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Use Of Dual Energy X-Ray Absorptiometry Measurements To Evaluate Total Body Volume When Compared To Air Displacement Plethysmography For Evaluating Body Composition In A Four Compartment Model

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EVALUATE TOTAL BODY VOLUME WHEN COMPARED TO AIR
DISPLACEMENT PLETHYSMOGRAPHY FOR EVALUATING BODY
COMPOSITION IN A FOUR COMPARTMENT MODEL

BY

LEE A. DOERNTE

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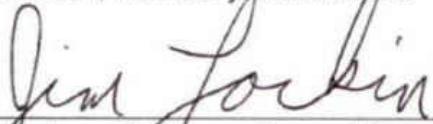
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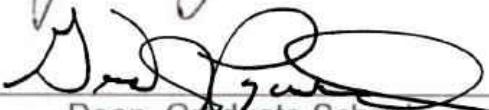
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A handwritten signature in black ink, appearing to read "Lee A. Doernte". The signature is fluid and cursive, with a horizontal line underneath it.

Lee A. Doernte

Date: April 4, 2018

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BY

LEE A. DOERNTE

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2018

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DEDICATION

This thesis is dedicated to my wife Alessandra, who supported me throughout my career change and decision to further my education, and April, who's tireless hours and support made this research project possible.

I couldn't have finished my thesis without the support of you both.

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- Shane Harris, for unending assistance throughout the years

ABSTRACT

Intro: Body composition is an important metric to evaluate overall health. Having reliable body composition testing methods are critical to ensure that an individual is receiving correct data in which to base health, nutrition, and lifestyle decisions. With technological improvements, there are more reliable, high-quality testing devices for body composition than in the past. However, there remains questions on the validity of the devices to properly measure body composition. There is a need for a more accurate, simplistic testing methodology.

Purpose: The purpose of this paper was to examine the use of dual energy x-ray absorptiometry (DEXA) based formulas to evaluate body volume (BV). The second purpose was to examine the validity of using predicted body volume measurements in four-compartment body composition models.

Methods: Subjects were tested on three devices designed for body composition metrics; DEXA for lean body mass, bone mineral content, and fat mass, BodPod for body volume, and a bioimpedance spectroscopy (BIS) device using dual electrode tabs (SFB7) for total body water. The measured metrics were used to compute two DEXA based predicted BV equations, Wilson, et al. (2012) and Smith-Ryan, et al. (2017). The results were then compared to measured BodPod BV. Second, this study calculated two different four-compartment model formulas, Withers, et al. (1998) and Wang, et al. (2005), using a DEXA calculated body volumes. These models were then compared to a four-compartment model using BodPod measured body volume.

Results: 90 healthy adults (50 females and 40 males), aged 18 to 66 years (median 23 years), BMI 18 to 34 (median 25), weight 45 to 115 kg (mean $73.64 \pm SD 14.35$ kg), height 150 to 191 cm (171.07 ± 9.98 cm), BodPod data was collected and used as a standard for comparison to the DEXA based body volume formulas; (Wilson et al. 2012) and (Smith-Ryan, et al., 2017). BodPod measured BV mean of $70.36 \pm SD 13.85$ L, Wilson, et al. (2012) 70.88 ± 13.54 L, and Smith-Ryan, et al. (2017) 70.02 ± 14.23 L. ANOVA yielded no statistical difference between the three groups ($p=.915$). Among the Withers, et al. (1998) formula, paired t-test of BodPod/Withers yielded a significant difference lower than Wilson/Withers ($z = -6.178$, $p \leq .001$) and Smith-Ryan/Withers yielded a significant difference lower than BodPod/Withers ($z = -5.052$, $p \leq .001$). Among Wang, et al. (2005) formula, Wilcoxon signed-rank test of BodPod/Wang yielded a significant difference lower than Wilson/Wang ($z = -5.816$, $p \leq .001$) and Smith-Ryan/Wang a significant difference lower than BodPod/Wang ($z = -4.690$, $p \leq .001$). These significant differences indicate the predicted BV equations are not equivalent to using measured BV and yielded inaccurate results

Conclusion: The use of DEXA based BV formulas is a viable replacement for other BV testing methodologies for use in four-compartment testing models. However, both the Wilson, et al. (2012) and Smith-Ryan, et al. (2017) formulas failed to be a viable replacement for measured BodPod values. A new formula, A new formula resulted from this study. Further studies may be needed before a formula can be utilized in four-compartment models.

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ABBREVIATIONS

1. Air Displacement Plethysmography BodPod
2. Bioelectrical Impedance Analysis BIA
3. Bioelectrical Impedance Spectroscopy BIS
4. Body Mass Index BMI
5. Bone Mineral Content BMC
6. Body Volume BV
7. Dual Energy X-Ray Absorptiometry DEXA
8. Fat-Free Mass FFM
9. Fat Mass FM
10. Lean Mass LM
11. Percent Body Fat %BF
12. Total Body Water TBW

CHAPTER I

INTRODUCTION

Body composition is an important metric to evaluate overall health. Having reliable body composition testing methods are critical to ensure that an individual is receiving correct data in which to base health, nutrition, and lifestyle decisions (Pescatello, 2014). With technological improvements, there are more reliable, high-quality testing devices for body composition than in the past. However, there remains questions on the validity of the devices to properly measure body composition. There is a need for a more accurate, simplistic testing methodology.

Body composition testing commonly consists of multiple separate metrics, typically first assessing total body weight, lean body mass, and fat mass, to calculate body fat percentage. Each of these metrics are important for evaluating body composition. There are other metrics that are underutilized and could provide a more accurate, complete model of body composition testing, such as bone mineral content and body volume. Utilizing these additional metrics to create a complete model would lead to higher accuracy in testing of body composition.

Body Composition and Health

Understanding the relationship between body fat percentage and lean body mass can be used for guidance towards fitness training and nutrition for

overall wellness purposes. According to the Centers for Disease Control and Prevention (2017), in America, 38% of the population is obese and another 33% are overweight. Over the years, it has become well established that obesity can lead to serious health problems. The CDC (2017) has linked obesity to “all causes of death (mortality), high blood pressure (hypertension), high LDL cholesterol, low HDL cholesterol, or high levels of triglycerides (dyslipidemia), type 2 diabetes, coronary heart disease, stroke, gallbladder disease, osteoarthritis (a breakdown of cartilage and bone within a joint), sleep apnea and breathing problems, some cancers (endometrial, breast, colon, kidney, gallbladder, and liver), low quality of life, mental illness such as clinical depression, anxiety, other mental disorders, body pain, and difficulty with physical functioning.” These issues are largely preventable if healthy body composition is maintained.

Body Composition Testing

A common method many individuals have traditionally used to gauge their body composition is to weigh themselves on a common bathroom scale. There are many problems with this method. The scales are often uncalibrated, low quality, and provide unreliable results. Body weight alone does not provide enough data to make informed decisions regarding health and fitness. While there are weight charts to give general guidelines, they do not account for the composition differences in body fat mass and lean body mass. This method also does not account for height, so a person with a height of 5'0" that weighs

200 pounds would rate the same on the weight chart as someone who is 6'6" and weighs 200 pounds, even though body fat and lean body mass composition could be drastically different. Without the composition knowledge, misinformation could mislead people to believe they are healthy, when in fact their body fat could be too high, or that they are overweight, when in fact, their body composition is ideal.

Another common method to address body composition is to use an individual's height, weight, and age to calculate body mass index (BMI). This method uses more data than weight alone. It has the additional benefit of using established standardized scales to provide knowledge on where an individual would fall within a spectrum (underweight to obese). BMI is calculated using a person's mass in kilograms divided by height in meters squared and reported as kg/m². Using this formula (BMI = kg/m²), the most common categories are as follows: (1) underweight -- BMI 18.5 kg/m² or less, (2) normal weight -- BMI between 18.5 kg/m² and 24.9 kg/m², (3) overweight -- BMI between 25 kg/m² and 29.9 kg/m², and (4) obesity -- BMI 30 kg/m² or greater.

This method is more effective than assessing body weight alone, but still has flaws. One major flaw is the numbers are not translatable into true body composition. The value from the equation helps to guide the general population to a recommended weight range for height, but it does not provide information on body composition. BMI only provides information regarding how far someone is from a range considered healthy by the BMI, but does not evaluate fat percentage, or how far they are from a desirable body fat percentage. Another

flaw is that BMI, not only fails to inform individuals of their body fat percentage but has no method to distinguish lean body mass from body fat mass. A person with a larger lean mass might be calculated to be overweight or obese using the BMI formula, even if their body composition is actually normal, or even lean. This method misinforms many athletes and muscular people into the belief they are overweight or obese, creating the illusion they are unhealthy (a false positive).

Bioelectrical impedance analysis (BIA) devices are another common method used to test body composition and provide a technological edge in evaluation of body composition. These devices can be hand-held or stand-on varieties. BIA devices provide more information about body composition than BMI. BIA estimates body composition by measuring electrical resistance of the human body to transmission of 800 microamps at 50KHz (using an electrical impedance plethysmograph) into the deep tissues of the body (Lukaski, Johnson, Bolonchuk, & Lykken, 1985). This data is then calculated with a manufacturer's formula to statistically estimate body fat percentage and lean body mass, providing the beginnings of a body composition model. Some models additionally predict total body water but are considered unreliable with single frequency scanning (Rallison, Kushner, Penn, & Schoeller, 1993). While these devices are affordable and readily available, there is a concern about the accuracy and reliability. Many studies have examined BIA and found their reliability and accuracy to be questionable, and possibly unfit to be used as a

measure of body composition (Dehghan & Merchant, 2008; Peterson, Repovich, & Parascand, 2011).

Bioelectrical Impedance Spectroscopy

A more advanced model of BIA is the bioimpedance spectroscopy (BIS). BIS devices scan 256 frequencies between 4 kHz and 1000 kHz. The additional scanning provides a validated metric unavailable by single frequency BIA, the evaluation of total body water (Higgins, Reid, Going, & Howell, 2007). The addition of an accurate body water evaluation, through the evaluation of intracellular fluid and extracellular fluid, along with lean body mass and fat mass, allows for an additional metric to be calculated into a body composition model. The additional metric provides increased accuracy in estimating percent body fat.

Underwater Weighing

For many years the “gold standard of analyzing percent body fat has been underwater weighing, also known as hydrostatic weighing or hydrodensitometry (McArdle, Katch, & Katch, 2011). Underwater weighing is done by first weighing a subject before entering a water tank. The subject is then placed on a scale, lowered into the water tank and asked to expel all air from the lungs. The difference in weight is then used to calculate percent body fat (Moon, et al., 2011). To get an accurate measurement, an average of 9 measurements is needed using underwater weighing (Zamora, Jakicic,

Hortobágyi, & O'brien, 1995). The displacement of the water can be used to calculate the volume of the subject. Using the formula density = mass x volume, density can also be calculated from underwater weighing, allowing more complete body composition measurements. Part of the challenge with this method is that it is very time consuming to do a valid percent body fat measurement using this method. Also, a large water tank and special scale are required, which can be very costly and space consuming. With the amount of time and space needed, underwater weighing is an inconvenient method compared to newer technologies.

Air Displacement Plethysmography

Air displacement plethysmography (BodPod) uses whole body densitometry to determine body composition in a method similar to underwater weighing. The BodPod measures body mass using a precise, calibrated scale. The subject then has their body volume measured by sitting in the BodPod as it adds small amounts of air and measures the difference in pressure (McArdle, Katch, & Katch, 2011). The resulting pressure difference is calculated to compute the body volume. The volume in the lungs can either be predicted or measured directly through breathing apparatus attached to the BodPod. The resulting volumes and mass measurements are then used to calculate density, which in turn is used to calculate lean mass and body fat. This method is easier, faster, and equally reliable to the traditional method of underwater weighing (Fields, Goran, & McCrory, 2002).

Dual Energy X-Ray Absorptiometry

One of the newest technologies for measuring body composition is dual energy X-ray absorptiometry (DEXA). DEXA operates by passing X-ray energy through a body and detecting the energy not absorbed on the other side. The results are used to derive bone mineral content, fat mass, and lean mass. DEXA machine is a fast method (typically taking only 6-10 minutes) to measure body composition. Besides being fast, DEXA is also accurate, equal in validity to underwater weighing and proving to have even greater reliability (Morrison, et al., 1994).

Body Composition Compartment Models

Growing technology trends lead to newer, more valid and reliable methods for testing body composition. Technology has improved to include the use of x-ray technology, air displacement, and improvements to traditional bioimpedance methods. With the use of these new methods, body composition is becoming much more reliable, but there are still issues with a practical method of accurate testing.

To compensate for this, multi-compartment models are used to increase the accuracy of body composition testing. Multi-compartment models use several factors to increase the accuracy of body composition testing, such as lean mass, fat mass, total body water, bone mineral content, and residual protein. Historically, most testing methods accounted for two of these (lean mass and fat mass), creating a two-compartment model. Few, such as the

DEXA can account for three (fat mass, lean mass, and bone mineral content) creating a three-compartment model. The use of a four-compartment model would increase the validity of body composition measurements and allow a more accurate percent body fat (%BF) evaluation.

While many of these technologies are accurate, they are also very time consuming and impractical when dealing with a four-compartment model. The need to create a practical and valid way of establishing a multi-compartment model without the use of several testing modalities has not been established. The ability to remove multiple testing devices to create an accurate model would not only save time, but also cost, reducing the overall expense per test and the cost of needing several testing machines. It would also appeal to many universities as a way to save space, creating a more effective lab in a much smaller space. The need for the large space demands of underwater weighing chambers and BodPod could be freed up for other research applications.

Gap in Literature

A gap in the literature is created from the lack of research on an efficient way to create a four-compartment model without the need for many testing devices. While many four-compartment models exist (mostly using a BodPod for body volume, BIA or BIS for total body water, and a DEXA for bone mineral content, lean mass, and fat mass), few have explored the option of comprising a testing modality to using only DEXA and BIS only to create a four-compartment model of testing body composition. Millard-Stafford, et al. (2001)

explored the possibility of using BodPod in place of DEXA or underwater weighing as a method to compute a four-compartment model, but this study found evidence that BodPod was not a valid method of estimating body density in place of underwater weighing, making it an unacceptable method of evaluating a four-compartment model. The next progression in research to reduce the quantity of required testing methods came when Wilson, et al. (2012) developed a method to use DEXA as a four-compartment model, through the calculation of body volume (BV), with only the use of one additional testing methodology, BIA, to evaluate total body water, but it was only in the theoretical stages. Smith-Ryan (2017) further explored methods and calculations of using DEXA to evaluate a four-compartment model by using lean mass, bone mineral content, and fat mass calculated from DEXA scans, a BIA for calculation of TBW, and expanded research in using body volume derived from calculations of the DEXA results. The results showed promise, but no validation of this method has been conducted. The need to explore a multi-methodology four-compartment model compared to a DEXA predicted BV four-compartment model still exists. Also, the validation of which formula, Wilson, et al. (2013) or Smith-Ryan, et al. (2017), for deriving body volume with a DEXA, needs to be explored and compared.

