EVALUATION OF BODY COMPOSITION IN OVERWEIGHT AND OBESE
SUBJECTS: THREE-COMPARTMENT MODEL AND ULTRASOUND
COMPARISONS

Sarah Nicole Fultz

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in
partial fulfillment of the requirements for the degree of Master of Arts in the department
of Exercise and Sport Science in the College of Arts and Sciences (Exercise Physiology).

Chapel Hill
2013

Approved By:

Abbie Smith-Ryan, PhD

Claudio Battaglini, PhD

Eric Ryan, PhD

# ABSTRACT

SARAH FULTZ: Evaluation of Body Composition in Overweight and Obese Subjects:
Three-Compartment Model and Ultrasound Comparisons.
(Under the direction of Dr. Abbie E. Smith-Ryan)

Identifying valid field methods to measure body composition in overweight and obese individuals is essential for quantifying percent body fat (%BF), fat mass (FM) and fat free mass (FFM) and the associated concomitant health consequences. The purpose of this study was to compare the validity and reliability of an A-mode ultrasound (US) to the criterion three compartment model (3C) for the measurement of body composition in overweight and obese subjects. Body composition testing was performed on forty-seven overweight and obese subjects via the ultrasound, air displacement plethysmography and bioelectrical impedance spectroscopy, on two separate days. The US was not found to be a valid measurement of body composition in overweight or obese individuals; %BF and FM was significantly under-predicted, while FFM was over-predicted when compared to the criterion 3C model. The US was found to be reliable when compared to 3C for measuring %BF.

**ACKNOWLEDGEMENTS**

First and foremost, I would like to thank everyone who helped to make this thesis project a success. I would first like to thank my advisor, Dr. Abbie Smith-Ryan, your guidance, knowledge and patience has, not only helped me to complete this document, but has taught me what it means to be a responsible and productive researcher. I would also like to thank my committee members, Dr. Claudio Battaglini and Dr. Eric Ryan, for taking the time to review my document and for providing advice which would guarantee a quality thesis. I would finally like to thank my classmates for your time and assistance with data collection and your endless support throughout this process.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

CHAPTER I

INTRODUCTION

Currently, about 68% of adults in the United States, over the age of 20, are overweight or obese (1). Obesity is quickly becoming the number one health problem in the country (2). Excess weight is linked with an increased occurrence of cardiovascular disease, type II diabetes mellitus (DM), hypertension, stroke, sleep apnea, dyslipidemia, osteoarthritis, some cancers, depression and early death (1, 3). One method of categorizing individuals as overweight or obese is by the use of body mass index (BMI), which is calculated as weight in kilograms divided by the square of height in meters (2). Individuals with a BMI between 25 and 29.9 kg·m$^2$ are categorized as overweight, and individuals with a BMI greater than 30kg·m$^2$ are classified as obese. Obesity can further be classified into different stages; stage I (BMI 30.0-34.9kg·m$^2$), stage II (BMI 35-39.9kg·m$^2$), or stage III (BMI ≥40.0kg·m$^2$) (2). It has been suggested that BMI may not be the most accurate technique to classify individuals as either overweight or obese. In a study of 909 Turkish outpatients, Bozkirli et al. (2007) found that the World Health Organization's (WHO) BMI cutoff points for obesity significantly underestimated the frequency of obesity in this group (4). Other studies have also noted a difference between BMI and percent body fat in varying ethnic groups (5-7). These differences demonstrate that BMI may not be the most accurate in defining overweight and obese classifications. Body mass index also does not take into consideration subcutaneous or visceral fat mass when classifying individuals. Studies have observed that abdominal obesity, in particular

visceral adipose tissue, is linked with the pathogenesis of numerous metabolic risk factors (8, 9). This data suggests the differentiation between subcutaneous and visceral adipose tissue may be a critical factor in identifying health implications of obesity. A way to determine an individual's health risk, as well as distribution of body fat, is by accurately measuring body composition, the components that make up the body such as fat mass, fat free mass and percent body fat (%BF). Accuracy in classifying the body composition of these overweight and obese individuals is critical for clinical practice, as well as for the evaluating the effectiveness of weight loss and health intervention programs.

Overview of Body Composition Measurement Techniques

Due to the increasing prevalence of obesity in the United States, measuring body composition is of critical importance to researchers, medical personnel, and clinicians. Methods to measure body composition are either considered to be "reference" or "prediction" techniques. Reference methods are the most accurate techniques for assessing body composition, and are employed as the criterion against which other techniques are compared (10). Reference methods include measuring certain components of the body and include methods such as densitometry, hydrometry and dual x-ray absorptiometry (DXA) (11). Other reference methods include cadaver dissection, multi-compartment models and medical imaging, such as computed tomography scan (CT Scan) and magnetic resonance imaging (MRI) (10). Prediction methods, such as skinfold thickness and bioelectrical impedance spectroscopy (BIS), use regression analyses to approximate the outcome of one or more reference techniques, usually total body water (TBW) measurements from BIS and body density (Bd) from skinfold thickness (12). Unfortunately, methods used to measure body composition are limited by the generalized

assumption(s) that must be applied across the entire population (13). To date, the gold standard of body composition is reported to be the hydrodensitometric model, which divides the body into two compartments (2C) of constant densities, fat mass (FM) and fat free mass (FFM). It assumes that the relative amounts of other fat free components (water, protein, bone mineral and non-bone mineral) are fixed (14). Thus, hydrodensitometric measurements may be inappropriate for obese individuals who fall outside these fixed or assumed values. More recently, the use of multi-compartment models have become widely acceptable as the method to provide the most accuracy when calculating body fat. Multi-compartment models are more generalizable because they are not known to be age, gender, race, or health status dependent (13). The three compartment model (3C model) of body composition measurement includes FM, FFM and TBW. The 3C model uses aid displacement plethysmography (ADP) to obtain body density (Bd) and BIS to obtain TBW measurements. However, it does assume a constant ratio of protein to mineral (the fat free dry matter in this model) (15). A more sophisticated technique has been found using the four compartment model (4C model), which includes FM, FFM, TBW, and bone mineral mass; however, Withers et al. determined that the means and variances for the relative %BF differences between the two and three compartment models (2.2 ± 1.6% BF) were significantly greater than (P<0.02) than those between the three and four compartment models (0.2 ± 0.3% BF) (16). Therefore, this indicates that additional control for interindividual variability in bone mineral mass attained via the four compartment model achieves little extra accuracy (16). The 3C model is simple, safe, and not as laborious, expensive or time intensive as the 4C model (13).

Air Displacement Plethysmography

Air Displacement Plethysmography (ADP) is another simple method used to measure body volume (BV) and Bd (17). Air displacement plethysmography is quick (usually taking 5-10 minutes), requires minimal compliance by the subject, and requires minimal technician skill to operate (17). Measuring body composition with ADP estimates BV from Boyle's law ($P_1/P_2=V_2/V_1$), which describes the inverse relationship between air volume (V) and pressure (P) under isothermal conditions (18). Body weight divided by body volume yields Bd, and from Bd percent body fat (%BF) can be estimated (19). Body density can be used in any 2C or multi-compartment model to estimate %BF, however, the BodPod® software defaults to the Siri 2C model equation. It is also possible to select the Brozek et al and Schutte et al. 2C equations, as well as the Siri 3C and Selinger 4C equations (17). The BodPod, introduced by Dempster and Aitkens is the most common method of measuring ADP.

There are many advantages of using the BodPod to assess body composition; it provides a quick, easy, and noninvasive assessment, and is a beneficial alternative to hydrodensiometry because it overcomes some of the methodological and technical constraints of traditional Bd assessment methods (20). The BodPod is also able to accommodate both obese (~159 kg) and very tall subjects (~2m) (21). There are however a few disadvantages of using the BodPod; it is possible that some subjects may become claustrophobic while inside. Overweight and obese patients may be uncomfortable in swimsuits and therefore may need to wear alternative clothing, such as skin tight shorts or a medical gown, which could potentially skew the results. Another limitation of the BodPod associated with subjects is moisture on the hair and skin which could affect

compressibility of air next to the surface of the skin. This would lead underestimation of the %BF (10). A final limitation using the BodPod is located in the capsule. Changes in ambient pressure through windows or doors may cause the system to require recalibration; therefore the capsule requires a separate room for storage (10).

Bioelectrical Impedance Spectroscopy

Bioelectrical Impedance Spectroscopy (BIS) is an inexpensive, portable, noninvasive, and technically simple method of assessing body composition, which has a wide application in research laboratories, hospitals, health centers, and private clinics (22). Since the 1990s, use of BIS as a common method of body composition measurements has increased due to the portability and safety of the equipment; additionally, the procedure is simple and noninvasive, and the results are reproducible and rapidly obtained (23). Bioelectrical impedance is based on the following principle: when a current is applied to the body, the resistance or impedance ($\Omega$) of the body to that current is measured (24). The electrical current through the body is based on the length of the conductive path, the volume of the conductive material, and the resistivity of the conductive material (23). In the human body, only water, containing dissolved electrolytes, will conduct a current (22). Therefore, TBW can be estimated by measuring impedance to the flow of a current, based on the assumption that resistivity of the conductive material is constant, and from estimating length of the conductive path from an individual's height (23).

Advantages of using the BIS include its low cost after purchase, ease of use, noninvasive measurement, portability and safety (22, 25, 26). Some disadvantages or limitations of using the BIS include the violation of the assumption that TBW is constant

at about 73% water content of FFM (23). Some factors affect the validity of the BIS method in the overweight and obese state; such as: an increased relative TBW (underestimating %BF), different body geometry (overestimating %BF), and increased relative extracellular water (underestimating %BF) (27). Another factor that could skew measurements made by the BIS is the actual body structure of the overweight or obese subject. In obese subjects, a greater proportion of water, (and therefore FFM), is located in the trunk, which would lower total body impedance, resulting in an underestimation of FFM (22). The empirical relation between TBW (FFM) and the impedance index depends on body water distribution. In subjects with a comparatively high amount of extracellular water, may lead to an overestimation of FFM by formulas developed in a population with a normal distribution of body water (27). In the overweight and obese population a further limitation of using the BIS is the lack of population specific equations (28). Nevertheless, in a study done by Fuller et al. (1992), it was determined that 3C and 4C models are not compromised by errors arising from individual techniques (15). This shows that, it is still accurate to combine individual measurements into a compartment model without suffering extreme error.

Ultrasonography

Ultrasound (US) may be used to measure body composition in subjects by assessing subcutaneous adipose tissue thickness. The US is based on the pulse-echo technique and can be used to measure the thickness of fat between the skin and the muscle (10, 29). The US works by using a transducer probe to emit, through the skin, an ultrasonic beam which travels at the speed of sound into the tissue (10); part of the beam is reflected back at the fat muscle interface. Once the propagation velocity of the waves

in body fat and the time of flight are known, the thickness of the fat is then easily calculated using a linear relationship (29). The US is able to analyze fat patterning well, and it may be expected that estimates of total body fat or total subcutaneous FM, will be more accurate than skinfolds and BIA (10). Appropriate protocols for US estimates have not yet been standardized (10).

Advantages of the US relate to its low cost, ease of operation, and portability (29). The US has the ability to take many measurements in the vicinity of a given site and the average of the values can be estimated, rather than viewing a single-point measurement (10). However, a limitation of the US is that it requires specific equipment to operate (30).

Limitations of Body Composition Estimates in Overweight Population

There are several limitations when attempting to measure body composition of overweight, obese, or severely obese patients using traditional laboratory methods. Several traditional reference methods are inaccurate for this population, or are inappropriate for practical reasons. As discussed above, obese individuals also have different levels of hydration, and therefore FFM and TBW measured by the BIS may be inaccurate in this group. Some field methods, such as skinfold calipers used to measure body composition are not valid or reliable for the obese population (31). Skinfold may not be valid for a few reasons: the fat-muscle interface cannot always be palpated, skinfold thickness is impossible to measure at some sites due to the skin exceeding the maximum opening of certain types of calipers, elastic properties of both fat and skin tissues vary with age, from one individual to another, and variations in the depth at which the caliper tips can be placed may yield significantly thicker or thinner skinfold

8

measurements (31). Three compartment models (FM, FFM and TBW) are theoretically more accurate because it overcomes some uncertainties concerning the hydration fraction of fat-free mass (15).

In summary, due to the large increases in obesity, developing accurate methods for body composition assessment is important to evaluate the health status of an individual. Therefore it is important to evaluate more field methods to measure body composition in overweight and obese subjects. The US device may provide a valid and reliable non-invasive alternative to other commonly used field methods. However, more studies on the validity and the reliability of US in this specific population are needed. A need also exists for the validation of a portable body composition technique to accurately measure visceral adipose tissue. It is important to accurately classify subjects based off %BF due to its high association with cardiovascular risk factors and other diseases (1, 3).

Purpose

1. The primary purpose of this study was to assess the validity and reliability of the ultrasound (US) to the criterion three compartment model for the measurement of body composition in overweight and obese subjects. Additional exploratory analysis was done in order to evaluate validity differences stratified by sex and BMI.

2. The secondary purpose of this study was to determine if visceral adipose tissue (VAT) is independently predicted by: body mass index, percent body fat determined by the three compartment model, body density and total body water in the overweight and obese population.

