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Validation of B-Mode Ultrasound Imaging as a Body Composition Measurement in Division I Football Players

Keagan Kiely
Georgia Southern University

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VALIDATION OF B-MODE ULTRASOUND IMAGING AS A BODY COMPOSITION MEASUREMENT IN DIVISION I FOOTBALL PLAYERS

by

KEAGAN KIELY

(Under the Direction of Stephen Rossi)

ABSTRACT

The purpose of the current study was to validate the use of B-mode ultrasound as a method to estimate body composition in Division I football players. Body composition was estimated using ultrasound, 7-site skinfold and a 4 compartmental model (4C) using Bioimpedance spectroscopy (BIS) to estimate total body water (TBW) and air-displacement plethysmography (BODPOD®) to determine body density (Db) and dual x-ray absorptiometry (DEXA) for bone mineral content. Pearson's product-moment correlation analyses were run to determine if there was a correlation between Σ Skinfold and Σ Ultrasound and between Σ Ultrasound and criterion 4C-model. Strong positive correlations were observed between Σ Skinfold and Σ Ultrasound ($r = .926$; $p < 0.001$). A Strong positive correlation was observed between Σ Ultrasound and %BF from 4C ($r = 0.961$, $p < 0.001$). A linear regression was used to generate the following prediction equation: $\%BF = 6.504 + (1.957 * \Sigma Ultrasound)$, with a standard estimate error (SEE) of 2.14%. A Cross-validation was run using the sample of 45 football players withheld from the derivation of the equation. The mean predicted %BF was 19.63% compared to the criterion %BF of 19.91%. The constant error (CE) value of 0.285% was not significant ($p > .05$). SEE and validity coefficient (r) were 1.81% and 0.91, respectively, with a total error (TE) value of 2.32%. Conclusion: This study supports the use of B-mode ultrasound as a method to predict body composition in Division I football players.

INDEX WORDS: Ultrasound, Body composition, 4-compartment, skinfold

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MEASUREMENT IN DIVISION I FOOTBALL PLAYERS

by

KEAGAN KIELY

B.S., Georgia Southern University, 2014

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KEAGAN KIELY

Major Professor: Stephen Rossi

Committee: Jim McMillan

John Dobson

Electronic Version Approved:

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Introduction

Body composition assessment has become more widely used in the sports population. Body composition, or the delineation between fat mass and fat-free mass, of an individual is a predictor of physical health and chronic disease (Dempster and Aitkens, 1995). Studies have also shown a relationship between body composition and athletic performance (Abe et al., 2011, De Lorenzo et al., 2000). There is a strong relationship between cross sectional area (CSA) size of various elite athletes and the amount of force the athlete could produce (Häkkinen and Keskinen 1989). While increases in muscle mass can improve performance, research shows that increased levels of body fat have a negative effect on sprinting, vertical jump and other performance test in Division I football players (Abe et al., 2011, Miller, 2002). Furthermore, high levels of body fat can have detrimental effects on the athlete's health. Therefore, assessment in body composition in football players is of importance for both health and performance reasons.

Body composition is often assessed for health reasons and can be assessed in various ways. More precise and expensive equipment are used in clinical or laboratory setting. Of these clinical assessments, hydrostatic weighing (HW) has long been considered the "gold standard" of body composition assessment, but its drawn-out methodology does not make it practical when testing a large sample (Lukaski, 1987). Another assessment tool is air displacement plethysmography (ADP), which uses Archimedes' principles of air displacement to derive body composition. ADP is deemed an accurate measure and when compared to HW, and has been shown to have roughly a 1% overall error (Fields et al., 2002). Ball et al. (2004) showed that ADP produced similar results for athletes and nonathletes when compared to DEXA. DEXA

allows research to examine bone mineral density simultaneously. Studies have shown DEXA has lower body fat percentages than ADP or HW, but closer values to 4C-model, which is considered a more accurate measure when compared to 2C-models (Ball and Altena, 2004, Khort, 1998, Prior et al., 1997). DEXA has also been shown to be more accurate regardless of race, gender and athletic status (Prior et al., 1997).

