared

to older BIA equations. Furthermore, the validity of BIS equations for predicting both segmental and TBMM has not been investigated and warrants additional research. The InBody720 MFBIA does not appear to be valid in any population studied to date. Additionally, the current classifications of sarcopenia are not standardized, and the methods used for calculating these standards have not been compared. Future research should investigate new BIA and BIS equations, as well as determine the appropriateness of multiple sarcopenia classification methods.

CHAPTER III

METHODS

Participants

Seventy-four healthy (32 men and 42 women) Caucasian older adults (65 and older) participated in this investigation. Descriptive characteristics of the subjects are presented in Table 1. This study was approved by The University of Oklahoma Institutional Review Board for Human Subjects, and all participants completed a written informed consent (Appendix E). All participants were ambulatory and not using a pacemaker and were considered healthy by evaluating a self-reported health history questionnaire (Appendix F). Typical validation studies utilize at least thirty participants per group, and several studies in multiple populations have utilized fewer subjects than the current investigation and have been published in high-impact journals such as *Medicine and Science in Sports and Exercise* and the *Journal of Applied Physiology* (4, 7, 8, 46, 49, 50, 52, 53, 87, 89). Therefore, the number of participants in the current investigation meets or exceeds similar published validation studies.

Research Design

All body composition assessments were performed on the same day in no particular order following a twelve-hour fast (*ad libitum* water intake was allowed up to one hour prior to testing). Participants were instructed to avoid exercise for at least twenty-four hours prior to testing. Hydration status was determined using specific gravity via handheld refractometry (Model CLX-1, precision = 0.001 +/- 0.001, VEE

GEE Scientific, Inc. Kirkland, Washington) prior to all body composition measurements. Specific gravity values indicated all subjects were properly hydrated (>1.004, <1.029) (2, 3). Subject characteristics are presented in Table 1.

Variables

Variables were classified as either a predictor or a criterion variable. Predictor and criterion variables included the following: total body water (TBW), total body fat-free mass (FFM), total body skeletal muscle mass (TBMM), appendicular lean mass (ALM), leg muscle mass (MM), arm MM, and relative skeletal muscle index (RSMI). Predictor variables were calculated using bioimpedance analysis using two devices (Imp™ DF50, and InBody720) and bioimpedance spectroscopy using one device (Imp™ SFB7). Criterion variables were calculated using deuterium oxide (D_2O) a four-compartment (4C) model and dual-energy X-ray absorptiometry (DXA). Criterion RSMI was calculated using ALM values from DXA, while criterion FFM was calculated using the 4C model.

Bioimpedance Spectroscopy (BIS)

Bioimpedance spectroscopy (BIS) was used to estimate muscle mass (MM) and FFM following the procedures recommended by the manufacturer (Imp™ SFB7, ImpediMed Limited, Queensland, Australia) as reported by Moon et al. (51, 52). After resting in a supine position for 5 to 10 minutes, total body water estimates were taken while the subjects lay supine on a table with their arms ≥ 30 degrees away from their torso with their legs separated. Prior to analysis, each subject's height,

weight, and sex were entered into the BIS device. Each pair of total body electrodes was connected by a non-conductive strip allowing for a distance of 5 cm between electrode centers. Segmental electrodes were similar in size and shape as the total body electrodes with the exclusion of the non-conductive strip. After hair removal and cleaning with alcohol, segmental and whole body electrodes were placed on the right and left side of the body. Total body electrodes were placed at the wrist (dorsal surface at the ulnar styloid process) and ankle (dorsal surface between the malleoli) with the connection strip and connected electrode 5 cm distal from the wrist and ankle. Segmental electrodes were placed using the locations described by Kaysen et al. (35). Using a range of frequencies (1-1000 kHz), the BIS generates complex Cole plots in the shape of an inverted "U", allowing for calculations of the resistance of electrical current through the body at both zero and infinite frequencies (45). These resistance values are used to calculate extracellular water (ECW) and intracellular water (ICW) and summed to equal TBW. Total body water was calculated internal to the BIS device using Cole modeling and the Hanai mixture theory (13, 26). Coefficients used for men (zero/extracellular = 273.9, infinite/intracellular = 937.2) and women (zero/extracellular = 235.5, infinite/intracellular = 894.2) were the same used in the investigation by Moon et al. (52). Total body water was used to calculate FFM using the two-compartment (2C) model of Pace and Rathbun (55) (Appendix A) Previous test retest assessments of 11 men and women measured 24-48 hours apart resulted in an SEM = 0.40L, ICC = 0.99 for TBW. Additionally, because muscle contains the majority of ICW, ICW and ECW can be used to calculate muscle or lean mass (45). The equations of Kaysen et al. (35) and Tengvall et al. (73)

were used to calculate TBMM using intracellular resistance (Ri) or Ri and extracellular resistance (Re) (Appendix A). The average of two trials was used to represent the subject's Ri, Re, and TBW. Previous test retest assessments of 11 men and women measured 24-48 hours apart resulted in an SEM = 49.65, ICC = 0.96 for Ri and an SEM = 10.12, ICC = 0.98 for Re. Segmental MM was estimated using Ri and the equations of Kaysen et al. (35). Previous test retest assessments of 11 men and women measured 24-48 hours apart resulted in an SEM = 33.07, ICC = 0.97 for arm Ri and an SEM = 39.27, ICC = 0.88 for leg Ri.

Bioelectrical Impedance Analysis (BIA)

Bioelectrical impedance analysis (BIA) was used to estimate lean mass following the procedures recommended by the manufacturer (Imp™ DF50, ImpediMed Limited, Queensland, Australia; InBody 720, Biospace, Beverly Hills, California). The protocol for the Imp™ DF50 was identical to the protocol used for the BIS (Imp™ SFB7). However, the frequency used in the Imp™ DF50 was a single 50 kHz rather than the range of frequencies for the BIS (1-1000 kHz). No prediction equations exist for the prediction of arm or leg muscle or lean mass in older adults, so the raw resistance (R) and reactance (Xc) values were used for comparison. Total body FFM was estimated using several predictions equations and the Imp™ DF50 (Appendix A). Total body muscle mass and ALM were estimated using BIA prediction equations and the Imp™ DF50 (Table 2). Previous test retest scans of 11 men and women measured 24-48 hours apart resulted in an SEM = 8.91, ICC = 0.99 for R and an SEM = 2.55, ICC = 0.74 for Xc.

Procedures for the InBody 720 differed from the Imp™ DF50. The InBody 720 required subjects to stand on a scale with electrodes on the surface of the feet at the heel and ball of the foot. After height and age were entered into the device, body mass was determined by the built-in scale. Subjects then lightly grasped handles with electrodes touching the palms and thumbs separately. Subjects were instructed to abduct their arms around 15-20 degrees. Using frequencies at 1, 5, 50, 250, 500, and 1000 kHz the InBody 720 measured R and Xc and calculated total body and segmental body composition values via predetermined manufactures equations internal to the device. Output values included FFM, total body muscle mass (TBMM), and arm and leg lean mass. Previous test retest scans of 11 men and women measured 24-48 hours apart resulted in an SEM = 0.68kg, ICC = 0.99 for FFM, an SEM = 0.44kg, ICC = 0.99 for TBMM, an SEM = 0.13kg, ICC = 0.99 for arm lean mass, and an SEM = 0.13kg, ICC = 0.99 for leg lean mass.

Air-Displacement Plethysmography

Body volume determined from air-displacement plethysmography was assessed using the BOD POD® (BP), which was calibrated before each test using the manufacturer's instructions with the chamber empty and using a cylinder of known volume (49.558 L). Subjects, in spandex shorts and swimming cap only, then entered and sat in the fiberglass chamber. The BP was sealed, and the subject breathed normally for 20 seconds while body volume (BV) was estimated. After this, the subject was connected to a breathing tube internal to the system to measure thoracic

gas volume. The subject resumed tidal breathing cycles; a valve in the circuit momentarily occluded the airway, during which subject gently "puffed". This effort produced small pressure fluctuations in the airway and chamber that were used to determine thoracic gas volume. This value was used to correct body volume for thoracic gas volume. All BV measurements were performed by a BOD POD-certified investigator who had previously demonstrated a SEM of 0.36 liters with an ICC > 0.99 for BV in 11 men and women measured 24-48 hours apart.

Deuterium Oxide (D_2O)

Criterion TBW estimations were conducted using D_2O (99.8% D_2O, Cambridge Isotope Laboratories, Inc., Andover, MA, USA) following the standard procedures reported by Moon et al. (51, 52). Prior to D_2O ingestion, urine samples were collected from all subjects. Subjects were instructed to void their bladders as much as possible. After voiding the bladder completely, subjects ingested ≈ 11 grams of ^2H along with a 100ml rinse of tap water. The exact amount of D_2O ingested for each subject was recorded. After a four-hour equilibration period subjects were instructed to provide a post-urine sample. Urine-diluted D_2O was analyzed in triplicate using an isotope-ratio mass spectrometer. Isotope abundances in the urine were calculated following the method of Wong et al. (92). TBW was then calculated from the dilution of isotopic water and corrected for the exchange of D_2O with nonaqueous tissue (67). Reliability measurements from 11 men and women for D_2O in one urine sample measured in triplicate resulted in a SEM value of 0.33 L with an ICC > 0.99.

Dual-Energy X-ray Absorptiometry (DXA)

DXA (software version 10.50.086, Lunar Prodigy Advance, Madison, WI) was used to estimate total body bone mineral content, total body lean mass, and segmental lean mass. Bone mineral content (BMC) was converted to total body bone mineral (Mo) using the following equation: Mo = total body BMC x 1.0436 (29). Lean mass values were calculated using the DXA software. Each day prior to testing, a quality assurance phantom was performed and passed. Before each test, the subjects' height, weight, sex, and race were entered into the computer program. The subjects were positioned supine on the DXA table with hands pronated and flat on the table. Total body mode was selected for each scan, and scanning thickness was determined by the DXA software. All DXA scans were performed by a certified enCORE^{TM} software operator. The sum of lean soft tissue for both arms and legs (ALST) estimated from DXA was used to calculate relative muscle mass index [RSMI = appendicular lean mass (kg)/HT(m)^2)] and used to classify sarcopenia using the standards of Baumgartner et al. (6) (Sarcopenic = RSMI < 7.26 kg/m^2 for men, < 5.45 kg/m^2 for women). Additionally, TBMM was estimated using the validated equation by Kim et al. (38) (MM = (1.13 x ALST) – 0.02 x age) + (0.61 x sex [men = 0, women =1]) + 0.97). Skeletal muscle index (SMI) was calculated using the equation reported by Janssen et al. (33) (SMI = TBMM / body mass x 100) and used to classify sarcopenia (Class I Sarcopenia, SMI 31% - 37% men, SMI 22% - 28% women; Class II Sarcopenia, SMI < 31% men, < 22% women). Previous test retest scans of 11 men and women measured 24-48 hours apart resulted in an SEM = 0.05kg, ICC = 0.99 for Mo, an SEM = 0.605kg, ICC = 0.99 for total body lean mass,

an SEM = 0.04kg, ICC = 0.99 for TBMM, an SEM = 0.016kg, ICC = 0.99 for arm lean mass, and an SEM = 0.029kg, ICC = 0.99 for leg lean mass.

Four-Compartment Model (4C model)

Criterion FFM was estimated using the 4C model described by Wang *et al.* (85). The equation includes measurements of BV, TBW, Mo, and body mass (BM). The equations for FM and FFM density are:

FM (kg) = 2.748(BV) – 0.699(TBW) + 1.129(Mo) – 2.051(BW)

FFM = BM-FM

FFM Density = 1/[(TBW/0.9937)+(Mo/2.982)+(Residual/1.404)] (48)

Residual = BM-BF-Mo-TBW

Propagation of Error

While multi-compartment models are recommended over 2C models for assessing body composition, the potential propagation of errors due to the inherent measurement error of each device used to assess each variable may offset the improved accuracy of 4C model estimates of body composition (86). Wang *et al.* (86) suggested calculating the propagated error, sometimes referred to as the total error of measurement (*TEM*) (31, 50) to account for the accuracy of the 4C equation. The standard errors of measurement (*SEM*) from the reliability data for the measurement of BV, TBW, and Mo were used to calculate propagated errors for %fat (86). In the current study, the *TEM* was 0.49%fat, which is similar (less than 1%fat) to values reported for the 4C and 5C models in other laboratories (0.70 -

0.89%fat) (69, 89). The *TEM* for the 4C model was calculated from the following equation (86):

4C *TEM* = (TBW SEM^2 + BV SEM^2 + Mo SEM^2)$^{1/2}$

4C *TEM* = ($0.33^2 + 0.36^2 + 0.05^2$)$^{1/2}$

4C *TEM* = 0.49 %fat

Statistical Analyses

Data were analyzed using a custom built LabVIEW Program version 8.2.1 (National Instruments, Austin, TX, USA) and Microsoft® Excel® 2007 version 12.0.6504.5001, SP1 MSO 12.0.6320.5000 (Microsoft Corporation Redmond, WA, USA). The validity and comparisons of prediction equations was based upon the evaluation of predicted values versus the criterion or actual values from D_2O TBW, the four-compartment model, DXA lean mass, or DXA-derived skeletal muscle mass by calculating the constant error (CE = *actual – predicted*), r value (Pearson product moment correlation coefficient), standard error of estimate (SEE), and total error (TE = $\sqrt{\sum[predicted - actual]^2/n}$) (31). The mean differences (CEs = constant errors) between criterion and predicted values were analyzed using dependent t-tests with Bonferroni alpha adjustments. The method of Bland and Altman was used to identify the 95% limits of agreement (LOA) between the criterion and predicted values (9).

CHAPTER IV

RESULTS

Total Body Water

Both methods [Bioimpedance spectroscopy (BIS) and the InBody720 multi-frequency bioelectrical impedance analyzer (MFBIA)] used to predict total body water resulted in similar findings (Table 2). Compared to deuterium oxide D_2O, BIS and the MFBIA produced valid total body estimations. Both methods produced r values greater than 0.92 and SEE values less than 1.57 L. All slopes (< 0.909) were significantly different ($p < 0.05$) than the line of identity (slope = 1) with the exception of BIS in all subjects. In all subjects, the y-intercept was only significantly different ($p < 0.05$) than zero for BIS (0.982). For both methods, the y-intercept was not significantly different ($p > 0.05$) than zero in the men but was significant in the women (y-intercept > 4.9, $p < 0.05$). All groups produced significant ($p < 0.025$) CE values (> -2.02 L) compared to D_2O. Total error values were less for BIS (TE < 2.77 L) compared to the MFBIA (TE > 3.35 L) for all subjects and the men, while the MFBIA produced a lower TE value (TE = 2.84 L) compared to BIS (TE = 2.93) in the women. Individual errors, represented by the limits of agreement, were less for BIS (< ± 3.04 L) compared to the MFBIA (± > 3.02 L) for all subjects and the men, while the MFBIA produced lower LOA's (± 2.37 L) compared to BIS (± 2.65 L) in the women. Significant trends were observed in the women for both methods and for the MFBIA in all subjects.

Bioimpedance Spectroscopy (BIS) Total Body Equations

Results from the total body equations using BIS are presented in Table 3. Equation 1 produced high r values (> 0.88) and low SEE values (< 2.17 kg) compared to four-compartment (4C) model fat-free mass (FFM) values. However, there was a significant CE ($p < 0.05$) for all groups (CE > -2.89 kg). All y-intercepts were significantly different ($p < 0.05$) than zero. When stratified by sex, equation 1 produced a slope not significantly ($p > 0.05$) different than 1. Total error values were greater for women (TE = 4.23 kg) compared to the men (TE = 3.62 kg). However, the limits of agreement were larger for the men (± 4.33 kg) compared to the women (± 4.08 kg). Only the women produced a significant trend (-0.228, $p < 0.05$).

All total body muscle mass (TBMM) equations produced high r values (> 0.70) and low SEE values (< 2.28 kg). Significant ($p < 0.0125$) CE values were found for all equations for men and women (CE > 0.67 kg) with the exception of equation 2 in the women (CE = -0.43 kg). For all equations, slope values were significantly different ($p < 0.05$) for the women but not for the men ($p > 0.05$) compared to the line of identity. Y-intercepts were not significantly different than zero for all equations in the men (< 4.2). Equations 2, 3, and 5 produced significant y-intercepts in the women compared to a y-intercept of zero. Total errors values were lower in the women (TE < 2.40 kg) than the men (TE > 2.25 kg) for all equations except for equation 4 (TE men = 2.24 kg, TE women = 3.58 kg) when comparing the same equations in men and women independently. Equation 4 produced the tightest LOAs for both the men (± 2.97 kg) and the women (± 2.27 kg) compared to

equations 2, 3, and 5 (± > 2.66 kg). In women only, equations 2 and 3 produced significant trends (> -0.39, $p < 0.05$).

Bioimpedance Spectroscopy (BIS) Segmental Equations

Results from the segmental equations using BIS are presented in Table 4. Significant slopes and y-intercepts ($p < 0.05$) were found for both the arms and legs compared to the line of identity and a y-intercept of zero. Significant CE values ($p < 0.05$) were found for all groups in the arms and the legs (CE > -1.20 kg) compared to dual-energy x-ray absoptiometry (DXA) lean mass values. However, when the arms and legs were combined, there was no significant CE for the men (CE = 0.12) compared to DXA. The combination of arms and legs (Total AMM) resulted in a higher r value for the women ($r = 0.78$) compared to the legs and arms alone ($r < 0.75$). However, the lowest SEE, TE, and CE values were found in the arms compared to the legs and Total AMM for the women. The largest r value ($r = 0.91$) and lowest SEE (0.41 kg) and TE (1.43 kg) values were found in the arms for the men compared to legs and Total AMM ($r < 0.78$, SEE > 1.41kg, TE > 1.77 kg). The LOAs were the tightest in the arms for both men and women (± < 1.20 kg) compared to legs and Total AMM (± > 2.20 kg). However, the arms produced significant ($p < 0.05$) trends (> -0.23).