Purpose

The purpose of this research was to examine the use of dual energy x-ray absorptiometry (DEXA) based measurements to evaluate body volume. The

formulas established by Wilson et al. (2012) and Smith-Ryan et al. (2017) were both evaluated for validity in comparison with known measurements by air displacement plethysmography (BodPod), to establish the validity of using DEXA based measurements to establish body volume. Second, the validity of using predicted body volume measurements from DEXA in four-compartment body composition models by Withers, et al. (1998) and Wang, et al. (2005) was compared to a multi-system four-compartment body composition model for validity utilizing BIA, BodPod, and DEXA (Kuriyan, Thomas, Ashok, Jayakumar, & Kurpad, 2014).

It was hypothesized that (1) the predicted body volume equations will be statistically equivalent to the measurements of BodPod; (2) the Ryan-Smith, et al. (2017) formula will have a smaller variation when compared to BodPod values than the Wilson et al. (2012) formula; (3) predicted body volume four-compartment modes will be statistically equivalent to the multi-system model; and (4) there will be statistical difference between DEXA measured body fat compared to the Withers, et al. (1998) formula and the Wang, et al. (2005) formulas, using both predicted and measured body volumes.

Results provided important information that will be useful to modify body composition testing for future subjects to ensure accurate data is presented to all subjects, allowing for more informed decision making regarding their body composition in a more time efficient manner. This information should make body composition testing faster and more economical when utilizing multi-compartment body composition models. Further, this research will provide

evidence to a potential alternative to the replacement of body volume measurement devices in favor of DEXA based formulas.

Limitations

A limitation to this study was the subjects unable to pass the measured thoracic volume portion of the BodPod. Of the 90 subjects, 30 had to use the predicted volume setting of the BodPod due to inability to pass measured thoracic volume portion. Research cited has shown the predicted thoracic model is statistically equivalent to measured thoracic volumes, however exact measurements would be more precise (Wagner, 2015).

A second limitation is that fasted states, workouts, and medical statuses were self-reported. Self-reporting in a study is a limitation because there is no proof of the action and no guarantee that the subject is being honest. If the subject did not report honestly, measurements could be misrepresented. While all attempts are taken to avoid subject misrepresentation, honest reporting can never be guaranteed.

Unknown menstrual cycles of female subjects can be an additional limitation. Shifts in fluid retention have been linked to female hormonal changes. Research has shown that total body water and percent body fat increases during the mid and end of a menstrual cycle (Hicks, 2017). Changes in menstrual cycle during testing period will affect body composition testing.

Due to subject availability, this study was also highly skewed toward college-aged, European-American adults, providing little availability to explore age and

racial differences among populations at similar mass with regards to density. This population is also not representative of the national census, resulting in a formula skewed toward a single population, misrepresenting other volumes. The age was also heavily skewed left on the histogram, toward college aged adults. This was expected as a sample of convenience was utilized. The study was open and encouraged to all ages of over 18, with a need for at least 20 of the subjects needing to be from the over-40 age category. The desire was to get an equal distribution for age and gender, however there wasn't an opportunity among age. The study was conducted at a university and, consequently, the median age was only 23, with a mean age of 28.58 ± 12.53 years and a range of 18 to 66. This was due to the availability and willingness to participate of college age adults versus the over-40-aged adults. Attempts were made to normalize this through recruitment as much as possible.

Delimitations

Underwater weighing was not utilized in this study due to time constraints and difficulty of use compared to BodPod. BodPod is easier, faster, and equally reliable to the traditional method of underwater weighing (Fields, Goran, & McCrory, 2002). There was also limited use to an underwater chamber, while there was unlimited access to a BodPod.

Selected exclusion criteria to this study included a BMI below 18 or above 35. BMI's outside this range report body volumes incorrectly. The BodPod under estimates people with BMI's lower than 18 up to 15% and BMI's higher

than 35 by 8.51% (Lowry & Tomiyama, 2015). The cut off for urine specific gravity was no greater than 1.030. Normal kidney function is between 1.000 to 1.030. Values outside of this range can indicate health issues and irregular total body water (Sommerfield, et al., 2016). Subjects were also eliminated from participation due to metabolic disease, kidney disease, heart disease, tachycardia, or hypertension. Irregular health status can result in incorrect body composition measurements (Powers, Choi,, Bitting, Gupta, & Buchowski, 2009).

CHAPTER II

REVIEW OF LITERATURE

Body Composition and Health

Body fat has long been associated with negative health effects. Chang, et al. (2012) conducted a systemic review of older adults and the association of body fat to morbidity and mortality. They found as individuals age, they lose key components such as bone mineral density and lean body mass, while their abdominal fat increases. This increase in abdominal fat is most commonly visceral fat, which is highly associated to adverse health effects (Chang, Beason, Hunleth, & Colditz, 2012). Many conditions such as metabolic syndrome, inflammation, dyslipidemia, insulin resistance, type-2 diabetes, cardiovascular diseases, and cancer are associated with high body fat (Cefalu, et al., 1995; Després & Lemieux, 2006; Steinberger & Daniels, 2003; Kuk, et al., 2006). Chang et al. (2012) first looked at common methods of analyzing body composition. The first common method was BMI. Chang et al, (2012) showed that BMI does not distinguish between lean mass and fat mass in weight, making it unreliable as a measure of proper body composition. Anthropometric measurements add more data and give a better calculation, but still have a high user error and provide unreliable data (Chang, Beason, Hunleth, & Colditz, 2012).

Metabolic Syndrome

Visceral adipose fat places individuals at greater risk for metabolic syndrome. Metabolic syndrome is a condition postulated to be from underlying insulin resistance that leads to other conditions, such as polycystic ovary syndrome, fatty liver, gallstones, sleep disturbances, asthma and some forms of cancer (Grundy, Brewer, Cleeman, Smith, & Lenfant, 2004). The development of metabolic syndrome may further exacerbate the collection of visceral fat, increasing many risk factors associated with metabolic syndrome, including dyslipidemia and hypertension (Després & Lemieux, 2006; Chang, Beason, Hunleth, & Colditz, 2012). The development of metabolic syndrome becomes a primary risk factor for other diseases, such as cardiovascular disease and diabetes (Chang, Beason, Hunleth, & Colditz, 2012).

Cardiovascular Disease

The likelihood of developing cardiovascular disease from metabolic syndrome becomes a high probability and leads to severe health consequences. Isomaa et al. (2001) studied 4,483 subjects for cardiovascular risk associated with metabolic syndrome. The results after follow-up showed subjects with metabolic syndrome were three times more likely to develop cardiovascular disease than those without metabolic syndrome. This leads to a direct link that increased body fat leads to a marked increase in risk for cardiovascular disease. Malik et al. (2004) found similar results in a study connecting metabolic syndrome to mortality via coronary heart disease,

cardiovascular disease, and all causes in United States adults. The results from were consistent with previous studies, finding coronary heart disease, cardiovascular disease, and total mortality are significantly higher in US adults with metabolic syndrome than in those without metabolic syndrome. The research was further expanded by Veronica and Esther (2012) with research involving aging, cardiovascular disease, and metabolic syndrome. The findings were that metabolic syndrome not only lead to a higher prevalence of cardiovascular disease, but other pathological conditions such as increased peroxidation of nitric oxide, generation of free radicals, and increased lipoperoxidation, which are precursors to cancer.

Type 2 Diabetes

Increased body fat is also linked to insulin resistance and type 2 diabetes. Steinberger and Daniels (2003) conducted a study reviewed by the American Diabetes Association and containing recommendations from the American Diabetes Association's Clinical Practice Recommendations. Weight loss is associated with improved insulin sensitivity, while obesity is associated with significantly more insulin resistance. Luckily, the link between diabetes type 2 and obesity is one that is reversible. Steinberger and Daniels suggest that just a 10% to 15% decrease in weight can have massive benefits in the treatment of diabetes type 2. Type 2 diabetes is emerging as a major health emergency, making the need for accurate body composition testing and education paramount.

Hypertension

Hypertension is also highly linked with body fat. Hayashi et al (2003) found in a study of 563 Japanese-Americans that intra-abdominal fat was associated with hypertension as a significant predictor of morbidity, even after adjustments for total subcutaneous fat area, abdominal subcutaneous fat area, body mass index, or waist circumference. Hypertension is a strong risk factor for stroke, coronary heart disease, congestive heart failure, and mortality. Currently hypertension affects 25% of all Americans, and as many as 90% at some point during their lifetime (Wang & Vasan, 2005). The use of proper body composition monitoring and treatment could help reduce the risk of this condition.

Body Composition Testing

Duren, et al. (2008) analyzed methodology and components of body composition in a study to address the growing epidemic of obesity and related diseases, such as type 2 diabetes. After analyzing multiple methods, Duren, et al. (2008) concluded the most important aspect for the benefit of health analysis is body fat. Increased body fat was cited as an important factor that leads to insulin resistance and has a dramatic impact on metabolism. Accurate assessment information is very important to assess body fat. The use of total body water and fat free mass is one way suggested to evaluate body composition. There is a need to find the optimal method of testing for body fat.

Bioelectrical Impedance Spectroscopy

Bioelectrical impedance spectroscopy (BIS), also known as multi-frequency bioelectrical impedance, is widely used as a measurement of body composition. BIS is similar to BIA in that it estimates body composition by measuring resistance of the human body to electrical transmission. However, while BIA only uses a single frequency, BIS uses multiple frequencies to provide a more accurate and reliable estimation of body fat, lean mass, and total body water.

While many methods exist for calculating total body water. Traditionally, the gold standard is using underwater weighing, however studies have been conducted to create a more practical method of evaluating total body water using bioelectrical impedance. A study of 36 healthy males, with cross validation analysis on two random subsets, yielded evidence supporting total body water calculations by BIA as a valid method (Segal, et al., 1991).

Goncalves, et al. (2015) conducted a similar study utilizing 32 athletes. Measurements included: total body water, extracellular fluid, and intracellular fluid using deuterium and bromide dilution techniques as criterion against BIA. When comparing the different methods, no significant difference was found between BIA and the criterion measures. These results lead to the conclusion that bioelectrical impedance is a valid non-biased assessment of TBW and may well be the new standard (Gonçalves, Matias, Santos, Sardinha, & Silva, 2015).

Underwater Weighing

Underwater weighing, or hydrostatic weighing, has historically been the “gold standard” of body composition testing. Using the Archimedes principle, which states an “object’s loss of weight in water equals the weight of the volume of water it displaces, thus making the specific gravity the ratio of the weight of an object in air divided by its loss of weight in water. The loss of weight in water equals the weight in air minus the weight in water” (McArdle, Katch, & Katch, 2011). This method is known to be reliable, but very time inefficient, causing time delays and reducing the number of subjects that can be tested for body composition at a given time.

A study was conducted to determine the minimum number of trials necessary to establish “true” underwater weight during body density measurements on 86 college females. Nine to ten trials of underwater weight assessment were recorded for each subject. The group was used as a matter of convenience. The method used was to conduct underwater weighing on each subject 9-10 times per person. What they found was that as people became accustomed to the methods of expiration during the weighing that their weight continued to increase. This increased body density values by .001–.003 density units. These results were anticipated pre-investigation. In 42% of the assessments, the subject’s highest underwater weights were observed during the first five trials. The magnitude of error associated with these trials was considerably higher than for the last several trials. This was due to an 85% reduction in within-individual variability (Katch, 2013). This study shows that, not

only is a single test time consuming, but to have reliability in the testing method, ten tests must be conducted, and the first five will have a high error level, making them unusable. With this delay in time, a new gold standard needs to be established.

Air Displacement Plethysmography

Other methods have been compared with underwater weighing to seek a new standard in body composition testing. One popular method is air displacement plethysmography (BodPod). Fields et al. (2012) conducted a systematic review of the validity of air displacement plethysmography, more commonly known as a BodPod. The BodPod was compared to DEXA and hydrostatic weighing. Results of percent body fat indicated the BodPod and hydrostatic weighing results are within 1% for adults and children, whereas the BodPod and DEXA results are within 1% for adults and 2% for children. This study was conducted because few studies have compared BodPod results with multicompartment models results. The studies that have done a comparison suggest an underestimation of \approx 2–3 %BF by both the BodPod and hydrostatic weighing. Compared with four-compartment models, the BodPod underestimates percent body fat by \approx 2–3 % in adults and children. The conclusion found BodPod is a reliable and valid technique that can quickly and safely evaluate body composition in a wide range of subject types but lacks the accuracy of a four-compartment model.

Another study conducted by Utter et al. (2003) compared hydrostatic weighing in a collegiate wrestling population in hydrated and acutely dehydrated states. The study methods were to examine body composition by BodPod, hydrostatic weighing, and three-site skinfolds. The subjects were 66 NCAA Division I collegiate wrestlers. The subjects were tested before and after acute dehydration (2.6% reduction in body mass). The results yielded no statistically significant differences between BodPod and hydrostatic weighing for body density, percent body fat, and fat-free mass in the hydrated or the dehydrated states. This study found body density, percent body fat, and fat-free mass from the BodPod are similar compared with hydrostatic weighing during hydrated and acutely dehydrated states. This study provided evidence that BodPod is a reliable replacement for hydrostatic weighing in various conditions.

Another study examined the effect of covert subject actions on percent body fat measured by BodPod. The reason why they were examining this is the belief some athletes were using methods such as changing their breathing to manipulate the readings and to examine how these manipulations might be affecting other measurements. Subjects underwent body composition analysis by BodPod following the standard procedure using the manufacturer's guidelines. The subjects then underwent eight more measurements while performing the following intentional manipulations: 4 breathing patterns altering lung volume, foot movement to disrupt air, hand cupping to trap air, and heat and cold exposure before entering the chamber. The results demonstrated that subjects were able to covertly change their estimated BodPod body

composition value by altering breathing when compared with the standard condition (Tegenkamp, Clark, Schoeller, & Landry, 2011).

To accurately establish body volume (BV), air displacement plethysmography (BodPod) provides a standard comparably equivalent to hydrostatic weighing (Lohman & Going, 1993). This allows for a much more practical and less time-consuming measurement of assessing BV. One question is the validity of a BV measurement when measured thoracic gas is not achievable, either by subject error or lack of equipment. McCrory et al. (1998) analyzed the validity of BodPod using measured thoracic gas volume against BodPod using predicted thoracic volume equations. Studying 50 subjects, no significant differences were found between measured and predicted groups (mean difference \pm SE, 53.5 ± 63.3 ml). With these findings, it allows for the collection of many more subjects using predicted thoracic volume assessment (when measured thoracic volume could not be established).

