

DXA REFERENCE STANDARDS FOR PERCENT BODY FAT AND LEAN BODY MASS IN
ADULTS

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ABSTRACT

Thesis: DXA Reference Standards for Percent Body Fat and Lean Body Mass in Adults

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Purpose: Dual energy x-ray absorptiometry (DXA) provides accurate measurements of percent body fat (%BF) and lean body mass (LBM). No universal references of body composition exist using DXA measurements. The purpose of this study was to develop a set of reference data for DXA-derived %BF and LBM and to characterize the agreement between classifications of obesity based on the BMI (≥ 30 kg/m²) and %BF ($\geq 25\%$ for men and $\geq 30\%$ for women).

Methods: A de-identified sample of 2,761 subjects was obtained through Ball State University's Clinical Exercise Physiology Program (Muncie, IN). Subjects were aged 20-94 years and were scanned from July 2003 until February 2013 using either the GE Medical Systems Lunar Prodigy DXA or the GE Medical Systems Lunar iDXA. **Results:** Normative reference tables displaying select percentiles and mean values were created for %BF and LBM across defined age groups for both males and females. Mean %BF and LBM closely resembled the means of the National Health and Nutrition Examination Survey (NHANES) in both men and women. Agreements between BMI and %BF were 97% when identified as obese and 33% when identified as non-obese. Kappa statistics showed fair agreement (0.236) in men and slight agreement (0.185) in women. Forty-seven percent were misclassified as non-obese using BMI while meeting %BF standards for obesity. **Conclusion:** Future research should consider creating a national registry for DXA-derived measurements. This national registry would allow clinicians to identify those who

are at an increased risk to health problems associated with an increase in %BF and decrease in LBM.

Chapter I

Introduction

Obesity has been linked to numerous chronic diseases and comorbidities including increased risk in developing diabetes, hypertension, heart disease, stroke, cancer, dyslipidemia, sleep apnea and/or respiratory problems (43, 51, 92, 99). Sarcopenia has been associated with negative health factors such as a decrease in functional capacity, quality of life, resting metabolic rate, insulin sensitivity, and an increased risk of injury (66, 135). Due to the breadth of negative health factors, both obesity and sarcopenia have been associated with higher costs in health care (42, 66) as well as an increase in mortality when compared to those in whom these diseases are not present (43, 111). Rates of obesity in adults aged 20-74 years have increased from 45% in 1960 to 65% in 2000 (99). Over 20% of adults aged 60-70 years are diagnosed with sarcopenia, with rates increasing to 50% in adults over 75 years of age (18).

Most studies revealing the burdensome nature of obesity utilized body mass index (BMI) to classify obesity. BMI is a simple calculation of an individual's weight in kilograms divided by their height in meters squared (kg/m^2). The current BMI classifications set by the National Institutes of Health (NIH), the Center for Disease Control (CDC), and the World Health Organization (WHO) are: underweight ($\text{BMI} < 18.5 \text{ kg}/\text{m}^2$), healthy weight ($\text{BMI} 18.5\text{-}24.9 \text{ kg}/\text{m}^2$), overweight ($\text{BMI} 25\text{-}29.9 \text{ kg}/\text{m}^2$) and obese ($\text{BMI} \geq 30 \text{ kg}/\text{m}^2$). Due to its low cost, simplicity as a measure and its application in a vast amount of epidemiological studies, BMI is

the recommended screening tool in the initial clinical assessment of obesity (6, 28). Although BMI is readily used to characterize an individual's body composition in clinical settings, it has several major limitations. BMI cut points were developed assuming independence of age, gender ethnicity and race (28). However, more recent studies have provided evidence to suggest otherwise (59, 62, 103, 107). What may be most concerning is that BMI significantly underestimates adiposity. A cross-sectional study by Shah and Braverman in 2012, found that when comparing BMI to percent body fat (%BF) 39% of the population was misclassified as non-obese based on BMI standards, while meeting obesity criteria based on %BF ($\geq 25\%$ for men and $\geq 30\%$ for women). Overall, 48% of women and 25% of men were misclassified as non-obese by BMI but were found to be obese by %BF (115). Since current BMI standards have been proven to be ineffective for measuring excess adiposity, there has been a growing interest in using other methods for more accurately determining %BF. One such method is dual energy x-ray absorptiometry (DXA).