InBody720 (MFBIA) Segmental and Total Body Analysis

Results from segmental and total body analyses using the InBody720 MFBIA are presented in Table 5. Significant slopes and y-intercepts ($p < 0.05$) were found

for FMM compared to the line of identity and a y-intercept of zero compared to the 4C model in men and women. In all groups, significant ($p < 0.05$) CE values were discovered for both FFM (> -3.52 kg) and TBMM (> -5.17 kg) compared to the 4C model and DXA, respectively. However, both FFM and TBMM produced high r values (> 0.84) and low SEE values (< 2.14 kg). TBMM produced tighter LOAs (± < 3.96 kg) than FFM (± > 3.61 kg) when comparing groups. Significant trends (> 0.07, $p < 0.05$) were found for all groups comparing FFM to the 4C model, while a significant trend was found in the women (-0.413, $p < 0.05$) for TBMM compared to DXA.

Segmental results produced similar r values (0.78-0.88) for both the arms and legs in the men and women compared to DXA. Women produced lower SEE values (< 0.62 kg) than the men (SEE > 0.62 kg) in the arms and the legs compared to DXA. Total error values were lower in the arms (TE < 0.82 kg) than the legs (TE > 1.05 kg) for both women and men compared to DXA. Y-intercepts were significantly different ($p < 0.05$) than zero for both arms and legs in both the men and women compared to DXA, while slopes were significantly different ($p < 0.05$) than zero for the arms and legs (> 0.535) in the women and in the arms (0.715) for the men compared to DXA. For men and women, the LOAs were tighter in the arms (± < 1.37 kg) than in the legs (± > 1.56 kg) compared to DXA. Significant ($p < 0.05$) trends were found in the women for both arms and legs compared to DXA.

Bioimpedance Analysis (BIA) Fat-Free Mass Equations

Results from BIA FFM analysis using the DF50 are presented in Table 6. Significant slopes and y-intercepts ($p < 0.05$) were found for equations 8-11 (slopes > 0.702, y-intercept > 6.808) for the women compared to the line of identity (y-intercept of zero, slope of 1) using the 4C model. A significant slope (0.816, $p < 0.05$) was also found in equation 12 for the women compared to the 4C model. Significant CE values ($p < 0.00625$) were found in equations 8-14 (CE > 1.83 kg) for the men and in equations 8-13 for the women (CE > 1.11 kg). All equations produced high r values (> 0.81) and low SEE values (< 3.22 kg) for men and women compared to the 4C model. Total error values ranged from 2.20 to 6.84 kg in the men and from 1.71 to 4.90 kg in the women compared to the 4C model. The LOAs ranged from 3.44 to 6.44 kg in the men and 3.37 to 4.58 kg in the women compared to the 4C model. Equations 13-15 produced significant trends (>0.125, $p < 0.05$) for the men, and equations 14 and 15 produced significant trends (> 0.285, $p < 0.05$) for the women compared to the 4C model.

Bioimpedance Analysis (BIA) Total Body Muscle Mass Equations

Results from BIA TBMM analysis using the DF50 are presented in Table 7. Significant slopes and y-intercepts ($p < 0.05$) were found for equations 16 and 17 (slopes > 0.633, y-intercept > 2.190) for the women compared to the line of identity (y-intercept of zero, slope of 1) using DXA as the criterion. However, equations 16 and 17 produced higher r values (> 0.89), lower SEE values (< 0.85 kg), lower TE values (< 1.42 kg), lower CE values (< -0.64), and tighter LOAs (< 2.52 kg) in

women than in men ($r < 0.89$, SEE > 1.51 kg, TE > 1.97 kg, CE > -1.30, LOA > 2.93) compared to DXA. Both equations produced significant CE values ($p < 0.025$) in men and women compared to DXA. Equation 17 produced a significant CE value ($p < 0.05$) in women (-0.361).

Appendicular Lean Mass

Results from appendicular lean mass ALM analysis using the DF50 BIA and InBody720 BIA are presented in Table 8. Significant slopes and y-intercepts ($p < 0.05$) were found for equations 18 and 19 and for the MFBIA (slopes > 0.770, y-intercept > 2.672) for the women compared to the line of identity (y-intercept of zero, slope of 1) using DXA as the criterion. The MFBIA produced significant slopes and y-intercepts ($p < 0.05$) for the men (slopes = 0.774, y-intercept = 5.519) compared to the line of identity (y-intercept of zero, slope of 1) using DXA as the criterion. Equation 19 produced a significantly different ($p < 0.05$) y-intercept (5.432) compared to a y-intercept of zero using DXA as the criterion. In men and women, r values were similar (0.80-0.88). Women produced lower SEE (0.80-0.90 kg) and TE (0.91-1.58 kg) values than men (SEE = 1.34-1.67 kg, TE = 1.49-5.39 kg) compared to DXA. Significant CE values ($p < 0.0167$) were found using equation 19 in the men and women (CE > 0.33 kg) and using the MFBIA in the women (CE = -1.14). The LOAs were tighter in women (< 2.20 kg) than men (> 2.57 kg) compared to DXA. The MFBIA produced a significant ($p < 0.05$) trend in the women (-0.324).

Relative Skeletal Muscle Index (RSMI)

Results from RSMI predicted using the DF50 BIA, InBody720 MFBIA, and SFB7 BIS are presented in Table 9. Significant slopes and y-intercepts ($p < 0.05$) were found for equations 6 +7 (slopes > 0.541, y-intercept > 1.977) in the men and women, and for equation 18 (slopes 0.683, y-intercept = 1.737) and the InBody720 (slopes = 0.613, y-intercept = 1.924) in the women compared to the line of identity (y-intercept of zero, slope of 1) using DXA as the criterion. Additionally, equation 19 produced a significantly different ($p < 0.05$) y-intercept (1.189) compared to a y-intercept of zero using DXA as the criterion. In the men and women, r values were similar (0.67-0.80). However, SEE values were lower in the women (0.33-0.35 kg/m^2) than in the men (0.47-0.58 kg/m^2) compared to DXA. Total error values ranged from 0.50 to 1.78 kg/m^2 in the men and from 0.36 to 1.19 kg/m^2 in the women. Significant CE values ($p < 0.0.125$) were observed in equation 19 in the men (CE = 1.72 kg/m^2) and equations 19 (CE = 0.14 kg/m^2), 6+7 (CE = -1.101 kg/m^2), and the MFBIA (CE = -0.42 kg/m^2) for the women. The LOAs were tighter for the women (0.66 to 0.91 kg/m^2) than the men (0.91 to 1.14 kg/m^2) compared to DXA. Significant trends ($p < 0.05$) were observed in equations 18 (0.294), 19 (0.397), and using the MFBIA (0.415) for the men and in equation 6+7 (-0.366) for the women compared to DXA.

Accuracy of Relative Skeletal Muscle Index (RSMI) and Skeletal Muscle Index (SMI) Predictions

Accuracy of RSMI predictions used to identify sarcopenia estimated via the DF50 BIA, InBody720 MFBIA, and SFB7 BIS are presented in Figures 1-3. Of the seventy-four subjects, sixteen were classified as sarcopenic using the RSMI classifications (6). In both men and women, equation 17 was the most accurate and correctly classified 94% of the subjects with sarcopenia and incorrectly classified 3% of the subjects as sarcopenic when they were not based on DXA values. Equation 18 produced the same 94% accuracy as equation 17 but misclassified 34% of the subjects as sarcopenic. The InBody720 correctly classified 44% of the subjects with sarcopenia and misclassified 2% of the subjects. The combination of equations 6 and 7 was 25% accurate at classifying sarcopenia and misclassified 3% of the subjects without sarcopenia.

Accuracy of SMI predictions used to identify sarcopenia estimated via the DF50 BIA and DXA are presented in Figures 4-6. Of the seventy-four subjects, forty-four were classified as sarcopenic using the SMI classifications of Janssen et al. (33). Comparing the Kim et al. (38) DXA-based TBMM equation to the Janssen et al. (32) BIA-based TBMM equation as the criterion, DXA correctly classified 95% of the subjects who were considered either class I or class II sarcopenic (n = 44). However, DXA was incorrect in 27% of the subjects, classifying them as sarcopenic when the Janssen et al. (32) BIA-based TBMM equation did not classify them as sarcopenic. DXA reported a total accuracy of 69% in all subjects compared to the Janssen et al. (32) equation. Of the forty-four classified as sarcopenic by the Janssen

et al. (33) standards, eleven were also classified as sarcopenic using the RSMI classifications (6) indicating an 18% agreement between methods when calculated from all sixty subjects who were classified by both methods combined (Figure 7). Additionally, including the agreement between non-sarcopenic subjects, the total agreement between methods for classifying non-sarcopenic and sarcopenic subjects was 24%, indicating that less than one out of four individuals with or without sarcopenia would be classified by both methods.

CHAPTER V

DISCUSSION

Total Body Water

In accordance with our hypothesis, both the SFB7 bioimpedance spectroscopy (BIS) and the InBody720 MFBIA resulted in valid total body water (TBW) estimations compared to deuterium oxide (D_2O). The results of the current study suggest that both methods are valid laboratory methods for predicting TBW in older men and women. However, the SFB7 BIS produced greater accuracy in the men while the MFBIA was more accurate in the women. To the best of our knowledge, this is the first investigation to evaluate TBW estimations in older adults using either the BIS or the MFBIA. Still, in agreement with previous literature in various populations, both methods produced high r values > 0.92 and low SEE values (< 1.57 L) (51, 52, 57, 80, 81). Surprisingly, both methods in the current investigation produced lower SEE values (0.96 – 1.56 L) and higher r values (0.93 – 0.98) compared to the studies by Moon et al. (51, 52), which used the SFB7 to predict TBW in healthy, overfat, and obese young (18 – 44 yr) men and women (SEE = 1.50 – 2.89 L, r = 0.70 – 0.98). In contrast to the investigation by Moon et al. (52) in non-overweight or obese subjects using the SFB7 BIS, the current investigation produced significant ($p < 0.025$) constant error (CE) values (> - 2.01 L) estimated via the SFB7 BIS and the InBody720 MFBIA. Specifically, both devices overestimated TBW compared to D_2O. These findings are similar to the investigation by Moon et al. (51), which found the SFB7 BIS overestimated TBW in overfat and

obese men and women (CE > -1.98 L). Still, compared to the investigations by Moon et al. (51, 52) (LOA = > ± 4.17 L in men, > ± 3.67 L in women), the current study produced tighter limits of agreement (LOA = < ± 3.49 L in men, < ± 2.66 L in women). These findings could partially be explained by the fact that TBW errors increase with an increase in body mass (BM) (51). Specifically, the subjects in the current investigation had lower BM values (mean 69.49 kg) compared to the investigations by Moon et al. (50, 51) (mean BM = 72.8 kg and 82.45 kg). However, the slight difference in body weight may not account for all the improvements in TBW prediction accuracy. The complete explanation for the improved LOAs and SEE values remains unclear. It is hypothesized that there may be less resistivity variability between older adults compared to younger adults. Specifically, younger populations may have more diverse resistivity constants accounting for greater individual variability. However, more research is needed to determine why older adults produce less individual variability when predicting TBW via BIS or a MFBIA compared to younger healthy adults. Additionally, there was a significant overestimation in TBW for both devices suggesting mean resistivity constants are not the same as in younger populations. However, since the r values were high and the SEE values were low, correcting for these mean differences would allow for more accurate TBW estimations for both devices. Specifically, TBW estimated by the BIS in older men should be adjusted by subtracting 2.02 L and by subtracting 2.61 L for the women. Similarly, the MFBIA TBW estimations should be adjusted by subtracting 3.52 L in men and 2.58 L in women. While there is a lack of literature utilizing the current methods used to predict TBW in older adults, the current results

support the use of these methods. Recently, it has been suggested that BIS and MFBIA devices that use raw impedance values to calculate TBW should use TBW adjusted equations rather than developing resistivity coefficients for every population (51). This is particularly important considering total body resistivity can vary with age, sex, ethnicity, and body mass (51). Still, more research is needed to identify the dissonant findings compared to younger adults. Nonetheless, both methods appear to be valid for use in older men and women, and correcting for the CE values for each device may produce more accurate results. Furthermore, if ease of use is important, the InBody720 MFBIA is suggested, while the SFB7 BIS is suggested if portability is desired.

Bioimpedance Spectroscopy (BIS) Total Body Equations

In accordance with our hypothesis, total body fat-free mass (FFM) estimated using the Pace and Rathbun (55) equation and TBW predicted via the SFB7 BIS resulted in larger errors than comparing muscle mass equations to DXA muscle mass. However, FFM predicted by the SFB7 BIS resulted in high r values (> 0.88) and low SEE values (< 2.17 kg) acquiring subjective ratings of "ideal" for both men and women (31). Still, a significant ($p < 0.05$) CE was observed for both the men (-2.90) and women (-3.70) resulting in TE values with subjective ratings of "good" for the men and "poor" for the women (31). Since the equation of Pace and Rathbun (55) is based on TBW and the constant FFM hydration status of 0.732, variations in this ratio and inaccurate TBW estimations could have accounted for the significant CE values. However, since one-sample t-tests revealed no significant ($p > 0.18$)

differences between 0.732 and the hydration status of FFM for men (mean ± SD, 0.7354 ± 0.0176) or women (mean ± SD, 0.7339 ± 0.0158), the significant CE values are most likely due to inaccurate TBW estimations. Due to the known overestimations in TBW when using the SFB7 BIS in an older population, as discussed above, correcting for the CE values when using this method to predict TBW should reduce the TE and CE values when converting TBW to FFM. However, when the two-compartment model (2C) of Pace and Rathbun (55) using D_2O was compared to a four-compartment (4C) model, CE values ranged from -0.14 kg for men and -0.46 kg for women (89); Yet this study used a FFM hydration status of 72% not 73.2%. Nonetheless, due to the known individual errors when predicting TBW via the SFB7 BIS, the accuracy of FFM predictions using the equation of Pace and Rathbun (55) will always be less than when using dilution techniques. Still, if the CE values can be corrected the accuracy of this technique would be considered "ideal". Specifically, subtracting 2.90 kg from FFM in the men and subtracting 3.70 kg for women, the Pace and Rathbun (55) 2C model would be an acceptable method. However, more research is required before this method is suggested for use in older men and women.

Contrary to our hypothesis, the BIS-based elderly population specific muscle mass equations of Tengvall et al. (73) resulted in larger errors than the muscle mass equations developed in hemodialysis patients by Kaysen et al. (35). To the best of our knowledge, this is the first investigation to validate equations 2-5 in an older population that was not used to develop an equation. Results indicated that the equations of Tengvall et al. (73) were more accurate than the equations of Kaysen et

al. (35) in women but not in the men. Specifically, the Tengvall et al. (73) equations (2 and 3) produced TE values less than 1.70 kg in the women, and equation 2 was not significantly different than dual-energy X-ray absorptiometry (DXA) muscle mass (MM) estimations, while equations 2 and 3 produced TE values over 4.16 kg in the men and both equations produced significant ($p < 0.0125$) CE values (>3.76 kg). Furthermore, equations 2 and 3 produced lower SEE values in the women (< 0.85 kg) than in the men (> 1.72 kg). These results suggest that equations 2 and 3 are appropriate for use in women but not for men. Of equations 2 and 3 in the women, equation 2, utilizing BM, produced the most accurate TBMM predictions and the tightest agreement (± 2.67 kg). Dissonant findings for the men could be related to the BIS device used and the method for calculating intracellular resistance (Ri) and extracellular resistance (Re). Specifically, past literature has shown that different BIS devices produce variable results (52). However, since the same device was used for the women, who produced accurate results, the inaccurate findings in the men may be related to other factors. Another factor could be related to the DXA TBMM equation used. While equations 2 and 3 were developed using an equation of Kim et al. (37), the authors used a more recent but less complex model than the model used in the current investigation. Nonetheless, equations 2 and 3 were accurate in women, so the criterion method may not be the only reason for the inaccuracy of these equations in the men. It is hypothesized that both the device used and the criterion method affected the outcome of the men's results. Still, if the CE values could be corrected in the men, equations 2 and 3 may produce accurate results; yet, based on the SEE values (< 1.84 kg), equations 2 and 3 may not be as accurate in men as they

are in women. More research is needed before either equation 2 or 3 is suggested for use in older men. Equation 2 is suggested for use in older women.

Equations 4 and 5, developed in hemodialysis patients (33-73 yr), compared to magnetic resonance imaging (MRI), produced low SEE values (< 1.94 kg) for both men and women. Total error values were less (< 2.85 kg) than equations 2 and 3 (> 4.16 kg) for the men but resulted in significant ($p < 0.0125$) CE values (> 3.76 kg). However, equation 4, based on intracellular water (ICW) only produced an SEE of 1.54 kg, which is over half as low as in the original equation (SEE = 3.28 kg). Furthermore, equation 5 produced an SEE of 1.93 kg which is slightly greater than in the original equation (SEE = 1.85 kg). Still, both equations 4 and 5 significantly ($p < 0.0125$) underestimated TBMM by more than 2.12 kg. However, due to the low SEE values, correcting for the CE values could produce accurate estimations in older men. Nonetheless, more research is needed before equations 4 or 5 are suggested for use in older men or women.

Bioimpedance Spectroscopy (BIS) Segmental Equations

In accordance with our hypothesis, segmental BIS analysis revealed low SEE (< 1.68 kg) values, large TE values (> 1.42 kg), and significant ($p < 0.05$) CE values (> 1.20 kg). To the best of our knowledge, this is the first investigation to compare the segmental BIS equations of Kaysen et al. (35) in older adults. Compared to the original investigation (SEE arms = 0.63 kg, legs = 2.03 kg), SEE values were lower in both men (arms = 0.41 kg, legs = 1.42 kg) and women (arms = 0.31 kg, legs = 0.92 kg). However, r values were lower in the legs for both men (0.69) and women

(0.70) and in the arms for the women (0.74), compared to the original investigation (r = 0.83) of Kaysen et al. (35). Still, considering the original investigation developed equations 6 and 7 in non-hemodialysis patients (33-73 yr), the current results support the validity of equations 6 and 7 in non-hemodialysis populations. However, corrections for significant CE values should be made. In the current population, arm TBMM was underestimated while leg MM was overestimated. When the arms and legs were combined to equal appendicular MM, in men, equations 6+7 were more accurate than alone and produced no significant ($p > 0.05$) CE (0.12 kg) with an SEE of 1.67 kg and a TE of 1.78 kg compared to appendicular lean mass (ALM) estimated by DXA. Yet this was not the case in women; the combination of equations 6+7 resulted in an increased SEE (0.99) compared to the arms (0.31 kg) and legs (0.92 kg) alone. Nevertheless, individual errors represented by the LOA were larger when equations 6 and 7 were summed compared to each equation alone. Therefore, segmental analysis alone is preferred over summing the arms and legs. However, the sum of equations 6 and 7 may produce accurate appendicular muscle mass (AMM) estimation in older men but not older women. Still, more research is needed before equations 6 or 7 are suggested for use in place of DXA in older men or women. Nonetheless, the current population produced lower SEE values in both men and women for both arms and legs compared to the original investigation. Therefore, adjusting the current ICW-based segmental MM equations for specific populations, or developing new ICW-based segmental MM equations may allow for more rapid assessments of MM and potentially be used in place of DXA. However,

the appropriateness of equations 6+7 for the classification of sarcopenia based on the relative skeletal muscle index (RSMI) standards remains unclear.