Researchers and clinicians may also use a combination of these clinical tests to form a compartment model that estimates body composition. In the previously mentioned test, the body was broken up into 2 compartments, fat mass and fat free mass. Fat mass is considered chemically extractable fat, is assumed to have a density of 0.9007 g/cm^3 . While fat free mass is assumed to have a density of 1.1000 g/cm^3 and a water content of 73.72% (Withers et al., 1998). Most of the discrepancy from 2C models is not from technical accuracy of the measurements but instead from the previously mentioned assumptions that were created based off three male cadavers (Withers et al., 1998). When fat free mass is broken into its further constituents (water, bone mineral and protein), a more accurate method of estimating body composition is created. Withers et al. (1998) found that a 3C model controlling for total body water was more accurate than the 2C, with the 4C adding even further accuracy. The more compartments that are controlled require multiple testing methods, making it advantageous to researchers and clinicians to validate a single method that correlates with a more accurate 4C-model.

On the other end, field testing is a more convenient and cost efficient way to assess body composition, especially among a large sample. One of the most common field tests is the measurement of subcutaneous fat using skinfold thickness of various sites of the body. The sum of the skinfold thickness (SF) is then plugged into an equation to derive the body density of an individual. Jackson and Pollock (1978, 1980) developed logarithms using the skinfold

measurements of 7 sites (chest, tricep, subscapula, abdomen, thigh, and suprailiac) to estimate D_b in men and women. The Jackson and Pollock (1978) prediction equation was shown to have only a 3% error when compared to %BF estimations of 82 male athletes by DEXA (Stewart and Hannan, 2000). In the same study, the use of the Durnin and Womersley (1974) prediction equation gave a much larger error, supporting the use of Jackson and Pollock (1978) prediction equation in the athletic population (Stewart and Hannan, 1999).

A second field test which also provides hydration information is bioelectrical impedance analysis (BIA). BIA uses the resistance of various tissues to electrical current and is directly related to its fluid content (Bracco et al., 1996). With this information, BIA can indirectly estimate intra and extracellular water, allowing an estimation of total body water (TBW) (Sivapathy et al., 2013). When assessing body composition of 25 male wrestlers, BIA repeatedly underestimated %BF when compared to HW. Furthermore, research shows that BIA may lose accuracy when used on individuals with higher %BF compared to their leaner counterparts, which may be problematic when assessing body compositions in sports that have larger athletes (Segal et al. 1988). Research supports the use of BIA as a practical method to accurately assess TBW in healthy individuals at rest but does not support its use as an accurate predictor of %BF (Kavouras, 2002).

In the past years, Ultrasound (US) has become a novel method to assess body composition. US is a noninvasive technique that measures subcutaneous fat thickness, similar to SF, where thickness is then plugged into an equation to find %BF. US uses piezoelectric crystals to produce waves, which travel through the skin and reflect back to the transducer, as an echo, when contact with interface tissue surface is made (Wagner, 2013). The echoes are then processed by the transducer to create an image (Wagner, 2013). There are two types or “modes”

of US, A-mode and B-mode, depending on the type of transducer used. A-mode, or amplitude-mode, uses a narrow beam to scan tissue discontinuity and produce a spike on the graph, while B-mode, or brightness modulator, combines A-mode signals from various directions to create a 2-dimensional image (Wagner, 2013). Research has shown the efficacy of US, one study reported B-mode US was more accurate than SF, when both methods were compared to HW (Fanelli et al., 1984). A study using A-mode US found that US measurements were correlated ($r = 0.97$, standard error of the estimate = 1.79) to those of DEXA in an athletic population (Pineau et al., 2009), yet Smith-Ryan et al. (2014) found that A-mode US was not accurate in overweight or obese population. Hyde et al. (2015) found that B-mode ultrasound was highly correlated to 7-site SF ($r = .984$) and a 3C model ($r = 0.878$) to estimate %BF in a racially-mixed sample of Division 1 football players, further supporting the use of B-mode US. With unique feature, such as, the ability to save images and software for more in depth processing, US may be a more advantageous way to assess body composition. All this considered, it would be advantageous for both, researchers and clinicians, to validate the use of ultrasound as a body composition.

With its distinctive ability to save images, ease of transportation, relatively low cost, and lack of literature comparing ultrasound to a 4C criterion, it is advantages to validate ultrasound's ability to predict %BF. Therefore, the purpose of this study was to validate ultrasound and its ability to predict %BF as a body composition measurement by examining the relationship of a B-mode ultrasound, skinfold measurements, and a four compartment model criterion in a Division I football team.

