BODY FAT PERCENTAGE VIA DUAL ENERGY X-RAY ABSORPTIOMETRY FOLLOWING MULTIPLE DIFFERENT APPROACHES COMPARED TO A LABORATORY-BASED 3-COMPARTMENT MODEL

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A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Kinesiology
in the Graduate School of
The University of Alabama

TUSCALOOSA, ALABAMA

2019

ABSTRACT

The purpose of this study was to examine the accuracy of using dual energy X-ray absorptiometry (DXA) derived body volume (BV) equations in a multi-compartment model for estimates of body fat percentage (BF%) in comparison to traditional under water weighing (UWW) measures of BV. BF% was estimated using three-compartment (3C), two-compartment (2C) models, and the DXA. The 3C-Siri equation with UWW for BV and deuterium oxide (D_2O) for total body water (TBW) was used as the criterion. One hundred twenty-nine adults (55 men and 74 women) volunteered to participate (age = 30 ± 13 years). DXA-derived BV was determined with the recent equations from Smith-Ryan et al. and Wilson et al. and then incorporated into multi-compartment models (i.e., $3C_{Siri}$-SR and $3C_{Siri}$-W). The $3C_{Siri}$-SR and $3C_{Siri}$-W DXA-derived BV values were highly correlated with UWW measured BV (74.24L, 74.30L, and 71.97L, respectively, and r=.999 for both). However, the mean BF% was overestimated in all multi-compartment models regardless of which DXA-derived BV equation was used. These results were consistent for the total sample and when stratified by sex, with the observed error ranging from 4.92% to 17.75% (effect size [ES] = .61 to 1.96, all $p<.001$). The correlation between the DXA-derived BV and 3C-criterion BF% was strongest for both Smith-Ryan et al. and Wilson et al. when utilized in the Siri model (i.e., $3C_{Siri}$-SR and $3C_{Siri}$-W) in the total sample, (r= .979 and .964, respectively) for men, (r= .974 and .971, respectively) and for women (r= .981 and .973, respectively). The $3C_{Siri}$-SR yielded the best accuracy in the total

sample, as well as when stratified by men, and women as indicated by the smallest SEE of all methods (1.91%, 1.83%, and 1.76% respectively), although it overestimated BF% by 6% in both sex-specific subgroups. These data indicate that both DXA-derived BV equations are strongly correlated with UWW, however do not provide an accurate measure when incorporated in a 3-compartment model for estimation of BF%. This is likely due to the higher BV values produced by the DXA-derived equations (roughly 2 liters), which overestimated BF% by roughly 5%.

LIST OF ABBREVIATIONS & SYMBOLS

2C	two-compartment
3C	three-compartment
4C	four-compartment
5C	five-compartment
ADP	air displacement plethysmography
BD	body density
BIA	bioelectrical impedance analysis
BIS	bioelectrical impedance spectroscopy
BF%	body fat percentage
BM	body mass
BMC	bone mineral content
BV	body volume
CE	constant error
DXA	dual energy x-ray absorptiometry
ES	effect size
FFM	fat-free mass
FM	fat mass
LOA	limits of agreement
Mo	bone mineral
SEE	standard error of estimate

TBW	total body water
USG	urine specific gravity
UWW	underwater weighing

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CHAPTER 1

INTRODUCTION

Body composition refers to the relative proportions of all tissues that comprise the human body, such as fat mass [FM], fat-free mass [FFM], total body water [TBW], and bone mineral content [BMC]. Overweight and obesity are commonly assessed using body mass index (BMI) using thresholds of ≥ 25 kg·m^2 and ≥ 30 kg·m^2 respectively, and are characterized by high amounts of body fat in relation to overall lean body mass. Body composition is an important health characteristic that is related to a number of chronic diseases, including hypertension, type II diabetes mellitus, dyslipidemia, some cancers, sleep apnea, arthritis, musculoskeletal problems, and cardiovascular disease (1-3). Due to the unfavorable consequences of having higher levels of relative adiposity, accurate assessment of body composition to 1) identify individuals at increased risk of disease and 2) accurately monitor changes in body composition over time is a paramount (4, 5). For example, tracking changes of body composition through interventions could show a decrease in one's BF%, and in turn decrease their chances of developing the aforementioned diseases (6). Therefore, selecting valid and reliable body composition assessment methods should be of high priority for clinicians and other health/fitness professionals.

As the body is commonly partitioned in to various tissues, these "compartments" can each be measured or estimated using a variety of techniques of varying invasiveness and difficulty. The most common compartments used in body composition assessment are body mass (BM), body volume (BV), TBW, total body bone mineral content (Mo), and total body soft

tissue mineral (Ms). Dual energy X-ray absorptiometry (DXA) technology assesses three different body compartments: BMC, as well as fat-, and lean-mass. This can be accomplished by using very low current X-rays at two energy levels that can image tissues of various densities. For the measurement, participants lay supine on a scanning bed as a mechanical arm moves over the body and receives an array of x-ray signals from within the machine. DXA is commonly utilized in clinical and research settings for the assessment of body composition (7-9), despite research from Moon et al. (10) and Nickerson and Tinsley (11) that revealed the large error from DXA-derived body fat percentage (BF%). DXA measurements often yield large systematic bias and individual error (i.e., overestimation of BF%), which could put someone in a different BF% category which may be misleading in risks of predisposition to previously mentioned diseases, when compared to multi-compartment models that include a measure of TBW (10), as DXA-derived estimates of BF% assume a constant hydration of FFM (i.e., 73%) (12) despite substantial individual variability (i.e., 68 to 81%) (10, 13).

As previously mentioned, multi-compartment models combine various techniques to more accurately determine the amount of various tissues within the body. However, the complexity of the multi-compartment model is usually dependent upon access to additional equipment and resources to accurately measure each compartment. It is well recognized that 2C models can be improved through expansion to a three-compartment (3C) model or four-compartment (4C) model by accounting for TBW and BMC in addition to BM and BV. 3C models are also commonly accepted and used as a criterion method (14-16). Although numerous equations exist, the most accurate 3C models are those that include an estimate of TBW (10, 17, 18).