Other researchers found similar results showing no significant differences between using predicted and measured thoracic volumes with BodPod measurements. Wagner (2015) conducted a similar study on 33 collegiate athletes and found predicted thoracic gas volume was not significantly different ($p = 0.343$) from measured thoracic gas volume. These results show BV measurements with a BodPod using predicted thoracic measurements are statistically equivalent to measured thoracic volumes.

Dual Energy X-Ray Absorptiometry

A newer trend that has been compared to underwater weighing is dual energy X-ray absorptiometry (DEXA). DEXA is quickly becoming the reference standard in body composition testing due to its ease of use, speed of testing, and high level of reliability and validity. Duren et al. (2008) stated the use of dual energy X-ray absorptiometry (DEXA) is the most popular method for calculating fat mass, lean mass, and bone tissue.

One study was completed to compare estimates of body composition in two ethnic groups, 31 black and 38 white females, 10 through 16 years of age, to establish accurate and precise laboratory standards for field measures of body composition. The method used was to examine DEXA scan values against corresponding values of fat-free mass and percent body fat from underwater weighing. These were determined using the two-compartment model of Siri, and these were corrected using the model of Lohman for white girls only. The results were the two-compartment model overestimated fat-free mass compared to estimates from DEXA for black girls, as did the corrected Lohman model for white girls. The two-compartment model significantly overestimated percent body fat in both white and black girls compared to corresponding estimates from DEXA. Because of this fact, DEXA values of percent body fat are typically greater than those from underwater weighing for subjects under approximately 24% body fat, but the converse occurs above 25% body fat. The inability of underwater weighing, using the two-compartment model, to account for the body composition in these girls can be corrected in

part by measuring the variables for a multicompartment model or more easily by using DEXA estimates of body composition. The results of the inaccuracies of underwater weighing method provide evidence for the need to replace underwater weighing as the “gold standard” and give DEXA the recognition as the more valid method and the new “gold standard” for body composition testing (Morrison, et al., 1994).

Glickman et al. (2004) conducted a study to examine the validity of DEXA for body composition. DEXA was originally only used as a method to measure bone density and total body composition. After improvements in software, DEXA can now determine abdominal fat mass. For this study, 65 adults aged 18-72 participated with DEXA to have their abdominal fat measured. Results from DEXA were then compared to computed tomography for abdominal fat mass in the L1-L4 region. DEXA showed excellent reliability among three different operators to determine total, fat, and lean body mass in the L1-L4 region. The DEXA was found to be a reliable and accurate method to determine abdominal obesity. This study lends further credit to DEXA as the new “gold standard” of body composition testing.

Body Composition Compartment Models

Siervo and Jebb (2010) reviewed the importance of various aspects of body composition in relation to establishing accurate body fat percentage. A framework was established with various models of body composition, all with limitations on their own. However, the importance of collecting as much

accurate data as possible was established in order to derive the most accurate percent body fat. The use of a multi-compartment model was shown to be more accurate than any single testing method. In order to derive a multi-compartment model, various data must first be collected. This may include fat mass (FM), fat-free mass (FFM), total body water (TBW), extracellular water (ECW), intracellular water, bone mineral content, and residual protein mass. This data can then be utilized by various multi-compartment models to derive accurate percent body fat (Siervo & Jebb, 2010).

Kopper et al. (1998) examined a three-compartment model against underwater weighing, deuterium oxide dilution, skinfold thickness measurements, bioelectrical impedance analysis, and a prediction equation based on the body mass index. Body fat was calculated using a three-compartment body composition model derived from body density and total body water percentage. The results showed correlation coefficients between the different methods were high and significant. This study shows that the single predictive methods have considerable mean and individual biases compared with the three-compartment model and all predictive methods underestimated body fat in the studied subjects. This information leads to the conclusion that a fully developed multi-compartment model would be the most accurate method of testing body composition as all single methods are far less valid. Future studies need to include the development of population-specific prediction formulas.

In a study that compared body fat percentage obtained from a four-compartment model with percent body fat from hydrodensitometry (using -deuterium) in 291 subjects, results showed there are differences between percent body fat measured by the four-compartment and two-compartment models. When validated against the reference four-compartment model, two-compartment models were found to be unsuitable for accurate measurements of percent body fat. These further provides evidence that an accurate four-compartment model is required to measure a valid and reliable percent body fat, and two-compartment models are too unreliable. (Deurenberg-Yap, Schmidt, Staveren, Hautvast, & Deurenberg, 2001)

Withers et al. (1998) compared two, three, and four-compartment models for analyzing body composition. The two-compartment model study consisted of fat mass (FM) and fat free mass (FFM). The three-compartment model consisted of fat mass, total body water, and fat free dry mass. The four-compartment model was comprised of fat mass, total body water (TBW), bone mineral content (BMC), and residual mass. These models were compared using equal groups of highly trained men (n=12), sedentary men (n=12), highly trained women (n=12), and sedentary women (n=12). For this study, all experiments were conducted when the subjects were fasting (twelve hours since last meal), normally hydrated, and had not exercised for 24 hours. To minimize fluid retention in women, they were not tested for seven days preceding menstruation or during menstruation. In order to minimize within-subject biological variability, the bone density and total body water tests were

administered on the same morning. Most of the subjects (n=34) had the DEXA measurements conducted the same morning as the other two tests, however 13 were rescheduled the following morning. One woman was not tested until 13 days later. Hydrodensitometry was used to evaluate bone density percent body fat via underwater weighing at residual volume. Fat free mass was estimated using the formula percent body fat (%BF) = (497.1/Body Density) - 451.9 (Brozek, Grande, Anderson, & Keys, 1963). DEXA scans were additionally taken and compared to underwater weighting results. Correlation coefficients between DEXA and underwater weighing of 1.0 were found for bone mineral content, 0.996 for fat, and 1.0 for lean tissue mass. Total body water was calculated with a deuterium dilution derived from a saliva sample collected from subjects. Their fat free mass was calculated using the assumption that 72% of the fat free mass is comprised of water in a normally hydrated person using the formula FFM (kg) = TBW (kg) / 72 * 100 (Withers, et al., 1998).

The two-compartment model was evaluated using fat mass and fat free mass. The assumptions were made that fat mass has a density of 0.9007 g/cm³ and fat free mass has a density of 1.1000 g/cm³ at 36°C. Percent body fat was then calculated using the formula %BF = (497.1/Body Density) – 451.9. Three compartment models add density to the calculation with the addition of total body water, assumed to have a density of 0.9937 g/cm³ and fat free mass becomes fat free dry mass, with density modified to 1.569 g/cm³ and percent body fat is calculated using the formula %BF = (211.5/Body Density) - 78.01 (TBW/body mass) - 134.8, where body density is defined as body volume /

body mass. The four-compartment model incorporates the additional use of bone mineral content, assumed at a density of 2.982 g/cm³ and residual mass, assumed a density of 1.404 g/cm³. The formula for the four-compartment model of percent body fat became %BF = (251.3 / Body Density) - 73.91 * (TBW / body mass) + 94.7 * (BMC / body mass) – 179.0 (Withers, et al., 1998).

The results for the comparison of the models revealed the overall mean of FFM to be 1.1075 g/cm³, significantly greater (P<0.001) than the two-compartment density assumption of 1.1000 g/cm³. Individual FFM densities ranged from 1.0974 g/cm³ to 1.1177 g/cm³. This resulted in overestimations of 0.9% and underestimations of 5.9% body fat. The results yielded evidence to support that two-compartment models compared to three-compartment models, for all groups, resulted in significantly greater means and variances (P<0.02) than those found between the three and four-compartment models (Withers, et al., 1998). No significant differences were found in the three-compartment versus the four-compartment models. Given these results, it is reasonable to infer the two-compartment model is significantly less accurate than a three or four compartment model, and thereby less useful for evaluating body fat percentage. The lack of significant differences between the three and four compartment models leads to the conclusion that the division of fat-free dry mass into residual mass and bone mineral content leads to little or no improvement in measurement of body fat percentage (Withers, et al., 1998).

Wang, et al. (2005) advanced research in the body composition field methodology and created a new formula to evaluate body composition. Wang et

al. created the formula $FM(kg) = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(\text{Body Mass})$ where Mo is equal to total body BMC $\times 1.0436$, a metric which is measured by DEXA. This method is able to utilize readily available metrics to create an accurate four-compartment model to evaluate fat mass in kilograms. This formula incorporates the same metrics used by Withers, et al. (1998) using body volume, total body water, bone mineral content, and body mass to derive a four-compartment model but removes body density in favor of only body volume, removing the redundancy of using body mass twice. The formula can easily be rewritten to $FM (kg) = 2.748 (BV) - 0.699 (TBW) + 1.129 (BMC \times 1.0436) - 2.051 (\text{Body Mass})$ for use with DEXA derived data. This formula can also be used to calculate body fat percentage where $\%BF = (FM / \text{Body Mass}) \times 100$ (Wang, Shen, Withers, & Heymsfield, 2005).

The Withers, et al. (1998) formula of $\%BF = (251.3 / \text{Body Density}) - 73.91 * (TBW / \text{body mass}) + 94.7 * (BMC / \text{body mass}) - 179.0$ and the Wang, et al. (2005) formula of $FM(kg) = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(\text{Body Mass})$ lend themselves to direct comparison. With body density equal to body volume / body mass and Mo equal to total body BMC $\times 1.0436$, both formulas incorporate the same metrics, BMC, body mass, BV, and TBW.

A study to assess the agreement of body fat and fat-free mass measured by simpler methods against the four-compartment model used 60 obese schoolchildren (defined by body mass index $\geq 95^{\text{th}} \text{ percentile}$) between the ages of 8y and 13y. Multicompartamental body composition was estimated using isotopic dilution, BodPod, DEXA, and anthropometric equations and compared

the results against a four-compartment model. The results showed isotopic dilution and anthropometric equations underestimated body fat in boys; while DEXA, BodPod, and anthropometric equations overestimated body fat in boys. All the equations underestimated body fat in girls. Isotopic dilution and DEXA two-compartment methods had the best agreement with the four-compartment model for both body fat and fat-free mass (Vergara, et al., 2014). This study lends further evidence that a four-compartment model is far more valid than the use of any single testing modality.

Methods for Evaluating Four Compartment Models

Wilson et al. (2012) expanded on the methodology of using a four-compartment model. Their objective was to simplify the process of establishing the four-compartment model by eliminating the need for deuterium and underwater weighing by instead measuring body protein using DEXA and BIA. The protein estimate from direct calibration protein derived from BIA water, bone mass, and body volume was compared to the Lohman (1993) and Wang (2005) equations—which derived protein content calculated from the data collected from the DEXA and BIA, and then was compared to the neutron activation analysis, which is considered the gold standard for measurement in vivo total body protein. The results of this study were that neutron activation analysis had the highest correlation, lowest root mean squared error, and fewest outliers with direct calibration protein, compared with the Lohman (1993) and Wang (2005) equations—derived protein. This evidence shows there are

simpler, and equally efficient methods of calculating body fat by using a four-compartment model without the complications and expense of underwater weighing and chemical compounds, such as deuterium.

With this new data, Wilson et al. (2012) were able to calculate a new method to assess body volume using the DEXA as well. With the lean mass, fat mass, and bone mineral content derived from the DEXA, Wilson et al. was able to derive the equation of body volume (with a GE-DEXA) = Fat/0.87+ Lean/1.072 -BMC/2.283 + 1.504, which converts the GE DEXA mass in kilograms to volume in liters (Wilson, et al., 2012). The use of this equation to evaluate BV without a separate testing modality would eliminate the need for an underwater weighing chamber or BodPod when utilizing a four-compartment body composition model.

Tinsley (2017) examined equations for estimation of body volume from DEXA scans to be used for body composition evaluation in modified four-compartment models. The design of the study used 48 recreationally active males and females who completed two pairs of identical assessments, which included a DEXA scan and single-frequency bioelectrical impedance analysis. Body volume and four-compartment equations were applied to the results to establish body composition. The results showed both body volume equations demonstrated excellent reliability but there was a significant difference between equations when a four-compartment model equation was used. The difference was 4.3 kg for lean mass and fat mass and 6.9% for body fat percentage. These results showed promise in the use of DEXA to establish

a single testing unit that produces a four-compartment model. Future studies are needed and include the continued study of body volume measurements.

Smith-Ryan et al. (2017) further developed a method for creating a four-compartment body composition model using DEXA for percent body fat, fat mass, and lean mass. These researchers sought to derive a new method of estimating body volume (Smith-Ryan, et al., 2017). BodPod is an industry standard in establishing body volume and a valid alternative to underwater weighing for the use of establishing body volume, arguably becoming the new gold standard for volume measurements (Lohman & Going, 1993). The Smith-Ryan et al. (2017) study focused on the validity and reliability of using DEXA for calculating body volume in comparison to BodPod and improving the calculation formula for using DEXA. When analyzing the body volume results of the DEXA and comparing it to results from BodPod, no significant differences were found with the Wilson et al. (2012) equation. Using the data from sub samples, Smith-Ryan et al. (2017) used inverse density coefficients and derived the formula of DEXA BV (L) = Fat/0.84+ Lean/1.03 -BMC/11.63 – 3.12 based on the formula of Wilson et al. (2012). With the additional research of validity and reliability of body volume calculated by DEXA being statistically equivalent to BodPod, there is opportunity to eliminate the need for underwater weighing and BodPod to calculate a four-compartment model using only DEXA and a total body water test. Using the DEXA to evaluate volume for a four-compartment model could potentially be the most accurate model, eliminating the need for any other tests

and saving hours of time per subject, as well as the expense of additional materials and equipment.

Further research would be needed to validate the use of a DEXA predicted BV model against a traditional four-compartment model. Both the Wilson, at al. (2012) formula and the Smith-Ryan (2017) formula need to be examined against BodPod results for validity and for use in the Withers, et al. (1998) and the Wang, et al. (2005) four-compartment body composition models.

CHAPTER III

METHODOLOGY

Experimental Design

Subjects were tested on three devices designed for obtaining body composition metrics: DEXA for lean body mass, bone mineral content, and fat mass; BodPod for body volume; and a bioimpedance spectroscopy (BIS) device using dual electrode tabs (SFB7) for total body water to examine the validity of two separate DEXA predicted body volume (BV) equations, Wilson, et al. (2012) and Smith-Ryan, et al. (2017), compared to measured BodPod BV. This study also compared two different formulas for establishing a four-compartment model, Withers, et al. (1998) and Wang, et al. (2005), using DEXA calculated body volumes. These models were compared to a four-compartment model using BodPod measured body volume.