Methods

Experimental Design

This was a cross-sectional study to assess the body composition of Division 1 football players. All testing took place during a single session and consisted of: BODPOD® BP, multifrequency BIA, DEXA, skinfold, and Ultrasound. Skinfold was done by researchers who will have been validated in reliability. Also, all ultrasound measurements were done by a single researcher to reduce inter-reliability error. The relationship of %BF from skinfold, US, and 4C criterion was assessed.

Participants

All participants were Division I varsity football players who volunteered for this study. Upon arrival to the lab, anthropometric measurements (height, weight, hips, and waist) were taken of each participant. Due to the nature of the test, participants were told to arrive at the lab in a fasted (>8hrs) and hydrated state and refrain from exercise 24h prior. All procedures were approved by the Georgia Southern University Institutional Review Board.

Procedures

Air Displacement Plethysmography (BODPOD®)

Before testing, BODPOD went through the necessary pre-testing protocol according to the manufacturer's instructions. Prior to each individual test, BODPOD went through a two-point calibration in which the first calibration was done with an empty chamber and the following used a cylinder with a known volume (50.0097L). During calibration, participants stepped on a

calibrated scale to measure their mass to the closest 0.001kg. Participants were instructed to wear minimal and tight fitting clothing and place a swim cap over their hair and remove any jewelry. After stepping on the scale, the BODPOD finished calibrating and the cylinder was removed. Researchers used the prediction equation given by the BODPOD to estimate lung volume. Participants will then enter the BODPOD and instructed to sit as still as possible and breathe normally. The BODPOD performed two consecutive measurements, a third was performed if variability exceeds 150 ml from the first two measurements. After all measurements were taken, and confirmed as accurate, the participant exited the BODPOD. All data displayed on the screen and participants were offered a print-out of all information.

Bioelectrical impedance analysis (BIA)

Multifrequency BIA was used to estimate total body water (TBW) following standard procedures (Bodystat Quadscan 4000: Bodystat LTD, Douglas, UK). This technique uses a range of frequencies (5KHz-200KHz), encompassing both low and high ranges that allow electrical current to pass around and through each cell. The participant was instructed to lay supine on a flat surface for a minimum of 5 minutes to allow time for fluid to equally distribute throughout the body. Electrodes were placed 5 centimeters apart at the wrist (dorsal surface at the ulnar styloid process) and ankle (dorsal surface between the malleoli). Height, weight, gender, age and hip/waist measurements were entered in the BIA and wires were attached to the electrodes. Resistance values were used to calculate extracellular water (ECW) and intracellular water (ICW) and summed to equal TBW. All measurements appear on the screen and were recorded by researchers.

Dual- energy X-ray Absorptiometry (DEXA)

Dual-energy X-ray absorptiometry was used to assess the bone mineral density of each participant. All DEXA calibration and scans were performed by a researcher who were properly certified to perform DEXA scans. DEXA was calibrated using the GE lunar software.

Participants were only instructed to remove clothing if it contained any metal parts. Participants laid supine on the DEXA with limbs as close to their bodies as possible. Researchers then positioned them in the appropriate position for testing and restrain their legs at the ankles to minimize movement during the test. Participants were instructed to not move or talk until the DEXA has scanned the whole body. All tests were saved on the DEXA software.

Skinfold

A designated researcher performed a Jackson-Pollack 7-site skinfold test and measurements were taken on the right side of the body from the: chest, tricep, subscapular, midaxillary, suprailium, abdominal, and anterior/mid thigh. The researcher made marks at all aforementioned sites to ensure all skinfold and ultrasound measurements were taken at the same spot. The researcher then pinched and pulled skin directly above marks and measured fold of skin with calipers. The researcher allowed the calipers to settle for about 2 seconds and the measurement was recorded to the closest 0.5 mm. After measurements had been taken from all sites, the researcher took a second measurement, a third measurement was taken if the previous two were not within 2 mm. Body density (Db) values were calculated using the generalized skinfold equation of Jackson et al (Chest, Midaxillary, Triceps, Subscapular, Suprailliac, Abdomen, Thigh) (1978) . Percent body fat was calculated from Db using the formulas of Brozek and Schutte (Brožek, Grande, Anderson, & Keys, 1963; Schutte et al., 1984).