Multi-compartment models that include a measure of total body water (TBW, in liters) are recommended over DXA when available to quantify FFM hydration. Nickerson and Tinsley (11) recently revealed three-compartment (3C) models are comparable to more advanced four- and five-compartment models (4C, 5C) when accounting for TBW in a sample of as physically active participants (n=141, 18-40 years of age), with very small SEE (ranging from 0.01% to 0.59%) and 95% limits of agreement (ranging from 0.02 to 1.16%). These results are similar to findings from Moon et al. (10) who assessed a sample of 29 NCAA Division I athletes. The 3C and 4C models produced nearly identical estimates when compared to a 5C model ($r>0.99$ and $TE<0.40\%$), further supporting the use of a 3C equation as a criterion. However, these studies were performed in high-level athletes and physically active adults, and may not be extended to the general population.

The assessment of TBW can be obtained from isotope dilution, and accurately predicted with bioelectrical impedance analysis (BIA) or bioelectrical impedance spectroscopy (BIS) (19). When using a dilution technique to estimate TBW, individuals are asked to consume a small amount of water containing an isotope tracer. Although the specific time varies depending on the tracer chosen, the concentration of the isotope in the urine is measured after allowing adequate time for absorption.

To measure TBW using BAI or BIS, electrodes are attached to the surface of the skin on either limb (hands and feet), or by asking participants to place their hands and feet on metal electrode plates. An undetectable electric current passes through the body (from one side to the other/one set of electrodes to the other) and the partner electrodes receive the flow of current. The conduction of this current is highly dependent on electrolyte and water distribution of the tissues, as FFM contains a greater concentration of water and, as a result, has higher

conductivity. In contrast, FM has a lower water concentration, produces higher impedance characteristics, and diminished current flow through the tissue. This technique produces TBW estimates based on the currents' encounter of resistance, impedance, and reactance, which then in prediction equations estimates their BF%. BIA and BIS differ based on the amount of frequency and strength of the current produced by the device. A higher frequency current (though still undetectable) has the capability to measure extra- and intra-cellular water which produces a value for TBW. However, if the current is not strong enough to fully penetrate the cells of the tissue only extra-cellular water is captured then manufacturer equations are used to estimate intra-cellular and total body water.

Although a multi-compartment model is recommended in research settings (20), the combination of multiple methods to measure BV, BMC, and TBW increases the potential time burden to participants. To simplify the process, DXA-derived BV equations were developed and yielded excellent agreement with traditional BV methods (21, 22). For instance, DXA-derived BV yielded near-perfect accuracy when compared to ADP ($r=\geq 0.99$) in a random sample subset ($n=40$, 35.8±9.4 years, 31.4±5.5 $kg \cdot m^2$), with no differences observed between measures (94.55±17.74L versus 94.33±17.78L for DXA and ADP, respectively, $p>.05$) (21). In addition, DXA-derived BV was strongly correlated with ADP measures ($r=0.98$ to 0.99) in a sample of predominantly normal weight participants ($n=22$, 37.6±15.5 years) at baseline and following a 6-month follow-up period (22).

While the DXA-derived BV yields comparable accuracy when compared to traditional UWW and ADP methods, replacing either of these methods in a multi-compartment model has yielded inconsistent results. Previous research indicated that multi-compartment models including DXA-derived BV yield near-perfect test-retest reliability ($r=\geq 0.99$) (23), and similar

accuracy in a multi-compartment model using ADP-derived BV in overweight and obese participants (24). In contrast, the observed error when substituting DXA-derived BV for UWW-derived BV (SEE=4.2%) has led some researchers to question the utility when estimating BF% (25). To the authors' knowledge, DXA-derived BV has yet to be validated against UWW-derived BV in multi-compartment models using the "gold standard" of D_2O as a measure of TBW. As such, the purpose of this study will be to examine the accuracy of using DXA-derived BV in a 3C body composition model when compared to traditional UWW measures.

CHAPTER 2

MATERIALS AND METHODS

Participants

Participants were recruited from Tuscaloosa, Alabama and the surrounding areas between May 2017 and May 2018. The inclusion criteria were simply all men and women between the ages of 18-90 years of age and apparently healthy. Individuals classified as being at high risk according to American College of Sports Medicine (ACSM) guidelines were not allowed to participate. High risk was defined by ACSM as having one or more signs/symptoms of or diagnosed cardiovascular, pulmonary, and/or metabolic disease (26). Women who were pregnant or previously pregnant within the previous 12 months were excluded as well due to potential radiation exposure from the DXA scan and changes in body composition during pregnancy. Data for this cross-sectional study were collected during a single visit to the Exercise Physiology Laboratory. Prior to their testing visit, participants were asked to 1) avoid moderate-vigorous intensity physical activity for 24 h, 2) abstain from alcohol for 24 h, 3) to arrive in a fasted state, with no food consumption within the previous 12 h, and to 4) cease consumption of water within 2 h prior to arriving for the study. Because body composition was the primary outcome of interest, an accurate measure of BM was needed for the 3C model. As such, a strict standardization protocol was followed to ensure that potential error was not introduced because of additional clothing, or recent fluid and food consumption remaining in the stomach after voiding the bladder. Furthermore, alcohol consumption was restricted prior to testing to ensure all participants were adequately hydrated prior to the measure of TBW. Participants provided

written informed consent prior to data collection, as well as completed a brief medical history questionnaire and 24 h dietary recall to ensure compliance with the study protocol (both pictured in **Appendix**). This study was approved by the Institutional Review Board at the University of Alabama (**Appendix**).

Procedures

Hydration status was assessed from a urine sample using a handheld refractometer (Atago SUR-NE, Atago Corp Ltd., Tokyo, Japan). This is accomplished by first collecting urine from the participant, then pipetting a small sample of the urine onto the handheld refractometer. Light traveling through the refractometer determines the Urine Specific Gravity (USG) based on the clarity of the sample (dehydrated or impurities of the urine). In order to be considered euhydrated, participants' USG value had to be < 1.030 (27, 28). If an individual's values are >1.030 they were given fluids, then waited for 30 minutes to then be re-evaluated. If USG was still elevated the participant was rescheduled and tested on a different day. After hydration confirmation, participants provided a nude body mass (BM) measurement (to the nearest 0.1 kg) with a calibrated digital weighing scale (Tanita BWB-800, Tanita Corporation, Tokyo, Japan). Next, participants were asked to change into shorts and t-shirt, and their height was measured to the nearest 0.1 cm with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany).