Subjects

Subjects were informed, prior to arrival, to be fasted for at least: (1) 8 hours before testing, (2) 2 hours without water, (3) 24 hours without alcohol, (4) 24 hours without intensive exercise, and (5) be normally hydrated (calculated by urine specific gravity less than 1.030). At least 24 hours prior to any testing, subjects were given information and instruction about each of the different body composition methods they were to participate in.

Initial Assessment

Subjects arrived at the lab and completed informed consent documents (see Appendix B) and health history questionnaires to ensure all subjects were healthy as defined by meeting all inclusion criteria (see Appendix C). Female subjects signed documentation denoting they were not currently pregnant and that there was no possibility they could be pregnant prior to their scan (see Appendix A). Subjects with elevated resting heart rates (over 100 beats per minute) or high blood pressure (greater than 140/90) were disqualified from participation in the study. Subjects who self-reported a history of metabolic diseases, previous kidney, heart, or hydration issues were also disqualified from participation in the study. Subjects were then questioned to confirm they did not (1) have a large meal within eight hours of their visit, (2) consume alcohol within 24 hours of their visit, (3) drink any fluids for two hours prior to testing, or (4) participate in any hard, physical activity for 24 hours before testing.

Upon arriving to the lab, subjects self-obtained a urine sample in a standard medical-grade specimen cup for analysis by the research team. They were instructed to catch approximately half the cup mid-stream. The urine sample was tested for urine specific gravity and color to ensure normal hydration (defined as specific gravity less than 1.030). During the initial bathroom visit, the subjects were also asked to void their bowels (if possible) so that an accurate body mass could be measured. Subjects then had their height measured with a wall mounted measuring tape and speed square. Height data was recorded on data sheets with all measurements in centimeters (cm) (see

Appendix D). Subjects next had their weight measured via the BodPod calibrated scale and recorded on the data sheet in kilograms (kg).

Body Composition Testing

The body composition metrics of the subjects were measured utilizing a variety of different testing methods. Air displacement plethysmography (BodPod) was utilized to measure total body volume with both measured and predicted thoracic volumes. Dual energy x-ray absorptiometry (DEXA) was utilized to measure fat mass, lean mass, and bone mineral content. Finally, bioelectrical impedance spectroscopy (BIS) was utilized to measure total body water.

Bioelectrical Impedance Spectroscopy

Subjects were tested for body composition using bioelectrical impedance spectroscopy attached to silver chloride dual wet electrodes via SFB7 device (ImpediMed Limited, Queensland, Australia) (see Appendix E). Each contact point for wet electrode pads was: (1) shaved to be hair free, ensuring proper conduction, and (2) cleaned utilizing isopropyl alcohol. Subjects were positioned lying supine on a nonconductive athletic training table. Electrodes were placed on each limb, with the dual electrode placed at the styloid process and extended to the lunate on both hands and the distal tibia and talus of both feet allowing measurement of right whole-body water and left whole body water (see Appendix D). Measurements were immediately repeated. Dual-tabs were then

removed, and the site was cleaned with isopropyl alcohol. New dual-tabs were then applied, and measurements repeated twice more. Measurements were analyzed by Impedimed (see Appendix E) analytical software. Results were recorded (see Appendix D) and averaged to calculate TBW.

Air Displacement Plethysmography

Body composition assessment via air displacement plethysmography was measured using a Cosmed BodPod (COSMED USA, INC, Concord, CA) (see Appendix E). Subjects wore standardized, gender appropriate, compression garments and a swim cap (per manufacturer recommendations). Subjects were instructed to remove all metallic objects and jewelry for the remainder of the testing session. Subjects were then weighed on a calibrated BodPod scale and body mass was recorded. Subjects were then instructed to remain still and breathe normally while being tested. Additionally, lung volumes were measured during the BodPod test, utilizing the measured lung capacity scan settings. Subjects were instructed to remain still and breath according to the prompts on the computer guiding the lung measurement. Subjects that were unable to complete the measured thoracic body volume in five attempts had their predicted thoracic volume measurements recorded using BodPod Siri settings (as defined by manufacturer recommendations for subject population). Scans were repeated if body volume measurements differed by more than 25 ml. Total body mass, predicted thoracic volume or measured thoracic volume, first body volume, second body volume, and total body volume were recorded.

Dual Energy X-Ray Absorptiometry

Dual energy X-ray absorptiometry (DEXA), measurements were conducted utilizing a GE Lunar Prodigy Advance Bone Densitometry scanner (General Electric Company, Cincinnati, OH) (see Appendix E). Subjects wore standardized clothing (medical scrubs). Subjects were again reminded to ensure all metal and jewelry had been removed. All scans were performed utilizing the total body scan. Subjects were placed upon the table symmetrically with feet and knees secured together with Velcro straps. Subjects were scanned using the total body option from the top of their head to the bottom of their feet. Data of total body mass, lean body mass, and bone mineral content were recorded (see Appendix D).

Data Collection

Data was collected following each individual test. Data was stored on each individual testing device for future recall, as well as collected on a data sheet, and stored in electronic data Microsoft Excel (Microsoft Corp. Seattle, WA, version 2016) (see Appendix E) sheets (see Appendix D). Data for the bioelectrical impedance spectroscopy (BIS) included left body water, repeated measurement, right body water, repeated measurement, reposition measurements, and reposition repeated measurements. Data for the BodPod included total body mass, predicted thoracic volume or measured thoracic volume, first body volume, second body volume, and total body volume. Data

for the DEXA scanner included total body mass, lean body mass, and bone mineral content.

Tester Reliability

Test/re-test reliability was conducted with each device. Reliability testing for DEXA was conducted on 16 subjects, tested on three occasions in a single week at the same time of day, Monday, Wednesday, and Friday for lean mass, fat mass, percent body fat, and bone mineral content. Intraclass correlation coefficients (ICC) were calculated using the two-way random effects model with absolute agreement for all four variables tested: LM, FM, %BF, and BMC [ICC (3,1)]. The ICC of $r = 0.99$ indicated excellent test/re-test reliability for all variables. SFB7 measurements were conducted then immediately repeated. Electrodes were removed and replaced, and measurements were again conducted then immediately repeated during the same session. All four values were recorded for analysis with an average being generated for each measurement. Intraclass correlation coefficients (ICC) were calculated using the two-way random effects model with absolute agreement [ICC (2,1)]. The ICC of $r = 0.99$ indicated excellent test/re-test reliability. Reliability testing for the BodPod was conducted on 16 subjects, tested on three occasions in a single week at the same time of day, Monday, Wednesday, and Friday. Intraclass correlation coefficients (ICC) were calculated using the two-way random effects model with absolute agreement [ICC (3,1)]. The ICC of $r = 0.99$ indicated excellent test/re-test reliability.

Data Analysis

Data was extracted from each of the body composition testing devices for total body mass, lean body mass, body fat, and body fat percentage. For devices that did not include a body fat percentage result, the formula of body fat divided by total body mass was used to calculate body fat percentage. To test for continuity on the same device, the formula of 1 - (lean body mass divided by total body mass) to calculate percent body fat was utilized.

All statistical tests were conducted using IBM SPSS Statistics software (Armonk, NY, version 23) and formatted using Microsoft Excel 2016 (Seattle, WA, version 2016) (see Appendix E). Variables were analyzed for normality using a Shapiro-Wilk normality test. Values found to be normally distributed were defined by normality test $p > .05$. Summary statistics for normally distributed demographic items were analyzed using descriptive data statistics and reported as means and standard deviations. These included height, weight, Wilson, et al. (2012) body volume formula, Smith-Ryan, et al. (2017) body volume formula, and BodPod measured body volume values. Data analysis for Wilson, et al. (2012) body volume formula, Smith-Ryan, et al. (2017) body volume formula, and BodPod measured body volume values was conducted using a single factor ANOVA for variance analysis and paired two sample t-Test to identify paired differences.

Values found to not be normally distributed were defined by normality test $p \leq .05$. Summary statistics for not normally distributed demographic items were analyzed using descriptive data statistics and reported as medians and

range. These included age, BMI, total body water, Withers, et al. (1998) formula utilizing BodPod measured values, Withers, et al. (1998) formula utilizing Wilson, et al. (2012) predicted values, Withers, et al. (1998) formula utilizing Smith-Ryan, et al. (2017) predicted values, DEXA measured percent body fat, Wang, et al. (2005) formula utilizing BodPod measured values, Wang, et al. (2005) formula utilizing Wilson, et al. (2012) predicted values, Wang, et al. (2005) formula utilizing Smith-Ryan, et al. (2017) predicted values, and DEXA measured fat (kg). Data analysis for Withers, et al. (1998) formula utilizing BodPod measured values, Withers, et al. (1998) formula utilizing Wilson, et al. (2012) predicted values, Withers, et al. (1998) formula utilizing Smith-Ryan, et al. (2017) predicted values, DEXA measured percent body fat, Wang, et al. (2005) formula utilizing BodPod measured values, Wang, et al. (2005) formula utilizing Wilson, et al. (2012) predicted values, Wang, et al. (2005) formula utilizing Smith-Ryan, et al. (2017) predicted values, and DEXA measured fat (kg) was conducted using Friedman test for variance analysis and Wilcoxon signed-rank test to identify paired differences.

CHAPTER IV

RESULTS

Normality

A normality test was conducted on all applicable variables and formula results pertaining to this study (Table 1). Results indicated that all variables and formulas were normal, as defined by a significance greater than .05, with the exception of age, BMI, total body water, %BF of Smith-Ryan/Withers, DEXA based %BF, and all FM (kg) measurements.

Table 1 - Normality

Tests of Normality	Kolmogorov-Smirnov^a	Shapiro-Wilk
	Sig.	Sig.
Age	.000	.000
BMI	.200*	.047
Weight	.200*	.258
Height	.101	.095
Total Body Water	.000	.001
DEXA BV (L) = (Wilson, et al. 2012)	.200*	.306
DEXA BV (L) = (Smith-Ryan, et al. 2017)	.200*	.318
BodPod Measured	.200*	.224
%BF = (BodPod/Withers)	.200*	.188
%BF = (Wilson/Withers)	.200*	.079
%BF = (Smith-Ryan/Withers)	.008	.004
Dexa %BF	.200*	.031
FM(kg) = (BodPod/Wang)	.003	.001
FM(kg) = (Wilson/Wang)	.001	.000
FM(kg) = (Smith-Ryan/Wang)	.000	.000
Dexa Fat (kg)	.000	.000

*. This is a lower bound of the true significance.

Shaded regions fail to show normality

Subject Data

Subject data included 90 healthy adults (Table 2), 50 females and 40 males, aged 18 to 66 years (median 23 years), BMI 18 to 34 (median 25), weight 45 to 115 kg (mean 73.64 ± SD 14.35 kg), height 150 to 191 cm (171.07 ± 9.98 cm), total body water 25 L to 63 L (median 37.58 L), who volunteered from a sample of convenience.

Table 2 – Subject Descriptive Data

Total Subject Descriptive Data					
	<i>Age (yrs)</i>	<i>BMI</i>	<i>Weight (kg)</i>	<i>Height (cm)</i>	<i>Total Body Water</i>
Mean	28.59	25	73.64	171.07	39.99
Median	23	25	73.08	169.23	37.58
Standard Deviation	12.53	3	14.35	9.98	9.68
Range	48	16	69.33	40.64	37.70
Minimum	18	18	45.31	149.86	25.09
Maximum	66	34	114.63	190.50	62.79
Count	90	90	90	90	90
Female Only Descriptive Data					
	<i>Age (yrs)</i>	<i>BMI</i>	<i>Weight (kg)</i>	<i>Height (cm)</i>	<i>Total Body Water</i>
Mean	28.10	25	66.34	163.94	33.18
Median	22.5	24	63.99	163.83	33.47
Standard Deviation	12.88	4	11.82	6.04	4.31
Range	48	16	51.84	27.94	18.78
Minimum	18	18	45.31	149.86	25.09
Maximum	66	34	97.14	177.80	43.87
Count	50	50	50	50	50
Male Only Descriptive Data					
	<i>Age (yrs)</i>	<i>BMI</i>	<i>Weight (kg)</i>	<i>Height (cm)</i>	<i>Total Body Water</i>
Mean	29.20	26	82.77	179.97	48.51
Median	23	26	80.88	180.34	48.49
Standard Deviation	12.21	3	11.86	5.92	7.53
Range	47	13	53.77	26.04	30.38
Minimum	19	19	60.86	164.47	32.41
Maximum	66	32	114.63	190.50	62.79
Count	40	40	40	40	40

Body Volume Results

All subjects were measured in the BodPod on measured thoracic settings. Of the 90 subjects, 60 completed measured thoracic volumes and 30 were unable to complete within five attempts. The 30 that were unable to successfully complete measured thoracic volume utilized the predicted Siri model (as defined by manufacturer recommendation for the population). BodPod data was collected and used as a standard for comparison to the DEXA based body volume formulas; DEXA BV (L) = Fat/0.87 + Lean/1.072 - BMC/2.283 + 1.504 (Wilson et al. 2012) and DEXA BV (L) = Fat/0.84 + Lean/1.03 - BMC/11.63 - 3.12 (Smith-Ryan, et al., 2017). BodPod measured body volume (Table 3) yielded a mean of 70.36 ± 13.85 L, Wilson, et al. (2012) yielded 70.88 ± 13.54 L, and Smith-Ryan, et al. (2017) yielded 70.02 ± 14.23 L.

Table 3 – Body Volume Descriptive Data

Body Volume Descriptive Data			
	BodPod Measured	Wilson, et al. 2012	Smith-Ryan, et al. 2017
Mean	70.36	70.88	70.02
Standard Deviation	13.85	13.54	14.23
Count	90	90	90

An ANOVA (Table 4) was conducted comparing the three body volume groups; BodPod measured, the results of the formula from Wilson, et al. (2012), and the results of the formula from Smith-Ryan, et al. (2017). Results of the ANOVA yielded a P-value of .915, indicating no significant variance among the three body volume groups.

Table 4 – ANOVA: Body Volume

Anova: Single Factor - Body Volume						
SUMMARY						
Groups	Count	Sum	Average	Variance		
BodPod Measured	90	6332.14	70.36	191.91		
DEXA BV (L) (Wilson, et al. 2013)	90	6379.41	70.88	183.29		
DEXA BV (L) (Smith-Ryan, et al. 2017)	90	6301.52	70.02	202.45		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	34.22	2	17.11	0.09	0.915	3.030
Within Groups	51411.10	267	192.55			
Total	51445.31	269				

A paired t-Test (Table 5) was then conducted to compare both the Wilson, et al. (2012) and Smith-Ryan, et al. (2017) formulas to the measured BodPod values. The paired difference between BodPod measured and Wilson, et al. (2012) showed Wilson, et al. (2012) higher than BodPod by 0.53 ± 0.89 L. The paired difference between BodPod measured and the Smith-Ryan, et al. (2017) formula showed BodPod measured body volumes 0.34 ± 0.85 L higher than the Smith-Ryan, et al. (2017). The paired difference between Wilson, et al. (2012) and the Smith-Ryan, et al. (2017) formula showed Wilson, et al. (2012) body volumes 0.87 ± 0.71 L higher than the Smith-Ryan, et al. (2017). The significance levels between all pairs were approximately $p \leq 0.001$, indicating there was a significant difference between each pairing.