4-compartment model

The 2C-model used to estimate %BF holds FFM density at a constant 1.1000 g/cm³ (Withers et al, 1998). This constant was derived using only three male cadavers (Withers et al, 1998). The largest source of variance between FFM densities was the hydration status of the FFM (Withers et al. 1998). While the 3C-model controls for density and hydration of FM, it keeps mineral:protein ratio at a constant of .35 (Withers et al. 1998). Due to the ability to control for these variables and increased accuracy this study will utilize a 4C-model criterion. The equation used was developed by Lohman. The equation includes measurements of Db, TBW, BMD, and body mass (BM), all found by the aforementioned test. The equation for percent %BF was as follows:

$$\%BF = [(251.3/BD) - (73.9 * (BM/TBW)) + (94.7 * (BMD/BM)) - 179]$$

Ultrasound

Ultrasound measurements were taken using a Terason T3200 B-mode device (Burlington, MA, USA) to measure subcutaneous fat thickness. All ultrasound measurements were taken in the same spot as the skinfold measurements (Jackson Pollack 7-site 1978). A transmission gel was applied to the transducer and the transducer was placed lightly on each of the seven sites. Great caution was taken to not over compress the subcutaneous fat, inadvertently altering the measurements. The transducer was slightly manipulated around the site until a clear image can be seen. The image was then captured, labeled and was saved. A minimum of two images were captured for each of the seven sites and measurements were averaged between the images and all seven sites were summed. After all data was collected, researchers calculated subcutaneous fat thickness using the electronic calipers associated with the T3200 software.

Statistical Analysis

Pearson's product-moment correlation analyses was ran to determine the strength of the relationship between Σ Ultrasound and Σ Skinfold, and the strength of relationship between Σ Ultrasound and the criterion %BF from 4C.

Results

Means and standard deviations of all variable can be found in Table 2. A positive relationship was observed between Σ Skinfold and Σ Ultrasound ($r = .926$; $p < 0.001$). A statistically significant and positive correlation was observed between Σ Ultrasound and %BF from 4C ($r = 0.961$, $p < 0.001$). Based on the significant correlation analysis, Σ Ultrasound was entered into a regression equation. The following equation was developed to predict %BF from Σ Ultrasound:

$$\%BF = 6.504 + (1.957 * \Sigma\text{Ultrasound}); \text{Standard error of the estimate (SEE)} = 2.14\%$$

Based on the sample of 45 football players withheld from the derivation of the equation, the mean predicted %BF was 19.63% compared to the criterion %BF of 19.91%. The constant error (CE) value of 0.285% was not significant ($p > .05$). The SEE and validity coefficient (r) were 1.81% and 0.91, respectively. The total error (TE) value was 2.32%

Discussion

The major finding of this study was B-mode ultrasound predicted %BF in Division I football players similar to 4C. Additionally, B-mode ultrasound measurements were positively correlated with skinfold measurements. These findings align with previous research examining the validity of ultrasound in an athletic population (Pineau et al., 2009, Hyde, 2015, Muller et al., 2013). Pineau et al. (2009) found that US measurements correlated with DEXA, which was

stated earlier to be accurate when testing an athletic population (Prior et al., 1997). Similar to our findings, Hyde (2015) found that 7-site B-mode ultrasound measurement positively correlated with 7-site skinfold and predict %BF in collegiate football players. Muller et al. (2013) showed that 8-site measurements of skinfold and US were correlated in female athletes. Pineau et al. (2009) found A-mode ultrasound could produce accurate %BF while only taking measurements at two sites (abdomen and thigh) in a sample of mixed gender and sport. Conversely, Loenneke et al., (2014) stated that A-mode ultrasound was unable to accurately predict %BF using 1-site and 3-site. Conclusions are hard to draw between studies due to the fact that the sample size used in Loenneke's study was much smaller than the aforementioned study (11 vs. 93), and only consisted of college non-athletes. Comparable to the study done by Hyde (2015), Smith-Ryan et al. (2014) compared US to a 3C criterion. Although the findings do not agree between studies, Smith-Ryan et al. (2014) used an obese population and stated that, although the %BF produced were lower, there was minimal error supporting US use for tracking %BF changes over time.

The secondary purpose of this study was to develop a prediction equation that will allow accurate prediction of %BF using B-mode ultrasound. The regression produced had a SEE of 1.99% deeming it an accurate way to predict %BF. This finding matches similar studies which reported B-mode ultrasound predicts body composition similar to the given criterion (Muller et al., 2013, Hyde et al., 2015). Although the sample population was different, both studies demonstrated the use of B-mode ultrasound using 3C as criterion for predicting %BF. Muller and colleagues (2013) compared B-mode ultrasound with the 8-site International Society of Advancement of Kinanthrometry (ISAK) standard against a 3C criterion. Similarly, after showing B-mode ultrasound measurements were correlated with skinfold, Hyde et al. (2015), produced a predication equation with an SEE of 2.14% in a football population similar to the

current study. Conversely, Smith-Ryan et al. (2014) found that ultrasound was not accurate at predicting %BF and tended to underestimate. This may be in part due to the fact that A-mode ultrasound was used and subjects were non-athletes and obese population (Smith-Ryan et al., 2014).