InBody720 (MFBIA) Segmental and Total Body Analysis

In accordance with our hypothesis, the InBody720 MFBIA produced significant CE values and low SEE values. With the exception of leg lean mass in the men (CE = 0.26 kg), all estimations resulted in significant ($p < 0.05$) CE values ranging from -0.29 to -5.23 kg. Significant overestimations were observed from all estimations with significant CE values. To the best of our knowledge, this is the first investigation to evaluate the InBody720 FFM estimates in an older population using a 4C model as criterion. However, investigations have been done using a wide range of ages (24, 83). An investigation by Gibson et al. (24) evaluated the InBody720 in men and women 18 to 82 years of age compared to a 4C model. However, Gibson et al. (24) only evaluated percent fat and not FFM. Still, results indicated much larger SEE values (> 4.83% fat) similar to a FFM SEE value greater than 4 kg (31). Additionally, Gibson et al. (24) reported no significant difference between methods for men (CE = 0.23 %fat, $p > 0.05$) and a significant difference for women (CE = 2.99 %fat, $p < 0.05$), which is similar to the current investigation (CE = -0.29 to 05.23, $p < 0.05$). Accurate percent fat values would provide accurate FFM values, thus, the discrepancies are not based on the data reported but other factors. Factors that may have contributed to discrepancies could include the age range used (18 to 82 yr), 4C model used, and varying ethnic groups. Specifically, the InBody720 does not use regression equations that include body weight, sex, age, or ethnicity. Volgyi

et al. (83) (37-79 yrs) compared a Tanita scale and the InBody720 to DXA and found differences between scales and between the InBody720 and DXA. Significant differences ($p < 0.05$) were reported in both men (3.2 kg) and women (3.4 kg) for FFM compared to DXA for the InBody720. These findings are similar to the FFM CEs in the current investigation (4.56 kg in men and 3.53 kg in women). Additionally, the current investigation and the investigation by Volgyi et al. (83) discovered significant overestimation by the InBody720. Furthermore, the authors determined that age and sex were factor in the dissonant findings between the InBody720 and the Tanita scale. When age and sex were adjusted for, there was no difference between the two devices (83). These findings suggest that sex- and age-adjusted equations for the InBody720 may provide more accurate estimations of FFM. In addition, the current study found large TE (> 5.30 kg) and CE (> 5.17 kg) values comparing the InBody720 TBMM values to DXA TBMM values. However, r values were high (> 0.84) and SEE values were low (< 1.71 kg) and considered "ideal" (31). These data suggest a systematic deviation between the InBody720 and DXA TBMM values using the Kim et al. (38) TBMM equation, and correcting for these deviations (CE values) may reduce the errors between methods. However, more research is needed before the InBody 720 can be recommended for predicting TBMM in older men or women. Still, the current investigation found low SEE, TE, and CE values for both men and women for the arms and legs lean mass compared to DXA. These data suggest that, although the InBody720 may not be valid at estimating FFM compared to a 4C model or TBMM compared to the DXA-based TBMM of Kim et al. (38), the InBody720 is a valid method for predicting segmental

lean mass. Specifically, the InBody720 may be an alternative method for predicting RSMI and classifying sarcopenia. However, further investigation is required before the InBody720 is suggested as an alternative to DXA for classifying sarcopenia.

Bioimpedance Analysis (BIA) Fat-Free Mass Equations

In agreement with our hypothesis, in both men and women, all equations produced high r values (> 0.83) and low SEE values (< 3.22 kg) producing subjective ratings of "ideal" to "good" for the men and "ideal" to "very good" for women (31). Additionally, in agreement with our hypothesis, several equations produced significant CE values ($p < 0.00625$) and large TE values (1.71 – 6.84 kg). The most accurate equation (equation 15) was the only equation to produce non-significant ($p > 0.00625$) CE values and TE values (men 2.20 kg, women 1.71 kg) considered "ideal" (31). Surprisingly, equation 15 was developed using DXA as the criterion model. In agreement with the original investigation ($r > 0.84$) by Roubenoff et al. (62) our results produced similar r and SEE values ($r > 0.88$). However, our results produced lower SEE values (< 2.2 kg) than the original investigation (< 3.5 kg) (62). In agreement with the current SEE values, Kyle et al. (41) found an SEE of 1.8 kg when utilizing equation 15 in men and women 22 to 94 years of age compared to DXA FFM. Of equations 8 and 9 reported by Deurenberg et al. (19), equation 8 produced lower TE values (< 2.80 kg) compared to equation 9 (SEE > 4.13 kg). However, our results for both equations 8 and 9 produced similar r and SEE values ($r > 0.86$, SEE < 2.29) compared to the original investigation ($r > 0.91$, SEE < 2.85 kg). Still, significant ($p < 0.00625$) CE values were present, indicating a systematic

underestimation of FFM. Significant CE values could be explained by the criterion method used to predict FFM. Specifically, equations 8 and 9 were developed based on a 2C model (71), which assumes constant hydration status, bone mineral content, and FFM density. Nonetheless, if the CE for equations 8 and 9 are adjusted for the current population, these equations could potentially be used in older men and women. Similarly, equation 10 was developed using the same 2C model (71) and produced high r values (> 0.81) and low SEE values (< 3.22 kg) consistent with the original investigation (r = 0.96. SEE = 2.5 kg) (18). In agreement with the current findings, Kyle et al. (41) discovered a high r value (0.96) and low SEE (2.4 kg) comparing equation 10 to DXA. Kyle et al. (41) also found a significant CE (2.9 kg) with equation 10, which is consistent with the current results (CE 1.69 – 1.84 kg). Equation 11 also produced similar values (r 0.84 – 0.9, SEE 2.00 – 2.71 kg) to the original investigation (r = 0.91, SEE = 2.51 kg) (5), as well as the investigation by Kyle et al. (41) (r = 0.94, SEE = 2.8 kg). Both the current investigation (CE -1.91 - -4.35 kg) and the investigation by Kyle et al. (41) (CE = -2.9 kg) indicated equation 11 overestimates FFM compared to either a 4C model or DXA. However, equation 12, developed by Kyle et al. (41), produced the largest CE values (-5.39 to -6.53 kg) in the current sample of older men and women. Still, the current r (0.89 – 0.99) and SEE (1.69 – 2.01 kg) values were consistent with the internal cross validation by Kyle et al. (41) (r = 0.97, SEE = 1.7 kg). Though, other than the current investigation, the final regression equation (equation 12) has not been validated internally or externally in any population. A more recent equation (equation 13) by Dey et al. (20) was developed using a 4C model. With the exception of significant (p

< 0.00625) CE values (- 2.35 to -4.32) the current investigation (r 0.88 – 0.98, SEE 1.74 – 2.19 kg, LOA ± < 4.38 k) produced more accurate estimations of FFM than the original investigation (r 0.95, SEE = 2.64 kg, LOA ± 5.21 kg) (20). Significant CE values could be explained by the different 4C models used and different populations. Specifically, Dey et al. (20) utilized a 4C model consisting of total body potassium and TBW, while the current investigation utilized a more resent model utilizing TBW, bone mineral content, and body volume. Additionally, equation 13 was developed in a Swedish population of older men and women and the current study utilized an American population. Still, equation 13 can be considered valid if the systematic deviations (CEs) are adjusted. However, more research is needed before equation 13 can be suggested for use in older American men and women. Another study utilizing a 4C model to predict FFM using BIA produced similar findings to the current investigation (88). Equation 14 produced high r values (> 0.87) and low SEE values (< 1.86 kg) comparable to the original investigation (r = 0.96 men, 0.87 women; SEE = 1.5 men, 1.5 kg women) (88). A nonsignificant (p > 0.00625) CE was observed in the women (-0.72 kg) but not in the men (3.71 kg). In addition, women produced a TE value (1.71 kg) considered “ideal”, while men produced a TE value (4.10 kg) considered “fairly good” (31). Nonetheless, in the men, equation 14 has the tightest LOAs (± 3.44 kg). These data suggest that equation 14 is a valid alternative to a 4C model in older women and could possibly be a valid method in men if the systematic deviations (CEs) are adjusted. However, more research is needed before equation 14 can be suggested in older men. Overall, all equations for women (± 3.64 – 4.58 kg) had tighter LOAs than the same equations in

men (± 4.06 – 6.44 kg), though all equations produced acceptable SEE values for both men and women, suggesting equations 8-15 could potentially be used in older men and women with more research. Currently, only equation 15 for older men and women and equation 14 for women are suggested for use over more complicated FFM methods such as a 4C model, 2C model, or DXA.

Bioimpedance Analysis (BIA) Total Body Muscle Mass Equations

Contrary to our hypothesis, equation 16, developed using MIR, predicted TBMM more accurately than equation 17, developed using DXA TBMM, compared to DXA TBMM for both women and men. Equation 16 produced a higher r value (0.98), lower SEE (1.17 kg) and TE (1.50 kg) value, a lower CE value (-0.86 kg), and tighter LOAs (± 2.44 kg), than equation 17 ($r = 0.95$, SEE = 2.13 kg, TE = 2.77 kg, CE = 1.14 kg, LOA ± 4.98 kg). Not surprisingly, when the investigators who developed equation 17 (73) compared equation 16 to their population using DXA, there was a significant CE (men -4.05 kg, women -1.02 kg, $p < 0.03$) in both men and women. Similarly, the current results showed a significant CE (men 3.45 kg, women -0.63 kg, $p < 0.03$) in both men and women for equation 17. Still, the current findings ($r = 0.95$, SEE = 2.13 kg) are similar to the original investigation for equation 17 ($r = 0.96$, SEE = 1.59 kg) (73). Discrepancies in these findings could be related to the DXA TBMM equation used and the population used. The study by Tengvall et al. (73) for equation 17 utilized a slightly different equation by Kim et al. (37) compared to the current investigation (38) and sampled subjects from a Swedish

population not American. Nonetheless, results for equation 17 indicate validity in older women and potential validity in older men, with a CE correction.

More extensive research has been conducted using equation 16 (11, 33, 73). Specifically, the original investigation (32) utilized two separate laboratories for equation development. However, the final prediction equation was not validated until recently in Swedes and Taiwanese (11, 73). Compared to the original investigation (r = 0.94, SEE = 2.6 and 2.7 kg), equation 16 produced similar results compared to DXA TBMM in the current investigation (r = 0.98, SEE = 1.17 kg). Similar to the findings from equation 16, equation 17 produced more accurate results than the original investigation. However, results from Chien et al. (11) were more comparable to the current findings. Chien et al. (11) found a CE of -0.44 kg, an r value of 0.98, an SEE of 1.56 kg, and LOAs approximately ± 3 kg comparing equation 16 to magnetic resonance imaging, which are similar to the current results in all subjects (r = 0.98, SEE = 1.17 kg, LOAs ± 2.44 kg). Both equations 16 and 17 appear to be valid in women, while only equation 16 appears to be valid in men. However, equation 17 could potentially be valid in men if the systematic deviation (CE) is corrected. Still, more research is needed before equation 17 is suggested for use in older men.

Appendicular Lean Mass

Contrary to our hypothesis, the more recent equation of Macdonald et al. (44) (equation 19) produced less accurate appendicular lean mass (ALM) predictions compared to the original BIA ALM equation of Kyle et al. (40) (equation 18).

Validity statistics from equation 18 provided comparable results to the original investigation (40). Current results indicated an r value of 0.98, an SEE of 1.21 kg, a CE of 0.15 kg and, LOAs of ± 2.4 kg, which are similar to the Kyle et al. (40) findings (r = 0.95, SEE = 1.12 kg, CE = 0.1 kg, LOA ± 1.1 kg). However, Macdonald et al. (44) found that equation 18 overestimated ALM significantly ($p < 0.001$) by 2.3 kg and produced a lower r value (0.89) and larger SEE value (2.49 kg) compared to the original investigation and the current results. In agreement with Macdonald et al. (44), Tengvall et al. (73) found a significant ($p < 0.03$) CE of -1.23 kg in men and -0.64 kg in women. Considering these findings, the Macdonald et al. (44) equation (equation 19) should have produced dissonant findings compared equation 18. This was the case; equation 19 had a significant ($p < 0.0167$) CE for all subjects (2.46 kg), men (5.23 kg), and women (0.34 kg). Still, equation 19 produced a similar r value (0.96) and SEE value (1.57 kg) compared to the current findings (r = 0.94, SEE = 1.91 kg) for all subjects, suggesting systematic deviations in equation 19 are the main contributing factor to the lack of agreement between with the subjects in the present study. Nonetheless, equation 19 could potentially be used for classifying sarcopenia if the accuracy of RSMI is acceptable. Segmental lean mass values from the InBody720, as stated earlier, were found to be valid in men and women. However, the InBody720 MFBIA results for ALM were found to be valid in men only compared to DXA. These results suggest that classifying sarcopenia based on RSMI predicted using the InBody720 MFBIA may not be valid.

Relative Skeletal Muscle Index (RSMI)

To date, this is the first investigation to compare methods for predicting RSMI based on BIA-based ALM predictions in older men and women. In accordance with our hypothesis, the most accurate ALM equation produced the most accurate RSMI predictions. Equation 18 was the most valid for predicting RSMI and ALM compared to equations 19 and 6+7 and DXA ALM. More specifically, equation 18 resulted in no significant ($p > 0.0125$) CE values (< 0.21 kg/m^2) for all subjects, men, or women. Individual errors (LOAs) were less than $\pm$ 0.92 kg/m^2). Still, equations 19, 6+7, and the InBody720 had high r values (> 0.73), low SEE values (< 0.77 kg/m^2), low TE values (< 1.79 kg/m^2), low CE values (< 1.73 kg/m^2), and low LOAs ($< \pm 1.75$ kg/m^2). However, the impact of these errors on sarcopenia classifications is not known.

Accuracy of Relative Skeletal Muscle Index (RSMI) and Skeletal Muscle Index (SMI) Predictions

Accuracies of RSMI and SMI predictions are presented in Figures 1-8. Compared to DXA RSMI, equation 18 was the most accurate at classifying sarcopenia with a total accuracy of 91% in all the subjects, 100% for the men, and 85% for the women (Figure 3). The next best equation was 19 with an overall total accuracy of 60% in all subjects, 71% in women, but only 3% in men. The InBody720 was less accurate than equations 18 and 19, and equations 6+7 were less accurate than all equations. These data suggest that equation 18 could be used as an alternative to DXA for classifying sarcopenia in older men and women with an

accuracy of 91% in men and women. However, equations 19, 6+7, and the InBody720 are not accurate enough for the classification of sarcopenia. Regarding SMI predictions, since equation 16 has been used to classify sarcopenia, the accuracy of the Kim et al. (38) DXA-based TBMM equation was used to calculate SMI and compared to the SMI values from equation 16. Total accuracy comparing these methods was less (< 73%) than using RSMI and equation 18. Therefore, the Kim et al. (38) DXA-based TBMM equation cannot be used as an alternative for equation 16. However, equation 16 was not developed using DXA but using MRI, and the appropriateness of using the more complex method of DXA in place of BIA is nonsensical. Nonetheless, these data support the idea that TBMM values differ between techniques and cannot be used interchangeably for the classification of sarcopenia. Currently, there are currently two accepted body composition-based methods for the classification of sarcopenia in older American men and women: using the Janssen et al. (32) BIA equation to predict TBMM and then calculating SMI; or calculating RSMI using DXA and incorporating the Baumgartner et al. (6) standards. However, to date, no investigation has discerned how well these methods compare for the classification of sarcopenia. Figures 7 and 8 illustrate the agreement between classification methods. Using the Janssen et al. (33) standards from BIA-predicted TBMM, the number of subjects classified as sarcopenic was over double that of the DXA-based ALM Baumgartner et al. (6) standards, suggesting the Baumgartner et al. (6) approach is much more conservative than the Janssen et al. (33) BIA method. More importantly, only 11 subjects were classified as sarcopenic by both methods, indicating only an 18% agreement between the two. Total

agreement between both methods (sarcopenic and non-sarcopenic) was only 24%. Therefore, the above methods for classifying sarcopenia cannot be used interchangeably, and, due to the variations between methods, accurate classifications of sarcopenia warrant further research.

Conclusion

All methods and equations resulted in low SEE values and high r values. Several equations produced significant mean differences and are not suggest for use in older men or women without more research. However, based on regression analysis, data support the potential validity for all equations in this population. Definitively, equations 2, 15, 16, 17, and 18, as well as the InBody720 MFBIA for arms and legs, are the preferred methods in women, and equations 15 and 18, as well as the InBody720 MFBIA for arms and legs, are preferred in men. While it was not a focus of the current investigation, we thought it would be interesting to compare DXA FFM values to the 4C model. Significant ($p < 0.00001$) overestimations (CE -1.15 to -2.06 kg) were discovered for all groups, but high r values (> 0.91) and low SEE values (< 1.73 kg) were observed. Equation 15 produced more accurate TE (1.71 to 2.20 kg) values than DXA (TE = 1.78 to 2.66 kg) for all groups compared to the 4C model. Nonetheless, DXA produced lower LOAs (< ± 3.32 kg) and SEE values (< 1.72 kg) than all BIA FFM equations, indicating DXA is a valid method for estimating FFM in older adults but may overestimate FFM by one to two kilograms. Considering cost, radiation exposure, time, and training, equation 15 is suggested for use in older men and women over DXA for the estimation of FFM.