Underwater Weighing

UWW was used to determine BV for the 3C-criterion. All tests were performed in an apparatus specifically designed for densitometry measurements (Vacu-Med, Ventura, CA). Prior to the UWW measurement, participants changed into compression type clothing or a bathing suit. Participants entered a water tank, sat in a specialized seat, blew out all air from their lungs,

and lowered themselves under water in the tank. All body parts remained submerged until underwater weight was recorded (usually 5-10 seconds) in pounds or kilograms. This procedure was repeated several times to ensure the most accurate measure possible, the average of the three highest values (6 to 10 trials) was used as the representative underwater weight. Determination of lung volume was completed with the oxygen dilution technique via a nitrogen analyzer (Vacu-Med, Ventura, CA). Participants completed a minimum of two trials and the average of the closest two trials within 5% were used to represent RV. During this procedure, participants breathed through a 2-way valve connected to 100% oxygen on inspiration and a collection spirometer on expiration. The spirometer measured the volume of air and fraction of nitrogen expired with each breath. Once the fraction of nitrogen decreased below 1.5% for 3 consecutive breaths, the test was complete. The initial amount of nitrogen in the lungs was assumed to be equal to the total amount of nitrogen exhaled, from which the total residual volume was estimated.

Deuterium Oxide

A D_2O tracer was used as the criterion method to estimate TBW. Prior to D_2O ingestion, urine samples were collected from all participants. Participants were instructed to void their bladders completely. After voiding the bladder, participants ingested roughly 11 grams of D_2O along with a 100 ml of deionized water to rinse and to insure all D_2O was ingested. The exact amount of D_2O taken was recorded for each subject by a calibrated scale to the 0.001g. The principle of the technique is based on the belief that water is equally distributed in all parts of the body with the exception of body fat. D_2O is a non-radioactive isotope that equilibrates in the body roughly after 4 hours of ingestion, after which, the amount of D_2O can be measured from a urine sample using a Europa Hydra continuous flow isotope ratio mass spectrometer. By

comparing the pre and post values of D_2O (raw form and urine sample form) TBW was measured by the process of absorption. After a 4-hour equilibration period restricting any use of the bathroom and food and/or water ingestion, participants were instructed to provide a post-urine sample.Within 30 minutes of collection, all urine samples were pipetted into two cryogenic vials and stored at -80°C for later analysis (19, 28, 29). Multiple vials were used incase any issues arrised during the shipping process we would have a backup. All samples were packaged and mailed to (Metabolic Solutions, Inc., Nashua, NH.) in dry ice according to the United States Postal Service requirements. The urine-diluted D_2O was analyzed in triplicate using an isotope-ratio mass spectrometer at MSI, and the isotope levels in the urine were calculated following the method of Wong et al. (30). TBW was then calculated from the dilution of isotopic water and corrected for the exchange of deuterium with nonaqueous tissue (31). D_2O can be summarized in the following equation: C_1V_1/C_2; where C_1 = the concentration of D_2O used, V_1 = the volume of D_2O consumed, and C_2 = the concentration of D_2O in the urine (32).

Dual Energy X-Ray Absorptiometry

DXA (GE Lunar Prodigy, Madison, WI) was used to determine manufacturer derived BF% and BMC. A DXA scanner is a machine that produces two x-ray beams, each with different energy levels. One beam is high energy while the other is low energy. The amount of radiation that pass through the body is measured for each beam. This will vary depending on the thickness of the tissue. Based on the difference between the two beams, the bone density, and the density of FM and FFM can be measured. Participants were informed that there is a low dosage of radiation produced with a whole-body DXA scanner, it is less than 1/10 amount of radiation from a standard chest x-ray. Prior to each whole-body scan, DXA was calibrated according to the manufacturer's instructions via a standard calibration block. Participants removed shoes, socks,

as well as all metal and jewelry (underwire, clasps, buttons, zippers). Next, participants were instructed to lay supine on the scanning bed with hands by their sides. If the participant's shoulders were too wide to fit within the area of the scan (max width of 60cm), the participant was shifted to ensure the full right side of the body was scanned, and the left limbs were estimated from the right limbs according to manufacturer guidelines. This is utilizing the estimate feature of the DXA scanner where only half the body is needed to accurately account for the whole body and has been proven to be comparable to a whole body scan of the same person with a mean difference of 0.6% (33). During all body scans, participants were asked to remain motionless, while Velcro straps were situated around the ankles and knees. Scans lasted approximately 6 to 10 min. The same researcher positioned all participants on the DXA scanning bed.

The equations derived from Smith-Ryan et al. and Wilson et al. were utilized to estimate DXA-derived BV for the modified 2C- and 3C-models (21, 34). The modified 2C- and 3C-models were abbreviated as follows: (1) $3C_{Siri}$-SR, (2) $3C_{Siri}$-W, (3) $3C_{Lohman}$-SR, (4) $3C_{Lohman}$-W, (5) 2C-SR, and (6) 2C-W are shown and numbered in **Table 1**. The BV prediction equations were developed by estimations of FM, LM, and BMC and are accompanied by a different constant number which can indicate which variable holds more weight or accounts more towards an individuals' BV. The equations from Smith-Ryan et al. (21) and Wilson et al. (34) are numbered and described as follows:

Smith-Ryan et al. (7)

DXA Volume (L) = (FM/0.84) + (LM/1.03) + (BMC/11.63) - 3.12

Wilson et al. (8)

DXA Volume (L) = (FM/0.87) + (LM/1.072) + (BMC/2.283) + 1.504

The BMC values produced from the DXA were also converted to total body mineral (M) for the modified 3C models (i.e., $3C_{Lohman}$-SR, and $3C_{Lohman}$-W) using the following equations (35):

(9) M_o = total body BMC × 1.0436

(10) M = M_o × 1.235

BMC represents all the minerals found within bones, however, there are other minerals throughout the entire body that needs to be accounted for (i.e. M = total body mineral). As previously mentioned, this is also where the differences in multi-compartment models (4C and 5C) come into play by including M_S. BMC estimated by a DXA scan represents ashed bone, one gram of bone minerals is roughly 0.9582 grams of ash. This is not a 1:1 ratio due to water and carbon dioxide being given off during the burning process. Hence why BMC is converted to M_O (M_O= BMC x 1.0436) which is 1/0.9582 (13). We used M cause it represents all minerals within the body where M_O is only that of bones.

Equations Used for Valdiation

The calculation of the 3C model is described by Siri (36) and Lohman (37). The Siri equation was chosen because TBW was included as one of the three compartments, whereas Lohman uses M. By comparing two different 3C models, the researchers were able to determine if including TBW increased the accuracy of predicting BF%. If not, then the Lohman equation could be used to estimate 3C body compsotion based soley on values obtained from a single DXA scan. The equation is used to derive FM, which can subsequently be used to estimate BF% after accounting for the total BM of the participant (which is referenced below). The only difference between the two predcition models is that BV was derived using equations (7) and (8).