Table 5 – Volume t-Test Paired Differences

Body Volume Paired Samples Test	Paired Differences				Sig. (2-tailed)	
	Mean	Std. Deviation	Interval of the			
			Lower	Upper		
BodPod Measured - (Wilson, et al. 2012)	-0.53	0.89	-0.71	-0.34	.000	
BodPod Measured - (Smith-Ryan, et al. 2017)	0.34	0.85	0.16	0.52	.000	
(Wilson, et al. 2012) - (Smith-Ryan, et al. 2017)	0.87	0.71	0.72	1.02	.000	

Withers Percent Body Fat Formula

The Withers, et al. (1998) formula of %BF = (251.3 / BD) - 73.91 * (TBW / body mass) + 94.7 * (BMC / body mass) – 179.0 was used to evaluate percent body fat and compared to DEXA derived percent body fat for comparison standard. Body volume was utilized (as part of the body density) from the measured BodPod volumes, the Wilson, et al. (2012) formula, and Smith-Ryan, et al. (2017) formula. The resulting median (Table 6) of the DEXA derived percent fat was 26.37% with a range of 33.87%. The resulting median of the BodPod and Withers, et al. (1998) formula was 26.37% with a range of 35.87%. The Wilson, et al. (2012) formula and Withers, et al. (1998) formula median was 26.19% with a range of 39.79%. The Smith-Ryan, et al. (2017) formula and Withers, et al. (1998) formula median was 22.44% with a range of 41.02%.

Table 6 – Percent Body Fat Descriptive Statistics

%BF Descriptive Data				
	Dexa %BF	%BF (BodPod/Withers)	%BF (Wilson/Withers)	%BF (Smith-Ryan/Withers)
Median	26.37	25.09	26.19	22.44
Range	33.87	35.87	39.79	41.02
Minimum	11.95	8.34	8.78	3.01
Maximum	45.82	44.20	48.57	44.03
Count	90	90	90	90

A Friedman test (Table 7) was conducted to examine variance between the four groups; BodPod/Withers, et al. (1998), Wilson, et al. (2012)/Withers, et al. (1998), Smith-Ryan, et al. (2017) /Withers, et al. (1998) formulas and DEXA %BF. The results yielded an asymptotic significance of $\leq .001$, indicating there was a significant variance among the four %BF values.

Table 7 – %BF Friedman Test

%BF Friedman Ranks	Mean Rank	Test Statistics ^a	
Dexa %BF	3.08	N	90
%BF (BodPod/Withers)	2.14	Chi-Square	101.187
%BF (Wilson/Withers)	3.22	df	3
%BF (Smith-Ryan/Withers)	1.56	Asymp. Sig.	.000
		a. Friedman Test	

A Wilcoxon signed-rank test (Table 8) was then conducted to evaluate the BodPod/Withers, et al. (1998), Wilson, et al. (2012)/Withers, et al. (1998), Smith-Ryan, et al. (2017)/Withers, et al. (1998) and DEXA %BF in paired comparisons. The paired difference between DEXA %BF and BodPod/Withers was BodPod/Withers showed a significant difference lower than DEXA %BF ($z = -0.498$, $p \leq .001$). The paired difference between DEXA %BF and Wilson/Withers showed no significant difference ($z = -1.080$, $p = 0.280$). When comparing the paired difference between DEXA %BF and Smith-Ryan/Withers, Smith-Ryan/Withers showed a significant difference lower than DEXA %BF ($z = -7.281$, $p \leq .001$).

Among the formulas, the paired difference between Wilson/Withers and BodPod/Withers was calculated. BodPod/Withers showed a significant difference lower than Wilson/Withers ($z = -6.178$, $p \leq .001$). The paired difference between BodPod/Withers and Smith-Ryan/Withers showed Smith-Ryan/Withers a significantly lower than BodPod/Withers ($z = -5.052$, $p \leq .001$). The paired difference between Wilson/Withers and Smith-Ryan/Withers showed Smith-Ryan/Withers had a significantly lower than Wilson/Withers ($z = -7.567$, $p \leq .001$).

Table 8 – Percent Body Fat Wilcoxon Signed-Rank Test

%BF Wilcoxon Signed-Rank Test	Z	Asymp. Sig. (2-tailed)
%BF (BodPod/Withers) - Dexa %BF	-5.498 ^b	.000
%BF (Wilson/Withers) - Dexa %BF	-1.080 ^c	.280
%BF (Smith-Ryan/Withers) - Dexa %BF	-7.281 ^b	.000
%BF (Wilson/Withers) - %BF (BodPod/Withers)	-6.178 ^c	.000
%BF (Smith-Ryan/Withers) - %BF (BodPod/Withers)	-5.052 ^b	.000
%BF (Smith-Ryan/Withers) - %BF (Wilson/Withers)	-7.567 ^b	.000

a. Wilcoxon Signed Ranks Test
b. Based on positive ranks.
c. Based on negative ranks.

Wang Fat Mass Formula

The Wang, et al. (2005) formula of FM (kg) = $2.748 (\text{BV}) - 0.699 (\text{TBW}) + 1.129 (\text{BMC}) - 2.051 (\text{Body Mass})$ was used to evaluate body fat and

compared to DEXA derived body fat for a comparison standard. Body volume was used from the measured BodPod volumes, the Wilson, et al. (2012) formula, and Smith-Ryan, et al. (2017) formula. The resulting median (Table 9) of the DEXA derived fat mass was 17.74 kg with a range of 32.43 kg. The resulting median of the BodPod and Wang, et al. (2005) formula was 15.69 kg with a range of 33.49 kg. The Wilson, et al. (2012) formula and Wang, et al. (2005) formula median was 17.40 kg with a range of 34.20 kg. The Smith-Ryan, et al. (2017) formula and Wang, et al. (2005) formula median was 14.39 kg with a range of 39.17 kg.

Table 9 – Fat Mass (kg) Descriptive Statistics

FM (kg) Descriptive Data				
	Dexa Fat (kg)	FM(kg) (Bod Pod/Wang)	FM(kg) (Wilson/Wang)	FM(kg) (Smith-Ryan/Wang)
Median	17.74	15.69	17.40	14.39
Range	32.43	33.49	34.20	39.17
Minimum	9.62	4.69	6.29	1.14
Maximum	42.05	38.17	40.49	40.31
Count	90.00	90.00	90.00	90.00

A Friedman test (Table 10) was conducted to examine variance between the four groups; DEXA fat (kg), BodPod/Wang, et al. (2005), Wilson, et al. (2012)/Wang, et al. (2005), and Smith-Ryan, et al. (2017)/Wang, et al. (2005). The results yielded an asymptotic significance of $p \leq .001$, indicating there was a significant variance among the four FM (kg) values.

Table 10 – Fat Mass (kg) Friedman Test

FM (kg) Friedman Ranks	Mean Rank	Test Statistics^a	
Dexa Fat (kg)	3.43	N	90
FM(kg) (Bod Pod/Wang)	2.00	Chi-Square	135.693
FM(kg) (Wilson/Wang)	3.09	df	3
FM(kg) (Smith-Ryan/Wang)	1.48	Asymp. Sig.	.000
		a. Friedman Test	

A Wilcoxon signed-rank test (Table 11) was then conducted to analyze the BodPod/Wang, et al. (2005), Wilson, et al. (2012)/Wang, et al. (2005), Smith-Ryan, et al. (2017)/Wang, et al. (2005) and DEXA fat (kg) in paired comparisons. The paired difference between DEXA fat (kg) and BodPod/Wang was BodPod/Wang showed a significant difference lower than DEXA fat (kg) ($z = -7.132$, $p \leq .001$). The paired difference between DEXA fat (kg) and Wilson/Wang showed Wilson/Wang a significantly lower than DEXA fat (kg) ($z = -2.372$, $p = .018$). The paired difference between DEXA fat (kg) and Smith-Ryan/Wang was Smith-Ryan/Wang showed a significant difference lower than DEXA fat (kg) ($z = -7.941$, $p \leq .001$).

Among the formulas, the paired difference between Wilson/Wang and BodPod/Wang was BodPod/Wang showed a significant difference lower than Wilson/Wang ($z = -5.816$, $p \leq .001$). The paired difference between BodPod/Wang and Smith-Ryan/Wang showed Smith-Ryan/Wang a significantly lower than BodPod/Wang ($z = -4.690$, $p \leq .001$). The paired difference between

Wilson/Wang and Smith-Ryan/Wang showed Smith-Ryan/Wang a significantly lower than Wilson/Wang ($z = -7.381$, $p \leq .001$).

Table 11 – Fat Mass (kg) Wilcoxon Signed-Rank Test

FM (kg) Wilcoxon Signed-Rank Test	Z	Asymp. Sig. (2-tailed)
FM(kg) (Bod Pod/Wang) - Dexa Fat (kg)	-7.132 ^b	.000
FM(kg) (Wilson/Wang) - Dexa Fat (kg)	-2.372 ^b	.018
FM(kg) (Smith-Ryan/Wang) - Dexa Fat (kg)	-7.941 ^b	.000
FM(kg) (Wilson/Wang) - FM(kg) (Bod Pod/Wang)	-5.816 ^c	.000
FM(kg) (Smith-Ryan/Wang) - FM(kg) (Bod Pod/Wang)	-4.690 ^b	.000
FM(kg) (Smith-Ryan/Wang) - FM(kg) (Wilson/Wang)	-7.381 ^b	.000

a Wilcoxon Signed Ranks Test
b Based on positive ranks.
c Based on negative ranks.

CHAPTER V

DISCUSSION

Body Volume Values

The first goal of this research was to evaluate the merits of using a DEXA machine to establish body volume. The formulas evaluated to establish BV using a DEXA were the Wilson, et al. (2012) and Smith-Ryan, et al. (2017) formulas. These formulas were then compared to the measured values from the BodPod for significant differences. If the DEXA-based formulas for measuring BV were found statistically equivalent to the measurements of the BodPod, then logically the DEXA becomes a viable method to replace other BV devices, such as BodPod and underwater weighing. Creating an alternative methodology of measuring BV with only a DEXA would allow the use of predicted BV values in a four-compartment model and save time by eliminating the need for a second device for the measure of BV.

Hypothesis 1

The first hypothesis of this study was the predicted body volume equations would be statistically equivalent to the measurements of BodPod. Evidence was found to support this hypothesis. No significant differences were found between the three groups containing both predicted body volume equations and the BodPod measured volume. An ANOVA conducted between the three body volume groups indicating no statistical difference between the formulas and the

measured BodPod results ($p = 0.915$). This was consistent with the first hypothesis. These results were expected, as the formulas were well researched, accounted for predicted densities of each metric, and were derived from linear regression. The finding of no statistical difference granted merit to the possibility of replacing BodPod measured body volume with DEXA based formulas.

Hypothesis 2

The second hypothesis was the Ryan-Smith, et al. (2017) formula results would have a smaller variation when compared to measured BodPod values than the Wilson et al. (2012) formula results. Evidence was found to support this hypothesis. Paired sample t-Tests were conducted comparing the Wilson, et al. (2012) formula and the Smith-Ryan, et al. (2017) formula to the measured BodPod body volume. Wilson et al. (2012) formula body volume calculated 0.53 ± 0.89 L greater than the measured BodPod volume, while the Smith-Ryan, et al. (2017) formula calculated 0.34 ± 0.85 L less than measured BodPod values. As Smith-Ryan, et al. (2017) mean difference to the measured BodPod results were smaller compared to the Wilson et al. (2012) formulas results, the Smith-Ryan, et al. (2017) formula is a statistically better choice to replace measured BodPod values for use in four-compartment body composition models. These results are consistent with the second hypothesis.

The findings of this hypothesis followed a logical progression as Smith-Ryan, et al. (2017) researched the Wilson, et al. (2012) formula and expanded

upon it. Smith-Ryan, et al. (2017) did have a smaller mean difference, and the standard deviation put it within the same range of BodPod, as did the Wilson, et al. (2012) formula, but there was still a difference. In this study, it was not found that these formulas were a perfect match to BodPod measured body volumes. The difference in body volumes could be a result of sample of the constant needing further evaluation.

Statistically, it appeared that these formulas, particularly Smith-Ryan, et al. (2018), were viable replacements for BodPod measured body volume. It then became imperative to evaluate the use of these formulas in a practical setting. While no statistical variance was found, and the difference in measured and predicted body volumes was as low as 0.34 ± 0.85 L, there was still a difference. It remained to be seen if this small mean difference yields a clinical significance when utilized to evaluate body composition in a four-compartment body composition model.

Body Composition Formulas

The second goal of this research was to evaluate the predicted body volume values derived from the formulas and the measured BodPod values for use in two separate four-compartment body composition model formulas, Withers, et al. (1998) and Wang, et al. (2005). Validity of using predicted body volume measurements, to arrive at a statistically equivalent value compared to the multi testing modality that utilizes the BodPod, needed to be assessed. The results

from these formulas were compared to the body composition results given by the DEXA factory.

Both four-compartment models utilize the same metrics to evaluate a four-compartment model; bone mineral content, total body mass, body volume, and total body water. Utilizing similar formulas with the same metrics allowed each formula to be evaluated with only a BIS for TBW, DEXA for BMC, and a DEXA utilizing predicted BV formulas measured BodPod values. These results allowed evaluation of the statically equivalent predicted BV values for clinical significance and practical use.

Hypothesis 3

The third hypothesis of the research was that predicted body volume four-compartment models would be statistically equivalent to a multi-system four-compartment model. Evidence was found to reject this hypothesis. Examining the difference between the pairing of the Smith-Ryan/Withers formula and the BodPod/Withers formula yielded a statistically significant difference of Smith-Ryan/Withers formula (median = 22.44, range = 41.02) lower than the BodPod/Withers formula (median = 25.09, range 35.87) ($p < .001$). This variation is a direct result of the 0.34 ± 0.85 L BV difference between the Smith-Ryan, et al. (2017) formula and the measured BodPod values. All other values were consistent in the Withers, et al. (1998) formula.