However, the ability of DXA and equation 15 to track changes in FFM warrant further investigation.

Overall, the most accurate method and equation for both men and women was using the DF50 BIA and the ALM equation of Kyle et al. (40) (equation 18). Conveniently, this equation allowed for accurate calculations of RSMI allowing equation 18 to classify sarcopenia with a total accuracy of 91% in both men and women compared to using RSMI based on DXA ALM. Therefore, if the sarcopenia classifications of Baumgartner (6) are of interest, utilizing BIA equation 18 allows for an accurate, portable, fast, and economical alternative to DXA. Sarcopenia classification methods are not interchangeable and may result in differing classifications. At best, both methods agreed only 28% of the time with a total agreement of 24%. Currently, there is no ideal method for classifying sarcopenia, and more research is needed before individuals can be considered sarcopenic. Because the two accepted methods used in this investigation do not agree, utilizing either of these methods in a clinical setting is premature. However, tracking changes in RSMI or SMI may provide valuable feedback during an exercise or nutrition intervention. Still, more research needs to be conducted to evaluate the appropriateness of either method for tracking changes in muscle mass and subsequent sarcopenia status in older men and women.

REFERENCES

1. Armstrong, L.E., R.W. Kenefick, J.W. Castellani, D. Riebe, S.A. Kavouras, J.T. Kuznicki, AND C.M. Maresh. Bioimpedance spectroscopy technique: intra-, extracellular, and total body water. *Medicine and science in sports and exercise* 29: 1657-1663, 1997.
2. Armstrong, L.E., C.M. Maresh, J.W. Castellani, M.F. Bergeron, R.W. Kenefick, K.E. LaGasse, AND D. Riebe. Urinary indices of hydration status. *International journal of sport nutrition* 4: 265-279, 1994.
3. Armstrong, L.E., J.A. Soto, F.T. Hacker, Jr., D.J. Casa, S.A. Kavouras, AND C.M. Maresh. Urinary indices during dehydration, exercise, and rehydration. *International journal of sport nutrition* 8: 345-355, 1998.
4. Arngrimsson, S., E.M. Evans, M.J. Saunders, C.L. Ogburn, 3rd, R.D. Lewis, AND K.J. Cureton. Validation of body composition estimates in male and female distance runners using estimates from a four-component model. *Am J Hum Biol* 12: 301-314, 2000.
5. Baumgartner, R.N., S.B. Heymsfield, S. Lichtman, J. Wang, AND R.N. Pierson, Jr. Body composition in elderly people: effect of criterion estimates on predictive equations. *The American journal of clinical nutrition* 53: 1345-1353, 1991.
6. Baumgartner, R.N., K.M. Koehler, D. Gallagher, L. Romero, S.B. Heymsfield, R.R. Ross, P.J. Garry, AND R.D. Lindeman. Epidemiology of sarcopenia among the elderly in New Mexico. *American journal of epidemiology* 147: 755-763, 1998.
7. Bergsma-Kadijk, J.A., B. Baumeister, AND P. Deurenberg. Measurement of body fat in young and elderly women: comparison between a four-compartment model and widely used reference methods. *The British journal of nutrition* 75: 649-657, 1996.
8. Biaggi, R.R., M.W. Vollman, M.A. Nies, C.E. Brener, P.J. Flakoll, D.K. Levenhagen, M. Sun, Z. Karabulut, AND K.Y. Chen. Comparison of air-displacement plethysmography with hydrostatic weighing and bioelectrical impedance analysis for the assessment of body composition in healthy adults. *The American journal of clinical nutrition* 69: 898-903, 1999.
9. Bland, J.M. AND D.G. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-310, 1986.
10. Census, U.B.o.t. Special tabulations on aging-extensive data on mobility and self-care. Washington, DC: US Bureau of the Census, 1994.
11. Chien, M.Y., T.Y. Huang, AND Y.T. Wu. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. *Journal of the American Geriatrics Society* 56: 1710-1715, 2008.

12. Cole, K.S. Permeability and impermeability of cell membranes for ions. *Cold Spring Harb Symp Quant Biol* 8: 110-122, 1940
13. Cornish, B.H., B.J. Thomas, AND L.C. Ward. Improved prediction of extracellular and total body water using impedance loci generated by multiple frequency bioelectrical impedance analysis. *Physics in medicine and biology* 38: 337-346, 1993.
14. Cox-Reijven, P.L. AND P.B. Soeters. Validation of bio-impedance spectroscopy: effects of degree of obesity and ways of calculating volumes from measured resistance values. *Int J Obes Relat Metab Disord* 24: 271-280, 2000.
15. Dawson, D., G. Hendershot, AND J. Fulton. Aging in the eighties: functional limitations of individuals age 65 and over. In: *National Center for Health Statistics* Hyattsville, MD: Advance Data from Vital and Health Statistics, 1987.
16. Day, J.C. Population Projections of the United States by Age, Sex, Race and Hispanic Origin: 1995 to 2050. Current Population Reports, B. o. t. C. (Ed.) Washington, DC, 1996, p. 25.
17. De Lorenzo, A., A. Andreoli, J. Matthie, AND P. Withers. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. *J Appl Physiol* 82: 1542-1558, 1997.
18. Deurenberg, P., K. van der Kooij, P. Evers, AND T. Hulshof. Assessment of body composition by bioelectrical impedance in a population aged greater than 60 y. *The American journal of clinical nutrition* 51: 3-6, 1990.
19. Deurenberg, P., K. van der Kooy, AND J.G. Hautvast. The assessment of the body composition in the elderly by densitometry, anthropometry and bioelectrical impedance. *Basic life sciences* 55: 391-393, 1990.
20. Dey, D.K., I. Bosaeus, L. Lissner, AND B. Steen. Body composition estimated by bioelectrical impedance in the Swedish elderly. Development of population-based prediction equation and reference values of fat-free mass and body fat for 70- and 75-y olds. *European journal of clinical nutrition* 57: 909-916, 2003.
21. Evans, E.M., M.J. Saunders, M.A. Spano, S.A. Arngrimsson, R.D. Lewis, AND K.J. Cureton. Effects of diet and exercise on the density and composition of the fat-free mass in obese women. *Medicine and science in sports and exercise* 31: 1778-1787, 1999.
22. Forbes, G.B. AND E. Halloran. The adult decline in lean body mass. *Human biology; an international record of research* 48: 161-173, 1976.
23. Fricke, H. A mathematical treatment of the electrical conductivity and capacity of disperse system. II. The capacity of a suspension of conducting spheroids surrounded by a non-conducting membrane for a current of low frequency. *Physiological reviews* 26: 678-681, 1925.
24. Gibson, A.L., J.C. Holmes, R.L. Desautels, L.B. Edmonds, AND L. Nuudi. Ability of new octapolar bioimpedance spectroscopy analyzers to predict 4-

component-model percentage body fat in Hispanic, black, and white adults. *The American journal of clinical nutrition* 87: 332-338, 2008.
25. Hamerman, D. Toward an understanding of frailty. *Annals of internal medicine* 130: 945-950, 1999.
26. Hanai, T. *Electrical Properties of Emulsions in Emulsion Science, Chap. 5*. London-New York: Academic Press, 1968,
27. Hebert, R., C. Brayne, AND D. Spiegelhalter. Incidence of functional decline and improvement in a community-dwelling, very elderly population. *American journal of epidemiology* 145: 935-944, 1997.
28. Heymsfield, S.B., C. Nunez, C. Testolin, AND D. Gallagher. Anthropometry and methods of body composition measurement for research and field application in the elderly. *European journal of clinical nutrition* 54 Suppl 3: S26-32, 2000.
29. Heymsfield, S.B., J. Wang, S. Heshka, J.J. Kehayias, AND R.N. Pierson. Dual-photon absorptiometry: comparison of bone mineral and soft tissue mass measurements in vivo with established methods. *The American journal of clinical nutrition* 49: 1283-1289, 1989.
30. Heymsfield, S.B. AND Z.M. Wang. Measurement of total-body fat by underwater weighing: new insights and uses for old method. *Nutrition (Burbank, Los Angeles County, Calif* 9: 472-473, 1993.
31. Heyward, V.H. AND D.R. Wagner. *Applied Body Composition Assessments*. Champaign, IL: Human Kinetics, 2004,
32. Janssen, I., S.B. Heymsfield, R.N. Baumgartner, AND R. Ross. Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J Appl Physiol* 89: 465-471, 2000.
33. Janssen, I., S.B. Heymsfield, AND R. Ross. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society* 50: 889-896, 2002.
34. Katsiaras, A., A.B. Newman, A. Kriska, J. Brach, S. Krishnaswami, E. Feingold, S.B. Kritchevsky, R. Li, T.B. Harris, A. Schwartz, AND B.H. Goodpaster. Skeletal muscle fatigue, strength, and quality in the elderly: the Health ABC Study. *J Appl Physiol* 99: 210-216, 2005.
35. Kaysen, G.A., F. Zhu, S. Sarkar, S.B. Heymsfield, J. Wong, C. Kaitwatcharachai, M.K. Kuhlmann, AND N.W. Levin. Estimation of total-body and limb muscle mass in hemodialysis patients by using multifrequency bioimpedance spectroscopy. *The American journal of clinical nutrition* 82: 988-995, 2005.
36. Kent-Braun, J.A., A.V. Ng, J.W. Doyle, AND T.F. Towse. Human skeletal muscle responses vary with age and gender during fatigue due to incremental isometric exercise. *J Appl Physiol* 93: 1813-1823, 2002.

37. Kim, J., S. Heshka, D. Gallagher, D.P. Kotler, L. Mayer, J. Albu, W. Shen, P.U. Freda, AND S.B. Heymsfield. Intermuscular adipose tissue-free skeletal muscle mass: estimation by dual-energy X-ray absorptiometry in adults. *J Appl Physiol* 97: 655-660, 2004.
38. Kim, J., Z. Wang, S.B. Heymsfield, R.N. Baumgartner, AND D. Gallagher. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *The American journal of clinical nutrition* 76: 378-383, 2002.
39. Kotler, D.P., S. Burastero, J. Wang, AND R.N. Pierson, Jr. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex, and disease. *The American journal of clinical nutrition* 64: 489S-497S, 1996.
40. Kyle, U.G., L. Genton, D. Hans, AND C. Pichard. Validation of a bioelectrical impedance analysis equation to predict appendicular skeletal muscle mass (ASMM). *Clinical nutrition (Edinburgh, Scotland)* 22: 537-543, 2003.
41. Kyle, U.G., L. Genton, L. Karsegard, D.O. Slosman, AND C. Pichard. Single prediction equation for bioelectrical impedance analysis in adults aged 20--94 years. *Nutrition (Burbank, Los Angeles County, Calif* 17: 248-253, 2001.
42. Lipsitz, L.A., I. Nakajima, M. Gagnon, T. Hirayama, C.M. Connelly, H. Izumo, AND T. Hirayama. Muscle strength and fall rates among residents of Japanese and American nursing homes: an International Cross-Cultural Study. *Journal of the American Geriatrics Society* 42: 953-959, 1994.
43. Lohman, T.G. *Advances in body composition assessment*. Champaign, IL: Human Kinetics, 1992,
44. Macdonald, J.H., S.M. Marcora, M. Jibani, G. Roberts, M.J. Kumwenda, R. Glover, J. Barron, AND A.B. Lemmey. Bioelectrical impedance can be used to predict muscle mass and hence improve estimation of glomerular filtration rate in non-diabetic patients with chronic kidney disease. *Nephrol Dial Transplant* 21: 3481-3487, 2006.
45. Matthie, J.R. Bioimpedance measurements of human body composition: critical analysis and outlook. *Expert review of medical devices* 5: 239-261, 2008.
46. Millard-Stafford, M.L., M.A. Collins, E.M. Evans, T.K. Snow, K.J. Cureton, AND L.B. Rosskopf. Use of air displacement plethysmography for estimating body fat in a four-component model. *Medicine and science in sports and exercise* 33: 1311-1317, 2001.
47. Miller, C.W. Survival and ambulation following hip fracture. *The Journal of bone and joint surgery* 60: 930-934, 1978.
48. Minderico, C.S., A.M. Silva, K. Keller, T.L. Branco, S.S. Martins, A.L. Palmeira, J.T. Barata, E.A. Carnero, P.M. Rocha, P.J. Teixeira, AND L.B. Sardinha. Usefulness of different techniques for measuring body composition

changes during weight loss in overweight and obese women. *The British journal of nutrition* 99: 432-441, 2008.
49. Moon, J.R., J.M. Eckerson, S.E. Tobkin, A.E. Smith, C.M. Lockwood, A.A. Walter, J.T. Cramer, T.W. Beck, AND J.R. Stout. Estimating body fat in NCAA Division I female athletes: a five-compartment model validation of laboratory methods. *European journal of applied physiology* 105: 119-130, 2009.
50. Moon, J.R., H.R. Hull, S.E. Tobkin, M. Teramoto, M. Karabulut, M.D. Roberts, E.D. Ryan, S.J. Kim, V.J. Dalbo, A.A. Walter, A.E. Smith, J.T. Cramer, AND J.R. Stout. Percent body fat estimations in college women using field and laboratory methods: a three-compartment model approach. *J Int Soc Sports Nutr* 4: 16, 2007.
51. Moon, J.R., A.E. Smith, S.E. Tobkin, C.M. Lockwood, K.L. Kendall, J.L. Graef, M.D. Roberts, V.J. Dalbo, C.M. Kerksick, J.T. Cramer, T.W. Beck, AND J.R. Stout. Total body water changes after an exercise intervention tracked using bioimpedance spectroscopy: A deuterium oxide comparison. *Clinical nutrition (Edinburgh, Scotland)* doi:10.1016/j.clnu.2009.04.025, 2009.
52. Moon, J.R., S.E. Tobkin, M.D. Roberts, V.J. Dalbo, C.M. Kerksick, M.G. Bemben, J.T. Cramer, AND J.R. Stout. Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. *Nutrition & metabolism* 5: 7, 2008.
53. Moon, J.R., S.E. Tobkin, A.E. Smith, M.D. Roberts, E.D. Ryan, V.J. Dalbo, C.M. Lockwood, A.A. Walter, J.T. Cramer, T.W. Beck, AND J.R. Stout. Percent body fat estimations in college men using field and laboratory methods: A three-compartment model approach. *Dyn Med* 7: 7, 2008.
54. Nyboer, J. AND J.A. Sedensky. Bioelectrical impedance during renal dialysis. *Proceedings of the Clinical Dialysis and Transplant Forum*: 214-219, 1974.
55. Pace, N. AND E.N. Rathbun. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *Journal of Biological Chemistry* 158: 685-691, 1945.
56. Pahor, M., T. Manini, AND M. Cesari. Sarcopenia: clinical evaluation, biological markers and other evaluation tools. *The journal of nutrition, health & aging* 13: 724-728, 2009.
57. Patel, R.V., J.R. Matthie, P.O. Withers, E.L. Peterson, AND B.J. Zarowitz. Estimation of total body and extracellular water using single- and multiple-frequency bioimpedance. *The Annals of pharmacotherapy* 28: 565-569, 1994.
58. Petrella, J.K., J.S. Kim, S.C. Tuggle, S.R. Hall, AND M.M. Bamman. Age differences in knee extension power, contractile velocity, and fatigability. *J Appl Physiol* 98: 211-220, 2005.

59. Piccoli, A., G. Pastori, M. Codognotto, AND A. Paoli. Equivalence of information from single frequency v. bioimpedance spectroscopy in bodybuilders. *The British journal of nutrition* 97: 182-192, 2007.
60. Piccoli, A., G. Pastori, M. Guizzo, M. Rebeschini, A. Naso, AND C. Cascone. Equivalence of information from single versus multiple frequency bioimpedance vector analysis in hemodialysis. *Kidney international* 67: 301-313, 2005.
61. Pietrobelli, A., Z. Wang, C. Formica, AND S.B. Heymsfield. Dual-energy X-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration. *The American journal of physiology* 274: E808-816, 1998.
62. Roubenoff, R., R.N. Baumgartner, T.B. Harris, G.E. Dallal, M.T. Hannan, C.D. Economos, P.M. Stauber, P.W. Wilson, AND D.P. Kiel. Application of bioelectrical impedance analysis to elderly populations. *The journals of gerontology* 52: M129-136, 1997.
63. Sargeant, A.J. Human power output and muscle fatigue. *International journal of sports medicine* 15: 116-121, 1994.
64. Schneider, E.L. Aging in the third millennium. *Science (New York, N.Y* 283: 796-797, 1999.
65. Schneider, E.L. AND J.M. Guralnik. The aging of America. Impact on health care costs. *Jama* 263: 2335-2340, 1990.
66. Schoeller, D.A. Changes in total body water with age. *The American journal of clinical nutrition* 50: 1176-1181; discussion 1231-1175, 1989.
67. Schoeller, D.A., E. van Santen, D.W. Peterson, W. Dietz, J. Jaspan, AND P.D. Klein. Total body water measurement in humans with 18O and 2H labeled water. *The American journal of clinical nutrition* 33: 2686-2693, 1980.
68. Schwendner, K.I., A.E. Mikesky, W.S. Holt, Jr., M. Peacock, AND D.B. Burr. Differences in muscle endurance and recovery between fallers and nonfallers, and between young and older women. *The journals of gerontology* 52: M155-160, 1997.
69. Silva, A.M., C.S. Minderico, P.J. Teixeira, A. Pietrobelli, AND L.B. Sardinha. Body fat measurement in adolescent athletes: multicompartment molecular model comparison. *European journal of clinical nutrition* 60: 955-964, 2006.
70. Silva, A.M., J. Wang, R.N. Pierson, Jr., Z. Wang, J. Spivack, D.B. Allison, S.B. Heymsfield, L.B. Sardinha, AND S. Heshka. Extracellular water across the adult lifespan: reference values for adults. *Physiological measurement* 28: 489-502, 2007.
71. Siri, W.E. Body composition from fluid spaces and density. Analysis of methods. In: *Techniques for Measuring Body Composition*. Brozek, J. AND A. Henschel (Eds.) Washington, DC: National Academy of Sciences, 1961, pp. 223-244.
72. Spirduso, W.W. Physical development and decline. In: *Physical dimensions of aging* Champaign, IL: Human Kinetics, 1995, pp. 57-86.