Siri 3C

(11) FM (kg) = 2.118(BV) – 0.78(TBW) – 1.351(BM)

Lohman 3C

(12) FM (kg) = 6.386(BV) + 3.916(M) – 6.09(BM)

The calculation of a 2C prediction models was based on the DXA-derived BV determined based upon the body density convertion fomula of Siri (36). The only difference between the two predcition models is that BV was derived using equations (7) and (8)

Siri 2C:

(13) FM (kg) = 4.95(BV) – 4.50(BM)

After calculating FM, BF% was estimated by accounting for the total mass of the participant using the following equation:

(14) BF% = (FM/BM) × 100

Table 1: Abbreviations of Prediction Equations

1) $3C_{Siri}$-SR	3-compartment Siri model using the Smith-Ryan equation for BV
2) $3C_{Siri}$-W	3-compartment Siri model using the Wilson equation for BV
3) $3C_{Lohman}$-SR	3-compartment Lohman model using the Smith-Ryan equation for BV
4) $3C_{Lohman}$-W	3-compartment Lohman model using the Wilson equation for BV
5) 2C-SR	2-compartment Siri model using the Smith-Ryan equation for BV
6) 2C-W	2-compartment Siri model using the Wilson equation for BV

Notes: 2C-SR= two-compartment model when using Smith-Ryan et al. equation for body volume; 2C-W= two-compartment model when using Wilson et al. equation for body volume; $3C_L$-SR= three-compartment model by Lohman et al. when using Smith-Ryan et al. equation for body volume; $3C_L$-W= three-compartment model by Lohman et al. when using Wilson et al. equation for body volume; $3C_S$-SR= three-compartment model by Siri et al. when using Smith-Ryan et al. equation for body volume; $3C_S$-W= three-compartment model by Siri et al. when using Wilson et al. equation for body volume

Statistical analysis

The differences in mean BF% among the prediction models and the 3C-criterion were analyzed using a repeated measured ANOVA (SPSS version 24) with the Bonferroni-adjusted alpha level ($P \leq 0.00833$). A repeated measures ANOVA was used to simply protect ourselves from producing Type I errors which may occur when utilizing multiple t-tests since we were comparing multiple equations to a criterion. The following assumptions pertain to a repeated

measures ANOVA test: 1) the dependent variable should be measured at the continuous level, 2) there should be no significant outliers, the distribution should be normally distributed, and 3) the variance of all the differences are equal in all groups or sphericity. Data were normally distributed with no outliers, and equal varianecs between groups. The relative accuracy of the multi-compartment models were based upon the evaluation of predicted values versus the 3C-criterion by calculating the constant error (CE), effect size (ES), Pearson's correlation (*r*), standard error of estimate (SEE), proportional bias, and total error (TE).

The CE was determined as the mean differences between the DXA-derived BF% and the 3C-criterion BF% (i.e. $3C_{siri}$-SR – 3C-criterion). Cohens'd was utilized for the ES, with the magnitude of the ES qualitatievly described using the following thresholds (38): 0 to 0.19 = trivial, 0.2 to 0.59 = small, 0.6 to 1.19 = moderate, 1.2 to 1.9= large, >2.0 = very large. Regression procedures were used to determine *r* and SEEs, the following thresholds were used to describe the *r* values: 0 to 0.30 = small, 0.31 to 0.49 = moderate, 0.50 to 0.69 = large, 0.70 to 0.89 = very large, and 0.90 to 1.00 = near perfect. The method of Bland-Altman (39) was used to identify the 95% limits of agreement (LOA) of the BF% for the DXA-derived BV models and 3C-criterion. The Bland Altman method graphs a solid middle line to represent the average difference between the prediction equations and the criterion. The Y-axis represents the CE between the prediction equation and criterion (3CS-SR - 3C-criterion). The X-Axis represents the mean between the prediction equation and criterion ([3CS-SR + 3C-criterion]/2). The two dashed lines represents the upper and lower agreement range, stating the 95% confidence intervals, and the dotted dash line is the trend line which indicates proportional bias. Lastly, BF% was analyzed for the entire sample, as well as stratified by sex due to potential sex-related differences (40).

CHAPTER 3

RESULTS

Fifty-five men and seventy-four women (n=129) participated in this study. Participants' characteristics are shown in **Table 2**. The sample was predominantly female (57%) and comprised of mostly young adults (64% below age 30). In addition, the majority of participants self-identified as White/Caucasian (n=114, 88%), Black/African-American (n=6, 4%), Hispanic/Latino (n=3, 2%), Asian (n=3, 2%), and other (n=3, 2%). Also, this was a convenience sample of participants was recruited via print advertising and by word of mouth from the Department of Kinesiology, local fitness centers, and health clubs. Although aerobic capacity or muscular strength was not assessed as part of this study, in general participants were recreationally active and relatively fit.

Table 2 Descriptive Characteristics of Participants (Mean ± SD)

Characteristics	**All (N=129)**	**Men (n=55)**	**Women (n=74)**
Age (years)	30.29 ± 13.66	31.96 ± 14.16	29.05 ± 13.23
Height (cm)	170.90 ± 8.76	178.41 ± 6.22	165.32 ± 5.65
Body Mass (kg)	75.56 ± 17.03	85.87 ± 14.40	67.89 ± 14.67
BMI ($kg \cdot m^2$)	25.7 ± 4.8	26.9 ± 3.8	24.8 ± 5.3

BV, as measued using equations (7) and (8) correlated strongly with the UWW criterion measure (r=.999 and .999 respectively). In addition, the estimated BV from each are 74.2L, 74.3L, and 72.0L respectively. However, when included in a multi-compartment model all DXA-derived BV equations overestimated BF% for total sample (6.12% to 12.6% and ES=0.66 to

1.36), men (4.92% to 9.63% and ES=0.61 to 1.20), and women (6.02% to 17.75% and ES=0.67 to 1.96) **Table 2**. Furthermore, the correlation between the DXA-derived BV and 3C-criterion BF% were strong (0.711 to 0.981; **Table 2**).