The reason for this variation in the formula could be an intolerance to the variation of the body volume or the need of an updated four-compartment

model formula. Because the Withers, et al. (1998) formula is $\%BF = (251.3 / BD) - 73.91 * (TBW / \text{body mass}) + 94.7 * (BMC / \text{body mass}) - 179.0$, and BD is defined as body mass / BV, which can be redefined as $(251.3 * BV) / \text{body mass}$, as BV increases, the total value of $(251.3 / BD)$ increases, which causes a greater difference in the resulting $\%BF$. Because the Smith-Ryan, et al. (2017) mean is higher than the measured BodPod mean, results varied significantly. These results lead to the conclusion that Smith-Ryan, et al. (2017) is unsuitable for use in the Withers, et al. (1998) formula.

These results illustrated the clinical significance of the predicted body volume difference compared to measured BodPod values. While there was no statistical variance in the body volume measurements, there was a statistically significant difference once the body volume values were placed into practical use as a metric for calculating $\%BF$. When comparing the results from Smith-Ryan, et al. (2018) minimum values to those of the measured BodPod minimum values, the Smith-Ryan, et al. (2018) formula underestimated the $\%BF$ by 8% utilizing the Withers, et al. (1998) formula values, and 11% with the Wang, et al. (2005) formula values. At the minimum values, this could result in a healthy female of athletic or lean $\%BF$ being misdiagnosed and mistreated as dangerously underweight. Conversely, the same error in the upper $\%BF$ range could also result in a misdiagnosis of a slightly overweight individual, needing only mild nutritional and fitness changes, being diagnosed as morbidly obese and being prescribed unnecessary medications for weight loss.

Hypothesis 4

The fourth hypothesis stated there would be a statistical difference between DEXA measured body fat compared to the Withers, et al. (1998) formula and the Wang, et al. (2005) formula, using both predicted and measured body volumes. This hypothesis was based on the premise that if a four-compartment model was truly more accurate, then there would be a statistical difference compared to the DEXA. Further research revealed this assumption to be accurate. Research into the validity of DEXA based %BF versus a four-compartment model found a significant difference in that DEXA consistently underestimated %BF by a mean of 1.7% compared to a four-compartment model ($p < .001$) (Ploeg, Withers, & Laforgia, 2003). Evidence was found to accept this hypothesis for Smith-Ryan, et al. (2017) and measured BodPod, but not Wilson, et al. (2012).

Friedman Ranks test yielded a significant variance among the four groups ($p \leq .001$) which was consistent with the fourth hypothesis. The pairings of BodPod/Withers and Smith-Ryan/Withers were both found to be statistically significant when compared to DEXA %BF ($p < .001$). Both pairings were found to be statistically lower than DEXA %BF, indicating Smith-Wilson, et al. (2017) may be an acceptable substitution in a four-compartment model for the use of BV. However, no statistically significant difference was found between the Wilson/Withers pairing and the DEXA %BF ($p = .280$), which was not consistent with the fourth hypothesis. This result was anticipated as a likely possibility due

to the Wilson, et al. (2012) BV formula not being thoroughly tested. This formula may not be ready for use in a four-compartment model to test percent body fat.

Wilson, et al. (2012) Concerns

The Wilson, et al. (2012) formula was derived principally on scientific theory, based on previous density studies of body composition metrics but, while very sound in theory, it had not been thoroughly tested. This formula was preliminary work of an idea not previously created. However, it remained virtually untested, as it was created using known densities of body metrics and a thorough understanding of DEXA based measurements, but no traditional testing. The conformational study after the creation of the theoretical formula by Wilson, et al. (2012) consisted of only 11 subjects and the only data analysis conducted was a simple correlation. This formula had great promise but needed thorough study to verify the constants had real-world applications and consistency to applicable samples. This study was a significant beginning but needed more data to analyze the formula (that likely contributed to the variance shown in this study) that resulted in a body volume of 0.53 ± 0.89 L higher than the measured BodPod values.

Smith-Ryan, et al. (2012) Concerns

The Smith-Ryan, et al. (2017) formula originally had 127 subjects used to create the formula, but only 27 to cross-validate and 40 to verify reliability. Also, the mean age: 35.8 ± 9.4 years, which was 7.2 ± 3.1 higher than this study. This

age variance could account for the difference in body volume. As younger adults traditionally have approximately 7.5% less body fat at 28 than at 35 (St-Onge & Gallagher, 2010), the body volume would be lower at the same weight, as the density of lean muscle is $1.06 \times 10^3 \text{kg/m}^3$ and the density of the fat tissue is $9.30 \times 10^2 \text{kg/m}^3$ (Martin, Daniel, Drinkwater, & Clays, 1994). Also, the number of males and females was not reported separately. As men have higher lean mass and lower percent body fat on average than females, the distribution could be skewed due to one gender. In addition, the BMI distribution of the Smith-Ryan (2017) study was 19.9 to 45.6, allowing for morbidly obese subjects. It has been documented that in obese subjects, the BodPod error underestimates by 8.51% (Lowry & Tomiyama, 2015). Using this data to create the body volume formula would create a formula that was approximately equal near the normal BMI measured values, but the obese measurements would place additional erroneous low values in the data set and create a formula that would measure BV too high, as was the case in this research.

Hypothesis Results

After analyses among the hypotheses of this study, the results found:

1. The predicted body volume equations will be statistically equivalent to the measurements of BodPod. Evidence was found to accept.
2. The Ryan-Smith, et al. (2017) formula will have a smaller variation when compared to BodPod values than the Wilson, et al. (2012) formula. Evidence was found to accept.

3. Predicted body volume four-compartment modes will be statistically equivalent to the multi-system model. Evidence was found to reject this hypothesis.
4. There would be a statistical difference between DEXA measured body fat compared to the Withers, et al. (1998) formula and the Wang, et al. (2005) formulas, using both predicted and measured body volumes. Evidence was found to accept for Smith-Ryan, et al. (2017) and BodPod, but not for Wilson, et al. (2012).

Formula Changes

The mean difference between predicted body volume equations and measured BodPod body volume resulted in the predicted BV equations being unsuitable for use in a four-compartment model, creating a need to re-evaluate the formulas. The Smith-Ryan, et al. (2017) formula was selected to be adjusted due to it being the most current research and based from the previous research of Wilson, et al. (2012). Paired analysis between each subject's measured BodPod values and Smith-Ryan, et al. (2017) values were conducted and averaged. Median, mean, and mode were all evaluated. The constant was then adjusted and analyzed, comparing BodPod measured values to new predicted values, as well as new pairings utilizing the Withers, et al. (1998) and Wang, et al. (2005) formulas. The formula was modified from DEXA BV (L) = Fat / 0.84 + Lean / 1.03 – BMC / 11.63 – 3.12 and changed to DEXA BV (L) = Fat / 0.84+ Lean / 1.03 – BMC / 11.63 – 2.78. Statistical procedures were then

replicated for the new formula. Normality was first conducted yielding similar results as the previous formulas (Table 12).

Table 12 - Doernte Normality

Tests of Normality	Kolmogorov-Smirnov	Shapiro-Wilk
	Sig.	Sig.
BodPod Measured	.200*	.224
Doernte BV	.200*	.318
%BF BodPod/Withers	.200*	.188
%BF Doernte/Withers	.003	.004
FM(kg) Bod Pod/Wang	.003	.001
FM(kg) Doernte/Wang	.000	.000

*. This is a lower bound of the true significance.
Shaded regions fail to show normality□

Descriptive statistics (Table 13) comparing the new formula to measured BodPod values yielded BodPod with a mean of 70.357 ± 13.853 L and the new formula with a mean of 70.357 ± 14.228 L.

Table 13 – Doernte Paired Sample Statistics

Paired Samples Statistics	Mean	Std. Deviation	Std. Error Mean	N
BodPod Measured	70.357	13.853	1.460	90
Doernte BV	70.357	14.228	1.500	90

A correlation (Table 14) was then conducted between measured BodPod values and the new modified formula yielding a correlation of 0.999 indicating a very high correlation.

Table 14 – Doernte Correlation

	N	Correlation	Sig.
BodPod Measured & Doernte BV	90	.999	.000

Paired samples t-Test (Table 15) conducted between measured BodPod values and modified predicted BV yielded a difference of 0.000 ± 0.854 L ($p = 0.998$).

Table 15 – BodPod - Doernte Paired Differences

Paired Samples Test	Paired Differences				Sig. (2-tailed)	
	Mean	Std. Deviation	95%			
			Lower	Upper		
BodPod Measured - Doernte BV	0.000	0.854	-0.179	0.179	0.998	

A Wilcoxon signed rank test (Table 16) was then conducted between both Withers, et al. (1998) and Wang, et al. (2005) formulas, utilizing BodPod measured BV and reformulated predicted BV. There were no statistical differences in any formula between BodPod groups or reformulated groups.

Table 16 – Doernte Wilcoxon Signed-Rank Test

Wilcoxon Signed Ranks Test	%BF Doernte/Withers - BodPod/Withers	FM(kg) Doernte/Wang - Bod Pod/Wang
Z	-1.257 ^b	-.682 ^b
Asymp. Sig. (2-tailed)	.209	.495

b. Based on positive ranks.

Descriptive data (Table 17) of both four-compartment models, utilizing measured BodPod values and reformulated predicted BV, were conducted and listed below.

Table 17 – Doernte Formula Descriptive Statistics

Descriptive Statistics	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
%BF BodPod/Withers	90	24.824	8.549	8.337	44.204	18.675	25.092	29.764
%BF Doernte/Withers	90	24.552	9.416	4.644	45.244	17.484	23.811	29.358
FM(kg) Bod Pod/Wang	90	17.531	7.459	4.685	38.173	12.588	15.691	21.213
FM(kg) Doernte/Wang	90	17.527	8.521	2.074	41.249	12.113	15.326	21.262

Statistical analysis of the reformulated predicted body volume formula revealed it may be a better fit for use in four-compartment body composition models than the Wilson, et al. (2013) and Smith-Ryan, et al. (2017) formulas. Further studies would need to be conducted with repeated samples to evaluate the reformulated equation.

Future Studies

This study consisted of 90 participants, but out of that 90, only 18 were over the age of 40. Future subject populations should have more age variance. Future studies should also explore the creation of different formulas for various age groups to account for the changing body composition trends as subjects age. This study also had a subject bias of ethnicity being highly skewed toward European Americans. This could affect results by skewing bone density.

Research has shown that race has an effect on bone mineral content (BMC) (Peacock, et al., 2009) (Vásquez, Shaw, Gensburg, Okorodudu, & Corsino, 2013) (Ettinger, et al., 1997). Multiple formulas may also need to be conducted for various BMI groups of smaller ranges, instead of one universal formula. Previously sited research has shown that as BMI moves to underweight, measurements can be misrepresented by as much as 15% too high, while measuring 8.51% too low for the obese category (Lowry & Tomiyama, 2015). Further research should be conducted to compare the viability of a uniform formula versus the need for multiple formulas.

Future studies should also consider a much larger sample size. This study had a sample size of 90 and Smith-Ryan, et al. (2017) had a sample of 127 subjects. While this was a large enough sample size to be significant and create a formula statistically equivalent to measured BodPod values, it may not be large enough to be accurate enough as a metric for body composition models. A much greater sample size may be needed before a formula is refined enough to be utilized in a four-compartment model.

Further research should also examine body volume testing utilizing deuterium oxide and hydrostatic weighing. While research has found the BodPod to be statistically equivalent to underwater weighing, and in some cases argued to be the new standard, other research has found there are variances (Gibby, et al., 2017). This study has shown evidence that statistically equivalent body volume can still cause formula failure when utilized as compartment model metrics. Future studies should be conducted utilizing

underwater weighing for body volume measurements and re-evaluated against formulated body volumes.

Conclusion

The use of DEXA based body volume formulas is a viable replacement for other BV testing methodologies for use in four-compartment testing models. However, both the Wilson, et al. (2012) and Smith-Ryan, et al. (2017) formulas failed to be a viable replacement for measured BodPod values. A new formula, or multiple formulas, need to be developed before the predicted values are suitable for use in body composition formulas.

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APPENDICES

APPENDIX A: DEXA Consent

Eastern Kentucky University

Waiver of Liability, Assumption of Risk, and Indemnity Agreement

THIS IS A LEGALLY BINDING RELEASE, WAIVER, INDEMNIFICATION OF LIABILITY, AND EXPRESS ASSUMPTION OF RISK.

Please read it carefully, fill in all blanks and initial each paragraph before signing.

I, _____, hereby affirm that I have read this document in its entirety. By my signature below and by my initialing each paragraph, I agree to each and every term and condition of this document.

I UNDERSTAND THAT PARTICIPATION IN _____ (hereafter referred to as "Event"), which involves _____, CARRIES WITH IT CERTAIN INHERENT RISKS AND DANGERS. THESE RISKS INCLUDE, BUT ARE NOT LIMITED TO: PERSONAL DAMAGE, INJURY, PARALYSIS, LOSS, DEATH, OR PROPERTY DAMAGE OR LOSS. I understand that these risks are described by way of example only, and that there are numerous other risks inherent in this activity to which I may be exposed. In the event of possible injury, I give permission for EKU to authorize the administration of medical care.

IN CONSIDERATION OF BEING PERMITTED TO PARTICIPATE IN ANY WAY IN _____ at _____, on _____: I, on behalf of myself and anyone claiming interest through me, DO HEREBY INTENTIONALLY, KNOWINGLY, AND VOLUNTARILY RELEASE, WAIVE, DISCHARGE, INDEMNIFY, AND AGREE TO HOLD HARMLESS EASTERN KENTUCKY UNIVERSITY, and all its employees, regents, volunteers, and representatives FROM ANY AND ALL CLAIMS, ACTIONS, SUITS, PROCEDURES, COSTS, EXPENSES, DAMAGES, AND LIABILITIES brought as a result of my involvement in this event, whether such damage, injury, or loss results from NEGLIGENCE or some other cause, and to reimburse them for any such expenses incurred.

I understand that the University in no way represents, or acts as an agent for, any third party trip organizer, the transportation carriers, hotels, and other suppliers of service during this event. I understand and agree that the University is not responsible for losses or expenses due to sickness, weather, strikes, hostilities, wars, natural disasters, or other such causes or disruptions. Further, the University is not responsible for any disruption of travel arrangements, or any consequent additional expenses that may be incurred therefrom.

I am not pregnant, and have been informed of the risk and potential consequences of participating in this program while pregnant.

I HEREBY ASSERT THAT MY PARTICIPATION IS VOLUNTARY AND THAT I KNOWINGLY ASSUME ALL SUCH RISKS. I acknowledge that EKU has not required, coerced, or encouraged me to participate in this event. I understand that I signed this document as my own free act and deed; no oral representations, statements, or inducements, apart from the foregoing written statement, have been made.

I further agree that this document will be interpreted in accordance with the laws of the Commonwealth of Kentucky. If any term or provision of this document shall be held illegal, unenforceable, or in conflict with any law governing this document, the validity of the remaining portions shall not be affected.