Research Questions

1. Is the ultrasound a valid and reliable measurement of body composition in the overweight and obese population?

2. Can visceral adipose tissue be predicted independently by body mass index, percent body fat, body density and total body water in overweight and obese population?

Hypothesis

1. The ultrasound, using a 7-site scan, is a valid and reliable measurement of body composition compared to the three compartment model for overweight and obese populations.

2. Combined, body mass index, percent body fat, body density and total body water will predict visceral adipose tissue.

Delimitations

1. Participants were between the ages of 18 and 50.

2. Subjects had a minimum body mass index of 25 $kg \cdot m^2$ and a maximum body mass index of 50 $kg \cdot m^2$.

3. Subjects were healthy and did not suffer from any chronic diseases such as cardiovascular disease and cancer.

4. Subjects were not on medications known to affect body fluid such as diuretics or corticosteroids.

Limitations

1. Results may not be applicable to lean, young or elderly populations.

1. Subjects provided accurate health information on the health history questionnaire.

2. Subjects complied with the pre-testing instructions.

Operational Definitions

*Body mass index (BMI)* - A widely used clinical assessment of appropriateness of a person's weight. The value is calculated by dividing the weight in kilograms by height in meters squared (32)

*Overweight* – An individual having a BMI greater than or equal to $25\text{kg}\cdot\text{m}^2$

*Obese* – An individual having a BMI greater than or equal to $30\text{ kg}\cdot\text{m}^2$

*Air Displacement Plethysmography (ADP)* - A technique used for the measurement of body composition that uses the concept of body density as the ratio of body mass to body volume (32)

*Body Density (Bd)* - Total body mass expressed relative to total body volume (17)

*Bioelectrical Impedance Spectroscopy (BIS)* - A technique used to measure body composition based on the assumption that tissues high in water content conduct electrical currents with less resistance than those with little water (32)

*Ultrasound (US)* - A technique used to measure body composition by using a transducer probe to emit, through the skin, an ultrasonic wave, which part is reflected at the fat muscle interface. Once the propagation velocity of the waves in body fat and the time of flight are known, the thickness of the fat is then easily calculated using a linear relationship (29).

*Three Compartment Model (3C)* – equation developed by Siri (1961) that adjusts Bd for the relative proportions of water in the body. This model divides the body into three components, fat mass, fat free mass and total body water, and assumes a constant density for the protein-to- mineral ratio (17).

%BF= [(2.118 /Bd) – 0.78TBW/BM (kg) – 1.354] x 100 (33)

%BF= relative body fat

Bd = body density

TBW= total body water

BM –body mass

CHAPTER II

REVIEW OF LITERATURE

The increasing prevalence of overweight and obese individuals supports the pursuit of identifying accurate measures of body composition assessments. Several traditional reference and prediction methods for measuring body composition may be inappropriate to use for the overweight or obese population due to the limitations in the generalized assumption(s) that are applied across the entire population (13). Concerns with laboratory based methods include the sophisticated equipment required, the expense to manage and operate the equipment, the lack of portability, and the technician experience required. In order to make body composition measurements more readily available, field methods such as skinfold and BIS have been developed, but these too have limitations. It has also become generally accepted in the literature that multicompartment models used to measure body composition are more precise than two compartment methods (15, 26, 34, 35).

Air displacement plethysmography (ADP), commercially known as the BodPod is one laboratory method used to measure body composition. The BodPod estimates Bd from variations in pressure and volume while the subject sits in a sealed chamber (21). Air displacement plethysmography is an attractive tool for measuring body composition because it can accommodate both obese (~159 kg) and very tall (~2m) subjects (36). Vescovi et al. (2001) reported accurate measures of Bd by ADP for 29 overweight

subjects, defined by %BF, compared to under water weighing (UWW) as a reference (37). In a pilot study, Petroni et al. (2003) demonstrated the feasibility of using ADP to evaluate nine obese subjects ranging in BMI from 36.4-58.8 kg/m$^2$ (38). Finally a study by Ginde et al (2005) found that Bd measured via BodPod did not differ significantly from Bd measured by the traditional UWW method, even in obese subjects with a BMI of 58.4 kg/m$^2$ (39). The BodPod has many advantages due to the short time necessary, ease of operation, and accommodation of special populations, such as obese.

Bioelectrical impedance spectroscopy (BIS) is another common method used to measure body composition. In the overweight and obese population, BIS has been found to provide a good relative agreement with DXA, as indicated by high correlation coefficients (22, 25). However, studies suggest that due to wide limits of agreement with DXA, BIS as an alternative assessment of body composition, on an individual level, in overweight and obese populations is limited, although, it may be useful for group comparisons (22, 25, 40). Alternatively, Bosy-Westphal et al. (2008) concluded that BIS has a high potential as an accurate method of body composition analysis in public use, but in order to meet these requirements, population-specific equations are needed. They also concluded that for the assessment of body composition in individuals, tetrapolar electrode arrangement is needed for more accurate assessments. In a study by Moon et al. (2009) it was found that BIS was considered an accurate tool for tracking changes in TBW regardless of variations in BMI, FM, FFM, or age in both overweight and obese men and women (41). Likewise, Verdich et al. (2011) found that BIS may be used for assessing changes in body composition at a group level, but differs substantially from DXA on an individual level (42). This indicates that BIS and DXA cannot be used interchangeably. Overall, BIS is not the best field

method to use for measuring body composition in the obese population on an individual basis, but may be beneficial at a group level, as well as for tracking changes.

Ultrasound has been shown to be an accurate method for the assessment of body composition, %BF, FM and FFM, in the overweight and obese population (29, 30, 43). Due to the novelty of the US being used to measure body composition, few validation studies are available. Even though the US has been suggested to produce accurate results, currently there is little data demonstrating the validity compared to a multi-compartment criterion method. Some evidence suggests US may be superior to the skinfold caliper technique, in measuring subcutaneous fat, in obese subjects (31). However, these results differ from Borkan et al. (1982) who found that the use of the US was not a more effective means of assessing subcutaneous fat tissue as the skinfold technique (44). Similarly, Fanelli et al. (1984) found that in nonobese, white men, %BF was estimated with nearly the same degree of accuracy using either the skinfold caliper or the US (45). More recent data, as a potential result of improvements in technology, has demonstrated the accuracy of US to be more consistent and useful. In a recent study done by Utter et al. (2008) it was found that the US provided similar estimates of FFM when compared to the DXA (46). However, this study was done in a group of high school wrestlers, as opposed to obese subjects. Finally, a study done by Johnson et al. (2012) found that BIS and ADP were both significantly correlated with the IntelaMetrix US device (r = 0.862 & 0.879, respectively; p = 0.01) when used to measure body composition in a healthy, college age population (47). Since this study only included healthy, college-age subjects, results cannot be generalized to clinical populations. Therefore, additional studies need to be performed in order to assess the validity of the US in an obese population. Few

validation studies have been reported using the US (29, 47), with the studies available varying in population, device used and criterion method chosen for validation.

In order to establish validity, the current study compared US (BodyMetrix, Intelametrix, Livermore, CA) to the 3C model. In a study by Withers et al. (1998), the differences between two, three, and four compartment models of body composition analysis in highly trained and sedentary men and women were examined (16). They found that the means and standard deviation for the relative %BF differences between the two and three compartment models (2.2 ± 1.6 (SD) %BF; n=48) were significantly greater ($p \leq 0.02$) than those between the three and four compartment models (0.2 ± 0.3 %BF; n=48) for all groups. The research concluded that the 3C model is more valid than the 2C model, due to its control for biological variability in TBW, but additional control for inter-individual variability in bone mineral mass from the four compartment model achieves little extra accuracy (16). Additionally, it has been suggested that the 3C model is not compromised by errors arising from the individual techniques (15). This is beneficial due to TBW measurements being skewed in obese subjects. The current study used the Siri 3C model, which has been shown to provide the most accurate estimate of %BF (48). The 3C model utilizing Bd measurements from ADP, and TBW from BIS, can provide accurate measurements of %BF in the overweight and obese population (26).

The primary purpose of this study was to investigate the validity of the US method of body composition compared to the 3C model. The secondary purpose of this study was to determine if BMI, percent body fat as determined by the three compartment model and the components of the three compartment model (body density and total body

water) independently contribute to the prediction of visceral adipose tissue in overweight and obese subjects as measured by ultrasound.

*Air Displacement Plethysmography*

Ginde, S.R., Geliebter, A., Rubiano, F., Silva, A.M., Wang, J., Heshka, S., Heymsfield, & S.B., 2005 (39)

The purpose of this study was to compare Bd measured by ADP to Bd measured by underwater weighing (UWW) in subjects ranging from normal weight to severely obese. There were 123 subjects recruited for this study (89 men and 34 women; age, 46.5 ±16.9 years; BMI 31.5 ±7.3 kg/m$^2$); 15, 70, and 10 subjects were overweight, obese, and severely obese. Subjects had both body composition measurements done on the same day with the BodPod (Life Measurement Instruments, Concord, CA) being carried out first. A simple linear regression analysis was used to demonstrate no significant difference between Bd measured by UWW and ADP. The two measures were highly correlated (r = 0.94; Bd UWW = 0.96 × Bd ADP + 0.046; standard error of the estimate (SEE) = 0.0073 kg/L; p< 0.001). There were no significant differences in Bd measured by UWW and ADP for the subgroups of normal weight, obese, and severely obese subjects. Finally, percent fat estimates were highly correlated between UWW and ADP (r = 0.94, percent fat UWW = .95 × percent fat ADP + 1.22, SEE= 3.58%, p < 0.001). This study supports the hypothesis that ADP is an acceptable method of measuring percent body fat in overweight and obese subjects and its correlation with a "gold standard" of measuring Bd is high.

Petroli, M.L., Bertoli, S., Maggioni, M., Morini, P., Battezzati, A., Tagliaferri, M.A., Liuzzi, A., & Testolin, G., 2003 (38)

The purpose of this pilot study was to evaluate the feasibility of using ADP for body composition assessment in morbidly obese patients. Nine subjects were used for this study (6 males and 3 females) with a mean body mass index (BMI) of 46.6 ± 7.7 kg/m$^2$ (range 36.4-58.8). Body composition measurements were carried out using the BodPod instrument (Life Measurement Instruments, Concord, CA, USA). Instead of wearing a swim suit during the test, subjects work underwear kept to a minimum and a tightly fitting swim cap. Each subject was tested twice and if a difference between the two measurements were more than 150ml, a third volume measurement was made. All patients could fit into the instrument chamber and perform the maneuver for pulmonary plethysmography. The present study indicates that ADP is a suitable method to use to measure body composition in patients with a BMI greater than 40 kg/m$^2$ and produces realistic data.

Sardinha, L.B., Lohman, T.G., Teixeira, P.J., Guedes, D.P., & Going, S.B., 1998 (20)

The aim of this study was to compare ADP with DEXA and three other field methods for estimation of body composition, BMI, single-frequency BIA, multifrequency BIS, and the skinfold thickness equations of Jackson and Pollock (J-P) and Durnin and Womersley (D-W). Subjects included sixty-two, white men with a mean (+SD) age of 37.6 ± 2.9y and a BMI 27.8 ± 3.5 kg/m$^2$ (range 18.6-34.5 kg/m$^2$). Height and weight were measured and BMI was calculated from those measurements. Five site skinfold measurements were taken using a Lange caliper (Cambridge Scientific Instruments, Cambridge, MD). Jackson Pollock's 3-site (chest, triceps, and subscapular) generalized

equation and Durnin and Womersley 2-site (triceps, biceps, subscapular, and suprailiac) generalized equations were used to predict Bd. Siri's 2C model was used to convert Bd to %BF. Percent body fat measurements were also made using the DXA total body scanner (pencil beam mode, software version 5.67, enhanced whole-body analysis, QDR-1500; Hologic, Waltham, MA). Whole-body impedance measurements were performed via BIS analyzer (model 4000B; Xitron Technologies, San Diego). Air displacement plethysmography measurements were made with the (BodPod; Life Measurements Instruments, Concord, CA). Correlation analysis and a paired t test were used to analyze the plethysmograph's trail-to-trial precision. Multiple regression analysis was used to assess the significance of selected independent variables on %BF, FFM, and FM assessed by DXA. Results found a mean difference of 2.6% between DXA and ADP (95% CI: 1.91, 3.29; $t_{[60]} = 7.58$, P<0.05). Compared with DXA, the ADP underestimated %BF, with a total error of 3.7%BF. The correlation among all methods were significant (P< 0.01), ranging from 0.47 for the correlation between BMI and BIS- measured %BF to 0.95 for the correlation between %BF assessed by the J-P and D-W anthropometric equations. The second highest correlation was 0.93, between DXA-measured %BF and plethysmography- measured %BF. Overall, this study found that the plethysmography model performed better than the other models with DXA-measured %BF, FM, and FFM as dependent variables. As assessed by the highest adjusted $R^2$ (89.5) value and the lowest SEE (2.4%) value of this sample of middle-aged men, the plethysmography model, which included age, weight and height, was much more accurate than any other model analyzed. In conclusion, body composition was accurately estimated in middle-aged men with the ADP, although %BF was systematically underestimated.