DXA scans were originally designed to measure an individual's bone mineral density (BMD) to aid in the diagnosis of osteoporosis. DXA technology uses low dose x-rays to measure BMD as well as other components of body composition; specifically fat and lean mass tissue. DXA has been shown to be a valid and reliable tool for measuring body composition (2, 95, 121). While there has been increased use in DXA technique for body composition studies (15, 49, 68, 120), researchers have also benefited from additional information that DXA provides. Unlike other body composition methods, DXA provides information such as lean body mass (LBM) measurements, which aids in the detection of sarcopenia. DXA also discloses an individual's distribution of fat which is a predictor of adverse health complications and disease (74, 131). Although DXA provides an easy and effective way to determine %BF and LBM, there are no widely recognized standards for body composition; specifically for %BF and LBM.

Previous research has provided a range of %BF for what is considered appropriate for good health (10, 86). The American Bariatric Society and the NIH has defined obesity as a %BF of $\geq 25\%$ for men and $\geq 30\%$ for women (3, 5). A reference data set for %BF was also developed by the Cooper Institute (Dallas, TX). This reference set is published in the American College of Sports Medicine's (ACSM) Guidelines for Exercise and Testing Prescription 8th edition (1), and was derived from skinfold measurements. However, the lack of diversity in socio-demographic characteristics (e.g., education, income, ethnicity) in this data may limit its application to other populations (83). Most recently in 2010, a study by Borrud et al. (20) used data from the National Health and Nutrition Examination Survey (NHANES) to develop reference ranges for %BF derived from DXA measurements. This study was the first to use DXA-derived data to develop normative reference ranges for %BF. It was also the first study to develop normative reference ranges for LBM using any type of body composition method.

Significance of the Study

There has been a growing interest in research involving DXA to measure body composition, specifically %BF and LBM. The limitation of DXA-derived body composition is that there are currently no universally accepted reference ranges for %BF and LBM. The development of these normative values can be applicable in research, clinical practice, and policy development and aid in understanding the important role that %BF and LBM play in an individual's health.

Purpose of the Study

The primary purpose of this study was to develop a set of reference standards for %BF and LBM using data derived from DXA. The secondary purpose of this study was to compare the agreement of BMI and %BF obesity classifications.

Specific Aim 1: To develop reference standards of %BF and LBM measurements from DXA derived data for males and females across defined age groups.

Specific Aim 2: To characterize the agreement of BMI and %BF obesity classifications.

Delimitations

Total body measurements of %BF, LBM, and android and gynoid region fat were provided in a de-identified data set gathered from 2003 to 2013. Two DXA models were utilized in the creation of the data set. From 2003 to 2010, a General Electric (GE) Medical Systems (Madison, WI) Lunar Prodigy DXA was used. The second DXA scanner was a GE Medical Systems (Madison, WI) Lunar iDXA which was utilized starting in 2010. The individuals scanned participated in various research studies and/or were members of Ball State University's Adult Physical Fitness Program. Individuals who were scanned multiple times were included in the study allowing for a minimum of three months between the scans.

Limitations

A limitation of this study is its lack of ethnic diversity, with the majority of the sample population being non-Hispanic whites, which strongly reflects the population of East Central Indiana (7). Previous studies have demonstrated that there are ethnic differences in %BF (103). A second limitation of the study is the criterion for being scanned. Due to physical limitations of the DXA models, subjects were excluded from being scanned if they weighed over 300 pounds when the GE Lunar Prodigy was used, or if they weighed over 450 pounds when the GE Lunar iDXA was used. An additional limitation is the small sample size of the data set used, which may lead to difficulty in generalizing results.

Definition of Terms

1. *Android fat*: Amount of fat in the lowest 20% region of the top of the iliac crest to the bottom of an individual's head.
2. *Body Mass Index (BMI)*: An indicator of a normal weight relative to height; calculated by an individual's weight in kilograms divided by height in meters squared (kg/m^2).
3. *Dual-energy x-ray absorptiometry (DXA)*: A method for body composition measurement which uses x-ray energy to measure bone mineral density, fat mass, and lean mass.
4. *Gynoid body fat*: Amount of fat in the region that extends twice the distance of the android region downward from the top of the greater trochanter.
5. *Lean body mass (LBM)*: The amount of mass in the body minus the portion that is fat and bone.
6. *Obesity*: A condition with excessive adiposity accumulation in the body to the extent that health and well-being are adversely affected; defined as body mass index of $\geq 30\text{kg}/\text{m}^2$.
7. *Percent Body Fat (%BF)*: A person's total weight of fat divided by total weight.
8. *Sarcopenia*: The degenerative loss of skeletal muscle mass and strength associated with aging.