Evaluation of the results of the cross validation analysis were established according to previous research, including the following criteria: (a) the mean values for observed and predicted %BF should be comparable; (b) the TE should be calculated because it reflects the true difference between the actual and predicted values for %BF, whereas the SEE only gives an indication about the error associated with the regression between the variables; (c) the TE and SEE should be similar because this reflects the relationship between the regression line for actual vs. predicted %BF and the line of identity; (d) a low SEE is preferred over correlation coefficients due to the SEE not being sensitive to differences in means and is affected by differences between samples in variability of %BF; and (e) there should be no relationship between the CE and %BF (Sinning et al., 1985). There was minimal difference between observed %BF and those predicted by the regression equation, indicated by the SEE of the cross validation (1.81%). The TE was 2.32% supporting a strong relationship between observed and predicted values. The non-significant CE value indicates the associated regression equation is a valid measure for estimating %BF.

Future research is needed to add to the small pool of research examining US as a body composition measurement tool. A major need is to validate current equations produced from previous research. Another consideration that needs to be addressed is standardizing the methods used during the ultrasound. With different modes and samples being used in past research, it is important to standardize measurement protocols. More research needs to be done not only on US

ability to estimate body composition but also its ability to track changes in body composition over time. Research needs to continue to use various populations to examine US accuracy across gender, race, and body composition.

Practical application

This study supports 7-site ultrasound as a body composition measure in an athletic population. In comparison to the 4C criterion, US is a cost and time effective method for body composition. With its ability to save images, US may be superior to the skinfold test due to the decrease in interrater error. US can be used as an accurate way for coaches and clinicians to assess body composition.

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APPENDIX A

RESEARCH QUESTIONS, LIMITATIONS, DELIMITATIONS, AND ASSUMPTIONS

Research Questions

1. Will there be a significant positive correlation between the US measurement and skinfold measurement?
2. Will there be a significant positive correlation between the US measurement and 4C?
3. Will the sum of US accurately predict %BF when compared to 4C criterion?

Limitations

1. A sample of convenience was utilized.
2. Generalizability to an athletic population, other than Division I football players, will be difficult.
3. Urine specific gravity was not used to test hydration status

Delimitations

1. Only utilized male football players

Assumptions

1. Each participant was fasted, and caffeine free for a period of at least eight hours.
2. Each participant was adequately and normally hydrated.
3. Each participant had not exercised for a period of at least 24-hours prior to testing.

APPENDIX B

Review of Literature

Introduction

Body composition testing is intended to evaluate the proportion of chemically distinct compartments within the human body (e.g. bone, adipose and fat-free mass). The purpose of the following literature review is to provide a broad, yet encompassing, synopsis of the literature regarding body composition testing. Furthermore, it will display the need for future research validating ultrasound imaging as a reliable way to estimate body composition.

Effects of Body Composition on Athletic Performance

Evaluation of body composition is of great importance to estimate an individual's physical status. The ratio of fat-mass (FM) to fat-free mass (FFM) has been shown to be associated with obesity and various diseases (Dempster and Aitkens, 1994). In sports, body composition is assessed, not only for health reasons, but also for its effect on performance (Lorenzo et al., 2000). Accurate assessment of changes in body composition, especially in sports with weight classes, may help athletes achieve optimal performance (Pineau et al., 2009).

With the help of improved dietary guidance and strength programming, collegiate football players have been growing in size (Noel, Vanheest, Zaneteas, and Rodgers 2003). In recent decades overall collegiate football players have increased in body weight, while height has remained relatively constant (Noel, Vanheest, Zaneteas, and Rodgers 2003). When comparing positional body composition, differences are frequently exhibited across a football team. "Skill positions," such as defensive or offensive backs, tend to be leaner individuals; however, offensive and defensive lineman are much larger and may average 25% body fat, near obesity

levels (Noel, Vanheest, Zaneteas, and Rodgers 2003). These results, combined with those of Mathews and Wagner (2008), demonstrate that offensive and defensive linemen possess high levels of %BF placing them at higher risk of disease. With the growing size of football players at the collegiate level, more emphasis is needed on body composition assessment, for both health and performance reasons.