Vescovi, J.D., Zimmerman, S.L., Miller, W.C., Hildebrandt, L., Hammer, R.L., & Fernhall, B., 2001 (37)

The purpose of this study was to compare estimations of %BF using ADP and UWW in a heterogeneous sample. The secondary purpose of this study was to determine whether there was a difference between the two methods among lean, average and overweight subjects in the sample. Ninety-five subjects (27 men and 68 women) 29 lean, 34 average and 29 overweight were recruited for this study. Subjects participated in either a one or two day test, if two days were required then day one consisted of body fat being measured via the BodPod and day two consisted of body fat being measured via UWW. A paired student's t test was used to compare estimations of body density and %BF measured by the ADP and UWW. Mean Bd using ADP [1.048 ±0.016 g/ml] was not significantly different when compared to UWW [1.049 ± 0.017 g/ml] which corresponds to a non-significant different in %BF [22.5 ± 7.6% ADP compared to 22.0 ± 7.6% UWW]. However, data for the subsets revealed a significant overestimation of %BF measured by ADP compared to UWW for lean individuals while no difference was found in the average or overweight subsets. A significant difference between Bd and %BF was not found between the men, women or both genders combined. This study would suggest that estimating %BF using ADP is accurate compared to UWW for all individuals except lean. Due to the heterogeneity of this sample the accuracy of the entire sample was affected. The plot of residual error for this entire sample shows a 95% confidence interval of individual measuring error near ±7% BF which is much greater than the accepted ±3% BF. This study supports the hypothesis that ADP is an appropriate method of measuring

%BF in the overweight population; however, ADP may not be accurate compared to UWW for very lean individuals.

*Bioelectrical Impedance Spectroscopy*

Bosy-Westphal, A., Later, W., Hitze, B., Sato, T., Kossel, K., Gluer, C.C., Heller., M., & Muller, M.J., 2008 (25)

The aim of this study was to compare body composition determined by BIA against criterion estimates determined by whole body magnetic resonance imaging (MRI) and DEXA in healthy normal weight, overweight and obese adults. One hundred and six subjects were recruited for this study (54 females, 52 males, age 54.2 ± 16.1 y, BMI 25.8 ± 4.4 kg/m$^2$). Each subject had FM, skeletal muscle mass (SM), total body bone-free lean mass (TBBLM), and level of visceral fat mass (VF) were estimated by 3 single-frequency bipedal and one tretrapolar BIA device, and compared to body composition measured by MRI and DEXA. Analysis according to Bland and Altman were used to determine absolute agreement between the body composition variables assessed by criterion methods (MRI and DEXA) and BIA as well as between REE measured by indirect calorimetry and predicted from BIA results. The Tanita Inner Scan Model BC-532, Soehnic Body Balance, Omron BF-400, and Omron BF-500 were all used to assess body composition. Percent fat mass, and SM or TBBLM, determined by both Omron scales and the Tanita instrument, were highly related to that determined by DXA or MRI (r values between 0.92 and 0.96, p < 0.01; mean bias < 1.5% and <1 kg SM or TBBLM) but showed less relative and absolute agreement for another bipolar device (r$^2$ = 0.82 and 0.84, mean bias ~3%FM and ~3kg SM). The 95% limits of agreement (bias ± 2SD) were narrowest for the tetrapolar device (-6.59 to 3.92kg SM). Systematic biases for %FM

were found for all bipedal devices, but not for the tetrapolar instrument. This study shows a good relative agreement with DXA and MRI for all but one of the BIA consumer devices tested. This study shows that BIA has a high potential as an accurate method of body composition analysis in public use, however, to meet these requirements, population-specific equations are needed. Finally, tetrapolar electrode arrangements should be preferred for individual or public use.

Moon, J.R., Tobkin, S.E., Roberts, M.D., Dalbo, V.J., Kerksick, C.M., Bemben, M.G., Cramer, J.T., & Stout, J.R., 2008 (41)

The aim of this study was to examine the validity of the BIS (SFB7) for estimating total body water in college-age men and women compared to the 4000B and deuterium oxide ($D_2O$). Twenty-eight Caucasian subjects were recruited for this study (14 men, 14 women, $24 \pm 4y$; $174.6 \pm 8.7$ cm; $72.80 \pm 17.58$ kg). All subjects had their TBW estimated using the SFB7 (ImpediMed Limited, Queensland, Australia) and 4000B BIA (XiTRON technologies, San Diego, CA). A $D_2O$ was used as the criterion method to estimate TBW. The mean difference (CE) between the predicted (SFB7 and 4000B) and actual ($D_2O$) TBW values were analyzed using dependent t-tests with the Bonferroni alpha adjustment ($p \leq 0.025$). Both BIS devices produced similar standard error of estimate (SEE) and r values (SFB7, SEE = 2.12L, r = 0.98; 4000B, SEE = 2.99L, r = 0.96) when compared to $D_2O$, though a significant constant error (CE) was detected for the 4000B (2.26L, $p \leq 0.025$). The SFB7 produced a smaller total error (TE) and CE (TE = 2.21L, CE = 0.09L) when compared to the 4000B (TE = 3.81L, CE = 2.26L). It was concluded that both BIS devices are valid when compared to $D_2O$ to estimate TBW in college-age Caucasian men and women. Additionally, the SFB7 is more precise when

compared to the 4000B, which could potentially decrease the error when estimating TBW on an individual basis.

Moon, J.R., Smith, A.E., Tobkin, S.E., Lockwood, C.M, Kendall, K.L., Graef, J.L., Roberts, M.D., Dalbo, V.J., Kerksick, C.M., Cramer, J.T., Beck, T.W., & Stout, J.R., 2009 (34)

The aim of this study was to determine the validity of a BIS device (Imp SFB7) for tracking changes in overfat and obese individuals compared to the criterion deuterium oxide ($D_2O$). Sixty overweight and obese subjects ($27 \pm 8$ yr, $33.41 \pm 3.81\%$BF) participated in this study. Subjects had their TBW estimated using both BIS and $D_2O$ before and after undergoing a 10 week training intervention. A dependent t-test was used to analyze the mean difference between BIS and $D_2O$ TBW values. An increase was seen in pre and post intervention BIS TBW as BMI, FM and FFM increased ($p < 0.05$). Delta values were more accurate than pre and post TBW estimations (total error = 1.45L). Age had a significant influence on pre and post BIS- estimated TBW errors ($p < 0.05$) therefore a regression equation was developed to correct for pre and post TBW errors. It was concluded that BIS can be considered an accurate tool for tracking changes in TBW regardless of variations in BMI, FM, FFM, or age in both overweight and obese men and women.

Pateyjohns, I.R., Brinkworth, G.D., Buckley, J.D., Boakes, M., & Clifton, P.M., 2005 (22)

The primary purpose of this study was to compare the BIA (ImpediMed SFB7) of body composition using three different methods against DXA in overweight and obese men. Forty-three healthy, overweight or obese men (ages 25-60 years, BMI 28 to 43

kg/m2) underwent BIA assessment of body composition using ImpediMed SFB7 multifrequency mode, single frequency mode and the Tanita UltimateScale. The validity was assessed by comparison against DXA. Subjects completed all body composition measurements on the same day. An ANOVA was used to compare differences between the body composition values determined using the different techniques. The ANOVA showed a statistically significant main effect. A good relative agreement between DXA and all assessments using the BIS multifrequency (FM, $r^2 = 0.81$; FFM, $r^2 = 0.81$; BF%, $r^2 = 0.69$; all p< 0.0001). In absolute terms, compared to DEXA, Imp-MF underpredicted FM by 6.59 kg and BF% by 7.0%, and it over predicted FFM by 7.97 kg, with wide limits of agreement for each variable (FM, -14.25 to 1.06 kg; FFM, 0.83 to 15.12 kg; BF% -13.62 to -0.38%). In relative terms, both MF-BIA and SF-BIA assessments of body compositions provide good agreement with DXA for overweight and obese men. However, in absolute terms, MF-BIA substantially underestimated FM and BF% and overestimated FFM compared with DXA, whereas SF-BIA methods provided good absolute agreement. This study indicates that a multifrequency ImpediMed BIS method of body composition may not be valid or suitable for measuring body composition in this specific population.

*Ultrasound*

Johnson, K.E., Naccarato, I.A., Corder, M.A., & Repovich, W.E.S., 2012 (47)

The main aim of this study was to cross-validate three clinical grade measures of body composition using the BIA, octopolar TANITA BC-418 MA (Tanita Corporation of America, INC., Arlington Heights, IL), US via BodyMetrics Pro System (IntelaMetrix, Inc., Livermore, CA) and ADP by the BodPod (Life Measurement Instruments, Concord,

CA). The secondary purpose of this study was to compare the US scans of total abdominal, subcutaneous and visceral fat depths (mm) against the trunk %BF from the BIA. Twenty-six college-aged (22.9± 1.35y, 18 men, 8 women) were recruited for this study. Each subject had their body composition analyzed via each device. Additionally, total abdominal, subcutaneous and visceral fat were measured using the US and BIA. A Pearson's correlation and two 1-way ANOVA'S were performed for all variables. All three clinical grade machines were significantly correlated when reporting the %BF with high (>.85) r values. No significant differences were found using a 1-way ANOVA. When comparing all three fat depths to the trunk %BF via BIA, all variables were significantly correlated, however, the r values were well below .70. Significant differences were found between groups in a 1- way ANOVA (F = 14.659, p = 0.001). A Tukey post hoc test was conducted and showed significant differences between the BIA trunk %BF and both subcutaneous (p = 0.0001) and visceral US fat depths (p = 0.004) but not for total fat depth. This study demonstrated that all three methods of measuring body composition are significantly correlated and no significant differences were found between them. Therefore, they can be used interchangeably to measure body composition in this population. When comparing trunk measurements made by US and BIA, the low r values reported for visceral fat bring to question the magnitude of the correlation. Further research needs to be done to validate the US to measure body composition and trunk measurements in broader populations, as well as comparing it to a criterion multicompartment model.

Kuczmarski, R.J., Fanelli, M.T., Koch, G.G., 1987 (31)

The purpose of this study was to assess whether ultrasonics collectively overcomes some of the known shortcomings of the skinfold caliper. Forty-four white, obese males (n =13) and females (n =31), aged 26-69 (BMI 33.5± 4.6 kg/m$^2$ range 24.5-42.2) were recruited for this study. Each subjects had their skinfolds measured using a Lange skinfold caliper (Cambridge Scientific Instruments, Cambridge, MA) followed by the ultrasound ADR real-time scanner (Model 2130, ADR Ultrasound, Temple, AZ). Finally each subject underwent hydrostatic weighting. Pearson correlation coefficients were evaluated for: caliper Bd with subcutaneous-fat thickness measured at each body site by caliper and by ultrasound; and finally, percentage compression of subcutaneous fat as measured by the caliper, with ultrasonic measurements at each body site. Pearson correlation coefficients between measurements taken with the caliper and the ultrasound were significant at all sites. The best predictor of Bd were the thigh and biceps sites with ultrasound (r= 0.820) and the triceps site with the calipers (r= 0.633). The US proved to be superior to the caliper technique in measuring subcutaneous fat of obese patients. The US was also a better predictor of Bd than the skinfold caliper.

Leahy, S., Toomey, C., McCreesh, K., O'Neill, K., & Jakeman, P., 2011 (49)

The purpose of this study was to evaluate the ability of the US measurements of subcutaneous adiposity to accurately predict whole body and segmental body fat in young adult men and women. One hundred and thirty-five healthy young adults (52 women and 83 men) between the age of 18 and 29y (BMI 25± 3.3 kg/m$^2$, range 17.5-35.4kg/m$^2$) were recruited for this study. Subjects had subcutaneous adipose tissue (SAT) thickness assessed using a GE Logiq e B-mode ultrasound scanner (GE Healthcare, Chalfont St.

Giles, Bucks, UK). Subjects also underwent full body scans using the Lunar iDEXA scanner (GE Healthcare, Chalfont St. Giles, Bucks, UK). Scatter plots, Spearman's rho (p) correlations and Bland Altman plots were used to investigate level of agreement and bias between %BF measured by DXA and that predicted from US measurements of segmental SAT. The difference in body fat between the methods was analyzed by Wilcoxon signed ranks test. Spearman's rho (p) correlation was used to investigate the relationship between SAT and %BF at each body segment and also the relationship between SAT across all regions-of-interest. Results found that US measured of SAT thickness were strongly correlated to segmental fat mass and total %BF (r = 0.697-0.907, p < 0.01). Specific to the aim of the study, it was found that, SAT thickness at selected regions-of-interest was found to be highly correlated to the fat mass contained in that segment and to the percentage of total body fat measured by DXA for men and women. Prediction equations generated using quantile regression found SAT thickness at the abdomen and thigh to accurately predict %BF in men (SEE =1.9%, 95%LoA; -3.6% to + 3.8%) and SAT thickness at the abdomen and medial calf to accurately predict &BF in women (SEE= 3.0%, LoA; -6.5% to + 5.4%). The results of this study indicate that %BF can be accurately predicted from US measurements of SAT thickness in healthy young adults. However, in order to make these results more generalizable, the equations should be validated in different populations.