73. Tengvall, M., L. Ellegard, V. Malmros, N. Bosaeus, L. Lissner, AND I. Bosaeus. Body composition in the elderly: reference values and bioelectrical impedance spectroscopy to predict total body skeletal muscle mass. *Clinical nutrition (Edinburgh, Scotland)* 28: 52-58, 2009.
74. Theou, O., G.R. Jones, T.J. Overend, M. Kloseck, AND A.A. Vandervoort. An exploration of the association between frailty and muscle fatigue. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* 33: 651-665, 2008.
75. Thomas, D. Medicare data. In: *HCFA; HCFA; BDMS; OSDM; DDP; HCFA Central Records*. Janssen, J. AND K. Manton (Eds.), 1994.
76. Thomasett, A. Bio-electrical properties of tissue impedance measurements. *Lyon Medical* 207: 107-118, 1962.
77. Tothill, P., A. Avenell, J. Love, AND D.M. Reid. Comparisons between Hologic, Lunar and Norland dual-energy X-ray absorptiometers and other techniques used for whole-body soft tissue measurements. *European journal of clinical nutrition* 48: 781-794, 1994.
78. Tothill, P. AND W.J. Hannan. Comparisons between Hologic QDR 1000W, QDR 4500A, and Lunar Expert dual-energy X-ray absorptiometry scanners used for measuring total body bone and soft tissue. *Annals of the New York Academy of Sciences* 904: 63-71, 2000.
79. Van Loan, M.D. Estimates of fat-free mass (FFM) by densitometry, dual energy X-ray absorptiometry (DXA), and bioimpedance spectroscopy (BIS) in caucasian and Chinese-American women. *Applied Radiation & Isotopes* 49: 751-752, 1998.
80. Van Loan, M.D. AND P.L. Mayclin. Use of multi-frequency bioelectrical impedance analysis for the estimation of extracellular fluid. *European journal of clinical nutrition* 46: 117-124, 1992.
81. van Marken Lichtenbelt, W.D., K.R. Westerterp, L. Wouters, AND S.C. Luijendijk. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. *The American journal of clinical nutrition* 60: 159-166, 1994.
82. Visser, M., D. Gallagher, P. Deurenberg, J. Wang, R.N. Pierson, AND S.B. Heymsfield. Density of fat-free body mass: relationship with race, age, and level of body fatness. *The American journal of physiology* 272: E781-787, 1997.
83. Volgyi, E., F.A. Tylavsky, A. Lyytikainen, H. Suominen, M. Alen, AND S. Cheng. Assessing body composition with DXA and bioimpedance: effects of obesity, physical activity, and age. *Obesity (Silver Spring, Md* 16: 700-705, 2008.
84. Wang, Z., P. Deurenberg, W. Wang, A. Pietrobelli, R.N. Baumgartner, AND S.B. Heymsfield. Hydration of fat-free body mass: new physiological modeling approach. *The American journal of physiology* 276: E995-E1003, 1999.

85. Wang, Z., F.X. Pi-Sunyer, D.P. Kotler, L. Wielopolski, R.T. Withers, R.N. Pierson, Jr., AND S.B. Heymsfield. Multicomponent methods: evaluation of new and traditional soft tissue mineral models by in vivo neutron activation analysis. *The American journal of clinical nutrition* 76: 968-974, 2002.
86. Wang, Z., W. Shen, R.T. Withers, AND S.B. Heymsfield. Multicomponent molecular-level models of body composition analysis. In: *Human body composition*. Heymsfield, S. B., T. G. Lohman, Z. Wang, AND S. B. Going (Eds.) Champaign, IL: Human Kinetics, 2005, pp. 163-176.
87. Wang, Z.M., P. Deurenberg, S.S. Guo, A. Pietrobelli, J. Wang, R.N. Pierson, Jr., AND S.B. Heymsfield. Six-compartment body composition model: inter-method comparisons of total body fat measurement. *Int J Obes Relat Metab Disord* 22: 329-337, 1998.
88. Williams, D.P., S.B. Going, L.A. Milliken, M.C. Hall, AND T.G. Lohman. Practical techniques for assessing body composition in middle-aged and older adults. *Medicine and science in sports and exercise* 27: 776-783, 1995.
89. Withers, R.T., J. LaForgia, R.K. Pillans, N.J. Shipp, B.E. Chatterton, C.G. Schultz, AND F. Leaney. Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women. *J Appl Physiol* 85: 238-245, 1998.
90. Wolfson, L., J. Judge, R. Whipple, AND M. King. Strength is a major factor in balance, gait, and the occurrence of falls. *The journals of gerontology* 50 Spec No: 64-67, 1995.
91. Wong, J.C. AND M.R. Griffiths. Comparative evaluation of the DPX and DPX-IQ lunar densitometer systems following software upgrade. *J Clin Densitom* 5: 199-205, 2002.
92. Wong, W.W., L.S. Lee, AND P.D. Klein. Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. *The American journal of clinical nutrition* 45: 905-913, 1987.
93. Yee, A.J., T. Fuerst, L. Salamone, M. Visser, M. Dockrell, M. Van Loan, AND M. Kern. Calibration and validation of an air-displacement plethysmography method for estimating percentage body fat in an elderly population: a comparison among compartmental models. *The American journal of clinical nutrition* 74: 637-642, 2001.

#	Equation	Reference
1	FFM = 1.361 x total body water (L)	(55)
2	TBMM = -23.953 + 0.333 x HT + -0.004 x Ri + -0.010 x Re + -1.727 x Sex + 0.042 x BM	(73)
3	TBMM = -24.05 + 0.365 x HT + -0.005 x Ri + -0.012 x Re + -1.337 x Sex	(73)
4	TBMM = 2.074 + 1.064 x ICW(L)[1]	(35)
5	TBMM = 9.52 + 0.331 x ICW(L)[1] + 2.77*Sex + 0.18*BM-0.113*Age	(35)
6	Arm Muscle Mass = -0.774 + 1.1 x ICW(L)[2]	(35)
7	Leg Muscle Mass = 4.44 + 1.21 x ICW(L)[2]	(35)
8	FFM = 0.340 x (HT^2/R) + 0.288 x BM - 0.116 x Age + 3.5 x Sex + 0.097 x HT -3.79	(19)
9	FFM = 0.382 x (HT^2/R) - 0.135 x Age + 0.216 x BM + 4.5 x Sex + 0.107 x HT -3.95	(19)
10	FFM = 0.360 x 10^4 x HT(m)^2/R) + 4.5 x Sex + 0.359 x BM – 20 x T(m) + 7.0	(18)
11	FFM = 0.28 x (HT^2/R) + 0.27 x BM + 4.5 x Sex + 0.31 x T(cm) - 1.732	(5)
12	FFM = 0.58 x (HT^2/R) + 0.231 x BM + 0.130 x Xc + 4.429 x Sex - 4.104	(41)
13	FFM = 11.78 + (0.499 x HT^2/R) +(0.134 x BM)+(3.449 x Sex)	(20)
14	Men FFM = 0.54 x (HT^2/R) + 0.13 x BM + 0.13 x Xc - 0.11 x Age + 8.71	(88)
14	Women FFM = 0.37 x (HT^2/R) + 0.16 x BM + 11.94	(88)
15	Men FFM = 0.4273 x (HT^2/R) − 0.1926 x BM + 0.0667 x Xc + 9.1536	(62)
15	Women FFM – 0.4542 x (HT^2/R) + 0.1190 x BM + 0.0455 x Xc + 7.7435	(62)
16	TBMM = [(HT^2/R) x 0.401 + Sex x 3.825 + Age x -0.071] + 5.102	(32)
17	TBMM − -24.021 + 0.33 x HT + -0.031 x R + 0.083 x Xc + -1.58 x Sex + 0.046 x BM	(73)
18	ALM = -4.211 + (0.267 x HT^2/R) + (0.095 x BM) + (1.909 x Sex) + (-0.012 x Age) + (0.058 x Xc)	(40)
19	ALM = -11.626 + 0.292 x (HT^2/R) + 0.06983 x Xc + 0.08553 x HT + -2.092 x Sex − -0.05 x Age	(44)

FFM = Fat-Free Mass

TBMM = Total Body Muscle Mass

ALM = Appendicular Lean Mass

HT = Height (cm), equation 10 (m)

BM = Body Mass (kg)

Age = in years

Sex: Men = 1, Women = 0

Ri = Intracellular resistance calculated from the SFB7 BIS

Re = extracellular resistance calculated from the SFB7 BIS

ICW[1] – intracellular volume calculated by the SFB7 BIS

ICW[2] = 2 / 1000 (273.9 x L^2 / R_I); L = Segment length (cm); [Intracellular Resistance (R_I)] = (R_0 x R_{inf}) / R_0 - R_{inf}

T − Thigh circumference

R = Resistance from the DF50 BIA

Xc = Reactance from the DF50 BIA

Appendix B.

Table 1

Variable	Mean	*SD*
All Subjects (n = 74)		
Age (y)	72	6
Body weight (kg)	69.49	12.71
Height (cm)	167.5	8.5
TBW/FFM (%)	73.48	1.76
FFM Density (g/cc)	1.105*	0.007
Men (n = 32)		
Age (y)	72	5
Body weight (kg)	80.24	9.24
Height (cm)	175.0	6.0
TBW/FFM (%)	73.54	1.90
FFM Density (g/cc)	1.105*	0.006
Women (n = 42)		
Age (y)	72	6
Body weight (kg)	61.31	8.01
Height (cm)	161.5	5.0
TBW/FFM (%)	73.39	1.58
FM Density (g/cc)	1.106*	0.007

Descriptive characteristics of subjects, FFM Density compared to 1.100 g/cc, $p < 0.001$

Table 2

	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
								CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects	D_2O TBW	32.89 ± 7.80									
	BIS TBW	35.24 ± 7.81	0.982	-1.710†	0.98	1.46	2.76	-2.35* ± 2.86	0.50	-5.21	-0.001
	MFBIA TBW	35.87 ± 8.47	0.908†	0.319	0.97	1.34	3.36	-2.99* ± 3.02	0.04	6.01	-0.082†
Men	D_2O TBW	40.96 ± 4.06									
	BIS TBW	42.98 ± 4.51	0.846†	4.618	0.94	1.40	2.53	-2.02* ± 3.03	1.01	-5.05	-0.11
	MFBIA TBW	44.48 ± 4.66	0.806†	5.098	0.93	1.56	3.93	-3.52* ± 3.48	-0.04	-7.00	-0.14
Women	D_2O TBW	26.74 ± 2.52									
	BIS TBW	29.34 ± 3.29	0.710†	5.914†	0.93	0.97	2.93	-2.61* ± 2.65	0.04	-5.26	-0.27†
	MFBIA TBW	29.32 ± 3.08	0.758†	4.500†	0.93	0.96	2.84	-2.58* ± 2.37	-0.21	-4.95	-0.21†

* Represents significance at ($p \leq 0.025$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods.

Table 3

	Eq	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects		4C FFM	44.79 ± 10.68									
	1	BIS FFM	48.14 ± 10.67	0.980	-2.391†	0.98	2.16	3.98	-3.35†± 4.23	0.88	-7.58	-0.001
		DXA TBMM	21.41 ± 6.59									
	2	BIS TBMM	20.02 ± 4.83	1.287*	-4.363†	0.94	2.23	2.94	1.39* ± 5.11	6.50	-3.72	-0.319†
	3	BIS TBMM	19.35 ± 5.33	1.174*	-1.322	0.95	2.09	3.05	2.05* ± 4.45	6.50	-2.40	-0.217†
	4	BIS TBMM	22.61 ± 4.32	1.174*	-11.354†	0.95	2.10	3.08	-1.21* ± 5.58	4.38	-6.79	0.428†
	5	BIS TBMM	21.46 ± 4.88	1.271*	-5.873†	0.94	2.27	2.59	-0.05 ± 5.12	5.06	-5.17	0.309†
Men		4C FFM	55.82 ± 5.45									
	1	BIS FFM	58.72 ± 6.17	0.827*	7.265†	0.94	1.96	3.62	-2.90†± 4.33	1.43	-7.22	-0.127
		DXA TBMM	28.35 ± 3.15									
	2	BIS TBMM	24.58 ± 2.60	0.993	3.942	0.82	1.83	4.17	3.77* ± 3.53	7.30	0.25	0.209
	3	BIS TBMM	24.49 ± 2.59	1.019	3.398	0.84	1.74	4.21	3.86* ± 3.36	7.22	0.49	0.212
	4	BIS TBMM	26.68 ± 2.81	0.980	2.201	0.88	1.54	2.24	1.67* ± 2.97	4.64	-1.30	0.119
	5	BIS TBMM	26.22 ± 2.72	0.923	4.143	0.80	1.93	2.84	2.13* ± 3.75	3.75	-1.62	0.163
Women		4C FFM	36.39 ± 3.62									
	1	BIS FFM	40.08 ± 4.49	0.717*	7.643†	0.89	1.67	4.23	-3.70†± 4.08	0.38	-7.78	-0.228†
		DXA TBMM	16.11 ± 1.88									
	2	BIS TBMM	16.54 ± 2.78	0.610*	6.027†	0.90	0.83	1.41	-0.43 ± 2.67	2.24	-3.09	-0.404†
	3	BIS TBMM	15.44 ± 3.03	0.559*	7.490†	0.90	0.84	1.69	0.68* ± 3.08	3.76	-2.40	-0.490†
	4	BIS TBMM	19.51 ± 2.10	0.751*	1.461	0.84	1.04	3.58	-3.40* ± 2.27	-1.13	-5.66	-0.118
	5	BIS TBMM	17.83 ± 2.35	0.566*	6.027†	0.71	1.35	2.39	-1.72* ± 3.29	1.57	-5.01	-0.259

* Represents significance at ($p \leq 0.0125$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 4

	Eq	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects		DXA Total ALM	19.13 ± 5.57									
	6+7	BIS Total AMM	20.69 ± 4.37	1.198*	-5.655*	0.94	1.91	2.59	-1.56* ± 4.08	2.52	-5.65	0.248
		DXA Arms LM	4.92 ± 1.78									
	6	BIS Arms MM	3.34 ± 2.07	0.836*	2.123*	0.97	0.41	1.66	1.58* ± 1.04	2.61	0.54	-0.150*
		DXA Legs LM	14.21 ± 3.85									
	7	BIS Legs MM	17.35 ± 2.56	1.275*	-7.915*	0.85	2.06	3.80	-3.14* ± 4.24	1.10	-7.37	0.434*
		DXA Total ALM	24.97 ± 2.74									
Men	6+7	BIS Total AMM	24.85 ± 2.94	0.745*	6.447*	0.78	1.67	1.78	0.12 ± 3.54	3.66	-3.42	-0.078
		DXA Arms LM	6.76 ± 0.98									
	6	BIS Arms MM	5.44 ± 1.24	0.722*	2.839*	0.91	0.41	1.43	1.33* ± 1.04	2.37	0.29	-0.243*
		DXA Legs LM	18.21 ± 1.93									
	7	BIS Legs MM	19.42 ± 2.10	0.634*	5.906*	0.69	1.42	1.98	-1.21* ± 3.12	1.91	-4.33	-0.102
		DXA Total ALM	14.68 ± 1.62									
Women	6+7	BIS Total AMM	17.52 ± 1.93	0.670*	2.935*	0.78	0.99	3.07	-2.84* ± 2.29	-0.55	-5.14	-0.19
		DXA Arms LM	3.51 ± 0.46									
	6	BIS Arms MM	1.75 ± 0.66	0.510*	2.620*	0.74	0.31	1.82	1.77* ± 1.19	2.64	0.89	-0.414*
		DXA Legs LM	11.17 ± 1.27									
	7	BIS Legs MM	15.77 ± 1.56	0.572*	2.149	0.70	0.92	4.74	-4.61* ± 2.21	-2.40	-6.82	-0.240*

* Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 5

	Method	(x ± SD)	Slope	Intercept	r	SEE	TE	Agreement			
								CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects	4C FTM	44.79 ± 10.68									
	MFBIA FFM	48.77 ± 11.45	0.916*	0.101	0.98	1.98	4.53	-3.98* ± 4.29	0.31	-8.26	-0.071*
	DXA TBMM	21.41 ± 6.59									
	MFBIA TBMM	26.61 ± 6.87	0.934*	-3.452	0.97	1.52	5.43	-5.20* ± 3.09	-2.11	-8.29	-0.042
	DXA Arms LM	4.92 ± 1.78									
	MFBIA Arms LM	5.35 ± 1.84	0.924*	-0.019	0.95	0.54	0.69	-0.43* ± 1.08	0.65	-1.51	-0.033
	DXA Legs LM	14.21 ± 3.85									
	MFBIA Legs LM	14.50 ± 3.51	1.050	-1.020	0.96	1.11	1.15	-0.29* ± 2.20	1.90	-2.49	0.094*
Men	4C FTM	55.82 ± 5.45									
	MFBIA FFM	60.38 ± 6.35	0.793*	7.943*	0.92	2.13	5.17	-4.56* ± 4.86	0.30	-9.41	-0.16*
	DXA TBMM	28.35 ± 3.15									
	MFBIA TBMM	33.58 ± 3.79	0.703*	4.753	0.85	1.70	5.59	-5.23* ± 3.95	-1.28	-9.17	-0.202
	DXA Arms LM	6.76 ± 0.98									
	MFBIA Arms LM	7.20 ± 1.07	0.715*	1.616*	0.78	0.63	0.81	-0.43* ± 1.36	0.92	-1.79	-0.090
	DXA Legs LM	18.21 ± 1.93									
	MFBIA Legs LM	17.95 ± 1.96	0.781	4.181*	0.79	1.19	1.26	0.26 ± 2.45	2.70	-2.19	-0.017
Women	4C FTM	36.39 ± 3.62									
	MFBIA FFM	39.92 ± 4.18	0.777*	5.358*	0.90	1.61	3.97	-3.53* ± 3.62	0.09	-7.15	-0.151*
	DXA TBMM	16.11 ± 1.88									
	MFBIA TBMM	21.29 ± 2.51	0.674*	1.754	0.90	0.84	5.31	-5.18* ± 2.28	-2.91	-7.46	-0.300*
	DXA Arms LM	3.51 ± 0.46									
	MFBIA Arms LM	3.94 ± 0.66	0.536*	1.402*	0.78	0.29	0.59	-0.42* ± 1.193	0.82	-1.25	-0.413*
	DXA Legs LM	11.17 ± 1.27									
	MFBIA Legs LM	11.88 ± 1.65	0.681*	3.077*	0.88	0.61	1.06	-0.71* ± 1.57	0.86	-2.28	-0.27*

* Represents significance at ($p \leq 0.05$). Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of resicual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods.