Student Information *Required Field if EKU student

First Name: _____ *Last Name: _____ *Student ID: _____

Phone Number: _____ *E-mail Address: _____

Sign name

Print name

Date

Signature of Parent or Guardian (if under 18 years of age): _____

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Revised October 2015



APPENDIX B: Informed Consent

Consent to Participate in a Research Study

Hydration level effects on Body Composition Evaluation methods

Why am I being asked to participate in this research?

You are being invited to take part in a research study on the effects of hydration and body composition. You are being invited to participate in this research study because you are a healthy person. If you take part in this study, you will be one of about 100 people to do so. *You cannot take part in this study if you are pregnant, or could be pregnant.*

Who is doing the study?

The person in charge of this study is Dr. Michael Lane, an assistant professor in the Exercise and Sports Science department at Eastern Kentucky University with the assistance of Zachary Bell, Ryan Bean, and Lee Doernte who are graduate students in the Exercise and Sports Science department. There may be other people on the research team assisting at different times during the study.

What is the purpose of the study?

The purpose of this study is to examine the effects of hydration on body composition measured by a variety of devices. By doing this study, we hope to learn how fluid intake can positively or negatively affect body composition measurements.

Where is the study going to take place and how long will it last?

The research procedures will be conducted at Moberly 223. You will need to come in 3 times during the study. Each visit will take about 60-75 minutes. The total amount of time you will be asked to volunteer for this study is 3 hours over the next 1-2 weeks.

What will I be asked to do?

You are asked to come to the lab, dressed appropriately for physical activity. Your necessary information will be taken (first and last name, age, sex, race). The first session will be for body composition testing and instruction. During this time each subject will be screened for general health and drug/supplement consumption (done by a health history questionnaire), along with blood pressure and resting heart rate values. Subjects that have any major health conditions, such as high blood pressure or high resting heart rate (blood pressure >140/90mmhg, heart rate >100bpm). You will be scanned by the Bod Pod, DXA scanner, and multiple BIA machines. These scans will require you sit, stand, or lay still for a few minutes at a time. You will additionally urinate (pee) in to a cup that will then be analyzed for your hydration level. You will then be instructed on how to follow your diet for the 24 hours before your next visit and be given a certain amount of water to drink before your next visit, we ask that you abstain from any hard physical activity before your next visit.

On your second visit you will first urinate in to a cup and your urine will be analyzed for your hydration level. You will then undergo each of the body composition tests performed in the previous trial. You will then be instructed on how to follow your diet for the 24 hours before your next visit and be given a certain amount of water to drink before your next visit, we ask that you abstain from any hard physical activity before your next visit.

On your third visit you will first urinate in to a cup and your urine will be analyzed for your hydration level. You will then undergo each of the body composition tests performed in the previous trial. Afterwards you will be finished with your involvement with the study.

Some subjects will be asked to occasionally return for body composition measurement utilizing all of the body composition equipment, however, you will not need to have any changes to your hydration for this portion. You will be notified about this and can stop your participation at any time.

Are there reasons why I should not take part in this study?

If you don't feel comfortable with the tracking of your diet or having to have how much fluid you consume tracked you should not participate. If you are pregnant you cannot participate in this study.

If you have ever had any issues with hydration, kidney function, cardiovascular function, or heart function you should not participate in this study. Please ask the researcher if you have any questions over this statement.

What are the possible risks and discomforts?

To the best of our knowledge, although we have made every effort to minimize this, you may find some questions we ask you (or some procedures we ask you to do) to be upsetting or stressful. If so, we can tell you about some people who may be able to help you with these feelings.

When you are altering the normal amount of fluid that you take in each day you can have symptoms of either dehydration or hyper hydration.

Symptoms of dehydration include: headache, dizziness, amber colored urine, lightheadedness, rapid heart rate, fever, poor skin elasticity (skin slowly sinks back to normal position after being pinched), chest or abdominal pain, fainting, seizures, and/or confusion.

Symptoms of hyper hydration include: dizziness, fatigue, nausea, vomiting, cramping, muscle weakness, mental confusion, sleepiness, lightheadedness, and/or abnormality of walking.

In the event that you begin to suffer from any of those symptoms of dehydration, immediate drink water and contact medical help if you feel it is necessary. In the event that you begin to suffer from any of the symptoms of hyper hydration, abstain from drinking any more water and contact medical help if you feel it is necessary.

In general if you have any of the following symptoms: difficulty breathing, dizziness, chest pain, swelling (like feet, ankles or legs), sudden chest congestion, sudden weakness, irregular heartbeat, any feeling that concerns you should call 911 or contact your medical provider.

Will I benefit from taking part in this study?

We cannot and do not guarantee that you will receive any benefits from this study. If you complete all visits you will have the chance to receive \$60.00 in the form of a gift card for participation.

Do I have to take part in this study?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study and still keep the benefits and rights you had before volunteering.

If I don't take part in this study, are there other choices?

If you do not want to be in the study, there are no other choices except to not take part in the study.

What will it cost me to participate?

There are no costs associated with taking part in this study.

Will I receive any payment or rewards for taking part in the study?

You will receive \$60.00 for your participation in all three of the visits successfully, and a copy of your body composition results will be available.

Who will see the information I give?

Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers, we will write about this combined information. You will not be identified in these written materials.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from the information you give, and these two things will be stored in different places under lock and key.

However, there are some circumstances in which we may have to show your information to other people. For example, the law may require us to show your information to a court. Also, we may be required to show information that identifies you to people who need to be sure we have done the research correctly; these would be people from such organizations as Eastern Kentucky University.

Can my taking part in the study end early?

If you decide to take part in the study, you still have the right to decide at any time that you no longer want to participate. You will not be treated differently if you decide to stop taking part in the study.

The individuals conducting the study may need to end your participation in the study. They may do this if you are not able to follow the directions they give you, if they find that your being in the study is more risk than benefit to you.

What happens if I get hurt or sick during the study?

If you believe you are hurt or if you get sick because of something that is done during the study, you should call 911 or your medical provider. After that situation has been resolved please contact Dr. Michael Lane at 859-622-1890. It is important for you to understand that Eastern Kentucky University will not pay for the cost of any care or treatment that might be necessary because you get hurt or sick while taking part in this study. That cost will be your responsibility. Also, Eastern Kentucky University will not pay for any wages you may lose if you are harmed by this study.

Usually, medical costs that result from research-related harm cannot be included as regular medical costs. You should ask your insurer if you have any questions about your insurer's willingness to pay under these circumstances.

What if I have questions?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigator, Dr. Michael Lane at 859-622-1890. If you have any questions about your rights as a research volunteer, contact the staff in the Division of Sponsored Programs at Eastern Kentucky University at 859-622-3636. We will give you a copy of this consent form to take with you.

What else do I need to know?

You will be told if any new information is learned which may affect your condition or influence your willingness to continue taking part in this study.

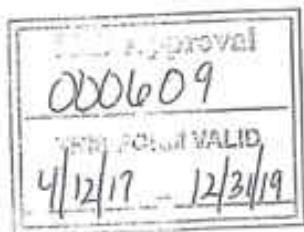
I have thoroughly read this document, understand its contents, have been given an opportunity to have my questions answered, and agree to participate in this research project.

Signature of person agreeing to take part in the study

Date

Printed name of person taking part in the study

Name of person providing information to subject



APPENDIX C: Subject History

**Hydration level effects on Body Composition Evaluation
methods: Data Collection Screening (CRF-001)**

Subject ID:

Eastern Kentucky University

EKUTAB-

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Date of Assessment:

— / — / —
(M M M / D D / Y Y Y Y)

Inclusion Criteria:		Yes	No
1.	Participant is willing and able to provide consent	<input type="checkbox"/>	<input type="checkbox"/>
2.	Participant is mentally and physically capable of complying with the protocol	<input type="checkbox"/>	<input type="checkbox"/>
3.	Participant is 18 years of age or older	<input type="checkbox"/>	<input type="checkbox"/>
4.	Participant has a BMI of no less than 18 and no greater than 35	<input type="checkbox"/>	<input type="checkbox"/>

If any of the above criteria is answered NO, the participant is NOT eligible for the study and must not be included in the study.

Exclusion Criteria:		Yes	No
1.	Participant has an active implantable (pacemaker, ICD), is connected to electronic life support, or has metallic implants (i.e. hip or knee joint)	<input type="checkbox"/>	<input type="checkbox"/>
2.	Participant is an amputee (of any type, limb, finger, etc)	<input type="checkbox"/>	<input type="checkbox"/>
3.	Participant is experiencing any swelling conditions (e.g. pregnancy, congestive heart failure, chronic/acute renal disease, cor pulmonale, nephrotic syndrome, nephrosis, liver failure, cirrhosis, pulmonary edema, thrombophlebitis, or deep vein thrombosis in arms)	<input type="checkbox"/>	<input type="checkbox"/>
4.	Participant is currently suffering from uncontrolled intercurrent illness, including: ongoing/active infection, unstable angina pectoris, or cardiac arrhythmia	<input type="checkbox"/>	<input type="checkbox"/>
5.	Participant has hypertension (140/90 or greater) on the day of testing, or self-report diagnosis	<input type="checkbox"/>	<input type="checkbox"/>
6.	Participant has resting tachycardia (BPM over 100) self-report/diagnosis	<input type="checkbox"/>	<input type="checkbox"/>

If any of the above criteria is answered YES, the participant is NOT eligible for the study and must not be included in the study.

Please list reason(s) for ineligibility on next page.

**Hydration level effects on Body Composition Evaluation
methods: Data Collection Screening (CRF-001)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Participant Eligibility Review:

Details of conditions that deem patient ineligible for study	
Details of amputation?	
Swelling Conditions?	
Other	

End of Screening Checklist:		Yes	No
1.	Does the participant satisfy the inclusion and exclusion criteria to date?	<input type="checkbox"/>	<input type="checkbox"/>
2.	Is the participant still willing to proceed in the study?	<input type="checkbox"/>	<input type="checkbox"/>

**Hydration level effects on Body Composition Evaluation
methods: Data Collection Screening (CRF-001)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Participant's eligibility Coordinator Sign-Off:

Is the participant eligible to take part in the Clinical Study?

Yes No

Coordinator's Signature

Coordinator's Name (Print)

Date: _____ / _____ / _____
(M M M / D D / Y Y Y Y)

Informed Consent:

Date subject signed written consent form:	_____ / _____ / _____ (M M M - D D - Y Y Y Y)
Date of first study related procedure:	_____ / _____ / _____ (M M M - D D - Y Y Y Y)
Name of person taking informed consent (print):	

Name of Person Completing Form: _____
(Print)

Signature of Person Completing Form: _____

Date Form Completed:

					/								
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**Hydration level effects on Body Composition Evaluation
methods: Data Collection Screening (CRF-002)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Date of Assessment: _____ / _____ / _____
(M M M / D D / Y Y Y Y)

Date of Data Collection Inclusion Criteria:		Yes	No
1.	Participant has been fasting for 8-12 hr	<input type="checkbox"/>	<input type="checkbox"/>
2.	Participant has not completed a heavy exercise session within past 24 hr	<input type="checkbox"/>	<input type="checkbox"/>
3.	Participant has not ingested alcohol for past 24 hr	<input type="checkbox"/>	<input type="checkbox"/>
4.	Participant has not ingested caffeine for past 8-12 hr	<input type="checkbox"/>	<input type="checkbox"/>
5.	Participant has not ingested any water for past 2 hr	<input type="checkbox"/>	<input type="checkbox"/>
6.	Participant is not pregnant (signed form confirming, and pregnancy test confirmed)	<input type="checkbox"/>	<input type="checkbox"/>

If any of the above criteria is answered NO, the participant is NOT eligible for the study and must not be included in the study today.

Reschedule at your discretion. Another CRF-002 to be completed on subsequent assessment date.

Name of Person Completing Form: _____
(Print)

Signature of Person Completing Form: _____

Date Form Completed:

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**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Date of Assessment: ____ / ____ / ____
(M M M / D D / Y Y Y Y)

Demographic Data:	
Age: _____ (yr)	Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female

(self report) Height: _____ (ft) _____ (in)	(self report) Weight: _____ (lbs)
Does the above weight indicate: a gain a loss no change in the past year?	
If a change, how many pounds? _____ (lbs)	

Ethnicity:			
American Indian	<input type="checkbox"/>	White	<input type="checkbox"/>
Alaskan Native	<input type="checkbox"/>	Asian	<input type="checkbox"/>
Black or African American	<input type="checkbox"/>	Hispanic or Latino	<input type="checkbox"/>
Jamaican	<input type="checkbox"/>	Haitian	<input type="checkbox"/>
Native Hawaiian or other Pacific Islander	<input type="checkbox"/>	Other	<input type="checkbox"/>
Do not care to respond			<input type="checkbox"/>
*If other, please specify: _____			

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID:

Eastern Kentucky University

EKUTAB-

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Alcohol Use:		Yes	No
1.	Has had an alcoholic drink in the past 72 hr	<input type="checkbox"/>	<input type="checkbox"/>
2.	Has had an alcoholic drink in the past 48 hr	<input type="checkbox"/>	<input type="checkbox"/>
3.	Has had an alcoholic drink in the past 24 hr	<input type="checkbox"/>	<input type="checkbox"/>
Alcohol Use details:			

Blood Pressure _____ / _____ (mmHg)	Resting Heart Rate: _____ (bpm)
-------------------------------------	---------------------------------

Joint-Muscle Status (select areas indicated by participant as problematic):			
Joints		Muscles	
Wrists	<input type="checkbox"/>	Arms	<input type="checkbox"/>
Elbows	<input type="checkbox"/>	Shoulders	<input type="checkbox"/>
Shoulders	<input type="checkbox"/>	Chest	<input type="checkbox"/>
Upper Spine and Neck	<input type="checkbox"/>	Upper Back and Neck	<input type="checkbox"/>
Lower spine	<input type="checkbox"/>	Abdominals	<input type="checkbox"/>
Hips	<input type="checkbox"/>	Lower Back	<input type="checkbox"/>
Knees	<input type="checkbox"/>	Buttocks	<input type="checkbox"/>
Ankles	<input type="checkbox"/>	Thighs	<input type="checkbox"/>
Feet	<input type="checkbox"/>	Lower Leg	<input type="checkbox"/>
Other	<input type="checkbox"/>	Feet	<input type="checkbox"/>
		Other	<input type="checkbox"/>

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Health Status (select areas indicated by participant as current):

High Blood Pressure	<input type="checkbox"/>	Acute Infection	<input type="checkbox"/>
Heart Disease or Dysfunction	<input type="checkbox"/>	Diabetes or Blood Sugar Level Abnormality	<input type="checkbox"/>
Peripheral Circulatory Disorder	<input type="checkbox"/>	Anemia	<input type="checkbox"/>
Lung Disease or Dysfunction	<input type="checkbox"/>	Hernias	<input type="checkbox"/>
Arthritis or Gout	<input type="checkbox"/>	Thyroid Dysfunction	<input type="checkbox"/>
Edema	<input type="checkbox"/>	Pancreas Dysfunction	<input type="checkbox"/>
Epilepsy	<input type="checkbox"/>	Liver Dysfunction	<input type="checkbox"/>
Multiply Sclerosis	<input type="checkbox"/>	Kidney Dysfunction	<input type="checkbox"/>
High Blood Cholesterol/Triglycerides	<input type="checkbox"/>	Phenylketonuria (PKU)	<input type="checkbox"/>
Allergic reactions to rubbing alcohol	<input type="checkbox"/>	Loss of Consciousness	<input type="checkbox"/>
		Kidney stones or hydration issues	<input type="checkbox"/>

If any of the above are selected, was physician health clearance obtained: Yes No

Physical Examination History:

Approximate date of last physical examination: ____ / ____ / ____
(M M M / D D / Y Y Y Y)

Physical problems noted at that time:

Has a physician ever made any recommendations relative to limiting your level of physical exertion?