Pineau, J.C., Guihard-Costa, A.M., & Bocquet, M., 2007 (29)

The aim of this study was to evaluate the accuracy of %BF estimates from a portable, non-traumatizing ultrasound device with high accuracy and reliability compared to the DXA. Eighty-nine healthy subjects (41 women, 48 men) aged 48.4 ± 17.7; with

BMI ($28.5 \pm 7.7$ kg/m$^2$) and body fat DXA ($29.6 \pm 10.8$ kg) were recruited for this study. Cross-validation between US techniques, DXA, ADP and BIA were developed in this study. Ultrasound measurements were made with a sonographic US Box in A-mode from Lecoeur Electronique Co, (Chuelles, France). Body fat and %BF measurements were also made using the DXA, Hologic QDR-4500 W. Bioelectrical impedance analysis was made using a dual-electrode portable impedance analyzer (IMP BO 1, France) and finally ADP measurements were made using the BodPod (Life Measurements Instruments, Concord, CA). A paired samples t-test was used to examine the relationships between DXA and %BF estimates according to the different techniques (US, BIA, ADP). Results show that all %BF estimates by US, BIA and ADP were significantly correlated with %BF by DXA ($r \geq .91$, p<0.01). Ultrasound estimates of %BF were better correlated with those of DXA in both males and females (r= 0.98, SEE =2.0) than with ADP (r = 0.94, SEE =3.7) or BIA (r=0.92, SEE=4.4). The US in both genders was better (TE= 1.0) than BIA (TE=2.6) and ADP (TE=3.0). The 95% limits of agreement were also better for the US (-2%; 2%) than with BIA (-5.1%; 4.9%) and ADP (-6.3%; 5.3%). The design of this study was set to compare the accuracy of %BF between US, BIA, and ADP verse DXA. This study indicates that %BF estimates by US versus DXA are more accurate than %BF estimates with BIA or ADP regardless of gender. The use of a new portable device based on a US produced a very accurate %BF estimate in relation to the DXA reference technique in this population. This study is very similar the current study, however, they use a higher grade ultrasound with better sonographic capabilities than the BodyMetrix Pro.

Ribeiro-Filho, F.F., Faria, A.N., Azjen, S., Zanella, M.T., & Ferreira, S.R.G., 2003 (30)

The purpose of this study was to compare several methods (BIA, DXA, and US) for the assessment of visceral fat with computed tomography (CT) and establish cutoffs to define visceral obesity based on such alternative methods. One hundred women (50.4 $\pm$ 7.7 years; BMI 39.2 $\pm$ 5.4 kg/m$^2$) had their percentage body fat estimated with a single-frequency BIA, their total body, trunk and leg fat measured by the DXA and visceral and subcutaneous fat measured by the US and MRI. Subcutaneous fat was defined as the distance between the skin and the external face of the rectus abdominis muscle, and visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta. A pearson coefficient was used to test the correlation between CT and the other methods of adipose tissue assessment. The best correlation coefficients of CT determined visceral fat were found with the US measurements ($r = 0.71$, $p < 0.001$), waist circumference ($r = 0.55$, $p < 0.001$), and WHR ($r = 0.54$, $p < 0.001$). Body mass index, DXA determined total body fat, and waist circumferences were shown to be more strongly associated with CT subcutaneous fat values than the other methods used. Total fat (visceral plus subcutaneous) determined by US correlated with visceral ($r = 0.63$, $p < 0.001$), subcutaneous ($r = 0.47$, $p < 0.001$), and total fat area determined by CT ($r = 0.65$, $p < 0.001$) as well as with waist circumference ($r = 0.75$, $p < 0.001$) and WHR ($r = 0.52$, $p < 0.001$). Linear regression indicated US visceral fat distance and WHR as the main predictors of CT-determined visceral fat (adjusted $r^2 = 0.51$, $p < 0.01$). This study shows that US seems to be the best replacement method for the assessment of intra-abdominal fat in obese women.

Rolfe, E.D.L., Sleigh, A., Finucane, F.M., Brage, S., Stolk, R.P., Cooper, C., Sharp, S.J., Wareham, N.J., & Ong, K.K., 2010 (50)

The aim of this study was to investigate whether ultrasound is a valid alternative method to MRI for the quantitative assessment of abdominal fat deposts in older individuals. Seventy-four white individuals (41 men and 33 women age 67-76 years) participated in the study. Subjects had their visceral and subcutaneous fat measured by both ultrasound and MRI. Visceral adipose tissue was defined as the depth from the peritoneal boundary to the corpus of the lumbar vertebra on longitudinal scanning at the end of a quiet expiration to avoid tensing and distorting the abdominal cavity. Subcutaneous fat was defined as the depth from the cutaneous boundary to the linea alba. Spearman rank correlation coefficients were calculated to describe associations between the different measures of abdominal fat. The proportion of variance between visceral adipose tissue and subcutaneous adipose tissue explained by the US was quantified using a linear regression analysis. Lastly, a Bland-Altman plot was used to assess the level of agreement in visceral and subcutaneous abdominal thickness between US and MRI. Ultrasound and MRI measures of visceral and subcutaneous fat were positively correlated ($r = 0.63$ and $0.68$ in men and women, respectively. When ultrasound measures are added to multiple regression models the prediction of visceral fat and subcutaneous fat in both men and women over and above the contribution of standard anthropometric variables is improved. This study supports the idea that ultrasound is a valid method to estimate visceral fat in epidemiological studies of older men and women when MRI and computed topography are not possible.

Stolk, R.P., Wink, O., Zelissen, P.M.J., Meijer, R., van Gils, A.P.G., & Grobbee, D.E., 2001 (43)

The purpose of this study was to examine the validity and reproducibility of a new abdominal ultrasound protocol for the assessment of intrabdominal adipose tissue. Nineteen overweight subjects (BMI 32.9 ± 3.7) had their abdominal adipose tissue assess by anthropometry, ultrasonography, CT and MRI. All measurements were made on the same day and then repeated after three months. Pearson's correlation coefficients were calculated in order to determine the associations between the different measures of intra-abdominal adipose tissue. Pearson correlation coefficient was 0.81 (p< 0.001) which showed a strong association between the CT and ultrasonographic measures. The correlation between ultrasound and waist circumferences was 0.74 (p< 0.001) and the correlation between CT and waist circumferences was 0.57 (p< 0.01). When measures were repeated three months later subjects had lost an average of almost 3 kg of body weight. The correlation coefficient between changes in intra-abdominal adipose tissue measured by the CT and ultrasound were 0.74 (p < 0.001), the mean difference 0.4 cm ±0.9 and the coefficient of variation was 5.4%. These measures indicate good reproducibility of the ultrasound measurements. This study supports the hypothesis that the ultrasound, using a strict protocol, is a valid and accurate method of measuring intra-abdominal adipose tissue and diagnosing intra-abdominal obesity in this population when compared to both the gold standard CT and the MRI.

Utter, A.C., & Hager, M.E., 2008 (46)

The purpose of this study was to evaluate the accuracy of ultrasound in assessing fat-free mass in comparison with hydrostatic weighing and skinfolds in high school

wrestlers. Seventy high school wrestlers (age 15.5 ± 1.5; height, 1.60 ±0.08 m; body mass, 65.8± 12.7 kg) had their Bd measured by the three different methods in the following order: skinfold analysis, ultrasound thickness, and hydrostatic weighing.  For each of the methods Bd was measured and converted to %BF using the Brozek equation. A multiple paired sample t-test with a Bonferroni's adjustment ($p$ <0.025) were performed to examine body composition differences. Researchers found no significant difference in mean FFM predicted by ultrasound (57.2 ± 9.7) and the criterion hydrostatic weighing (57.0 ± 9.9) and a significant correlation was found ($r = 0.97$). Even though a strong correlation ($r= 0.96$) was found between skinfold and hydrostatic weighing a significant underestimation ($p < 0.001$) was found for FFM predicted by skinfold (54.9 ±8.8) compared with hydrostatic weighing. A systematic bias was found for skinfold, as the difference between skinfolds and hydrostatic weighing significantly correlated with the FFM average of the two methods ($r = -0.38$, $p < 0.001$). This systematic bias was not found for ultrasound. This study demonstrates that ultrasound (IntelaMetrix BX-2000) systems estimate FFM within a suitable range when compared with hydrostatic weighting in high school wrestlers. This study implies that the IntelaMetrix ultrasound system could be used in lieu of hydrostatic weighing and skinfold measurement. Ultrasound may now be considered as an alternative field-based method of estimating FFM of high school wrestlers and possibly other populations.

*3 Compartment Model*

Das, S.K., Roberts, S.B., Kehayias, J.J., Wang, J., Hsu, L.K., Shikora, S.A., Saltzman, E., McCrory, M.A., 2003 (26)

One purpose of this study was to characterize the body composition of extremely obese subjects before and after gastric bypass surgery (GBP) and to measure the

32

composition of their weight loss. Researchers also evaluated several clinical and research body composition techniques for their ability to measure body composition and the composition of weight loss in severely obese subjects. Twenty extremely obese women (BMI 48.7 ± 8.8 kg/m2) had %BF and fat free mass measured using densitometry, isotope dilution and bioelectrical impedance analysis as well as the 3C criterion method using Bd by air displacement plethysmography and TBW by $H_2^{18}O$ dilution (3C-$H_2^{18}O$) during a stable weight period of extreme obesity and after a weight-reduction due to gastric bypass surgery. The 3C model of Siri modified by Modlesky et al. which incorporates Bd and TBW as a fraction of body weight (w) was used to calculate %BF: $2.1176/Bd - 0.78w - 1.351$. Fat free mass was calculated as the difference between body weight and fat mass. The FFM coefficient was calculated in standard fashion as TBW/FFM. An ANOVA and Bland-Altman analysis were used to test the agreement between the reference method and the test method. ANOVA with Dunnett's post hoc test were used to determine whether %BF by the alternative methods differed significantly from the reference method. Results show that %BF by $H_2^{18}O$ dilution and ADP differed significantly form %BF by 3C- $H_2^{18}O$ in extreme obesity (p <0.05) and 3C models using $^2H_2O$ or BIA to determine TBW improved mean %BF estimates over most other methods at both time points. All methods except BIA using the Segal equation were comparable to the reference method for determining changes over time. This study shows that a simple 3C model utilizing air displacement plethysmography and BIA is useful for clinical evaluation in this extremely obese population.

Fuller, N.J., Jebb, S.A., Laskey, M.A., Coward, W.A., & Elia, M., 1992 (15)

The primary purpose of this study was to establish the extent of agreement between the various methods of body composition analysis, with emphasis on DXA and the 3C and 4C models. Twenty-eight healthy adults (12 women, 16 men) with a BMI of 22.31±2.46 (range 17.10-28.10) volunteered to participate in this study. Subjects had their body composition assessed by the DXA, deuterium dilution, densitometry, $K^+$ counting and four prediction methods (skinfold thickness, bioelectrical impedance, near-IR. interactance and BMI). Three and four compartment models were constructed from combinations of the reference methods. Bias and 95% limits of agreement had been previously established in their laboratory (Fuller &Elisa) (Fuller et al., 1989). In many comparisons, such as those between the 3C and 4C model, there were no material difference in the bias and 95% limits of agreement between men and women. Precision (±SD) measurements were evaluated and associated with estimated amount so fat (kg) or FFM for a 70kg man using the 3C and 4C models were found to be ±.49kg and ±.54kg, when a 1%precision for water estimation was assumed. It was also found that DXA predicted the 3C and 4C model slightly less well than either densitometry or deuterium dilution. Results of this study suggest that both the 3C and 4C models of body composition are not compromised by errors arising from individual techniques.

Moon, J.R., Tobkin, S.E., Smith, A.E., Roberts, M.D., Ryan, E.D., Dalbo, V.J., Lockwood, C.M., Walter, A.A., Cramer, J.T., Beck, T.W., & Stout, J.R., 2008 (51)

The purpose of this study was to determine the validity of field and laboratory methods for estimating %BF compared to the Siri 3C model. Subjects consisted of 31 healthy college-age Caucasian men (22.5 ± 2.7 yrs; 175.6 ± 6.3 cm; 76.4 ± 10.3 kg).

Subjects reported to the laboratory and had all body composition methods performed on the same day. Body composition measurements included laboratory based methods, ADP via the BodPod and hydrostatic weighing and newly unvalidated field methods and devices including near-infrared interactance (NIR), four circumferences based military equations, Marine Corps (MC), Navy and Air Force (NAF), Army (A), and Friedle. Subjects also had their body composition measured via the bioelectrical impedance using the BodyGram® computer program (BIA-AK) and population-specific equation (BIA-Lohman). Dependent t-tests with a Bonferroni alpha adjustment (p <0.0055) were used to analyze the mean difference (CE) between the predicted (BP, HW, NIR, BIA-AK, BIA-Lohman, MC, NAF, A, and Friedl) and actual 3C %BF values. Percent body fat was calculated using the criterion 3C equation: $\%BF = [(2.118/Bd0 - (.78 \times TBW/Body\ Mass\ (kg)) - 1.354] \times 100$. Researchers found that all laboratory methods resulted in acceptable *TE* values ($\leq 2.7\%$ fat). Both BIA (BIA-AK, BIA-Lohman) field methods resulted in acceptable TE values ($\leq 2.1\%$ fat), while all military circumference-based equations (MC, NAF, A, Friedl) and NIR resulted in unacceptable TE values ($\geq 4.7\%$ fat). Furthermore, compared to HW, the MC, A, and Friedl, military circumference-based equations produced large but acceptable *TE* values ($\geq 3.5\%$ fat and $< 4.0\%$ fat). This study demonstrates that both BodPod and HW are acceptable methods compared to Siri 3C model method to estimate %BF in college aged white men. If laboratory based methods are not available then the only field methods, tested in this study, that are acceptable to use are BIA-AK and BIA-Lohm.