CHAPTER II

Literature Review

The prevalence of obesity has increased at an epidemic rate over the past four decades from 45% in 1960 to 65% in 2000 (99). With the population of older adults rising, the prevalence of sarcopenia has increased as well with reports that sarcopenia affects over 20% of 60-70 year-olds, and approaches 50% in those over 75 years (18). The increased prevalence of both obesity and sarcopenia has resulted in a growing interest to be able to effectively measure and classify body composition factors directly related to these diseases. Such composition factors include %BF and LBM, both of which can be effectively estimated using DXA. A major limitation in using DXA method to estimate %BF and LBM is that there are no universal reference standards for these measurements.

Body Composition and Risk of Disease

BMI and Risk of Disease

Obesity is a condition where excessive fat accumulates in the body (28). This excessive accumulation of body fat has an adverse effect on health and well-being, specifically an increased risk of developing metabolic syndrome, type II diabetes, hypertension, coronary artery disease, cancer, osteoarthritis, and liver and gall bladder disease (77). In addition to an increased risk in developing these diseases, obesity leads to an increase in mortality rate with estimates of

22% reduction in life expectancy in individuals who are obese compared to those who are not obese (45). Current weight classifications are defined using BMI. BMI is a simple calculation of an individual's weight in kilograms divided by their height in meters squared (kg/m^2), with underweight represented by a BMI of $< 18.5 \text{ kg}/\text{m}^2$, normal weight BMI of $18.5\text{-}24.9 \text{ kg}/\text{m}^2$, overweight BMI of $25\text{-}29.9 \text{ kg}/\text{m}^2$, and obese represented by a BMI of $\geq 30 \text{ kg}/\text{m}^2$. From 2009 to 2010 the CDC reported that 35.7% of the adult population in the United States was obese and 16.9% of the children and adolescents were obese (BMI \geq age and sex specific 95th percentiles of the 2000 CDC growth charts) (4).

In 2002, Wilson et al. (136) conducted a study to evaluate the validity of BMI standards as a determinant of cardiovascular risk. Members of the original Framingham cohort were used in the study, with an initial population sample that included 5,209 subjects aged 30 to 62 years old. The initial examination took place from 1948 to 1951 and subjects were reexamined every two years following their initial examination. The study was concluded 44 years later. Key health outcomes assessed included hypertension, hypercholesterolemia, diabetes mellitus, and total cardiovascular disease. Total cardiovascular disease was comprised of two subgroups: coronary heart disease with endpoints of either angina pectoris, myocardial infarction, or coronary death, and cerebrovascular disease which included strokes and transient ischemic attacks. Age-adjusted relative risk (RR) of hypertension was significantly greater in overweight men and women (men: RR, 1.46, 95% CI 1.24-1.75; women: RR, 1.75, 95% CI 1.54-2.0) as well as in obese men and women (men: RR 2.21, 95% CI 1.75-2.79; women: RR, 2.75, 95% CI 2.32-3.27). RR of diabetes mellitus was significantly higher in obese individuals (men: RR 2.12, 95% CI 1.52-2.96; women: RR 1.42, 95% CI 1.09-1.85) as well as overweight males (RR 1.33, 95% CI 1.02-1.73). In addition to demonstrating a strong relationship between BMI classifications and cardiovascular risk factors, the study demonstrated a strong association between BMI and cardiovascular disease, with RR for overweight individuals of 1.21 (95% CI 1.05-1.40) and 1.20 (95% CI 1.03-1.41) in

men and women respectively as well as in obese individuals, with a RR of 1.46 (95% CI 1.20-1.77) and 1.64 (95% CI 1.37-1.98) in men and women respectively. Although these authors demonstrated the strong relationship between the well-established BMI guidelines and cardiovascular disease and risk factors, many researchers have argued that because BMI is a measure of excess weight rather than excess fat, measures of %BF should be used instead to define obesity (28, 103, 115).