Body composition in sports may serve as more than just an indicator of overall health, it may also function to predict performance in numerous sports. A study by Miller et al. (2002) examined the correlation between %BF, among Division 1 football players, and a myriad of performance based tests (e.g. power clean, bench press, squat, vertical jump, 40-yd dash, 20-yd shuttle). While body weight of a player was positively related to bench press and power clean, %BF had a negative effect in relation to power clean, vertical jump, dash, and shuttle (Miller et al., 2002).

Additional assessments to determine athletic prowess may also include muscle cross sectional area (CSA), which has been positively associated with force production. Häkkinen and Keskinen (1989) demonstrated a direct relationship between CSA of various elite athletes and the amount of force the athlete could produce. These results, in conjunction with negative correlation of %BF and performance, support the importance of tracking body composition in collegiate football players.

Clinical Assessment of Body Composition

Clinical methods of estimation tend to have greater validity with a lower standard error of estimate (SEE), but can be more cumbersome, time consuming and expensive, as compared to field methods of estimation. Therefore, these methods are almost exclusively found in a clinical

or research, setting. There are various ways of assessing body composition within a clinical setting, consisting of: dual-energy X-ray absorptiometry (DEXA), air displacement plethysmography (ADP), hydrostatic weighing (HW) or the combination of multiple methods to create a multi-compartment model.

Two-compartment (2C) and four-compartment models (4C) are the foundation, upon which all body composition methods have been developed (Lukaski 1987). 2C estimation models employ the central premise of subdivision of the body into two chemically distinct compartments: fat mass (FM) and fat-free mass (FFM). Classification of the body into two distinct groups fails to account for the different constituents of FFM (e.g. water, protein, bone) and assumes uniformity across the population (Khort 1998). 2C models rely on several assumptions, including: (1) the separate densities of the body are additive, (2) densities of different body parts are constant between subjects, (3) the proportion of these parts are the same between subjects, (4) each person differs from a standard reference body only in the amount of fat they have (Roche, 1996). With these assumptions, the two major sources of variability are hydration and protein:mineral ratio, with variations of 1-3% and 2.1%, respectively (Lohman 1981, Lukaski 1987). As more research arises, the overall consensus is that 2C models do not adequately adjust for ethnicity, aging, gender, and excess adipose tissue. This lends to the idea that to accurately assess body composition the body must be broken into more than just two compartments.

Due to the many assumptions, and lack of direct measurement of 2C models, researchers have investigated other methods to more accurately estimate body composition while controlling for variables. Keys and Brozek (1953) divided the body into four separate groups, consisting of: 1) water, 2) protein, 3) bone mineral, and 4) fat. Siri (1956) created the 3 compartment model

which accounts for TBW, while mineral:protein ratio remains constant at 0.35. Recent development of DEXA for body composition made it possible to estimate bone mineral density (BMD). The ability to estimate these four chemically distinct compartments lead to the creation of the 4C-model for estimating body composition. This model, deemed more valid due to its ability to control for BMD and TBW, is considered to be a more accurate way to estimate body composition (Ellis, 2000).

HW is referred to as the “gold standard” for assessing body composition and estimates body density (D_b) using Archimedes’ principle (Lukaski, 1987). This principle states that the total volume of an object is equal to the volume of water that the object displaces (Lukaski, 1987). A study out of the University of Iowa demonstrated that HW was closely correlated with three different skinfold (SF) equations in Division I football players (Oppliger et al., 1992). However, a study found that the D_b derived from HW was lower than the more accurate 4C-model, giving a lower BF% by approximately 2-3% (Withers et al. 1998). This discrepancy may be primarily due to the assumption of FFM hydration during HW (Withers et al., 1998). A cumulative analysis of literature on HW found that D_b derived from this method has a <1% variability in the participants of the analyzed studies (Withers et al., 1998). Furthermore, acquiring accurate data can be difficult in a population that is apprehensive to being submerged in water and sustaining from breathing for prolonged periods of time. Although HW is deemed accurate and reliable, its procedures are tedious and time consuming. Furthermore, the required equipment can be expensive and difficult to properly maintain.