Yes No

If YES, what limitations were recommended?

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Female Reproductive History (Female only):

N/A: Patient is male

Began menses within the past year? Yes No

Consistent menstrual periods for the last 3 months? Yes No

Date of onset of last menstrual period

— / — / — —
(M M M / D D / Y Y Y Y) — —

Used a hormonal contraceptive within the last 3 months ? Yes No

Current Medication Usage:

Medication	Daily Dose	Condition/ Diagnosis	Length of Use (include units, yr, mo, wk, days)

Current Supplement Usage:

Supplement	Daily Dose	Reason	Length of Use (include units, yr, mo, wk, days)

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Caffeine Use:	Yes	No
Regular caffeine use	<input type="checkbox"/>	<input type="checkbox"/>
Caffeine consumed daily	Mgs	Cups
Caffeine consumed weekly		
What time of day take in the most caffeine?		
Main sources of caffeine:		

Source of Nutritional Information (RANK 1-6, 6 least used):	
Source	Rank (1-6)
Family	
Friends	
Coach	
Nutritionist/Dietician	
School	
Other	
If Other is predominant, describe:	

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID:

Eastern Kentucky University

EKUTAB-

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Heart and Kidney Function:	Yes	No
Diagnosed Heart Problem	<input type="checkbox"/>	<input type="checkbox"/>
Diagnosed Kidney Problem	<input type="checkbox"/>	<input type="checkbox"/>
Issues with Dehydration	<input type="checkbox"/>	<input type="checkbox"/>
Issues with Hyperhydation	<input type="checkbox"/>	<input type="checkbox"/>
Issues with Light headedness	<input type="checkbox"/>	<input type="checkbox"/>
Issues with Edema	<input type="checkbox"/>	<input type="checkbox"/>
Diagnosed Sweating issues	<input type="checkbox"/>	<input type="checkbox"/>
Issues with Heat illness	<input type="checkbox"/>	<input type="checkbox"/>
Diagnosed Diabetes or Blood Sugar problems	<input type="checkbox"/>	<input type="checkbox"/>
Ever been told to limit salt	<input type="checkbox"/>	<input type="checkbox"/>
Ever been told to limit water intake	<input type="checkbox"/>	<input type="checkbox"/>
Diagnosed Heart Failure	<input type="checkbox"/>	<input type="checkbox"/>
Trouble sleeping flat/on back	<input type="checkbox"/>	<input type="checkbox"/>
Currently being treated for Kidney Disease or injury	<input type="checkbox"/>	<input type="checkbox"/>
Take a diuretic or water pill	<input type="checkbox"/>	<input type="checkbox"/>
If yes to any of the questions above please elaborate on the information below:		
If yes to diagnosed heart or kidney problem please list the information below:		
If yes to diagnosed with a hydration problem such as dehydration or water Intoxication (hyperhydation) please list the information below:		

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID:

Eastern Kentucky University

EKUTAB-

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Physical Perceptions: Over the last 6 months	When Exercising	Regularly
Chest Pain	<input type="checkbox"/>	<input type="checkbox"/>
Heart Palpitations	<input type="checkbox"/>	<input type="checkbox"/>
Unusually Rapid Breathing	<input type="checkbox"/>	<input type="checkbox"/>
Overheating	<input type="checkbox"/>	<input type="checkbox"/>
Muscle Cramping	<input type="checkbox"/>	<input type="checkbox"/>
Muscle Pain	<input type="checkbox"/>	<input type="checkbox"/>
Joint Pain	<input type="checkbox"/>	<input type="checkbox"/>
Nausea	<input type="checkbox"/>	<input type="checkbox"/>
Light Headedness	<input type="checkbox"/>	<input type="checkbox"/>
Loss of Consciousness	<input type="checkbox"/>	<input type="checkbox"/>
Loss of Balance	<input type="checkbox"/>	<input type="checkbox"/>
Loss of Coordination	<input type="checkbox"/>	<input type="checkbox"/>
Extreme Weakness	<input type="checkbox"/>	<input type="checkbox"/>
Numbness	<input type="checkbox"/>	<input type="checkbox"/>
Mental Confusion	<input type="checkbox"/>	<input type="checkbox"/>
Other		
On average, how many hours of sleep, nightly:	hrs	
On average, how many hours of sleep, weekly:	hrs	

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID:

Eastern Kentucky University

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Family History (immediate only):	Yes	No
Heart Disease	<input type="checkbox"/>	<input type="checkbox"/>
Heart Attacks or Strokes (prior to age 50)	<input type="checkbox"/>	<input type="checkbox"/>
Elevated Blood Cholesterol or Triglyceride Levels	<input type="checkbox"/>	<input type="checkbox"/>
High Blood Pressure	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Sudden Death (other than accidental)	<input type="checkbox"/>	<input type="checkbox"/>
Do not know for any of the above	<input type="checkbox"/>	<input type="checkbox"/>

Exercise Status:	Yes	No
Current regular aerobic exercise	<input type="checkbox"/>	<input type="checkbox"/>
History of this exercise: _____ Yrs _____ Months		
Hours per week of this exercise: _____ hrs		
Current regularly lift weight	<input type="checkbox"/>	<input type="checkbox"/>
History of this exercise: _____ Yrs _____ Months		
Hours per week of this exercise: _____ hrs		
Current regularly play recreational sports:	<input type="checkbox"/>	<input type="checkbox"/>
History of this exercise: _____ Yrs _____ Months		
Hours per week of this exercise: _____ hrs		

**Hydration level effects on Body Composition Evaluation
methods: Demographics (CRF-003)**

Subject ID: Eastern Kentucky University

EKUTAB-

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Sports played regularly						
Sport	YES	NO	Times per week	Level		
				Varsity	Intramural	Recreational
Football	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Basketball	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cross Country	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Track and Field	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Soccer	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tennis	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rugby	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Baseball/Softball	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Golf	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Swimming	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cycling	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cheerleading	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Name of Person Completing Form: _____
(Print)

Signature of Person Completing Form: _____

Date Form Completed: ____/____/____

APPENDIX D: Data Collection

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID Eastern Kentucky University

EKUTAB-

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Date of Assessment: ____ / ____ / ____
(M M M / D D / Y Y Y Y)

Standing Height: _____ (in) nearest 1/4 in	Weight: _____ (lbs) nearest 0.000
Seated Height (head): _____ (in) nearest 1/4 in	Arm Length (left): _____ (in) nearest 1/4 in
Seated Height (AC joint): _____ (in) nearest 1/4 in	Arm Length (right): _____ (in) nearest 1/4 in
Tester Name: _____	

Urine Analysis:	
Tester Name: _____	
Color: _____	Specific Gravity: _____

SUPINE Assessments

Assessment 1: DXA:	Yes/ Attached	No
Tester Name: _____		
DXA Left Forearm	<input type="checkbox"/>	<input type="checkbox"/>
DXA Right Forearm	<input type="checkbox"/>	<input type="checkbox"/>
DXA APVA Spine Bone Density (Anterior/Posterior)	<input type="checkbox"/>	<input type="checkbox"/>
DXA Dual Femur Bone Density	<input type="checkbox"/>	<input type="checkbox"/>
DXA Total Body (Lean Tissue/Visceral Fat/Bone Density)	<input type="checkbox"/>	<input type="checkbox"/>

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

EKUTAB-

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Standing Height: _____(in)	Weight: _____(lbs)
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Assessment 2: SFB7 Bioimpedance (Supine): Appendix A

Tester Name:

Segment	Ro- 1	Rinf1	Ro- 2	Rinf2	ID+Extension
Right Arm (1)					IDs2dtsu1
Left Arm (2)					IDs2dtsu2
Left Arm Alt (3)					IDs2dtsu3
Right Leg (4)					IDs2dtsu4
Left Leg (5)					IDs2dtsu5
Left Leg Alt (6)					IDs2dtsu6
Right Whole Body (7)					IDs2dtsu7
Left Whole Body (8)					IDs2dtsu8
Right Trunk (9)					IDs2dtsu9
Left Trunk (10)					IDs2dtsu10

REPOSITION REPLICATIONS

Segment	Ro- 1	Rinf1	Ro- 2	Rinf2	ID+Extension
Right Arm (1)					IDs2dtsur1
Left Arm (2)					IDs2dtsur2
Left Arm Alt (3)					IDs2dtsur3
Right Leg (4)					IDs2dtsur4
Left Leg (5)					IDs2dtsur5
Left Leg Alt (6)					IDs2dtsur6
Right Whole Body (7)					IDs2dtsur7
Left Whole Body (8)					IDs2dtsur8
Right Trunk (9)					IDs2dtsur9
Left Trunk (10)					IDs2dtsur10

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

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Standing Height: _____(in)	Weight: _____(lbs)
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SITTING assessments

Assessment 3: BodPod		
Tester Name:		
BodPod Weight _____(lbs)	Body Fat %: _____	
L Vol Me:	L Vol Pre:	
Body Volume Final:	Body Volume 1:	Body Volume 2:

Assessment 6: Bioimpedance/SOZO: SITTING				
Tester Name:				
SOZO PROFILE	<input type="checkbox"/> FIRST NAME: EKUTAB+ID (# written out) <input type="checkbox"/> LAST NAME: SITTING <input type="checkbox"/> MRN: ID+si (Example: M23si)			
Measurement/Configuration	Ro-Right	Rinf- Right	Ro- Left	Rinf-Left
SOZO	Sitting- 1			
	Sitting-2			
	Sitting- reposition- 1			
	Sitting- reposition-2			

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

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10 of 10

Standing Height: _____(in) **Weight:** _____(lbs)

Assessment 5: SOZO Shell- SFB7- SITTING: Appendix B

Tester Name:

Segment	Ro-1	Rinf1	Ro-2	Rinf2	ID+Extension
Right Arm (1)					IDs1shsi1
Left Arm (2)					IDs1shsi2
Left Arm Alt (3)					IDs1shsi3
Right Leg (4)					IDs1shsi4
Left Leg (5)					IDs1shsi5
Left Leg Alt (6)					IDs1shsi6
Right Whole Body (7)					IDs1shsi7
Left Whole Body (8)					IDs1shsi8
Right Trunk (9)					IDs1shsi9
Left Trunk (10)					IDs1shsi10

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID Eastern Kentucky University

EKUTAB-

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Standing Height: _____ (in)	Weight: _____ (lbs)
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STANDING Assessments

Assessment 8: Anthropomorphic (Standing)

Tester Name:

SKINFOLD CALIPERS	Rep 1 (mm)	Rep2 (mm)	Rep3 (if needed)
Abdominal			
Triceps			
Biceps			
Chest			
Medial Calf			
Posterior Calf			
Midaxillary			
Subscapular			
Suprailliac			
Thigh			

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

EKUTAB-

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Standing Height: _____ (in)	Weight: _____ (lbs)
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CIRCUMFERENCE	Rep 1 (cm)	Rep2 (cm)	Rep3 (if needed)
Abdomen			
Arm			
Buttocks/Hips			
Calf			
Forearm			
Hips/Thigh			
Mid-Thigh			
Waist			

Assessment 9: Bioimpedance/SOZO: STANDING

Tester Name:

SOZO PROFILE		<input type="checkbox"/> FIRST NAME: EKUTAB+ID (# written out) <input type="checkbox"/> LAST NAME: STANDING <input type="checkbox"/> MRN: ID+st (Example: M23st)			
Measurement/Configuration		Ro-Right	Rinf- Right	Ro- Left	Rinf-Left
SOZO	Standing- 1				
	Standing-2				
	Standing- reposition- 1				
	Standing- reposition-2				

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

EKUTAB-

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Standing Height: _____ (in)	Weight: _____ (lbs)
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Assessment 8: SOZO Shell- SFB7- Standing: Appendix B

Tester Name:

Segment	Ro- 1	Rinf1	Ro- 2	Rinf2	ID+Extension
Right Arm (1)					IDs1shst1
Left Arm (2)					IDs1shst2
Left Arm Alt (3)					IDs1shst3
Right Leg (4)					IDs1shst4
Left Leg (5)					IDs1shst5
Left Leg Alt (6)					IDs1shst6
Right Whole Body (7)					IDs1shst7
Left Whole Body (8)					IDs1shst8
Right Trunk (9)					IDs1shst9
Left Trunk (10)					IDs1shst10

Hydration level effects on Body Composition Evaluation methods: Body Composition (CRF-004)

Subject ID

Eastern Kentucky University

EKUTAB-

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Standing Height: _____(in) **Weight:** _____(lbs)

Assessment 8 (cont.) SOZO Shell- SFB7- Standing Alternative: Appendix C

Tester Name:

Segment	Ro- 1	Rinf1	Ro- 2	Rinf2	ID+Extension
Right Arm (11)					IDs1shst11
Left Arm (12)					IDs1shst12
Left Arm Alt (13)					IDs1shst13
Right Leg (14)					IDs1shst14
Left Leg (15)					IDs1shst15
Left Leg Alt (16)					IDs1shst16
Right Whole Body (17)					IDs1shst17
Left Whole Body (18)					IDs1shst18
Right Trunk (19)					IDs1shst19
Left Trunk (20)					IDs1shst20

Name of Person Completing Form: _____
(Print)

Signature of Person Completing Form: _____

Date Form Completed: / /

APPENDIX E: Equipment

BioImp Body Composition Analysis Software (ImpediMed Limited, Queensland, Australia)

Cosmed BodPod (COSMED USA, INC, Concord, CA)

GE Lunar Prodigy Advance Bone Densitometry scanner (General Electric Company, Cincinnati, OH)

IBM SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.)

Microsoft Excel 2016 (Microsoft, Redmond, Washington)

SFB7 (ImpediMed Limited, Queensland, Australia)