Table 6

	Method	Eq	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects	4C FFM		44.79 ± 10.68									
	BIA FFM	8	43.09 ± 10.13	1.031	0.376	0.98	2.21	2.79	1.71* ± 4.35	6.06	-2.64	0.053†
		9	40.82 ± 10.35	1.014	3.408†	0.98	1.96	4.42	3.98* ± 3.84	7.81	0.14	-0.031
		10	43.04 ± 10.74	0.961	3.435†	0.97	2.76	3.26	1.76* ± 5.43	7.18	-3.67	-0.006
		11	48.09 ± 9.58	1.081†	-7.190†	0.97	2.63	4.27	-3.30* ± 5.34	2.05	-8.64	0.110†
		12	50.18 ± 11.70	0.899†	-0.345	0.99	1.83	5.81	-5.39* ± 4.25	-1.15	-9.64	-0.092†
		13	48.26 ± 9.48	1.108†	-8.669†	0.98	1.93	4.08	-3.46* ± 4.26	0.80	-7.73	0.120†
		14	43.59 ± 8.38	1.255†	-9.935†	0.99	1.85	3.05	1.20* ± 5.53	6.73	-4.33	0.243
		15	44.49 ± 10.12	1.038	-1.392	0.98	1.90	1.94	0.30 ± 3.77	4.02	-3.47	0.054†
Men	4C FFM		55.82 ± 5.45									
	BIA FFM	8	53.35 ± 5.28	0.942	5.594	0.91	2.28	3.33	2.47* ± 4.45	6.92	-1.97	0.034
		9	51.50 ± 5.01	1.007	3.940	0.93	2.09	4.77	4.32* ± 4.03	8.35	0.30	0.087
		10	53.98 ± 5.36	0.829	11.061	0.82	3.21	3.72	1.84* ± 6.44	8.28	-4.60	0.018
		11	57.74 ± 4.86	0.979	-0.702	0.87	2.71	3.25	-1.91* ± 5.22	3.31	-7.14	0.123
		12	62.36 ± 5.68	0.894	0.098	0.93	2.01	6.84	-6.53* ± 4.06	-2.47	-10.60	-0.043
		13	58.17 ± 4.44	1.128	-9.782	0.92	2.19	3.21	-2.35* ± 4.37	2.03	-6.72	0.213†
		14	52.11 ± 4.82	1.073	-0.103	0.95	1.75	4.10	3.71* ± 3.44	7.15	0.28	0.126†
		15	55.29 ± 4.38	1.152	-7.866	0.93	2.10	2.20	0.53 ± 4.25	4.78	-3.72	0.226†

* Represents significance at ($p \leq 0.00625$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 6 continued

	Method	Eq	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
Women	4C FFM		36.39 ± 3.62									
	BIA FFM	8	35.27 ± 4.11	0.766†	9.372	0.87	1.79	2.29	1.12* ± 3.95	5.07	-2.83	-0.138
		9	32.68 ± 3.86	0.824†	9.474†	0.88	1.75	4.14	3.71* ± 3.64	7.35	0.07	-0.068
		10	34.70 ± 4.33	0.703†	12.008†	0.84	1.98	2.86	1.69* ± 4.58	6.27	-2.89	-0.196†
		11	40.74 ± 4.17	0.726†	6.809†	0.84	2.00	4.90	-4.35* ± 4.48	0.13	-8.83	-0.154
		12	40.91 ± 3.93	0.816†	2.984	0.89	1.69	4.87	-4.53* ± 3.57	-0.95	-8.10	-0.087
		13	40.70 ± 3.15	1.009	-4.676	0.88	1.74	4.64	-4.32* ± 3.37	-0.95	-7.68	0.145
		14	37.10 ± 2.76	1.152	-6.374	0.88	1.75	1.89	-0.72 ± 3.48	2.76	-4.20	0.286†
		15	36.26 ± 2.74	1.172	-6.102	0.89	1.68	1.71	0.12 ± 3.37	3.50	-3.25	0.290†

* Represents significance at ($p \leq 0.00625$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 7

	Eq	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects		DXA TBMM	21.41 ± 6.59									
	16	BIA TBMM	22.26 ± 6.94	0.935†	0.581	0.98	1.17	1.50	-0.86* ± 2.44	1.58	-3.29	-0.051†
	17	BIA TBMM	20.27 ± 4.84	1.290†	-4.736†	0.95	2.13	2.77	1.14* ± 4.98	6.11	-3.84	0.315†
Men		DXA TBMM	28.35 ± 3.15									
	16	BIA TBMM	29.66 ± 2.88	0.960	-0.113	0.88	1.52	1.98	-1.31* ± 2.94	1.63	-4.25	0.093
	17	BIA TBMM	24.90 ± 2.61	0.986	3.792	0.82	1.83	3.88	3.45* ± 3.54	6.99	-0.08	0.204
Women		DXA TBMM	16.11 ± 1.88									
	16	BIA TBMM	16.62 ± 2.03	0.837†	2.200†	0.90	0.82	1.00	-0.51* ± 1.71	1.20	-2.22	-0.080
	17	BIA TBMM	16.74 ± 2.66	0.634†	5.491†	0.90	0.84	1.41	-0.63* ± 2.51	1.88	-3.14	-0.361†

* Represents significance at ($p \leq 0.025$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 8

	Eq	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects		DXA ALM	19.13 ± 5.57									
	18	BIA ALM	18.98 ± 5.21	1.045	-0.695	0.98	1.21	1.23	0.15 ± 2.40	2.55	-2.25	0.068†
	19	BIA ALM	16.67 ± 3.37	1.553†	-6.759†	0.94	1.91	3.61	2.46* ± 5.21	7.67	-2.76	0.505†
		MFBIA ALM	19.85 ± 5.27	1.017	-1.064	0.96	1.51	1.66	-0.72* ± 2.94	2.22	-3.66	0.056
Men		DXA ALM	24.97 ± 2.74									
	18	BIA ALM	24.39 ± 2.55	0.929	2.325	0.86	1.40	1.49	0.58 ± 2.73	3.31	-2.15	0.078
	19	BIA ALM	19.74 ± 2.43	0.990	5.432†	0.88	1.34	5.39	5.23* ± 2.58	7.8	2.65	0.128
		MFBIA ALM	25.15 ± 2.83	0.774†	5.519†	0.80	1.67	1.75	-0.18 ± 3.46	3.29	-3.64	-0.036
Women		DXA ALM	14.68 ± 1.62									
	18	BIA ALM	14.85 ± 1.76	0.771†	3.217†	0.84	0.90	0.98	-0.18 ± 1.91	1.73	-2.09	-0.090
	19	BIA ALM	14.33 ± 1.68	0.837†	2.673†	0.87	0.81	0.91	0.34* ± 1.66	2.01	-1.32	-0.040
		MFBIA ALM	15.81 ± 2.21	0.643†	4.509†	0.87	0.80	1.58	-1.14* ± 2.19	1.05	-3.32	-0.324†

* Represents significance at ($p \leq 0.0167$), † Represents significance at ($p \leq 0.05$). Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Table 9

	Eq	Method	(x ± SD)	Slope	Intercept	*r*	*SEE*	*TE*	Agreement			
									CE / Bias ± 2SD	Upper Limits	Lower Limits	Trend
All Subjects		DXA RSMI	6.72 ± 1.40									
	18	BIA RSMI	6.67 ± 1.26	1.061	-0.361	0.95	0.43	0.43	0.05 ± 0.85	0.90	-0.81	0.111†
	19	BIA RSMI	5.89 ± 0.69	1.758†	-3.647†	0.86	0.72	1.20	0.82* ± 1.74	2.56	-0.91	0.730†
		MFBIA RSMI	6.97 ± 1.21	1.078	-0.801†	0.93	0.52	0.58	-0.25* ± 1.02	0.77	-1.27	-0.152†
	6+7	BIS RSMI	7.33 ± 1.05	1.131	-1.571†	0.84	0.76	0.97	-0.61* ± 1.50	0.89	-2.11	0.315†
Men		DXA RSMI	8.14 ± 0.77									
	18	BIA RSMI	7.95 ± 0.59	1.039	-0.110	0.80	0.47	0.50	0.20 ± 0.91	1.11	-0.72	0.294†
	19	BIA RSMI	6.42 ± 0.54	1.122	0.936	0.78	0.49	1.78	1.72* ± 0.94	2.66	0.78	0.397†
		MFBIA RSMI	8.18 ± 0.54	0.958	0.303	0.67	0.58	0.56	-0.04 ± 1.11	1.08	-1.15	0.415†
	6+7	BIS RSMI	8.11 ± 0.90	0.654†	2.837†	0.77	0.50	0.57	0.03 ± 1.14	1.18	-1.11	-0.180
Women		DXA RSMI	5.63 ± 0.51									
	18	BIA RSMI	5.70 ± 0.57	0.683†	1.737†	0.76	0.33	0.38	-0.07 ± 0.74	0.67	-0.80	-0.121
	19	BIA RSMI	5.49 ± 0.48	0.809	1.189†	0.77	0.33	0.36	0.14* ± 0.66	0.80	-0.52	0.054
		MFBIA RSMI	6.05 ± 0.61	0.613†	1.924†	0.74	0.35	0.59	-0.42* ± 0.82	0.40	-1.24	-0.212
	6+7	BIS RSMI	6.73 ± 0.70	0.542†	1.978†	0.75	0.34	1.19	-1.101* ± 0.91	-0.19	-2.01	-0.366†

* Represents significance at ($p \leq 0.0125$), † Represents significance at ($p \leq 0.05$), Slope significance is compared to a slope of 1, CE / Bias = constant (mean) error, TE = total error, SEE = standard error of estimate, r = Pearson product-moment correlation coefficient, Limits = 95% limits of agreement [CE ± 1.96 SD of residual scores (actual-predicted)], Trend = relationship (r) between the difference of actual and predicted values, and the mean of both methods, Eq = equation number.

Appendix C.

Figure 1

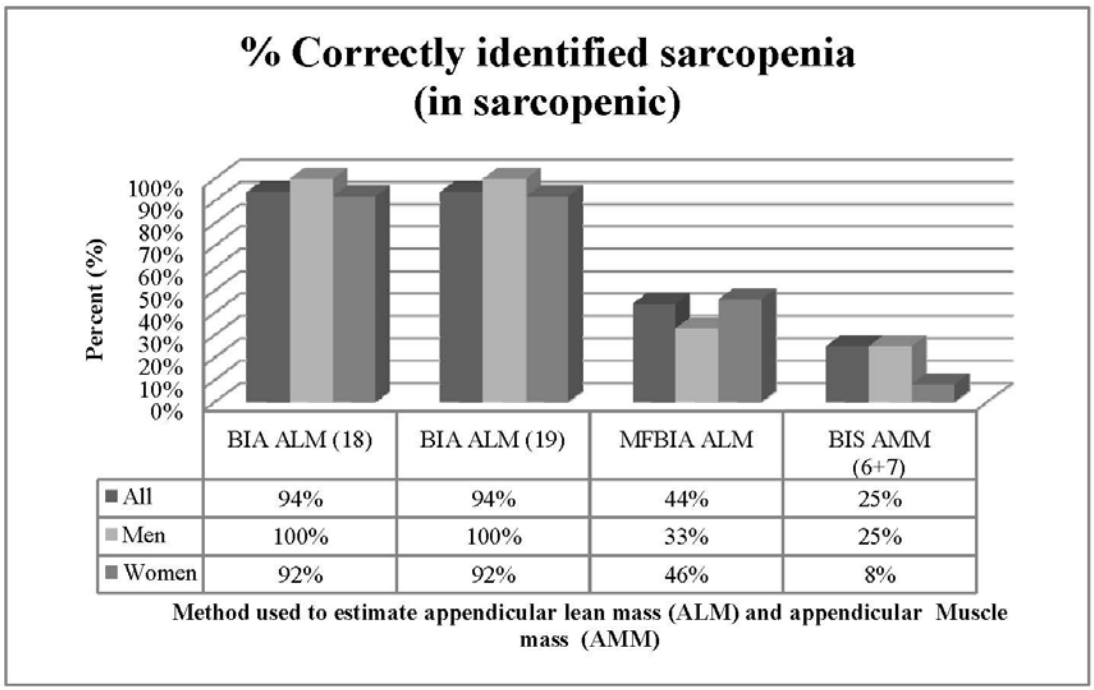


Figure 2

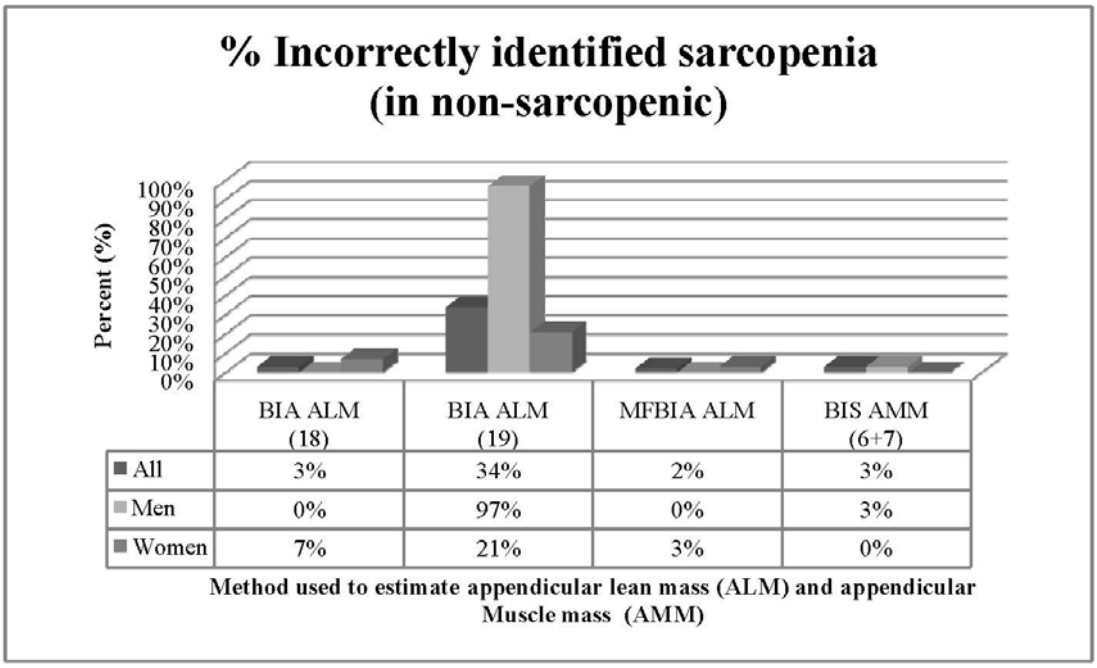

% Incorrectly identified sarcopenia
(in non-sarcopenic)
Percent (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BIA ALM (18)
BIA ALM (19)
MFBIA ALM
BIS AMM (6+7)
All 3% 34% 2% 3%
Men 0% 97% 0% 3%
Women 7% 21% 3% 0%
Method used to estimate appendicular lean mass (ALM) and appendicular Muscle mass (AMM)

Figure 3

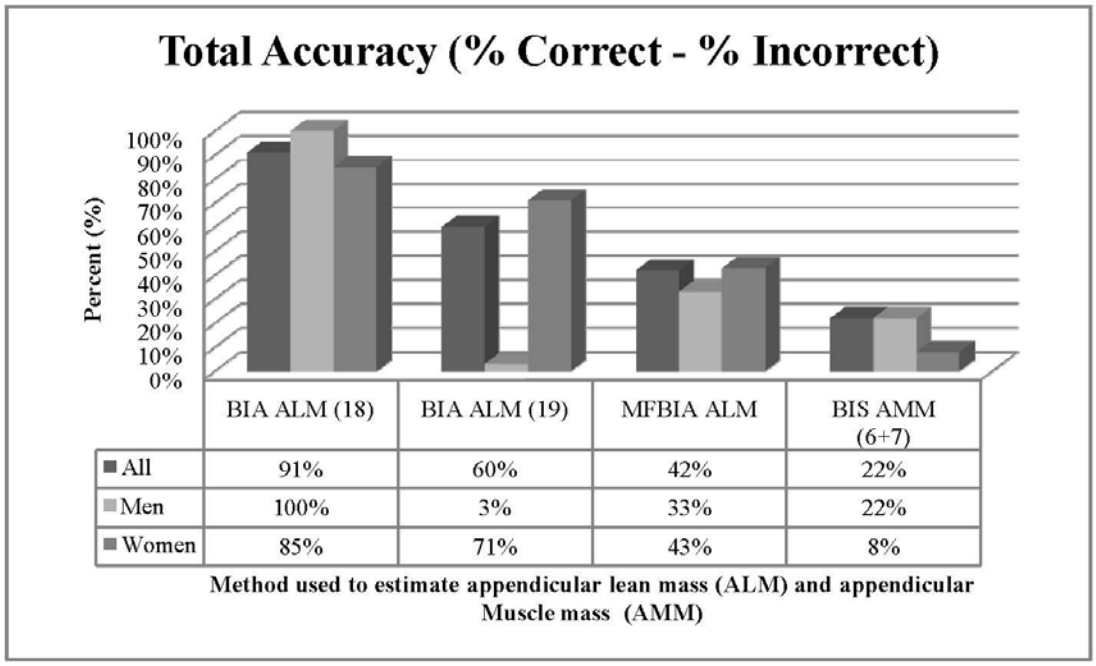

Total Accuracy (% Correct - % Incorrect)
Percent (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BIA ALM (18)
BIA ALM (19)
MFBIA ALM
BIS AMM (6+7)
All 91% 60% 42% 22%
Men 100% 3% 33% 22%
Women 85% 71% 43% 8%
Method used to estimate appendicular lean mass (ALM) and appendicular Muscle mass (AMM)