Percent Body Fat and Risk of Disease

%BF is an individual's total weight of fat divided by their total weight. While there are currently no universally accepted guidelines that classify obesity solely through the measurement of %BF, some organizations agree that a %BF of $\geq 25\%$ for men and $\geq 30\%$ for women would classify one as obese (5, 8). In a 2012 study using %BF obesity standards of $\geq 25\%$ for men and $\geq 30\%$ for women, Zeng et al. (141) examined 3,859 subjects (2,173 males and 1,686 females) aged 18-85 years without a history of cardiovascular disease and divided them into four groups: reference group [normal BMI ($< 25 \text{ kg/m}^2$) and normal %BF ($< 25\%$), $n=1,961$], group 1 [normal BMI, but abnormal %BF ($\geq 25\%$ in men, $\geq 30\%$ in women) $n=381$], group 2 [abnormal BMI ($\geq 25 \text{ kg/m}^2$), but normal %BF, $n=681$], and group 3 [abnormal BMI and abnormal %BF, $n=836$]. The odds ratio (OR) for cardiovascular risk factors in group 1 and group 3 were 1.88 (95% CI=1.45-2.45) and 2.06 (95% CI=1.26-3.35) times those in the reference group. The study also demonstrated that %BF was able to predict the probability of hypertension (OR=1.03, 95% CI=1.01-1.04, $p=0.001$), dyslipidemia (OR=1.05, 95% CI=1.02-1.09, $p=0.001$) and hyperglycemia (OR=1.03, 95% CI=1.00-1.07 $p=0.038$) whereas BMI failed to predict the probability of hypertension (OR=1.09, 95% CI=0.99-1.20, $p=0.076$), dyslipidemia (OR=0.97, 95% CI=0.87-1.07, $p=0.511$) and hyperglycemia (OR=1.09, 95% CI=0.99-1.20, $p=0.083$). In summary, the authors found that %BF, not BMI, was independently associated with

cardiovascular risk factors. This indicates that %BF is a better predictor for these risk factors and clinicians should pay closer attention to %BF in an attempt to forecast risk.

Sarcopenia and Risk of Disease

Sarcopenia was first defined by Irwin Rosenberg in 1989 to describe a recognized age-related decline in muscle mass among the elderly (108). Rosenberg noted that the involuntary loss of muscle tissue in the elderly was partially responsible for the age-related decline in functional capacity. While the current definition of sarcopenia includes a loss of muscle strength, it is still unclear whether a decline in functional capacity results from the loss of LBM or the qualitative impairment of the muscle tissue (113). In a 12-year longitudinal study (46), 12 healthy, sedentary men with an initial age of 65.4 years \pm 4.2, were studied for measurements of isokinetic muscle strength of the knee and elbow extensors as well as cross-sectional area (CSA) measurements of the thigh, quadriceps femoris, and flexor muscle (semimembranosus, semitendinosus, and the short and long heads of the biceps femoris) through the use of computed tomography (CT). Results of the study revealed significant reductions from baseline to follow-up ($p < .05$) in the CSA of the thigh (-12.5%), all thigh muscles (-14.7%), quadriceps femoris muscles (-16.1%), and flexor muscle (-14.9%). Significant reductions were also seen in muscle strength ranging from 23.7% to 29.8% in both extensors and flexors of the knee at both angular velocities (60°/s and 240°/s). The authors concluded that the quantitative loss in CSA is a major contributor to the decrease in muscle strength seen with aging. In total, the decrease in CSA attributes to 90% of the variability in strength from baseline to follow-up. The decrease in strength due to a decrease in CSA has been well-documented (53, 106, 125) with reduction of strength ranging from -1.4 to -2.5% per year (12, 13, 105, 137).

While a decrease in strength strongly impacts the physical capabilities of an individual, a decrease in LBM with aging has also been linked to an increased risk of injury and falls (71), a

reduction in the ability to carry out activities of daily living (47, 67), and a decline in resting metabolic rate (37, 101). A decrease in LBM, through either generalized muscle atrophy or sarcopenia, has also been associated with a loss in BMD, which leads to the development of osteopenia and its progression to osteoporosis (34). Low LBM has also been associated with physical disability (16, 63, 64) and plays a large role in the development of frailty which has been shown to be highly predictive of adverse events such as hospitalizations associated with morbidity and mortality (39, 104).