Equation (1) demonstrated the highest level of accuracy in the total sample, as well as when stratified by sex, as indicated by the smallest SEE of all methods (1.91%, 1.83%, and 1.76% respectively). In contrast, equation (4) demonstrated the lowest level of accuracy when compared to the criterion measure (SEE values for total sample, men, and women: 6.30%, 5.70%, and 5.68% respectively). The Bland-Altman plots comparing the agreement of difference between 3C-criterion and each DXA-derived BV prediction equation for BF% values for the entire sample are shown in Figures 1-6 (**Appendix**). In all cases we have a positive trend line which indicates as a participant's BF% is higher the DXA-derived BV equations will over predict BF% even more in comparison to the criterion. Finally, accounting for TBW in a multi-compartment model strengthened correlations and reduced observed error among the total sample, as well as when stratified by sex regardless of the BV method used, although the estimated BF% remained significantly different in comparison to the criterion (**Table 2**). This indicates that multi-compartment equations that include a measure of TBW should be used for more accurate estimations of BF%.

CHAPTER 4

DISCUSSION

The purpose of this study was to examine the validity of using DXA-derived BV in a 3C body composition model when compared to traditional UWW measures. Regardless of the predication equation, 3C models using DXA-derived BV overestimated BF% in the total sample and when stratified by sex. However, it should be noted that the observed error appeared to be smallest in men. Equation (1) demonstrated the highest level of accuracy of all the prediction equations compared in this study, although it overestimated BF% by approximately 6% independent of sex, indicating that these DXA-derived BV equations should not be used in multi-compartment models when estimating BF%.

The results of the current study are consistent with previous research that indicated the Smith-Ryan and Wilson DXA-derived BV equations produce near perfect correlations when compared to UWW. Although it was not used in the current study, DXA-derived BV is strongly correlated (r= 0.99) with ADP, with small differences observed between equation (7) and a 4C-criterion (96.6L vs. 95.7L, p= <0.001) (24). In addition, our findings are also consistent with previous research that found BF% values estimated using DXA-derived BV equations (equations 7 and 8) demonstrate large differences when used in multi-compartment models in estimating BF% when compared to the criterion (i.e., 32.25% and 32.97% vs 26.13%. p<.001) which suggests these two methods should not be used interchangeably. Tinsley (23) assessed the relative accuracy of equation (7) and (8). BV equations in a four-compartment model (i.e., 4C-SR and 4C-W), and demonstrated that both had excellent test-retest reliability when estimating

BV (ICC > 0.99). However, the DXA-derived BV equations resulted in large observed error, with equation (8) consistently predicting a higher BF% than equation (7) in two different multi-compartment equations (27.4% vs 20.6% and 28.1% vs 21.3%, respectively). McLester et al. (41) also tested the validity of the Smith-Ryan and Wilson BV equations using a 4C model to estimate BF% in normal weight, overweight, and obese adults, and concluded that the observed bias (CE) was larger in normal weight and overweight adults (4.8% and 4.5%, respectively) when using equation (8). These data indicate that the Smith-Ryan equations [i.e., (1), (3), and (5)] have lower group and individual errors in the total sample and among women, but only lower individual error in men, whereas the Wilson equation has lower group error when compared to the 3C-criterion in men.

Not all of our findings agree with previous research, that is, our results do not support the claims of a DXA-derived BV equation in place of traditional BV techniques. The correlation between the DXA-derived BV and 3C-criterion BF% were strong, but weaker than expected based on previous research (0.711 to 0.981; **Table 2**). Both Mclester et al. (41) and Nickerson et al. (42) tested these equations against a criterion and both showed that when comparing the individual level of BF% that equation (8) produced smaller SEE values than equation (7). However, the findings from Blue et al. (24) show smaller SEE (0.32%) when utilizing equation (7) in an overweight sample of participants. The current study indicates that equation (7) produced lower SEEs in all comparisons, with even greater differences observed when using the Siri equation. Additionally, when implemented into the $3C_L$ equations [i.e., equations (3) or (4)] SEEs increased roughly 3-4% which further supports previous research (10, 17, 18) in how important the factor of TBW is when using multi-compartment models for estimating BF%.

Although the results of this study are novel, they do come with limitations. First, the sample was predominantly Caucasian, and as a result the findings should not be extended to other racial groups, as differences in FFM characteristics and their subsequent impact on estimated BF% has yet to be thoroughly examined (43, 44). In addition, the sample ranged from 18 to 82 years of age, however 64% of the sample was under age 30. As such, the relative accuracy of these methods in assessing body composition in older adults has yet to be determined, due to the increase in FM, and concurrent decrease in FFM and BMC that occur as part of the normal aging process (45). In addition, the sample was limited to adults over 18 years of age and should not be generalized to children and adolescents. The rapid change in height and weight, as well as FM and FFM characteristics (i.e. higher water, lower protein, and mineral content) in this age group justifies the need to account for growth and development when estimating body composition (46, 47). The BMI ranged from 18-44 $kg \cdot m^2$, however most of the sample (56%) was classified as normal weight, so it is unclear how weight status and influences the accuracy of these body composition methods. Future research should stratify recruitment across age and race/ethnic groups to ensure even representation across all participant demographic characteristics. Lastly, participants who did not fit within the DXA scanner were included in the current analysis after applying the manufacturer "estimate" feature for their respective scans. Although the participants were shifted to ensure the full right side of the body was scanned, and the left limbs were estimated from the right limbs according to manufacturer guidelines. It is unclear how this strategy impacts the relative accuracy of DXA-derived BV and the subsequent impact on body composition estimated using a multi-compartment model.

The strength of the current study is bolstered by the use of D_2O and UWW in the assessment of TBW and BV, respectively, for the criterion. These methods are considered to be

the “gold-standard” in research settings, and specifically in validation studies for body composition assessment. Furthermore, the strict pretesting protocol restricted alcohol and food consumption, as well as strenuous exercise prior to each visit; with the hydration status of each participant was verified via urine-specific gravity. When accounting for TBW, the observed error of equations (1) and (2) significantly decreased, highlighting the importance of TBW in a multicompartment model regardless of the method used to assess BV. TBW was measured via D_2O for the 3C-criterion emphasizes why future research may seek to compare modified 3C models that may us DXA-derived BV measurements and either BIA or BIS for TBW estimates in comparison to a criterion that uses D_2O. Furthermore, the use of UWW as a criterion measure of BV allowed the authors to determine the relative accuracy of multiple DXA-derived BV equations. In addition, the sample was 57% female and allowed for generalization to both sexes, as we could examine any potential sex-related differences in the observed error. It has been shown that men have significantly higher FFM and TBW values when their female counterparts have higher BF% (40, 48).