Figure 4

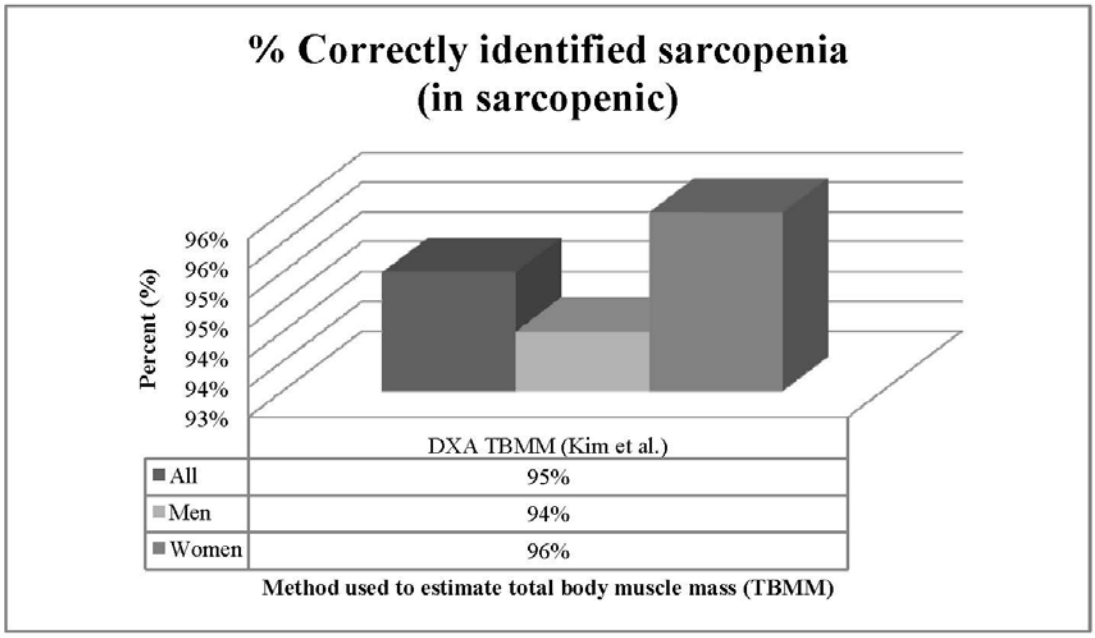

% Correctly identified sarcopenia
(in sarcopenic)
Percent (%)
96%
96%
95%
95%
94%
94%
93%
DXA TBMM (Kim et al.)
All
95%
Men
94%
Women
96%
Method used to estimate total body muscle mass (TBMM)

Figure 5

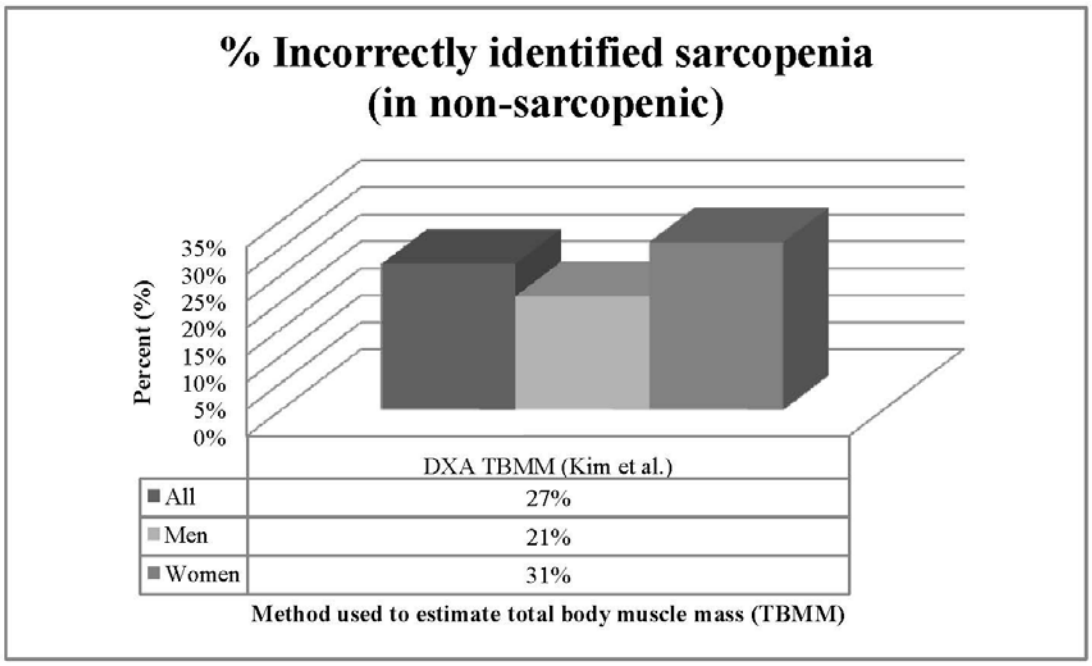

% Incorrectly identified sarcopenia
(in non-sarcopenic)
Percent (%)
35%
30%
25%
20%
15%
10%
5%
0%
DXA TBMM (Kim et al.)
All
27%
Men
21%
Women
31%
Method used to estimate total body muscle mass (TBMM)

Figure 6

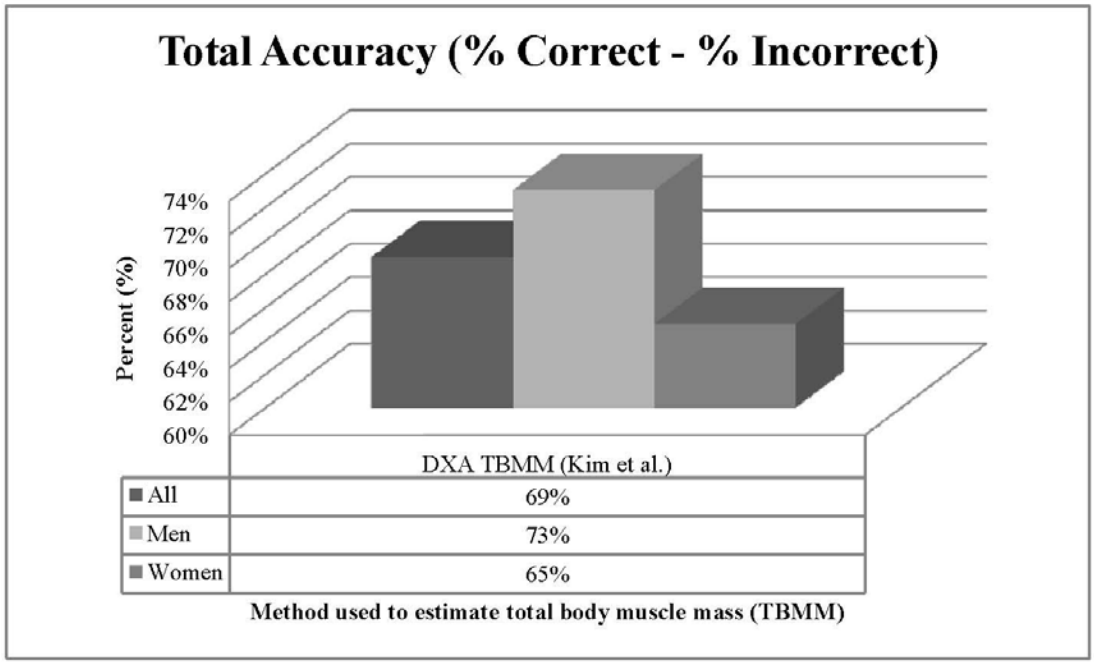

Total Accuracy (% Correct - % Incorrect)
Percent (%)
74%
72%
70%
68%
66%
64%
62%
60%
DXA TBMM (Kim et al.)
All
69%
Men
73%
Women
65%
Method used to estimate total body muscle mass (TBMM)

Figure 7

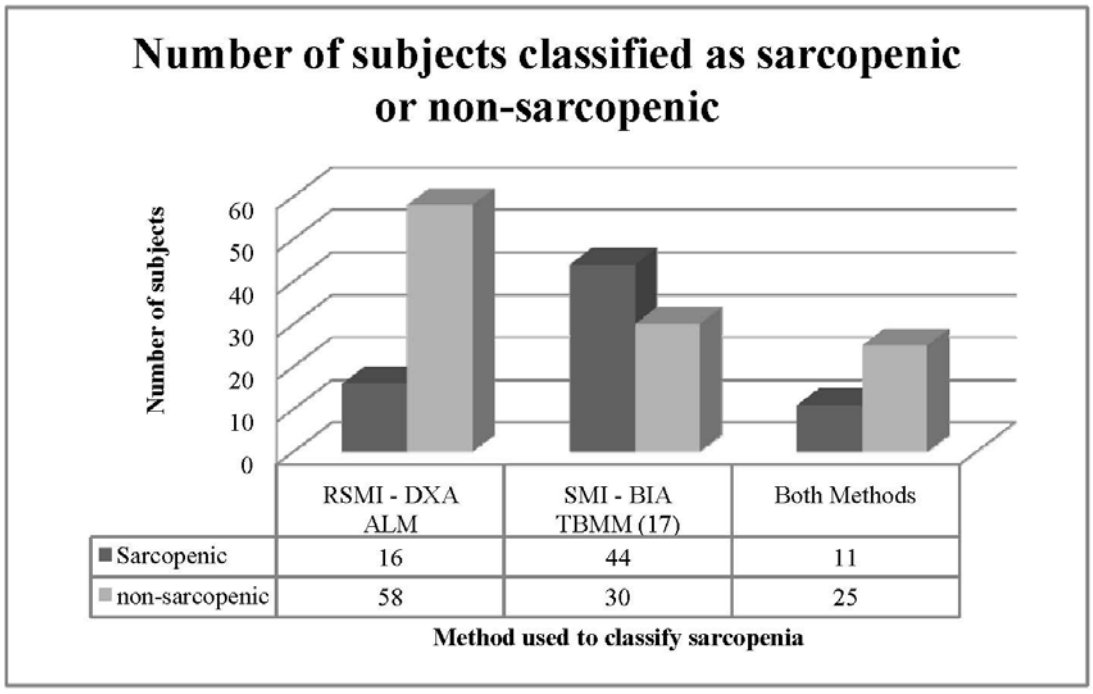
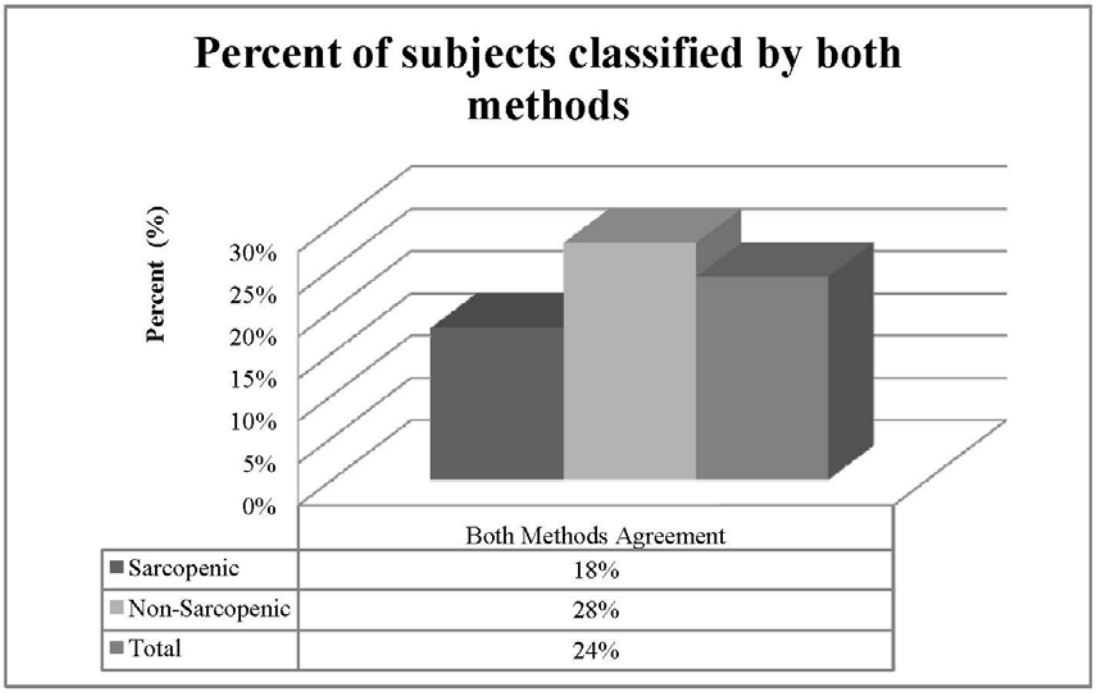

Number of subjects classified as sarcopenic or non-sarcopenic
Number of subjects
60
50
40
30
20
10
0
RSMI - DXA ALM
SMI - BIA TBMM (17)
Both Methods
Sarcopenic
16
44
11
non-sarcopenic
58
30
25
Method used to classify sarcopenia

Figure 8


Percent of subjects classified by both methods
Percent (%)
30%
25%
20%
15%
10%
5%
0%
Both Methods Agreement
Sarcopenic
18%
Non-Sarcopenic
28%
Total
24%

Appendix D.

FIGURE LEGENDS

Figure 1. Percent of correctly identified sarcopenic subjects comparing appendicular lean mass equations to DXA using the relative skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 2. Percent of incorrectly identified sarcopenic subjects comparing appendicular lean mass equations to DXA using the relative skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 3. Total accuracy in percent of identified sarcopenic subjects comparing appendicular lean mass equations to
DXA using the relative skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 4. Percent of correctly identified sarcopenic subjects comparing total body muscle mass equations to DXA muscle mass using the skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 5. Percent of incorrectly identified sarcopenic subjects comparing total body muscle mass equations to DXA muscle mass using the skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 6. Total accuracy in percent of identified sarcopenic subjects comparing total body muscle mass equations to DXA muscle mass using the skeletal muscle index classification method. Equations numbers are in parentheses.

Figure 7. Number of sarcopenic subjects classified by both the relative skeletal muscle index and the skeletal muscle index classification methods.

Figure 8. Total percent of agreement between the relative skeletal muscle index and the skeletal muscle index sarcopenia classification methods.

Appendix E.

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

University of Oklahoma
Institutional Review Board

Informed Consent to Participate in a Research Study Being Conducted Under the Guidance of the University of Oklahoma-Norman Campus

Project Title: Evaluation of AN777 in Elderly Subjects
Protocol Number: BK32
Sponsor: Abbott Nutrition, Abbott Laboratories
Columbus, Ohio
United States

Principal Investigator: Jeffrey R. Stout, PhD, FNSCA, FACSM, FISSN
Director Metabolic and Human Body Composition Laboratories
Department of Health and Exercise Science
University of Oklahoma
1401 Asp Avenue
Norman, OK 73019
Phone: 405-325-9023
Fax: 405-325-0594

This consent form may contain words that you do not understand. Please ask the study investigator or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

SUMMARY

- You are being asked to participate in a research study
- Your decision to be in this study is voluntary
- If you decide that you will be in this study and then you change your mind, you can leave the study at any time
- The care you receive in this study is not standard medical care. Your usual medical care from your doctor should not be replaced
- You will be in this study for about 6 months and have 4 study visits. You may receive reminder phone calls between visits. You may receive a follow-up phone call 7 to 10 days after you finish the study.
- If you agree to be in this research study, your medical records will become part of this research. They may be looked at or copied by the sponsor of this study or government agencies (including the U.S. Food and Drug Administration) or other groups associated with the study.
- If you are injured in this study, your medical insurance may be billed for any treatment you may need, or for standard medical care that you receive that is not part of this study
- The study product is a metabolite of leucine, an essential amino acid found in protein foods. Benefits of this product MAY include the following; increased

muscle tissue growth, and/or reduced muscle protein breakdown, increased immunity, decreased body fat, and lowering of blood cholesterol.

- Physician's clearance is necessary for participation in this study. Your physician will be contacted (via fax) with your approval. Typically this is free of charge, however, you will be responsible for paying any charges that a personal physician may implement to complete the release form or any charges for an office visit, if your physician requires that you make a personal visit in order to complete the release form. There is a study physician that may provide an exam for participation, free of charge.

More detailed information about this study is in this consent form. Please read this form carefully and ask any questions that you may have before agreeing to take part in this study.

You are being asked to volunteer for this research study. This study is being conducted at the University of Oklahoma-Norman Campus. You were selected as a possible participant because you are greater than or equal to 65 years of age, you agree to maintain your current activity level, you are able to walk on your own, and you have certain nutritional risk factors.

Purpose of the Research Study

The purpose of this study is to evaluate the effect of a study product compared to a control product on muscle mass change in the elderly population while on an adequate protein diet.

Number of Participants

About 60 people will take part in this study.

Procedures

If you agree to be in this study, you will be asked to do the following:

You will be asked to consume one packet of your assigned study product, with water, two times per day. The times of day in which you supplement are at your discretion. You cannot choose which study product you will get. This is decided by chance at Visit 1. You will have an equal chance of receiving the study product or the control product. Neither you nor your study investigator will know which study product you receive. Your study investigator can find this out in an emergency.

You will make four study visits to the study site, each of which will last about 4 hours, with the exception of the screening visit. Study staff may phone you between visits to see how you are doing, remind you of study visit preparations and record keeping, and/or clarify information you have previously shared. You may also receive a follow-up phone call 7 to 10 days after study exit, if you have an ongoing medical event at exit.

You will be required to visit the Human Performance Lab, located within the Huston Huffman Center, for all visits.