Factors Affecting Percent Body Fat and Lean Body Mass

While a large percentage of the adult population in the United States is affected by obesity and sarcopenia, there have been differences in %BF and LBM observed in subsets of the population. These subsets include differences in age, gender, ethnicity, physical activity status, nutrition, and genetics.

Age

It is well documented that age-related decline in strength is directly impacted by decreases in skeletal muscle mass (31, 46, 47, 65). Total muscle CSA has been shown to decrease by 40% between the ages of 20 and 60 (32, 102) with a steady decline of 1-2% per year after the sixth decade (129). Young et al. (138) found differences when comparing 12 young, healthy, male medical students (21-28 years old, mean age 25 years) and 12 healthy men in their seventies (mean age 75 years) who were leisurely active, with at least four hours per week of walking, bicycling, or other moderate physical activities. Older men were found to have a mean isometric strength of 39% less than the young controls and CSAs were 25% smaller when measured by ultrasound when compared to young controls. Similar results were seen in 25 healthy, older, women (71-81 years old, mean age 74.4 years) where mean isometric strength was 35% less than

the 25 healthy, young controls (20-29 years old; mean age 24.4 years). CSAs were also reduced in the older adults having a 33% smaller area than the young controls (139).

Unlike LBM which decreases with age, fat mass increases. In a cross-sectional study by Bazzocchi et al. (17), 25 healthy men and 25 healthy women were assigned to five age groups: 18-30 years, 31-40 years, 41-50 years, 51-60 years and 61-70 years, for a total of 250 subjects. Subjects' body composition was measured using a whole body DXA (Lunar iDXA, Madison, WI, USA; enCORE™ 2011 software version 13.6). Results demonstrated that total fat mass in men increased from age 18 to age 70, and women demonstrated an increase of total fat mass from age 18 to age 40, after which mean values of total fat mass remained steady.

Similar results were seen in a study by Kyle et al. (81) which measured 5,225 healthy adults (2,735 men and 2,490 women), aged 15-98 years and were divided into eight different groups according to their age (15-24 years, 25-34 years, 35-44 years, 45-54 years, 55-64 years, 65-74 years, 75-84 years and ≥ 85 years). Fat mass measurements were performed utilizing a 50-kHz bioelectrical impedance. Results showed that mean fat mass increased gradually from the youngest age group to the eldest age group in men (11.6 kg and 20.2kg) and women (15.6 kg and 22.0 kg).

As LBM decreases and fat mass increases with age, obese individuals are susceptible to having insufficient muscle strength relative to their body size. This condition has been termed sarcopenic obesity (118). With such large increases of fat mass and large decreases in strength, and in some cases mobility, sarcopenic obesity has severe implications which can lead to disability, morbidity, and even mortality in the elderly population (140).

Gender

Gender differences have also been observed in relation to LBM and %BF. A study by Janssen et al. (65) measured total skeletal muscle mass in 468 healthy individuals (268 men and 200 women) aged 18-88 years old (mean age: 40 years \pm 14 in men, 43 years \pm 16 in women) with whole body magnetic resonance imaging (MRI) (General Electric 1.5-T scanner, Milwaukee, WI). The authors found that men had significantly greater skeletal muscle mass in both absolute terms (33.0 kg vs. 21.0 kg) and relative to body mass (38.4% vs. 30.6%) when compared to women respectively ($p < .001$). Significant differences were also found in the distribution of skeletal muscle mass (upper body and lower body) between men and women, with men having 40% more upper body mass and 33% more lower body mass than women ($p < .001$). While it has been demonstrated that with an increase in age there is a decrease in muscle mass, men tend to experience larger age-related declines when compared to women (-14.8% vs. -10.8%), with absolute declines almost doubling those in women (50). The reason for the larger decreases in muscle mass in men compared to women is not fully understood, however, authors have suggested that it could be related to the decline in hormonal factors with aging, including growth hormone, insulin-like growth factor, and testosterone which are key contributors in building and preserving muscle (65).