Withers, R.T., LaForgia, J., Pillans, R.K., Shipp, N.J., Chatterton, B.E., Schultz, C.G., & Leaney, F., 1998 (16)

The primary aim of this study was to examine the differences between two, three and four compartment models of body composition analysis in highly trained and sedentary subjects. Forty-eight young (18-36y) nonobese, Caucasian subjects (24 men and 24 women) volunteered for this study. Subjects had their body composition measured by UWW, $H^2O$ and DEXA. Two, three and four compartment model equations were derived from the measurements taken. A dependent t test was run in order to compare the means and standard deviation between the 2C and 3C models compared with those of the 3C and 4C models. Results show that the means and standard deviations for the relative %BF differences between the two and three compartment models (2.2 ± 1.6 %BF) were significantly greater (P≤ 0.02) than those between the three and four compartment models (0.2 ± 0.3 %BF) for all groups. This data shows that the 3C model is more valid than the 2C hydrodensitometric model but additional control for differences in individual BMM achieved by the 4C model achieves little extra accuracy.

Wang, Z.M., Deurenberg, P., Guo, S.S., Pietrobelli, A., Wang, J., Pierson Jr, R.N., & Heymsfield, S.B., 1998 (48)

The aim of this study was to compare sixteen currently used total body fat methods to a six compartment (6C) criterion model based on *in vivo* neutron activation analysis. Twenty-three healthy adults (17 males and six females) ranging in age from 19-69y with body weight from 54-105kg. All subjects completed tritium dilution, UWW, Anthropometry, DXA, BIA, whole-body K counting and *in vivo* neutron activation analysis studies. Simple linear regression analysis was applied to describe the relationship

between total body fat measured by the 6C model and that estimated by the other 16 methods. The differences between the 6C model and the other methods being measured were compared to the 6C model as described by Bland and Altman. Results show strong correlations ($r^2 > 0.80$) between the 6C model and all 16 methods. Very high correlations ($r^2 > 0.97$) also existed between the 6C model and the Selinger, Baumgartner, Heymsfield, Cohn, Siri-3C, Pace and DEXA methods. The methods of Siri-3C (which will be used in the present study), Heymsfield, Selinger, Baumgartner, and Cohn gave results consistent with the 6C model. The study concludes that since it is difficult to implement the 6C model in research laboratories, the simpler and reduced radiation methods proposed by Baumgartner, Heymsfield, Selinger and Siri-3C may serve as alternative practical reference methods.

CHAPTER III

METHODOLOGY

<u>Subjects</u>

A group of 47 subjects (20 male and 27 female) between the ages of 18 and 50 were recruited from the University of North Carolina at Chapel Hill (UNC-CH) and from volunteer recruitment fliers hung around campus. Subjects were required to have a minimum BMI of 25 $kg \cdot m^2$ and a maximum BMI of 50$kg \cdot m^2$. Subjects were stratified into two BMI groups, overweight (OW: 25- 30$kg \cdot m^2$) and obese (OB: 30-50$kg \cdot m^2$). Exclusion criteria included: patients with known chronic diseases such as cancer, and coronary heart disease, subjects on medication know to influence hydration status such as diuretics and corticosteroids, subjects who are pregnant, and subjects who had a history of weight loss surgery. Before participating in the study, subjects were required to complete a written informed consent approved by the University's institutional review board and medical health history questionnaire.

<u>Research Design</u>

Subjects reported to the UNC-Chapel Hill Applied Physiology Laboratory and completed two body composition measurements on two separate days with the same measurements taken on both days. Body composition measurements were not performed in any particular order, but were performed by the same trained investigator, at the same time ($\pm$ 2 hrs) of the morning. Subjects were required to fast 8 hours before testing, as well as not participate in any physical activity 24 hours prior to testing. Subjects were

allowed to consume water 60 minutes prior to testing. Subjects had their body composition measured using the 3C model, BodPod and BIS. Ultrasound was also used to assess body composition. Height and weight were measured and BMI was calculated.

Instrumentation

Height was measured using a portable stadiometer (Perspective Enterprises, Portage MI) and weight measured using a mechanical scale (Detecto, Webb City, MO). Body composition was measured using three separate techniques: air displacement plethysmography, (BodPod®, Life Measurements Inc. Concord, California), bioelectrical impedance spectroscopy (IMP SFB7, Impedimed, San Diego, CA), and ultrasound (US; Body Metrix, Intelametrix, Livermore, CA).

Air Displacement Plethysmography

The BodPod was used to determine Bd. Prior to each test the BodPod was calibrated using a two point calibration according to manufacturer's instructions. It was first calibrated with the chamber empty and then with a known 50L volume cylinder. The scale was also calibrated according to the manufacturer using a 20kg weight. Subjects were asked to wear tightly fitted clothing (i.e. swimming suit or spandex) and a tightly fitted swim cap. All metal, including jewelry, watches and glasses were removed. Subject's body weight was measured to the nearest 0.01kg using the BodPod system's electronic scale. Two trials were performed on each subject and the two measurements were averaged if they were within 150ml. If the two measurements are not within 150ml of each other a third measurement was performed. The thoracic gas volume of the subject was predicted using the manufacturer's software, based off standard predictions equations.

Bioelectrical Impedance Spectroscopy

Total body water was measured using the BIS. Subjects laid in a supine position on a nonconductive surface, to ensure stabilization of body water. Since electrodes were placed on the right side of the body, subjects removed their right shoe and sock and all metals. Subject laid with their hands pronated and with arms and legs not touching any other part of their body. Before placing the electrodes, any excess hair on the hands or feet was shaved and the areas were cleaned with alcohol. Two electrodes were placed on the right hand and wrist, one on the dorsal surface of the hand 1 cm proximal to the third metacarpophalangeal joint, and the other centrally on the dorsal side of the wrist in line with the ulnar head (22). Two electrodes were placed on the right foot and ankle, one on the dorsal surface of the foot 1 cm proximal to the second metatarsophalangeal joint, and the other centrally on the dorsal surface of the ankle between the lateral and medial malleoli (22). All electrodes were placed 5 cm apart. Measurements were repeated twice and averaged.

Ultrasound

Ultrasound thickness measurements was made using the IntelaMetrix BX-2000 A-mode, using a 2.5- MHz transmitter and separate receiver to measure tissue thickness (46). All ultrasonographic procedures were performed by the same examiner. Measurements were made on the right side of the body with the subject standing. Skin thickness measurements were taken at 7-sites which included: triceps, subscapular, abdomen, suprailiac, midaxillary, chest and thigh. The ultrasound transducer was manually held perpendicular against the skin using ultrasound transmission gel. After a clear image was achieved the image was saved. Each site was measured approximately

three times and the average of the measurements was used as the final thickness measure. Body density was calculated with the following equation: Bd = (0.155 × log HT) - [0.191 × log (AB - N)] + 1.032 (51). Percent body fat was calculated using the Siri, 1961 three compartment model equation: %BF= [(2.118/Bd − (0.78 × TBW/Body Mass (kg)) − 1.354] × 100 (33). Visceral adipose tissue was measured by obtaining an image of the abdomen and tracking the thickness of the tissue using the BodyMetrix US.

Statistical Analysis

All statistical analyses were performed using SPSS 19.0 statistical analyses Software (IBM, Somers, NY, USA). This was a cross-sectional experimental design. A p-value of < 0.05 was considered significant. Descriptive statistics of mean ± SD were performed for all variables. A paired samples T test was performed in order to determine if there was a difference between ultrasound measurements of %BF, FM and FFM and the criterion 3C model. Intraclass correlation coefficients (ICC), standard error of the measurement (SEM), percent standard error of the mean (%SEM), and minimal difference (MD) were reported in order to determine reliability between US measurements, using a customized Excel (Microsoft Inc., Redmond, WA; Windows HP, 2007 Version 6.0.6002) spreadsheet. The ICC was calculated with the following equation (52):

$$ICC_{2,1} = \frac{MS_S - MS_E}{MS_S + (k-1)MS_E + \frac{k(MS_T - MS_E)}{n}}$$

$MS_S$ represents the mean square for subjects, $MS_E$ is the mean square error, $MS_T$ is the mean square for trial, $k$ represents the number of trials, and $n$ is the sample size. The SEM for this model was calculated using the following equation (53):

$$SEM = \sqrt{MSE}$$

Regression analysis was also performed in order to determine the relationship between VAT measured by the US, BMI, %BF as measured by the 3C model, Bd from BodPod and TBW from BIS. Finally, a one-way ANOVA was run in order to compare the difference between overweight and obese individuals, as well as men versus women.

CHAPTER IV

MANUSCRIPT

Introduction

Obesity-related health complications have gained increasing attention at both National and Federal levels due to the rising occurrence and associated medical costs. Cawley et al. (2012) estimate that an obese individual incurs $2,741 greater medical costs compared to non-obese, translating to $190.2 billion per year (54). National Health and Nutrition Examination Survey (NHANES) indicates that approximately 68% of US adults are overweight or obese (1), with 35.7% of those being classified as obese (55). Trends in obesity continue to climb; it is predicted that by the year 2030, there will be approximately 65 million more obese adults in the US (56). Additionally, combined medical costs associated with obesity-related diseases such as diabetes, heart disease, stroke and cancer will increase by $48-66 billion per year in the US (56). The obesity epidemic has given rise to the need for accurate measures of body composition, fat mass (FM) and fat free mass (FFM) at an individual level in order to better assess a patient's health risks. While body mass index (BMI) is a common field-based method used to classify individuals as overweight or obese (2), it has not accurately assessed health risks due to its inability to differentiate between tissues (50). Appropriate classification of body composition, specifically fat distribution, may allow for improved evaluation of an individual's overall health status (50, 57). More so, clinical settings, such as doctor's offices and weight loss facilities, would benefit from accurate field based measurements

of %BF in order to track weight changes over time, and to accurately identify health risks.

Multicompartment body composition measurement models have gained increasing support as criterion methods (13). Specifically, the three compartment (3C) model is considered a criterion due to its ability to account for variation in subject hydration by adding total body water (TBW) to Behnke's two-component model (58). Wang et al. (48) concluded that, when compared to a six-compartment model, the Siri 3C model was superior to hydrostatic weighing for predicting %BF (59). Furthermore, accounting for bone mineral content, obtained using a four-compartment model, yields little additional accuracy than the 3C model (15, 16). A common 3C model proposed by Siri et al (33) utilizes air displacement plethysmography via the BodPod to predict body density (Bd), and bioelectrical impedance spectroscopy (BIS) to predict TBW; this model has been shown to be accurate in predicting %BF in an overweight and obese population (26), a healthy population (48) and an athletic population (34). Additionally, this model has been used as a validation method for body composition devices such as BodPod and BIS (51, 60).

A-mode Ultrasound (US) technology has been reported to produce accurate measures of %BF in normal weight subjects (29, 47) and an athletic population (61). Furthermore, A mode US has been previously validated against dual energy x-ray absorptiometry (DXA) for determining %BF in healthy individuals (29, 61). While DXA is a commonly reported method for determining body composition, results demonstrate it is not the most accurate technique for %BF measurement (62) and may not be considered a gold standard. Although B- mode US technology has been utilized for decades to

estimate various soft tissues (31, 45, 63), more recently, an A-mode US device has been developed exclusively for the measurement of body composition. The BodyMetrix US is a field-based device, equipped with body composition software to measure FM, FFM, %BF and visceral adipose tissue (VAT). Currently, only two studies have demonstrated the accuracy of this device for estimates of %BF (47) and FFM (46). Furthermore, while other US equipment, M- mode, has been reported to be reliable (43), the sensitivity of the BodyMetrix US device for tracking changes has not yet been evaluated. To date, there is no available data validating this device against a criterion method for body composition variables (%BF, FM, FFM), nor has it been evaluated in an overweight or obese population. Therefore, the primary purpose of this study was to assess the validity and reliability of the ultrasound (US) for the measurement of body composition in overweight and obese subjects.

Methods

*Subjects*

Forty subjects (20 male, 27 female; mean ± SD; Age = 37.6 ± 11.6 years; Body mass = 94.1 ± 16.1 kg; Height = 172.9 ± 10.1 cm, BMI = 31.5 ± 5.2 kg·m$^2$), Table 1, were recruited to participate. Subjects read and signed the consent form approved by the Institutional Review Board. Subjects were not eligible for the study if they had any ongoing/untreated disease such as cancer or coronary heart disease, on medication known to affect hydration status; if they were pregnant or lactating, or if they had a history of weight loss surgery. All subjects used in the statistical analysis met the inclusion criteria: subjects were between the ages of 18 and 50 yrs and had a BMI between 25 and 50kg·m$^2$.