In conclusion, using DXA-derived BV as part of a 3C model introduced error and overestimated BF% in adults, regardless of the equation used. However, the $3C_S$-SR equation demonstrated better accuracy in comparison to all other DXA-derived prediction equations, especially in the total and women samples. Findings from this investigation indicate that both DXA-derived BV equations are strongly correlated with UWW-derived BV, although it does not provide an accurate measure when incorporated in a multi-compartment model for estimation of BF%. This is mostly due to the DXA-derived equations on average over predict by 2L, which can influence total BF% by almost 5%. As well as, the Bland Altman plots showed a positive proportional bias affect in all comparisons indicating that as a participant’s BF% is higher the

prediction equations will over predict BF% even more. Additional validation research is needed in larger and more racially diverse samples across the lifespan.

CHAPTER 5

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APPENDIX

Table 3 Comparison of BF% values (Mean ± SD) between modified 2C, 3C models and DXA when compared with 3Ccriterion (N=129)

									95% limits of Agreement		Linear Regression	
	Method	M ± SD	*P*	Cohen's D	r	SEE	TE	CE	Upper	Lower	Coefficient	Trend
Total (N=129)	3C-criterion	26.13± 9.27	---	---	---	---	---	---	---	---	---	---
	$3C_{Siri}$-SR	32.25± 10.20	<.001	0.66	.979	1.91	6.50	6.12	10.45	1.78	0.979	<.001
	$3C_{Siri}$-W	32.97± 9.81	<.001	0.74	.964	2.48	7.32	6.84	11.97	1.71	0.964	<.001
	$3C_{Lohman}$-SR	36.55± 12.09	<.001	1.12	.886	4.32	11.91	10.42	21.78	-0.95	0.886	<.001
	$3C_{Lohman}$-W	38.73± 12.38	<.001	1.36	.737	6.30	15.10	12.60	29.00	-3.82	0.737	<.001
	2C-SR	35.25± 11.05	<.001	0.98	.889	4.27	10.44	9.12	19.11	-0.87	0.889	<.001
	2C-W	36.94± 10.95	<.001	1.17	.784	5.78	12.77	10.81	24.2	-2.58	0.784	<.001
	DXA	29.92± 9.07	<.001	0.41	.888	4.29	5.76	3.79	12.32	-4.74	0.888	<.001
Men(n=55)	3C-criterion	22.12± 8.03	---	---	---	---	---	---	---	---	---	---
	$3C_{siri}$-SR	28.36± 8.23	<.001	0.78	.974	1.83	6.51	6.24	9.88	2.60	0.974	<.001
	$3C_{siri}$-W	27.04± 7.51	<.001	0.61	.971	1.92	5.28	4.92	8.70	1.15	0.971	<.001
	$3C_{Lohman}$-SR	31.75± 9.50	<.001	1.20	.817	4.67	11.06	9.63	20.38	-1.12	0.817	<.001
	$3C_{Lohman}$-W	27.78± 7.85	<.001	0.70	.711	5.70	8.23	5.66	17.48	-6.16	0.711	<.001
	2C-SR	30.91± 8.71	<.001	1.09	.822	4.61	10.11	8.79	18.66	-1.08	0.822	<.001
	2C-W	27.83± 7.15	<.001	0.71	.766	5.21	7.73	5.71	16.02	-4.60	0.766	<.001
	DXA	24.31± 6.98	.007	0.27	.839	4.41	4.85	2.19	10.76	-6.39	0.839	<.001
Women (n=74)	3C-criterion	29.12± 9.05	---	---	---	---	---	---	---	---	---	---
	$3C_{siri}$-SR	35.14±10.60	<.001	0.67	.981	1.76	6.50	6.02	10.82	1.23	0.981	<.001
	$3C_{siri}$-W	37.38±8.98	<.001	0.91	.973	2.11	8.52	8.26	12.39	4.14	0.973	<.001
	$3C_{Lohman}$-SR	40.12± 12.63	<.001	1.22	.899	3.99	12.50	11.00	22.74	-0.74	0.899	<.001
	$3C_{Lohman}$-W	46.87± 8.11	<.001	1.96	.782	5.68	18.64	17.75	28.99	6.51	0.782	<.001
	2C-SR	38.49± 11.54	<.001	1.04	.902	3.94	10.68	9.37	19.49	-0.76	0.902	<.001
	2C-W	43.72± 7.96	<.001	1.61	.820	5.22	15.49	14.60	24.82	4.38	0.820	<.001
	DXA	34.10± 8.18	<.001	0.55	.899	3.99	6.34	4.98	12.74	-2.78	0.899	<.001

Note. BF%= body fat percentage; DXA= dual energy X-ray absorptiometry; 2C-SR= two-compartment model when using Smith-Ryan et al. equation for body volume; 2C-W= two-compartment model when using Wilson et al. equation for body volume; $3C_L$-SR= three-compartment model by Lohman et al. when using Smith-Ryan et al. equation for body volume; $3C_L$-W= three-compartment model by Lohman et al. when using Wilson et al. equation for body volume; $3C_S$-SR= three-compartment model by Siri et al. when using Smith-Ryan et al. equation for body volume; $3C_S$-W= three-compartment model by Siri et al. when using Wilson et al. equation for body volume; *P*<.05= significant difference between mean BF% values of DXA-derived equations and 3C-criterion; Cohen's D: 0.2 to 0.59 = small, 0.6 to 1.19 = moderate, 1.2 to 1.9= large; Correlation threshold: 0.70 to 0.89= very large, and 0.90 to 1.00= near perfect; SEE= standard error of estimate; TE= total error; CE= constant error; Trend = correlation between the difference of the methods and their mean; Significant trend (p <.05).

FIGURE LEGENDS

Figure 1-6. Bland-Altman plots for body fat percentage (BF%) using all comparisons in the total sample.