DEXA is a noninvasive method to estimate total body or regional measurements of FM, FFM, and BMD. Although DEXA’s true validity has not been sufficiently proven using chemical analysis, its accuracy has been shown to be close to other in vitro and in vivo, methods

(Svendsen 1993). Two studies showed that when compared to ADP and HW, DEXA estimation was significantly lower than those of the 2C model (Khort, 1998, Ball and Altena, 2004). Results from Prior et al. also found that DEXA measurements were not similar to those using Db to estimate %BF. In contrast, the same study found that DEXA had similar results to the more accurate 4C-model (Prior et al., 1997). Prior and colleagues (1997) found that DEXA results were not statistically different when compared to the 4C-model in subjects of various gender, race, athletic status, and body size (Prior et al., 1997). The higher accuracy of DEXA, when compared to Db or a 2C model, further supports the idea that DEXA is a superior method for estimating body composition.

Another clinical method of body composition is ADP, which is commercially available as BOD POD (Cosmed, Rome, Italy). Similar to HW, ADP relies on Archimedes' principles of displacement, contrary to HW, ADP employs the use of air displacement to derive Db. When %BF was compared between ADP and HW there was a mean difference of around 1%, (Fields et al., 2002). Conversely, Collins and colleagues (1999) also showed a discrepancy in Db between ADP and HW in collegiate football players. In multiple studies, when ADP was compared to a 4C-model, %BF was underestimated by around 2-3% (Collins et al., 1999, Fields et al., 2001, Millard-Stafford et al, 2001). Despite the discrepancies, Ballard et al. (2003) compared ADP and DEXA in both Division II female athletes and nonathletes and found that ADP produced accurate %BF results when compared to the DEXA 3C method. Vescovi et al. (2001) found that there were no difference in mean Db or %BF between ADP and HW, but ADP underestimated Db when strictly looking at leaner individuals, leading to overestimated %BF. Utter et al. (2003) compared ADP, using BODPOD, to HW static weighing in 66 collegiate wrestlers in a hydrated

and dehydrated state. In both hydration states, there was no difference between Db, %BF and FFM between the two methods (Utter et al. 2003).

Field Assessment of Body Composition

Field tests are less expensive than most clinical assessments, use limited equipment and can be transported easily to various sites for testing. They also allow researchers to collect data from larger number of participants in shorter time periods. Common field tests consist of skinfold measurements (SF), bioelectric impedance analysis (BIA) and ultrasound (US).

A commonly utilized field assessment is skinfold measurement, wherein, Db is derived from an equation using subcutaneous fat thickness from different sites on the body. Jackson and Pollack (1978, 1980) developed logarithms using the skinfold measurement of 7 sites (chest, tricep, subscapula, abdomen, thigh, and suprailiac) to estimate Db in men and women. SF has about a 5% error when determining %BF, which may increase when applied on extremely lean or overweight individuals (Lukaski 1987). Stewart and Hannan (1999) compared SF measurements to DEXA in 82 male athletes and found that the Jackson and Pollack (1978) prediction equation had a mean difference of 3%. The Durnin and Womersley (1974) equation, used in the study, had a much larger error, supporting the use of Jackson and Pollack (1978) equation when testing athletes or physically active individuals (Stewart and Hannan 1999). Oppliger et al. (1992) compared SF to HW in 28 Division I football players and found that the 3-site SF had little difference compared to HW. Clark et al. (1994) compared 8 different SF equations in a racially mixed population of athletes and found that the Jackson and Pollack (1978) equation had the best correlation with HW, supporting the use of the Jackson and Pollack (1978) equation when assessing body composition of a racially mixed group of athletes. Hortobágyi et al. (1992) compared 7 site SF to HW in a racially mixed group of Division 1

football players and found no statistical significant difference in %BF between the two methods. Conversely, in the aforementioned study by Utter et al. (2003), they found that SF overestimated %BF in hydrated and dehydrated collegiate wrestlers. Although the difference was small (1.1%) the sample size was large enough to make it statistically significant (Utter et al, 2003).