*Protocol*

All body composition measurements were taken on two separate days. Measurements were not performed in any particular order, but were performed by the same trained investigator, at the same time (±2 hrs) of the morning. Subjects were asked to follow the same pre-testing guidelines for both sessions; which included an eight hour fast (*ad libitum)* water intake was allowed one hour prior to arrival), and abstention from exercise twelve hours prior to testing. Upon arrival subjects confirmed that they had followed pre-testing guidelines. Subjects had their height measured using a portable stadiometer (Perspective Enterprises, Portage MI), and weight measured using a mechanical scale (Detecto, Webb City, MO). The body density (Bd) of the subjects was determined using air displacement plethysmography (ADP) (BodPod®, Life Measurements Inc. Concord, California) and total body water (TBW) was determined using bioelectrical impedance spectroscopy (IMP SFB7, Impedimed, San Diego, CA) and were incorporated into the Siri three compartment model equation (33). Percent body fat was also measured using the ultrasound (US; Body Metrix, Intelametrix, Livermore, CA) from the standard seven-site Jackson and Pollock equation (64).

*Air Displacement Plethysmography*

Body density (Bd) was determined using the BodPod. The BodPod measures body volume based on the inverse relationship between air volume and pressure under isothermal conditions, which is represented via Boyle's Law ($P_1/P_2=V_2/V_1$) (18). Prior to testing the BodPod was calibrated using a two point calibration according to manufacturer's instructions. It was first calibrated with the chamber empty, and then with a known 50L volume cylinder. Preceding the measurement subjects were asked to remove all metal including jewelry, watches and glasses. Subjects also wore a swim suit

or tight fitting spandex as well as a swim cap. Body mass was measured to the nearest 0.01kg using the system's calibrated electronic scale. Subjects were then instructed to sit quietly in the chamber in an upright position, with hands folded on their lap, feet planted on the floor and breathe normally. A minimum of two trials were performed and if the measurements were not within 150ml of each other a third trial was conducted. The thoracic gas volume of the subjects was predicted using the manufacturer's software based off standard prediction equations.

*Bioelectrical Impedance Spectroscopy*

Bioelectrical impedance spectroscopy was used to estimate total body water (TBW) following the manufacture's recommendations. The BIS measured TBW by sending a current through the body and measuring the resistance or impedance ($\Omega$) of the body to that current (24). The BIS has been shown to produce valid estimates of TBW when compared to a criterion model, such as deuterium oxide (60, 65). Total body water measurements were taken while the subject was lying in supine position on a non-conductive surface with arms and legs not touching and after the subject had been resting for five minutes. Before placing the electrodes any excess hair was shaved and the area was cleaned with alcohol and gauze. Two electrodes were placed 5 cm apart on the right hand and wrist, the first was placed on the top of the hand 1 cm proximal to the third metacarpophalangeal joint, and the other centrally on the top of the wrist in line with the ulnar head (22). Two electrodes were placed 5 cm apart on the right foot and ankle, one on the top of the foot 1 cm proximal to the second metatarsophalangeal joint, and the other centrally on the top of the ankle between the lateral and medial malleoli (22). Measurements were repeated twice and the average was used to demonstrate the subject's

TBW. Once TBW was measured it was used as a variable in the Siri, 1961 three compartment model equation:

%BF= [(2.118/Bd – (0.78 × TBW/Body Mass (kg)) – 1.354] × 100 (33).

*Ultrasound*

The ultrasound was used in order to determine percent body fat. The ultrasound measurements were conducted using the Bodymetrix BX-2000 A-mode ultrasound (US; Body Metrix, Intelametrix, Livermore, CA), with a standard 2.5- MHz transmitter to measure subcutaneous fat thickness (46). The probe is used to transmit an ultrasonic beam into the tissue with part of the beam reflected back at the fat muscle interface. The thickness of the fat is calculated by using a linear relationship between propagation velocity and the time of flight (10, 29). Measurements were taken on the right side of the body while the subject was standing using 7 sites, which included: triceps, subscapular, abdomen, suprailiac, midaxillary, chest and thigh. Measurements were made by applying transmission gel to the probe and lightly placing the probe perpendicular to the site. Each site was measured approximately three times and the average of the trials was used to represent the final thickness measurement. Percent body fat was calculated internally by the ultrasound using a standard 7-site Jackson and Pollock equation (64). After the 7 sites were measured an abdominal scan was assessed distal to the xyphoid process, to determine visceral adipose tissue thickness (VAT).

*Statistical Analysis*

Descriptive statistics of mean ± SD were performed for all variables. A paired samples t-test was performed in order to determine if there was a significant difference between US measurements of %BF, FM and FFM and the criterion 3C model. Intraclass

correlation coefficients (ICC), standard error of the measurement (SEM), percent standard error of the mean (%SEM), and minimal difference (MD) were reported in order to determine reliability between US measurements, using a customized Excel (Microsoft Inc., Redmond, WA; Windows HP, 2007 Version 6.0.6002) spreadsheet. The ICC was calculated with the following equation (52):

$$ICC_{2,1} = \frac{MS_S - MS_E}{MS_S + (k-1)MS_E + \frac{k(MS_T - MS_E)}{n}}$$

$MS_S$ represents the mean square for subjects, $MS_E$ is the mean square error, $MS_T$ is the mean square for trial, $k$ represents the number of trials, and $n$ is the sample size. The SEM for this model was calculated using the following equation (53):

$$SEM = \sqrt{MSE}$$

Regression analysis was also performed in order to determine the relationship between VAT measured by the US, BMI, %BF as measured by the 3C model, Bd from BodPod and TBW from BIS. Finally, a one-way ANOVA was run in order to compare the difference between overweight and obese individuals, as well as men versus women. All statistical analyses were performed using SPSS 19.0 Statistical Analysis Software (IBM, Somers, NY, USA).

Results

*Validity*

All body composition variables measured from the US were significantly different compared to the 3C criterion model; %BF (p=0.001); FM (p=0.001); FFM (p=0.001) (Table 2). A significant difference was seen between the US and 3C model for all variables stratified by sex (Table 3); %BF (p=0.001); FM (p=0.001); FFM (p=0.001) and BMI (Table 4); %BF (p=0.001); FM (p=0.001); FFM (p=0.001). Differences between

%BF, FM and FFM measured from the US are plotted against the mean %BF, FM and FFM calculated by the 3C model. The agreement between US and 3C %BF, FM, and FFM for individual subjects are shown in Figures 1-3. The mean difference ± 2SD establishes limits of agreement that are between -2.91 and 11.71 for body composition variables. It appears that subjects with a lower amount of FM are closer to the mean indicating a closer agreement between the US and the 3C model for individuals with lower FM. Likewise, it appears that subjects with a higher amount of FFM are closer to the mean, indicating a better measurement agreement for higher FFM individuals.

*Reliability*

Intraclass correlation coefficient, $ICC_{2,1}$, (52) for US %BF was 0.98 indicating that 98% of the observed score was due to total variance of the subjects, while 2% was due to error of the US. The standard error of the measurement (SEM) was 2.2%BF. The percent standard error of the mean (%SEM) was 5.5% and the minimal difference (MD) needed to be deemed real was 4.3%BF, with a p-value of 0.284 demonstrating no significant difference between trials.

*Regression Analysis*

A multi-component regression analysis was run in order to predict visceral adipose tissue (VAT) from TBW, Bd, 3C%BF, and BMI. There was no significance found between any predictors and VAT (p=0.072). This indicates that VAT cannot be predicted by using TBW, Bd, 3C%BF, or BMI.  The regression equation is:

$$\gamma = 233.4 + -0.025(TBW) + -209.76(Bd) + -.459(\%BF) + 0.320(BMI)$$

*Exploratory Analysis*

An exploratory analysis was run using a one way ANOVA to determine if there was a difference between the US and 3C model for measuring %BF stratified by sex and BMI. There was a significant difference between the US and the 3C model for females ($p<0.001$), males ($p<0.001$), overweight ($p<0.001$) and obese ($p<0.001$) Table 5. This indicates that the US is not a valid measurement of %BF in any of the populations measured.

Discussion

To the best of our knowledge, this is the first study to investigate the validity and reliability of the A- mode US (US; Body Metrix, Intelametrix, Livermore, CA) in the overweight and obese population. The principle finding of this investigation demonstrated that there was not an agreement between the US and the 3C model, indicating that the US is not a valid measurement of %BF in an overweight and obese population. More so, when evaluated by sex and %BF classification, the US still did not produce valid results compared to the 3C criterion. Furthermore, there was no relationship between VAT and body composition variables. However, results demonstrated strong reliability for US when measuring %BF.

Validation of %BF against a 3C criterion has been shown to be an effective method for determining accuracy (34, 48). Few studies have identified valid and reliable methods for body composition measurement in an overweight and obese population. Ginde et al. (2005) found that ADP was a valid method of measuring Bd under water weighing (UWW) in an overfat population (overweight: $\Delta$ 0.004 ± 0.007, obese: $\Delta$ -0.001 ± 0.007, severely obese: $\Delta$ 0.001± 0.007) (39). Likewise, the BIS has been shown to be

valid in tracking changes in body composition, and is useful for group comparisons, but has not been shown to be valid in measuring body composition in the overweight and obese population (22, 25, 40-42). While these devices have been shown to be valid measures for estimating %BF in various populations, additional field based methods, such as the US could be clinically advantageous. The current study is the first to report data using the BodyMetrix US for measuring %BF, FM or FFM in an overweight and obese population. This device was not shown to be valid for %BF ($\Delta4.7 \pm 1.1\%$), FM ($\Delta10.4 \pm 1.7$kg) or FFM ($\Delta4.4 \pm 0.4$kg) when compared to the criterion. In contrast, previous studies have demonstrated validity of the US, but strictly with healthy (29, 47, 49, 61) and athletic (46, 61) populations, as well as with varying technologies. Johnson et al. (2012) reported that the BodyMetrix US was valid compared to ADP ($\Delta0.2 \pm 0.69\%$) and BIS ($\Delta0.4 \pm 3.29\%$BF) however their subjects consisted of healthy, college age individuals (47). Likewise, Pineau et al. (2007), using an A-mode US (Lecoeur Electronique Co.,Chuelles, France), found the device to produce accurate measures of %BF estimates in relation to the DEXA (29). Utter et al (2008) is the only other study to use the BodyMetrix US to measure FFM, supporting its validity compared to hydrostatic weighing ($\Delta0.2 \pm 0.1$kg) (46). However, the subjects measured were high school wrestlers, which could influence the generalizability to the population in the current study. More so, physiological differences in overweight and obese populations must be considered when validation data are compared. A greater amount of adipose tissue and greater inconsistencies within the tissue of the overweight and obese population could cause a slower pulse through the subcutaneous adipose tissue (SAT), initiating an uneven reflection of the pulse to return back to the probe (10, 29, 66). This in turn could skew the

image and measurement of tissue depth made by the US, and increase error in an overweight and obese population. Additional research is warranted to explore its validity.

The current study is the first to report the reliability of the BodyMetrix US for measuring %BF in an overweight and obese population. Results are similar to those of Stolk et al. (2001) who found US (ATL HDI 3000, M-mode, System, Bothell, Washington, USA) to be reliable in measuring intra-abdominal adipose tissue in a group of 19 obese subjects compared to computed tomography (CT) scan (p <0.001, $\Delta$= 0.4 ± 0.9cm, %CV= 5.4%). Data from the current study demonstrate strong reproducibility of measurements using the BodyMetrix US, indicating that the US can be used to track body composition changes in overweight and obese individuals. This device could be useful for field based evaluation, such as during a weight loss program or clinical testing where body composition will be measured multiple times. Although results show that the BodyMetrix US may not be valid in measuring %BF, it may be useful for multiple measurements and tracking changes over time, thereby giving an accurate picture as to the change in %BF for a given individual. Furthermore, while the same investigator performed all measurements for each subject in the current study, technician variability should have little influence in measurement due to the standard software readings.

This is the first study to investigate the measurement of visceral adipose tissue (VAT) using the BodyMetrix US. The evaluation of intra-abdominal fat using ultrasonography was first explored by Armellini et al. (67-69). Subsequent studies have been done  to validate the US against CT scans in an obese population (30, 43, 70, 71), and against magnetic resonance imaging (MRI) (50). Notably, VAT has been shown to be correlated with cardiovascular disease (CVD) and markers of metabolic syndrome (57,

72). Affordable and accurate estimates of VAT may provide additional insight regarding and individual's risk for CVD and other metabolic disorders. Inferring validity of ultrasonography to accurately detect VAT from Stolk et al. (43), Ribeiro-Filho et al. (30, 70) and Rolfe et al. (50), the present study aimed to predict VAT from other more standard measurement tools. However, using BMI, Bd, TBW, and 3C %BF as predictors were unable to account for enough variance to significantly predict VAT in this population. It could be advantageous for future studies to validate different US technologies (A- and B-mode) for measurements of VAT, as well as explore other feasible predictor variables, such as lipid markers, waist-to-hip ratio, and physical activity. Furthermore, a larger sample size should be used, as the current study used an exploratory pilot approach.

As with any study, limitations exist; due to the novelty of this US, there is minimal data to compare the US with other body composition devices. Due to a large amount of adipose tissue and tissue inconsistencies in an overweight and obese population, the US could underpredict %BF; which may be due to the beam being reflected back prematurely. Likewise, more studies are needed to investigate the validity of the US in a normal population (BMI< $25.5 \text{kg/m}^2$). A final limitation involved predicting VAT from BMI, Bd, TBW and 3C %BF; there was an insufficient sample size in order to establish a regression equation which would accurately predict VAT. If a larger sample size was used then perhaps a more practical prediction equation could be developed.