Figure A Example of brief medical history questionnaire

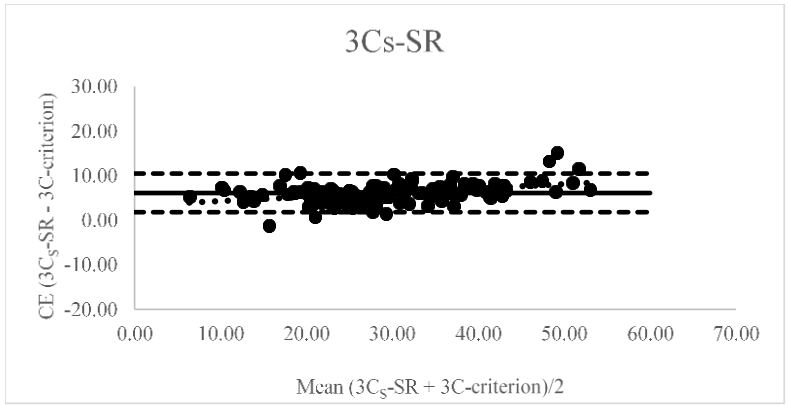
Figure B Example of past 24-hour dietary history questionnaire

Figure C Illustrated representation of Underwater-Weighing Technique

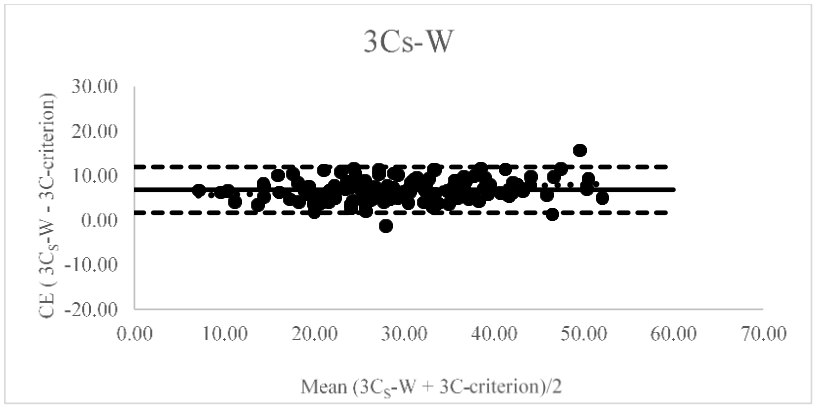
Figure D Illustrated representation of Dual Energy X-ray Absorptiometry Technique

Figure E IRB Approval Letter

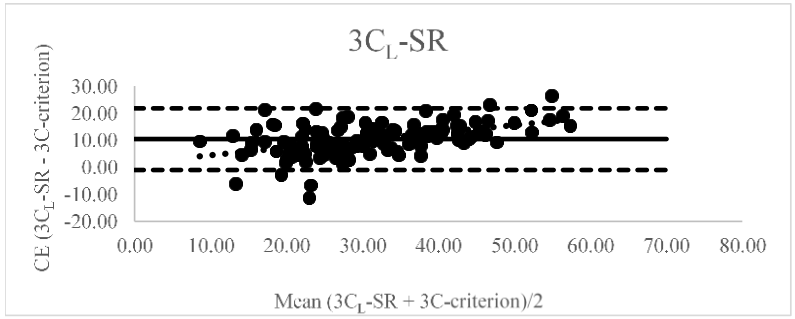
1)



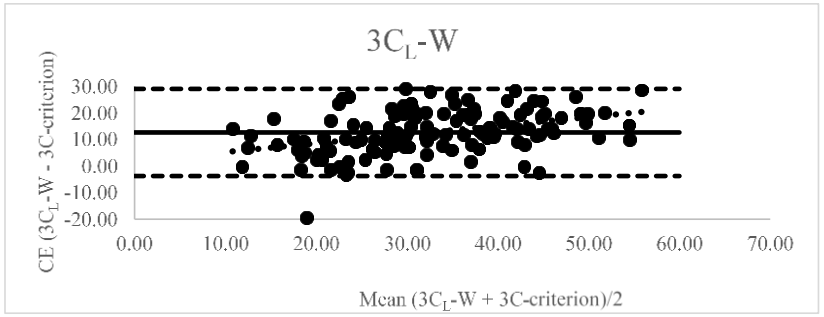
2)



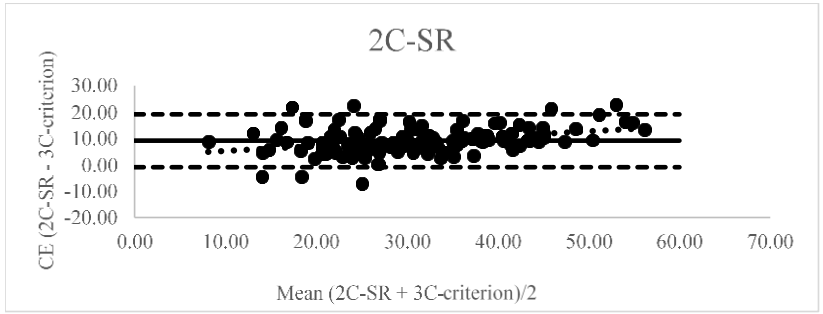
3)



4)



5)



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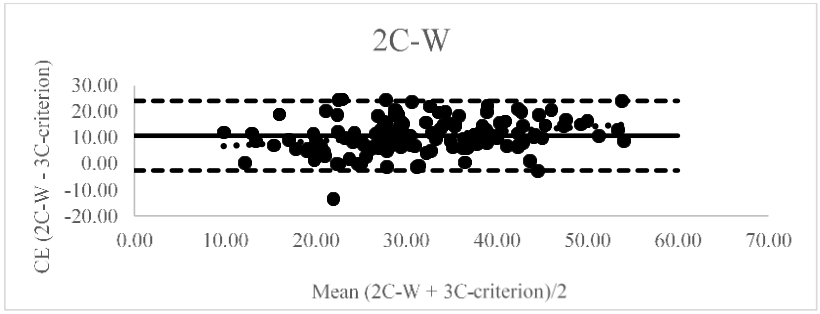


Note: The middle solid line represents the constant error between the DXA-derived BV multi-compartment equations and the 3C-criterion BF% values. The 2 outside dashed lines indicate the 95% confidence interval of the bias (difference) and their means. The dashed-dotted regression line represents the trend between the difference of the methods and their mean. Separate Bland-Altman plots depicting BF% in the DXA-derived models are shown for 1) 3C$_S$-SR; 2) 3C$_S$-W; 3) 3C$_L$-SR; 4) 3C$_L$-W; 5) 2C-SR; 6) 2C-W.