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

During the screening, visit 1 and the final visit/exit you will be escorted to the Goddard Health Center, located on the OU-Norman Campus. The visits to Goddard will last approximately 0.5-1.0 hours, and are for blood work to assess total cholesterol (low density lipoproteins and high density lipoproteins), complete blood counts (CBC) and a CHEM-20, to assess albumin and HMB levels. Each blood draw requires 11.5 mL of blood. These tests help evaluate the function of your liver and kidneys; the visit-1 test will be used to assess your inclusion within the study, whereas visit-6 testing will be used to assess health-related response to the test intervention and supplementation compliance. If blood draw results are abnormal, your will be referred to Goddard Health Center for follow-up.

Screening Visit:

You will be asked to read and sign this consent form before any study-related procedures are performed. During the Screening Visit, the following will be done:

- Your age, race and gender will be collected
- Your self-reported medical history
- Your medication use
- Your self-reported physical activity
- Your body measurements (weight, height, knee height, and body mass index) will be measured
- A sample of your blood will be drawn and analyzed to assess your current nutritional status
- Your diet history will be collected
- You will receive a 3-day food diary to document three days of intake in the week prior to your next visit
- You will be reminded to fast prior to your next visit and that the first morning urine void will need to be collected at the facility during your next visit. In case of emergency, you will be provided with a sample collection container and instructed on how to collect a "clean catch" urine sample.
- You will be contacted by phone by the study staff prior to your next visit to be reminded of the following:
    - Fast overnight (at least 8 hours) prior to the visit
    - Complete the 3-day diet diary in the week prior to the next visit
    - First morning urine void on the day of Visit 1 needs to be collected at the site

Visit 1, Visit 2 and Final Visit/Exit

Visit 1 will take place about 7 days after your Screening Visit. Visit 2 will take place about 12 weeks after Visit 1. The Final Visit/Exit will take place about 24 weeks after Visit 1. The following will be done at each visit:

You will be asked about your medical history since the last visit, including the following:

- Changes in your he

APPROVED
AUG 2 8 2008
OU-NC IRB
APPROVAL
AUG 0 6 2009
EXPIRES

- Use of any medications, vitamins/mineral supplements, liquid or bar nutritional supplements, or other dietary supplements

- You will be randomly assigned, by chance (like the flipping of a coin) to one of the two study products: (Visit 1 only)
  - Study product
  - Control product
- Your weight will be measured
- A fasting blood draw will be performed (Visit 1 and Final Visit/Exit only)
- A first morning urine void will be collected
- Your body composition measurements include the following:
  - After a 12-hour fast, with water consumption allowed up to one hour prior to testing, you will participate in a series of data collection stations; each station will be made private by the use of room dividers and/or separate closed rooms. A female research assistant will accompany female subjects, who are being tested by a male researcher, during each testing station. The complete body composition measurements will last approximately 4-4.5 hours, per visit. You will be required to wear either a bathing suit or tight-fitting clothing, such as Spandex, during these visits.
  - **Bioelectrical Impedance (BIA/BIS)** (15-20min) – (Body tissue impedance is measured when a small, harmless electrical signal is passed through your body, carried by water and fluids) You will be asked to lie flat on your back and will have two electrodes attached to your right foot and hand; at the ankle and wrist, and the toe and finger, respectively. This widely used and FDA-approved commercial device conducts a harmless and painless electrical current through your body.
  - **Dual X-Ray Absorptiometry (DEXA) (10-15min) – *(The DEXA is essentially a padded table with a mechanical arm that uses low-dose radiation to measure bone mineral density)*** You will be asked to lie flat on your back, with your arms at your sides, legs extended and feet together. The "arm" of the DEXA will then slowly move over your body, without contact. This test will be used to assess total bone mineral density and upper and lower body muscle mass. You will be exposed to very small levels of radiation – exposure that has been determined to be no greater than that which most Americans receive in several days from natural background radiation (~300 mrem/year) sources, such as radioactivity released from the soil. You will be required to wear either a bathing suit or tight-fitting clothing, such as Spandex, during this test.
  - **BOD POD Measurements (10-15min) – *(The BOD POD is an egg-shaped device for someone to sit in, and is used for measuring and tracking body fat and lean mass using patented air displacement technology)*** You will be weighed, given a swimming cap to cover your hair, and then asked to sit in the Bod Pod for approximately three minutes. You will be asked to breathe normally and not to move while the machine calculates your body volume.

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

- **Total Body Water (TBW) and Extra cellular Water (4hrs) – *(The amount of water within your body; both the water within and outside your body cells, as measured by a urine test after drinking a chemical solution)*** You will be asked to drink a solution containing 10mL of deuterium oxide (D2O). The substance is non-radioactive, but may taste quite salty. Prior to drinking the solution, you will be asked to urinate into an 8 fl-oz cup, filling the cup about halfway. You will then be asked to ingest the D2O solution and then refrain from consuming any food or beverage for the next four (4) hours. All other body composition stations will be completed during this 4-hour period. After the four hours have expired, you will be asked to again urinate in an 8 fl-oz cup, as described above.
- **Ultrasound Thickness Measurements (10-15 min) – *(The thickness of your skin and muscle tissue)*** This technique involves applying a thick layer of gel on the surface of your skin and a metallic transducer gently applied over the surface of your skin. During the measurement, the ultrasound transducer is slid back and forth along the surface of your skin. Measurements will be taken at the following eight sites: biceps, abdomen, thigh, calf, hamstring, front hip (female only), triceps (female only), and chest (male only).

- You will be asked to complete a questionnaire to assess activities of daily living
- Your 3-day food diary will be collected and reviewed by your study investigator
- You will receive study product and instructions on how to prepare the product (Visit 1 and Visit 2 only)
- You will receive a new 3-day food diary to document three days of intake in the week prior to your next visit (Visit 1 and Visit 2 only) and product intake forms to record daily study product intake (Visit 1 and Visit 2 only)
- You will be tested for strength and functionality within 1-3 days after the body composition tests as follows;
  - **Your upper body strength test** requires that you squeeze a handheld dynamometer handle as forcefully as possible for three to five seconds. You will be asked to do this three times using your dominant arm and the three tests will be averaged for your max strength score. **Your lower body strength** will be assessed using an isokinetic dynamometer and will require you to sit in an oversized chair with your knee joint aligned with the dynamometer axis of rotation, and straps will be positioned at the hips, shoulders, and over the right thigh to prevent extraneous movement. The fulcrum of the lever will be positioned on the shin, just above the ankle. You will be asked to perform three maximal effort knee extension and flexion movements at an angular speed of 60-degrees/second and 180-degrees/second. Three trials will be performed, with three minutes of rest in between trials, and the average of the three trials will be considered your max lower body strength. **The 'Get-up-and-Go' test** consists of timed measurements starting from a seated position of a chair, standing,

APPROVED
AUG 2 8 2008
OU-NC IRB
APPROVAL
AUG 0 6 2009
EXPIRES

walking forward 3 meters, turning around, walking back to the chair, and sitting down.

- You will be reminded to fast prior to your next visit and that the first morning urine void will need to be collected at the facility during your next visit. In case of emergency, you will be provided with a sample collection container and instructed on how to collect a "clean catch" urine sample.
- You will be contacted by phone by the study staff prior to your next visit to be reminded of the following:
  - Fast overnight (at least 8 hours) prior to the visit
  - Complete the 3-day diet diary in the week prior to the next visit
  - First morning urine void on the day of Visit 2 needs to be collected at the site
- You will return any remaining study product (Final Visit/Exit only)

7 Day Follow-up Phone Call:

If you have an ongoing medical event (such as a cold) at the time of study exit, you will be contacted 7 to 10 days after the exit visit. You will be asked if the medical event has resolved.

Length of Participation

You will be in this study for about 6 months and have 4 study visits. You may receive reminder phone calls between visits. You may receive a follow-up phone call 7 to 10 days after you finish the study.

Visit one (Screening visit) will last approximately 30-60 minutes, where you will complete the required forms, health history questionnaire and sign the informed consent. During this visit you will also be instructed on how to fill out the provided Nutrition Logs.

Visits 1, 2 and Final Visit/Exit will last approximately 4 hours. During these visits you will be assessed for body composition.

The visits for strength and functionality (within 1-3 days after the body composition tests) will last approximately 45-60 minutes.

This study has the following risks:

You will be asked health-related questions (i.e., allergies, current/recent medications, medical conditions, etc.) that will be recorded and used for screening purposes only. If you do not meet the inclusion criteria, as provided by the researchers, or are not cleared by your physician, you will not be allowed to participate.

Because the study product is considered investigational, there may be other risks to you that are unknown at this time. If you have any problems with the study product, you should contact the study investigator or study coordinator as soon as possible.

APPROVED
AUG 2 8 2008
OU-NC IRB
APPROVAL
AUG 0 6 2009
EXPIRES

- Pain, bruising, feeling faint, arm soreness or slight risk of infection from having your blood drawn. Blood draws will be performed by a phlebotomist at the Goddard Health Center and will require a single needle to puncture your skin. If any of the results are abnormal, you will be given a copy and advised to visit your personal physician or referred to Goddard Health Center for follow-up.
- Muscle fatigue, during and immediately following upper and lower-body maximal exercise tests and functionality tests
- Feelings of hunger from observing a 12-hour fast, on two test days
- Small amount of radiation from DEXA. Although the amount of radiation exposure received in the study is minimal, it is important that you are aware that the risk from radiation exposure is cumulative over a lifetime. If you participate in the study you will receive three DEXA scans (a type of x-ray), and thus be exposed to additional amounts of radiation that you would not have received otherwise. Women should always inform their physician or X-ray technologist if there is any possibility that they are pregnant. No complications are expected with the DEXA procedure.

May Possibly Occur:

- Emotional/psychological discomfort, distress and/or anxiety due to the type of clothing required and/or human-human contact and interaction required for accurate and successful completion of the body composition assessment. Tests, however, take only a few minutes to complete.
- Muscle soreness beginning within 24 hrs following maximal upper- and lower-body maximal testing and lasting for several days
- Anxiety resulting from closed-in spaces (BOD POD)

New Findings

Any new information that is discovered during the study, which may change your decision to continue participation in the study, will be made available to you in a timely manner.

Benefits of being in the study are

This is not a treatment study. You are not expected to receive any direct medical benefits from being in this study. The information from this research study may benefit others in the future.

The benefits of knowing your body composition, regional distribution of muscle mass, upper and lower-body strength, functionality, and general blood parameters (cholesterol, triglycerides, glucose, white blood cells and red blood cells, etc) are wide and numerous and include potentially identifying underlying problems that you may not currently be aware of.

The benefit of knowing your recommended protein intake may help you to understand healthier eating habits.

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

The study product is a metabolite of leucine, an essential amino acid found in protein foods. Benefits of this product MAY include the following; increased muscle tissue growth, and/or reduced muscle protein breakdown, increased immunity, decreased body fat, and lowering of blood cholesterol.

Alternate Procedures

This is not a treatment study. Your other option is to not participate in this study.

COSTS

The sponsor will provide study product. There are no charges for the study visits. You will be billed for all medical treatment that is not part of this study. These charges may include charges relating to your medical care (hospital and physician fees), which are not a part of this study.

SOURCE OF FUNDING FOR THE STUDY

The study investigator is being paid by Abbott Nutrition, Abbott Laboratories to conduct this research.

Injury

If you are injured as a direct result of participation in this study, contact the study investigator immediately. The study investigator will review the situation. If necessary, the study investigator will provide treatment or refer you for treatment.

If the study investigator determines that any complication, injury, or illness requiring emergency medical treatment is a result of participation in this study, appropriate acute medical care will be provided at no cost to you. Abbott agrees to pay all reasonable medical expenses necessary to treat such injury:

(1) to the extent you are not otherwise reimbursed by medical insurance, and
(2) provided you have followed the directions of the study investigator and/or study staff.

This agreement to provide medical treatment does not include complications, injuries, or illnesses you might get while in the study if these complications are not a result of the study product. There are no plans for additional payment for lost wages, pain and suffering, or for other losses.

The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

By signing this consent form, you will not give up any legal rights for yourself.

Compensation

You will be compensated for your participation. You will receive a stipend in the amount of $200 upon completion of the study, otherwise, a prorated amount will be awarded. Stipends will be prorated based on weekly participation. The total duration of the study

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

is 6 months (24 weeks). Weekly participation will be compensated $8.33. If you withdraw from the study you will be paid according the last completed week.

Voluntary Nature of the Study

Your participation in this study is voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your participation in this study may be stopped at any time by the study investigator or the sponsor without your consent if it is determined that it is in your best interest or in the best interest of this study.

If you are removed from the study prior to your final study visit, then you may be asked by the study investigator to return to the study site one last time to return study product, forms and answer questions about any changes in your health status.

Participation in this study should not replace routine medical care by your primary care physician or specialist.

Contacts and Questions

Contact **Jeffrey R. Stout, PhD** at **405-325-9023** or jrstout@ou.edu for any of the following reasons:

- If you have questions concerning your participation in this study,
- If at any time you feel you have experienced a research-related injury or reaction to the study product, or
- If you have questions, concerns, or complaints about the research.

You may also contact: Abbie E. Smith, MS at 405-325-5211 or abbiesmith@ou.edu
Jordan Moon, MS at 405-325-1368 or JordanMoon@ou.edu

If you have questions about your rights as a research subject, or if you have questions, concerns, or complaints about the research, and wish to talk to someone other than individuals on the research team or if you cannot reach the research team, you may contact:

The University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

If you agree to be in this study, then you will receive a signed and dated copy of this consent form for your records. If you are not given a copy of this consent form, please request one.

Statement of Consent

I have read the information in this consent form. All my questions about the study and my participation in it have been answered. I freely consent to be in this research study.

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

I authorize the use and disclosure of my health information to the parties listed in the authorization section of this consent for the purposes described above.

By signing this consent form, I have not given up any of my legal rights.

_____
Printed Name of Subject

_____ _____
CONSENT SIGNATURE: Date

_____
Printed Name of Legally Authorized Representative

_____ _____
Signature Legally Authorized Representative Date

_____
Authority of Subject's Legally Authorized Representative or Relationship to Subject (when applicable)

_____
Printed Name of Person Conducting Informed Consent Discussion

_____ _____
Signature of Person Conducting Informed Consent Discussion Date

APPROVED
AUG 2 8 2008
OU-NC IRB

APPROVAL
AUG 0 6 2009
EXPIRES

Appendix F.

University of Oklahoma

Evaluation of a leucine metabolite on muscle mass in elderly individuals

Health History & Exercise Status Questionnaire

Side A

Demographics:

Name: _____ Subject number: _____

Date: _____ Age: _____ Date of Birth: _____

Daytime phone: _____ Evening contact number: _____

Family History:

Has anyone in your immediate family had any of the following: Please circle **Yes** or **No.**

Heart disease	Yes	No	Diabetes	Yes	No
High blood pressure	Yes	No	Cancer	Yes	No
Stroke	Yes	No	Tuberculosis	Yes	No
Sudden Death (before 50)	Yes	No	Asthma	Yes	No
Epilepsy	Yes	No	Gout	Yes	No
Migraine Headaches	Yes	No	Marfan's Syndrome	Yes	No
Eating Disorder	Yes	No	Sickle Cell	Yes	No

Please explain all Yes responses; denoting relationship and age of onset/occurrence of the family member in question (if known):

_____

_____

_____

Personal History:

1. Have you ever been hospitalized? Yes No
   Have you ever had surgery? Yes No
   Are you presently under a doctor's care? Yes No
   Have you ever been diagnosed with a sleeping disorder or clinical depression? Yes No
   Please explain and give dates for all Yes responses: _____

2. Please list any medications you are currently taking and for what conditions: _____

3. Please list any known allergies: _____

4. Have you ever had a head injury / concussion? Yes No
   Have you ever been "knocked-out" or unconscious? Yes No
   Have you ever had a seizure, "fit" or epilepsy? Yes No
   Have you ever had a "stinger," "burner" or pinched nerve? Yes No
   Do you have recurring headaches or migraines? Yes No
   Please explain and give dates for all Yes responses:

5. Have you ever had the chicken pox? Yes No
   If Yes, at what age? _____

6. Have you ever had the mumps or measles? Yes No

7. Do you have a history of asthma? Yes No

8. Are you missing an eye, kidney, lung or testicle? Yes No

9. Do you have any problems with your eyes or vision? Yes No

BK32 Version 2, 8/12/08 Page 1 of 2

University of Oklahoma

Evaluation of a leucine metabolite on muscle mass in elderly individuals

Health History & Exercise Status Questionnaire

Side B

10. Have you ever had any other serious medical problems (mononucleosis, diabetes, anemia, etc)? Yes No

11. Have you ever taken any supplements for improved performance? Yes No

12. Are you presently taking any supplements for diet or performance (creatine, protein, etc.)? Yes No

13. What is the lowest bodyweight you have been at, within the last **3 months**? _____
Highest? _____ What is your ideal weight? _____

14. Do you have trouble breathing, or do you cough during or after exercise? Yes No

15. Have you ever had heat cramps, heat illness or muscle cramps? Yes No

16. Do you have any skin conditions (ex: itching, rashes, acne, rosacea, etc)? Yes No

Please explain all Yes responses for question 5 -16: _____

17. Have you ever fainted during or after exercise? Yes No
Have you ever been dizzy during or after exercise? Yes No
Have you ever had chest pain during or after exercise? Yes No
Have you ever had high blood pressure? Yes No
Have you ever been told you have a heart murmur? Yes No
Have you ever had racing of your heart or a skipped heartbeat? Yes No
Have you ever had an EKG or echocardiogram? Yes No

Please explain all Yes responses for question 17: _____

18. Have you ever sprained / strained, dislocated, fractured, or had repeated swelling or other injury of any bones or joints? Please explain all Yes responses.

Head / Neck	Yes	No	_____
Shoulder	Yes	No	_____
Elbow & Arm	Yes	No	_____
Wrist, Hand & Fingers	Yes	No	_____
Back	Yes	No	_____
Hip / Thigh	Yes	No	_____
Knee	Yes	No	_____
Shin / Calf	Yes	No	_____
Ankle, Foot & Toes	Yes	No	_____

19. Have you received any of the following procedures, within the last year? If yes, indicate how many.

Chest X-ray _____ Dental X-ray_____ Mammogram_____
PQCT scan _____ DEXA scan _____ Other _____

Please Sign:
I hereby state that, to the best of my knowledge, my answers to the above questions are correct.

Subject's Signature: _____ Date: _____