CHAPTER V

CONCLUSION

The comparison of %BF using the US against the 3C model demonstrated that the BodyMetrix US significantly underpredicted %BF and FM, and overpredicted FFM in an overweight and obese population regardless of sex or BMI; however, it was reliable across varying measurement time points. Due to the high reliability reported, the US may be useful in a clinical setting for tracking changes in body composition over time. Additionally, VAT tissue could not be predicted in the present sample, using a regression equation, from BMI, Bd, TBW or 3C %BF. Future research replicating the current study in a larger homogenous sample could be advantageous for exploring the validity of this affordable field-based body composition assessment. More so, a larger sample size could offer a more valuable prediction equation for VAT, providing greater insight into fat storage and health risks. Due to the many advantages of the US, affordability, portability and ease of use, it would be beneficial to use in a clinical setting, such as physician clinics, weight loss facilities or gyms, in order to obtain a better assessment of body composition than BMI. By using the US, rather than body weight, to accurately track body composition changes over time, a better indication of disease risks and the overall health of an individual could be achieved.

Table 1. Descriptive Statistics of all Subjects, Male, Female, Overweight and Obese subjects (Mean ±SD).

|  | Total (n=47) | Male (n=20) | Female (n=27) | Overweight (n=27) | Obese (n=20) |
|---|---|---|---|---|---|
| Age (yrs) | 37.6 ± 11.6 | 40.8 ± 10.8 | 35.2 ± 11.8 | 38.8 ± 11.3 | 35.9 ± 12.0 |
| BMI (kg·m2) | 31.5 ± 5.2 | 30.6 ± 4.4 | 32.2 ± 5.7 | 28.1 ± 1.32 | 36.2 ± 4.6 |
| Height (cm) | 172.9 ± 10.1 | 181.9 ± 7.5 | 166.3 ± 5.6 | 173.9 ± 10.8 | 171.7 ± 9.3 |
| Weight (kg) | 94.1 ± 16.1 | 101. 3 ± 13.3 | 88.8 ± 16.1 | 85.1 ± 11.2 | 106.3 ± 8.1 |
| Body Fat (%) | 33.7 ± 7.6 | 35.0 ± 8.1 | 32.8 ± 7.2 | 31.3 ± 6.2 | 36.9 ± 8.1 |

Table 2. Comparison of percent body fat (%BF), fat mass (FM) and fat free mass (FFM) between the ultrasound (US) and 3 Compartment model (3C) for subjects. (Mean ± SD)

| Method | %BF $\bar{x} \pm SD$ | P Value | FM $\bar{x} \pm SD$ | P Value | FFM $\bar{x} \pm SD$ | P Value |
|--------|------|---------|------|---------|------|---------|
| US | 29.0 ± 6.5 | 0.001 | 27.3 ± 8.1 | 0.001 | 66.7 ± 13.0 | 0.001 |
| 3C | 33.7 ± 7.6 | | 31.7 ± 9.8 | | 62.3 ± 12.6 | |

Table 3. Comparison of percent body fat (%BF), fat mass (FM) and fat free mass (FFM) between the ultrasound (US) and 3 Compartment model (3C) for male and female subjects. (Mean ± SD)

| | n | Method | %BF | | FM | | FFM | |
|---|---|---|---|---|---|---|---|---|
| | | | x̄ ± SD | P Value | x̄ ± SD | P Value | x̄ ± SD | P Value |
| Male | 20 | US | 22.8 ± 2.7 | 0.001 | 23.3 ± 4.8 | 0.001 | 78.0 ± 9.4 | 0.001 |
| | | 3C | 27.0 ± 5.0 | | 27.6 ± 7.3 | | 73.8± 9.3 | |
| Female | 27 | US | 29.1 ± 6.5 | 0.001 | 27.3 ± 8.1 | 0.001 | 66.7 ± 13.0 | 0.001 |
| | | 3C | 33.7 ± 7.6 | | 31.8 ± 9.8 | | 62.3 ± 12.7 | |

Table 4. Comparison of percent body fat (%BF), fat mass (FM) and fat free mass (FFM) between the ultrasound (US) and 3 Compartment model (3C) for overweight and obese subjects. (Mean ± SD)

| | n | Method | %BF | | FM | | FFM | |
|---|---|---|---|---|---|---|---|---|
| | | | $\bar{x} \pm SD$ | P Value | $\bar{x} \pm SD$ | P Value | $\bar{x} \pm SD$ | P Value |
| Overweight | 27 | US | 27.1 ± 5.7 | 0.001 | 22.4 ± 3.9 | 0.001 | 62.3 ± 11.7 | 0.001 |
| | | 3C | 31.3 ± 6.2 | | 26.2 ± 4.5 | | 58.8 ± 11.9 | |
| Obese | 20 | US | 31.7 ± 6.8 | 0.001 | 33.6 ± 8.1 | 0.001 | 72.6 ± 12.9 | 0.001 |
| | | 3C | 36.9 ± 8.3 | | 39.2 ± 10.2 | | 67.0 ± 12.5 | |

Table 5. Comparison of percent body fat (%BF) between the ultrasound (US) and 3 Compartment model (3C) for males, females, overweight and obese subjects. (Mean ± SD)

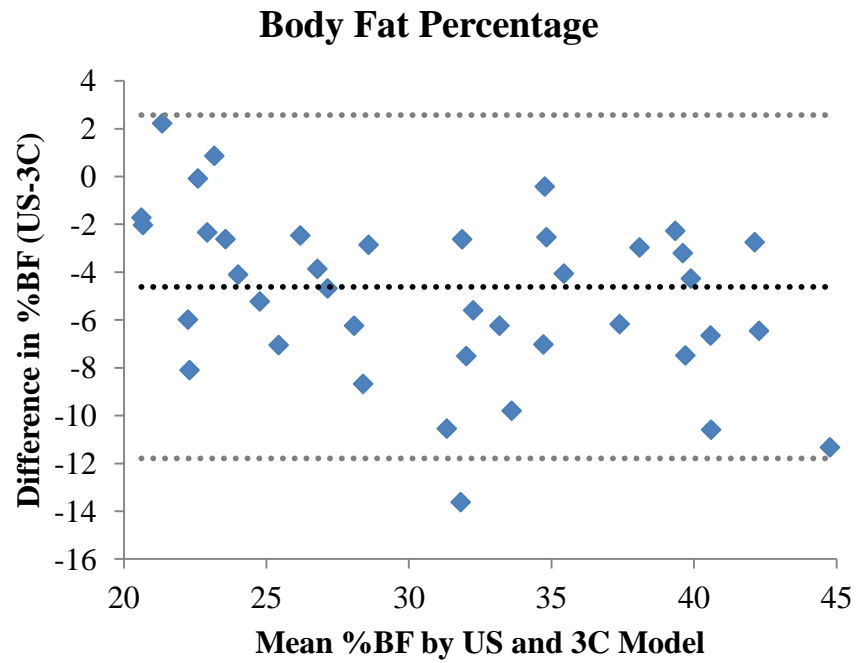| | Males | | Females | | Overweight | | Obese | |
|---|---|---|---|---|---|---|---|---|
| Method | x̄ ± SD | P Value | x̄ ± SD | P Value | x̄ ± SD | P Value | x̄ ± SD | P Value |
| US | 22.9 ±2.7 | 0.001 | 33.7 ± 4.2 | 0.001 | 27.0 ±5.7 | 0.001 | 31.7 ± 6.6 | 0.001 |
| 3C | 27.0 ± 5.0 | | 38.7 ± 4.9 | | 31.2 ±6.2 | | 36.9 ± 8.1 | |

Figure 1-Individual differences in percent body fat between the ultrasound (US) and the 3 compartment model (3C) methods compared with the mean percent body fat for both methods.
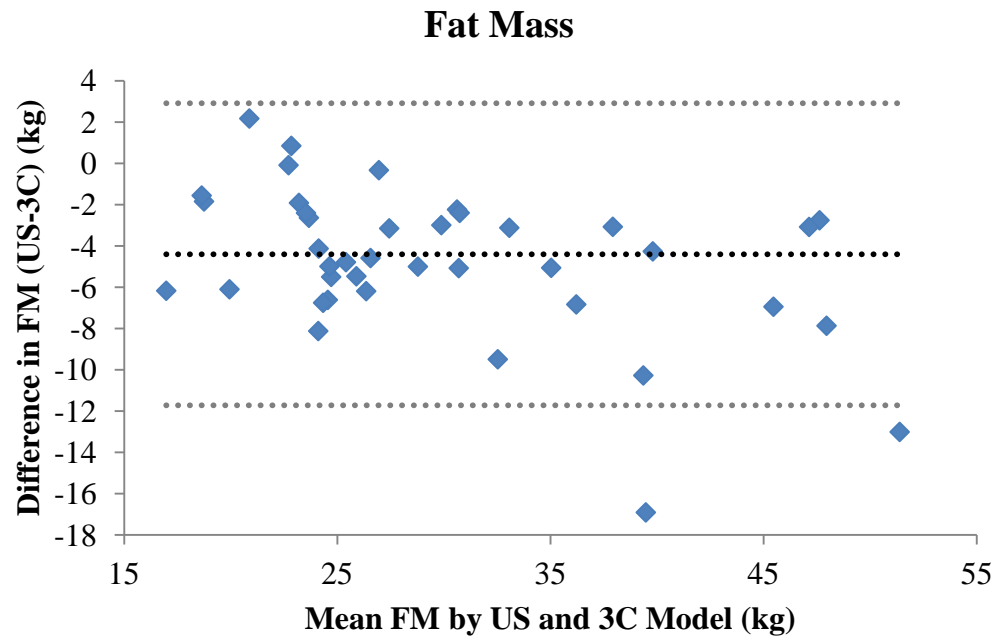
**Fat Mass**



Figure 2- Individual differences in fat mass (FM) between the ultrasound (US) and the 3 compartment model (3C) methods compared with the mean fat mass for both methods.
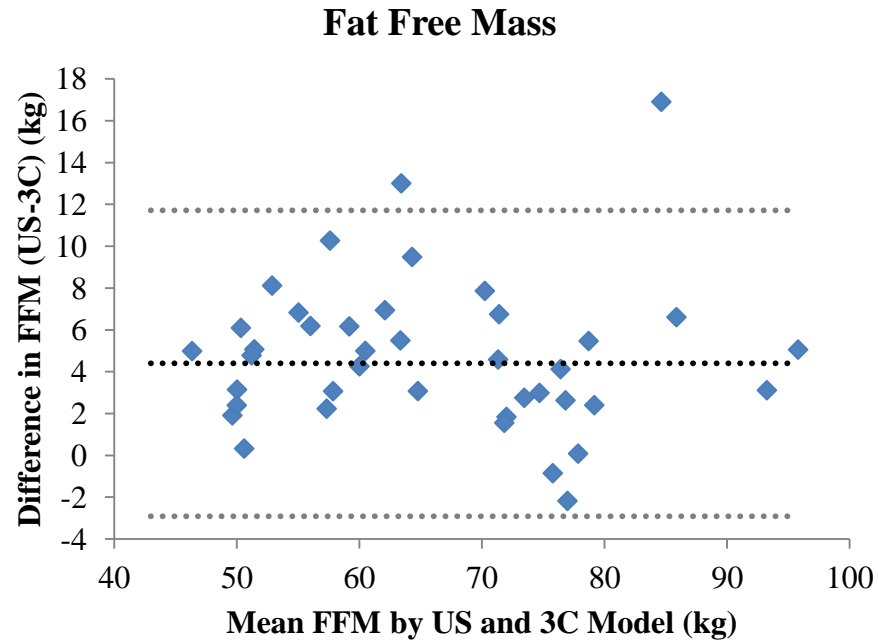
Figure 3- Individual differences in fat free mass (FMM) between the ultrasound (US) and the 3 compartment model (3C) methods compared with the mean fat free mass for both methods.

REFERENCES

1.      Flegal KM, Carroll, M.D., Ogden, L.C., & Curtin, L.R. Prevalence and trends in obesity among US adults, 1999-2008. JAMA. 2010;303(3):235-41.

2.      Wyatt SB, Winters KP, Dubbert PM. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Am J Med Sci. 2006 Apr;331(4):166-74.

3.      Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA. 1999 Oct 27;282(16):1523-9.

4.      Bozkirli E, Ertorer, M. E., Bakiner, O., Tutunca, N.B., & Demirag, N.G. The validity of the World Health Organization's obesity body mass index criteria in a Turksih population: a hospital-based study. Asian Pacific Journal of Clinical Nutrition. 2007;16(3):443-7.

5.      Deurenberg P. YM, Van Staveren, W.A. Body mass index and percent body fat: a meta analysis among different ethnic groups. int j obes. 1998;22:1164-71.

6.      Norgan RG. Population differences in body composition inrelation to BMI. European Journal of clinical Nutrition 1994;48:10-27.

7.      Prentice AM, Jebb SA. Beyond body mass index. Obes Rev. 2001 Aug;2(3):141-7.

8.      Despres JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis. 1990 Jul-Aug;10(4):497-511.

9.      Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. Obes Res. 1995 Sep;3 Suppl 2:187S-94S.

10.     Ackland TR, Lohman TG, Sundgot-Borgen J, Maughan RJ, Meyer NL, Stewart AD, et al. Current status of body composition assessment in sport: review and position statement on behalf of the ad hoc research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. Sports Med. 2012 Mar 1;42(3):227-49.