A)

SELF-ADMINISTERED PRE-EXERCISE MEDICAL HISTORY

Date _____

Name _____
Last First MI

Address _____
Street Apt #

_____
City State Zip Code

Contact _____
Home Phone Cell Phone Email

Date of Birth_____ Age_____ Sex_____ Ht_____ Wt_____
MM/DD/YYYY

Occupation _____

Emergency Contact_____
Name Relationship Phone

Race: (Select ONE of the following)

_____American Indian or Alaska Native
_____Asian (includes persons from the Indian subcontinent)
_____Black or African American
_____Hispanic or Latino: **Please also select one of the following**
  _____American Indian or Alaska Native
  _____Asian
  _____Black or African American
  _____Native Hawaiian or Pacific Islander
  _____White
  _____Two or more of the above _____
  _____None of the above
_____Native Hawaiian or Pacific Islander
_____White
_____I do not wish to disclose this information

Mark any of the following that apply to you (adapted from Preparticipation Health Screening and Risk Stratification. In: *ACSM's Guidelines for Exercise Testing and Prescription*, edited by W. R. Thompson, N. F. Gordon, and L. S. Pescatello. Philadelphia, PA: Wolters Kluwer; Lippincott Williams & Wilkins, 2010, p. 18-39)

_____Pain, pressure, or other discomfort in the chest, neck, jaw, arms, or other areas
_____Shortness of breath at rest or with mild exertion
_____Dizziness or syncope
_____Shortness of breath when you lie down or that wakes you up while sleeping
_____Swollen ankles, or any unexplained swelling
_____Your heart feels like it is skipping beats or racing while at rest
_____Pain when walking that is relieved with rest, especially pain in the calf or thigh muscle
_____Known heart murmur told to you by a physician
_____Unusual fatigue or shortness of breath with usual activities

---

B)

24-Hour History

ID _____
Date _____
Time _____

1. How many hours of sleep did you get last night? (please circle one)
   1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 (hrs)

2. How many hours of sleep do you normally get? (please circle one)
   1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 (hrs)

3. How many hours has it been since your last meal or snack? (please circle one)
   1 **1.5** 2 **2.5** 3 **3.5** 4 **4.5** 5 **5.5** 6 **6.5** 7 **7.5** 8 **8.5** 9 **9.5** 10 **10.5** 11 **11.5** 12 **12.5** 13 **13.5** 14 **14.5** 15 (hrs)
   List the items below:

4. When did you last have:
   · a cup of coffee or tea?

   · cigarettes?

   · drugs (including aspirin)?

   · alcohol?

   · herbal or dietary supplements?

5. How many glasses of water or other beverages have you consumed in the last 24 hours?
   1 2 3 4 5 6 7 8 9 10 11 12 13 14

6. When did you last consume water or another beverage? _____How much? _____ (glasses)

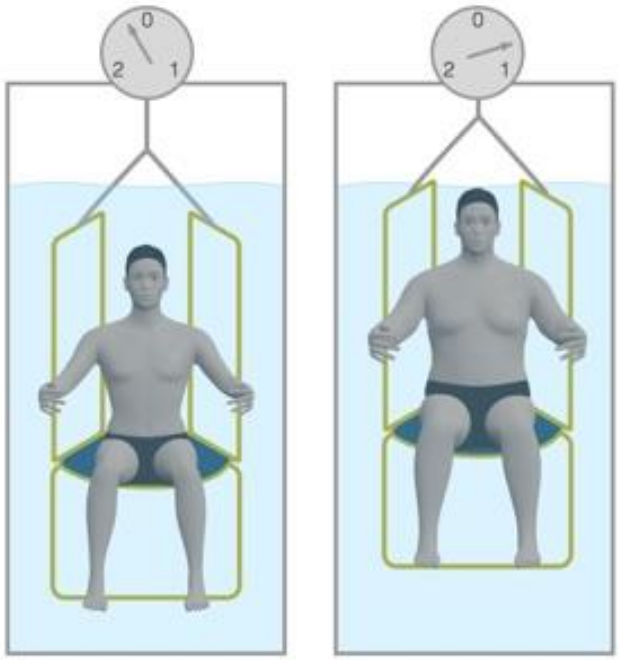
7. What sort of physical activity did you perform yesterday?

8. What sort of physical activity have you performed today?

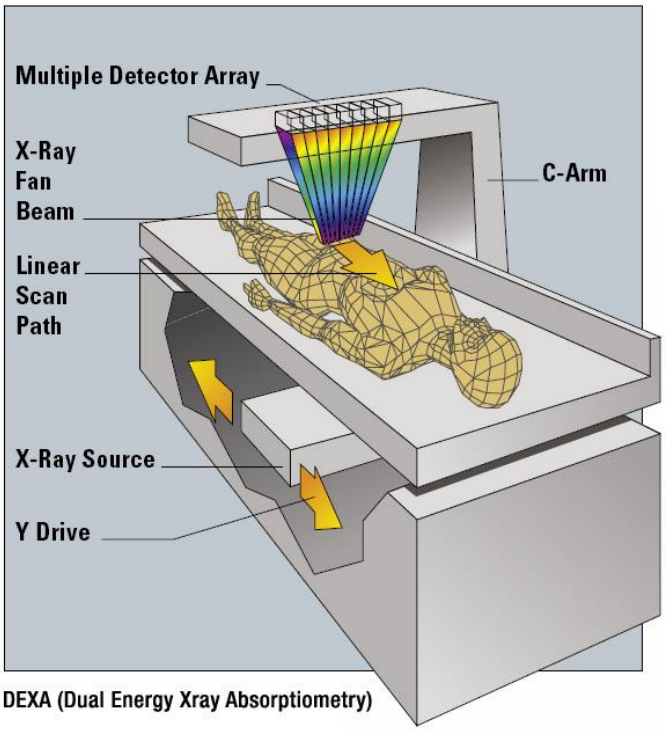
9. Describe your general feelings by checking one of the following:

_____excellent	_____good	_____very bad
_____very, very good	_____neither good nor bad	_____very, very bad
_____very good	_____bad	_____terrible

C) Depiction of Underwater-weighing technique



D) Depiction of Dual Energy X-ray Absorptiometry



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

December 18, 2018

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

Re: IRB Protocol # 15-019-ME-R3
"Development of a Novel Body Fat Prediction Equation: A Four-Compartment Model Approach"

Dr. Esco:

The University of Alabama Medical IRB recently met to consider your renewal application. The IRB voted to approve your protocol for a period of one year.

Your application will expire on November 7, 2019. You will receive a notice of the expiration date 90 days in advance. If your research will continue beyond this date, complete the renewal portions of the FORM: IRB Renewal Application. If you need to modify the study, please submit FORM: Modification of An Approved Protocol. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the FORM: Request for Study Closure.

Should you need to submit any further correspondence regarding this application, please include the above application number.

Good luck with your research.

Sincerely,

[redacted]
Medical IRB Chair