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) is a method of estimating body composition as well as estimate the hydration status of an individual. BIA is centered on the idea that the resistance of tissues to electrical current is directly related to its fluid content (Bracco et al., 1996). Based on this resistance to flow, BIA can indirectly estimate intra and extracellular water, allowing an estimation of total body water (TBW) (Sivapathy et al., 2013). Hoffer et al. (1969) demonstrated very early that BIA was highly correlated with tritium-dilution technique ($r = .92$) for TBW assessment. Later research demonstrated BIA was accurate at estimating TBW in both males and females, when compared to D₂O dilution (Lukaski et al., 1985, Kushner and Schoeller, 1986). Research supports the use of BIA as a practical method to accurately assess TBW in healthy individuals at rest (Kavouras, 2002). In addition to assessment of TBW, BIA has also been evaluated as a noninvasive and quick method of estimating %BF. Dixon et al. (2005) compared multiple methods of body composition estimation to HW in 25 Division III wrestlers. Results indicated that ADP and SF were reliable methods to estimate body composition, however, BIA repeatedly underestimated %BF. Research shows that BIA may lose accuracy when used on individuals with higher %BF compared to their leaner counterparts, which may be a problem when assessing body compositions in sports that have larger athletes (Segal et al. 1988).

Ultrasound as a Predictor of Body Composition

US, a relatively new method for assessing body composition, is a noninvasive technique that measures subcutaneous fat thickness, similar to SF. US uses piezoelectric crystals to produce waves from the transducer (Wagner, 2013). These waves travel through the skin and reflect back to the transducer, as an echo, when contact with tissue is made (Wagner, 2013). The echoes are then processed by the transducer to create an image (Wagner, 2013). The transducer of particular US equipment can vary depending on the systems mode, A-mode and B-mode. A-mode, or amplitude-mode, uses a narrow beam to scan tissue discontinuity and produce a spike on the graph (Wagner, 2013). While B-mode, or brightness modulator, combines A-mode signals from various directions to create a 2-dimensional image (Wagner 2013). Unique to US, the image can be saved and viewed by multiple clinicians or researchers, allowing for better control of inter-rater error.

Albeit, US and SF estimate %BF in a similar manner, early research demonstrated that US may be more accurate at measuring subcutaneous fat than SF, as compared to HW (Fanelli et al., 1984). Published literature has suggested that SF and US are equally effective at predicting %BF, but the equipment used with the US has features that may be beneficial for clinicians (Fanelli et al., 1984). An earlier study by Pineau et al. (2009) compared %BF estimated by A-mode US versus DEXA in 93, male and female, athletes. The %BF predicted by US were highly correlated ($r > .96$) to those of DEXA (Pineau et al., 2009). These results parallel with the findings of a previous study done by Pineau et al.(2007), which found that US %BF predictions were accurate when using DEXA as the criterion in healthy nonathletes. A study, with findings contradictory to those of Pineau, used 104 male participants to compare multiple techniques of %BF estimation (SK, US, BIA) against DEXA criterion (Duz et al., 2009). Duz et al. (2009)

findings showed that B-mode US underestimated %BF, which may be due to the different equations used to estimate %BF from Db. A more recent study sought to validate a 7-site B-mode US measurement in Division 1 football players (Hyde, 2015). This study utilized a 7-site SF and US as well as a 3C-model to estimate %BF on a racially-mixed sample of Division 1 football players (Hyde, 2015). This study found a high correlation between SF and US measurements and no significant difference between US and the 3C model (Hyde, 2015). Conversely, Loenneke and colleagues (2014) found that US 1- and 3-site was not highly correlated to SF measurements. Additionally, a study done by Smith-Ryan et al. (2014), showed that US did not predict %BF well in an overweight and obese population, when using 3C criterion. Although the previously mentioned studies have conflicting results, this may be explained by the use of A-mode US by Loenneke et al. (2014) and Smith-Ryan et al. (2014).

Conclusion:

There is clearly an abundance of literature pertaining to the validity of various estimation modes for assessing body composition. With clinical methods being cumbersome and complex, research has extended further into the uses of field methods. There is an anticipated lack in literature validating the use of B-mode ultrasound. This lends to the need of this study to help expand the deficient body of literature on the subject.

APPENDIX C

Table 1. Descriptive Characteristics.

	N	Min.	Max.	Mean	Std. Deviation
Age	90	18	26	20.42	1.31
Height (cm)	90	167.64	198.12	182.42	5.87
Weight (kg)	90	71.36	160	102.98	20.78

Table 2. Means \pm Std. Deviation of Variables

	n	Mean	Std. Deviation
Sum of Skinfold (mm)	90	127.41	64.8
Sum of Ultrasound (cm)	90	119.21	61.77
4C BF (%)	90	20.55	8.85
BMC (g)	90	5.56	0.88
TBW (L)	90	55.87	8.15
Db (kg/L)	90	1.06	0.022

Figure 1. Measurement of Subcutaneous Adipose Tissue.