11.     Heyward V. ASEP Methods recommendation: Body composition assessment. JEP online. 2001;4(4):1-12.

12.     Jebb SA, Cole, T.J., Doman, D., Murgatroyd, P.R., & Prentice, A.M. . Evaluation of the novel Tanita body –fat analyser to measure body composition by comparison with a four-compartment model. British J of Nutrition. 1999;83:115-22.

13.     Van der Ploeg GE, Withers, R.T., & Laforgia, J. . Percent body fat via DEXA: comparison with a four-compartment model. JAP. 2002;94:499-506.

14.     Brozek J, Grande, F., Anderson, J.T., & Keys, A. Densitometric analysis of body composition: revision of some quantitative assumptions. Annals of the NY Academy of Science. 1963;110:113-40.

15.	Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. Clin Sci (Lond). 1992 Jun;82(6):687-93.

16.	Withers RT, LaForgia, J., Pillans, R.K., Shipp, N.J., Chatterton, B.E., Schultz, C.G., &Leaney, F. Comparisons of two-, three-, and four-compartment models of body composition analysis in men and women. J Appl Physiol. 1998;85:238-45.

17.	Heyward VHW, D. R., editor. Applied Body Composition Assessment Champaign, IL: Human Kinetics 2004.

18.	Baracos V, Caserotti P, Earthman CP, Fields D, Gallagher D, Hall KD, et al. Advances in the science and application of body composition measurement. JPEN J Parenter Enteral Nutr. 2012 Jan;36(1):96-107.

19.	Oates MK, Puhl, S., Wacker, & W.K. . Total Body %Fat- comparison of DXA with other body composition methods. J Bone Miner Res. 2006;21(S117-S121).

20.	Sardinha LB, Lohman TG, Teixeira PJ, Guedes DP, Going SB. Comparison of air displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men. Am J Clin Nutr. 1998 Oct;68(4):786-93.

21.	Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. Med Sci Sports Exerc. 1995 Dec;27(12):1692-7.

22.	Pateyjohns IR, Brinkworth GD, Buckley JD, Noakes M, Clifton PM. Comparison of three bioelectrical impedance methods with DXA in overweight and obese men. Obesity (Silver Spring). 2006 Nov;14(11):2064-70.

23.	Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, et al. Bioelectrical impedance analysis--part I: review of principles and methods. Clin Nutr. 2004 Oct;23(5):1226-43.

24.	Lukaski HC, Johnson, P.E., Bolonchuk, W.W., & Lykken, G.I. . Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr. 1985;41:810-7.

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

26.	Das SK, Roberts SB, Kehayias JJ, Wang J, Hsu LK, Shikora SA, et al. Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery. Am J Physiol Endocrinol Metab. 2003 Jun;284(6):E1080-8.

27.	Deurenberg P. Limitations of the bioelectrical impedance method for the assessment of body fat in severe obesity. Am J Clin Nutr. 1996 Sep;64(3 Suppl):449S-52S.

28.	Das KD. Body composition measurements in severe obesity. Curr Opin Clin Nutr Metab Care. 2005;8:602-6.

29.     Pineau JC, Guihard-Costa AM, Bocquet M. Validation of ultrasound techniques applied to body fat measurement. A comparison between ultrasound techniques, air displacement plethysmography and bioelectrical impedance vs. dual-energy X-ray absorptiometry. Ann Nutr Metab. 2007;51(5):421-7.

30.     Ribeiro-Filho FF, Faria AN, Azjen S, Zanella MT, Ferreira SR. Methods of estimation of visceral fat: advantages of ultrasonography. Obes Res. 2003 Dec;11(12):1488-94.

31.     Kuczmarski RJ, Fanelli MT, Koch GG. Ultrasonic assessment of body composition in obese adults: overcoming the limitations of the skinfold caliper. Am J Clin Nutr. 1987 Apr;45(4):717-24.

32.     Howley ET, Franks, B.D. Fitness Professional's Handbook. 5 ed. Illinois: Human Kinetics 2007.

33.     Siri WE. Body composition from fluid spaces and density. Analysis of methods. Brozek J. HA, editor. Washington DC: National Academy of Sciences 1961.

34.     Moon JR, Eckerson JM, Tobkin SE, Smith AE, Lockwood CM, Walter AA, et al. Estimating body fat in NCAA Division I female athletes: a five-compartment model validation of laboratory methods. Eur J Appl Physiol. 2009 Jan;105(1):119-30.

35.     Wang Z, Pi-Sunyer FX, Kotler DP, Wielopolski L, Withers RT, Pierson RN, Jr., et al. Multicomponent methods: evaluation of new and traditional soft tissue mineral models by in vivo neutron activation analysis. Am J Clin Nutr. 2002 Nov;76(5):968-74.

36.     Heymsfield SB, Wang Z, Baumgartner RN, Ross R. Human body composition: advances in models and methods. Annu Rev Nutr. 1997;17:527-58.

37.     Vescovi JD, Zimmerman SL, Miller WC, Hildebrandt L, Hammer RL, Fernhall B. Evaluation of the BOD POD for estimating percentage body fat in a heterogeneous group of adult humans. Eur J Appl Physiol. 2001 Aug;85(3-4):326-32.

38.     Petroni ML, Bertoli S, Maggioni M, Morini P, Battezzati A, Tagliaferri MA, et al. Feasibility of air plethysmography (BOD POD) in morbid obesity: a pilot study. Acta Diabetol. 2003 Oct;40 Suppl 1:S59-62.

39.     Ginde SR, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S, et al. Air displacement plethysmography: validation in overweight and obese subjects. Obes Res. 2005 Jul;13(7):1232-7.

40.     Ramsey R, Isenring, E., Daniels, L. Comparing measures of fat-free mass in overweight older adults using three different bioelectrical impedance devices and three prediction             equations. J of Nutr. 2012;16(1):26-30.

41.     Moon JR, Smith, A.E., Tobkin, S.E., Lockwood, C.M., Kendall, K.L., Graef, J.L., Roberts,        M.D., Dalbo, V.J., Kerksick, C.M., Cramer, J.T., Beck, T.W., & Stout, J.R. Total body water changes after an exercise intervention tracked using bioimpedance spectroscopy: A deuterium oxide comparison. Journal of Clinical Nutrition. 2009;28:516-25.

42.     Verdich C, Barbe P, Petersen M, Grau K, Ward L, Macdonald I, et al. Changes in body composition during weight loss in obese subjects in the NUGENOB study: comparison of

bioelectrical impedance vs. dual-energy X-ray absorptiometry. Diabetes Metab. 2011 Jun;37(3):222-9.

43.     Stolk RP, Wink O, Zelissen PM, Meijer R, van Gils AP, Grobbee DE. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. Int J Obes Relat Metab Disord. 2001 Sep;25(9):1346-51.

44.     Borkan GA, Hults DE, Cardarelli J, Burrows BA. Comparison of ultrasound and skinfold measurements in assessment of subcutaneous and total fatness. Am J Phys Anthropol. 1982 Jul;58(3):307-13.

45.     Fanelli MT, Kuczmarski RJ. Ultrasound as an Approach to Assessing Body-Composition. American Journal of Clinical Nutrition. 1984;39(5):703-9.

46.     Utter AC, Hager ME. Evaluation of ultrasound in assessing body composition of high school wrestlers. Med Sci Sports Exerc. 2008 May;40(5):943-9.

47.     Johnson KE, Naccarato, I. A., Corder, M. A., & Repovich, W.E.S. . Validation of three body composition techniques with a comparison of ultrasound abdominal fat depths against an octopolar bioelectrical impedance device. int j of exercise science 2012;5(3):205-13.

48.     Wang ZM, Deurenberg, PL, Guo, S.S., Piertrobelli, A., Wang, J., Pierson, R.N., & Heymsfield,     S.B. . Six-compartment body composition model: Inter-method comparisions of total body fat measurement. int J Obes Relat Metab Disord. 1998;22:329-37.

49.     Leahy S, Toomey, C., McCreesh, K., O'Neill, C., & Jakeman, P. Ultrasound Measurement of Subcutaneous Adipose Tissue Tickness Accurately Predicts Total and Segmental Body Fat of Young Adults. Ultrasound in Medicine and Biology. 2012;38(1):28-34.

50.     Rolfe EDL, Sleigh, A., Finucane, F.M., Brage, S., Stolk, R.P., Cooper, C., Sharp, S.J., Wareham, N.J., & Ong, K.K. Ultrasound measurements of visceral and subcutaneous abdominal thickness to predict abdominal adiposity among older men and     women. Obesity. 2009;18:625-31.

51.     Moon JR, Tobkin, S.E., Smith, A.E., Roberts, M.D., Ryan, E.D., Dalbo, V.J., Lockwood, C.M., Walter, A.A., Cramer, J.T., Beck, T.W., & Stout, J.R. Percent body fat estimations in college men using field and laboratory methods: A three-compartment model approach. Dynamic Medicine. 2008;7(7).

52.     Weir JP. Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J Strength Cond Res. 2005 Feb;19(1):231-40.

53.     Hopkins WG. Measures of reliability in sports medicine and science. Sports Med. 2000 Jul;30(1):1-15.

54.     Cawley J, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. J Health Econ. 2012 Jan;31(1):219-30.

55.     Flegal KM, Carroll C.D., Kit, B.K., & Ogden, C.L. Prevalence of Obesity and Trends in the Distribution of Body Mass Index Among US Adults, 1999-2010. JAMA. 2012;307(5):491- 7.

56.     Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet. 2011 Aug 27;378(9793):815-25.

57.     Stolk RP, Meijer R, Mali WP, Grobbee DE, van der Graaf Y. Ultrasound measurements of intraabdominal fat estimate the metabolic syndrome better than do measurements of waist circumference. Am J Clin Nutr. 2003 Apr;77(4):857-60.

58.     Behnke AR, Feen, B.G., & Welham, W.C. The specific gravity of healthy men. Body weight divided by volume as an index of obesity. Med Assoc. 1942;118:495-8.

59.     Brozek J, Grande F, Anderson JT, Keys A. Densitometric Analysis of Body Composition: Revision of Some Quantitative Assumptions. Ann N Y Acad Sci. 1963 Sep 26;110:113-40.

60.     Moon JR, Hull HR, Tobkin SE, Teramoto M, Karabulut M, Roberts MD, et al. Percent body fat estimations in college women using field and laboratory methods: a three-compartment model approach. J Int Soc Sports Nutr. 2007;4:16.

61.     Pineau JC, Filliard JR, Bocquet M. Ultrasound techniques applied to body fat measurement in male and female athletes. J Athl Train. 2009 Mar-Apr;44(2):142-7.

62.     Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol. 1996 Dec;271(6 Pt 1):E941-51.

63.     Bullen BA, Quaade F, Olessen E, Lund SA. Ultrasonic reflections used for measuring subcutaneous fat in humans. Hum Biol. 1965 Dec;37(4):375-84.

64.     Jackson AS, Pollock ML, Gettman LR. Intertester reliability of selected skinfold and circumference measurements and percent fat estimates. Res Q. 1978 Dec;49(4):546-51.

65.     Matthie J ZB, De Lorenzo A, Andreoli A, Katzarski K, Pan G,, P: W. Analytic assessment of the various bioimpedance methods used to estimate body water. J Appl Physiol. 1998;84(5):1801-16.

66.     Bellisari A, Roche AF, Siervogel RM. Reliability of B-mode ultrasonic measurements of subcutaneous adipose tissue and intra-abdominal depth: comparisons with skinfold thicknesses. Int J Obes Relat Metab Disord. 1993 Aug;17(8):475-80.

67.     Armellini F, Zamboni M, Robbi R, Todesco T, Rigo L, Bergamo-Andreis IA, et al. Total and intra-abdominal fat measurements by ultrasound and computerized tomography. Int J Obes Relat Metab Disord. 1993 Apr;17(4):209-14.

68.     Armellini F, Zamboni M, Rigo L, Bergamo-Andreis IA, Robbi R, De Marchi M, et al. Sonography detection of small intra-abdominal fat variations. int j obes. 1991 Dec;15(12):847-52.

69.     Armellini F, Zamboni M, Rigo L, Todesco T, Bergamo-Andreis IA, Procacci C, et al. The contribution of sonography to the measurement of intra-abdominal fat. J Clin Ultrasound. 1990 Sep;18(7):563-7.

70.     Ribeiro-Filho FF, Faria AN, Kohlmann O, Jr., Ajzen S, Ribeiro AB, Zanella MT, et al. Ultrasonography for the evaluation of visceral fat and cardiovascular risk. Hypertension. 2001 Sep;38(3 Pt 2):713-7.

71.     Tornaghi G, Raiteri R, Pozzato C, Rispoli A, Bramani M, Cipolat M, et al. Anthropometric or ultrasonic measurements in assessment of visceral fat? A comparative study. Int J Obes Relat Metab Disord. 1994 Nov;18(11):771-5.

72.     Kim SK, Kim HJ, Hur KY, Choi SH, Ahn CW, Lim SK, et al. Visceral fat thickness measured by ultrasonography can estimate not only visceral obesity but also risks of cardiovascular and metabolic diseases. Am J Clin Nutr. 2004 Apr;79(4):593-9.