While these previous studies have revealed that mean lean muscle mass is higher in men, other studies have demonstrated that mean %BF is higher in women. In a study comparing air displacement plethysmography (ADP) to three other body composition techniques, hydrostatic weighing (HW), bioelectrical impedance (BIA), and dual-energy x-ray absorptiometry (DXA), the mean %BF measurements were similar among all four techniques with women having a higher %BF in each technique (ADP: 31.2% vs. 15.7%; HW: 29.2% vs. 18.6%; BIA: 29.0% vs. 17.2%; DXA 34.2% vs. 18.6%) (85). The significantly greater %BF in women has also been

observed throughout age as demonstrated by Kirchengast (72). The cross-sectional study examined a total of 513 adult women and 412 adult men aging between 19 and 92 years (mean 51.7 ± 15.2) using BIA or DXA. Subjects were grouped by age per decade except for the youngest group (19-29 years) and the eldest group (≥ 80 years). Significant differences ($p < .001$) were observed at every age group between women and men, with women having significantly higher %BF than men at each age group. Largest differences observed occurred in age group 50-59 years (15.3%), while smallest differences occurred with the oldest age group (8.5%).

Ethnicity

Additionally, ethnicity has been demonstrated to play a role in body composition. Lear et al. (82) examined differences in LBM measurements gathered using DXA (Norland XR-36 scanner, Norland Medical Systems, White Plains, NY). A total of 828 subjects (403 men and 425 women) from four ethnic backgrounds were measured: 196 American, 222 Chinese, 202 European, and 208 South Asian individuals. After adjustment for confounders South Asian men had less LBM than American [3.42 kg less; 95% confidence interval (CI) = 1.55-5.29], Chinese (3.01 kg less; 95% CI = 1.33-4.7), and European (3.57 kg less; 95% CI = 1.82-5.33) men. Women showed similar differences with South Asian women having less LBM than American (1.98 kg less; 95% CI = .045-3.5), Chinese (2.24 kg less; 95% CI = .81-3.68), and European (2.97 kg less; 95% CI = 1.67-4.27) women. Significant ethnic differences in LBM were also highlighted in the comparison of Blacks, Hispanics, and Whites in a study by Araujo et al. (14). Authors measured the 1,157 subjects (mean age 47.5 years) LBM using a DXA (QDR 4500 W densitometer (Hologic, Inc., Waltham, MA). Results revealed that LBM was significantly lower ($p < .05$) in Hispanics (51.82 kg) than both Blacks (56.35 kg) and Whites (55.43 kg).

Further ethnic differences in %BF have been demonstrated. A meta-analysis of the literature included 32 studies and consisting of 11,924 subjects measured using a variety of body

composition models including: HW, DXA, three and four compartment models, skinfolds (SF), BIA, and deuterium oxide dilution (30). Differences were found in the mean %BF between the studied ethnicities with Chinese having the lowest %BF (26.1%) followed by Blacks (26.6%), Caucasian (27.2%), Polynesian (28.2%), Indonesian (29.3%), Ethiopian (30.7%) and Thai having the largest %BF (32.0%).

Physical Inactivity

Physical inactivity is an important contributor to the loss of muscle mass and muscle strength at any age (78, 84, 93). A 1970 study by Kuta et al. (79) sought to examine muscle strength, anthropometric dimensions, and LBM, measured through HW, in 132 men aged 60-79 years. Subjects were split into three groups based on their past physical activity history. Group A was comprised of men who participated in at least 15 years of intense physical training and participated in contests. Group B was comprised of men who were involved in regular sports activities for at least 15 years, but only as recreation. Group C was comprised of men who were never engaged in physical training except during school years, military service, or only sporadically. The results revealed that those individuals who actively participated in intense physical activity had a significantly higher ($p < .05$) amount of LBM (60.4 kg) when compared to those who participated in physical activity recreationally (54.8 kg) or periodically (54.6 kg).

Like LBM, %BF has also been shown to be influenced by physical inactivity. Kohrt et al. (76) studied the influence of physical activity on %BF between younger (18-31 years old, $n=133$) and older (58-72 years old, $n=244$), men and women. Subjects were classified as either sedentary or trained based on their physical activity history. Sedentary subjects had not engaged in regular exercise training (≥ 30 min/day, ≥ 2 days per week), including walking, for at least two years, while the trained subjects varied in activity level from average to competitive in local age-group events. Mean %BF measured through HW was significantly lower ($p < .01$) in the trained group

when compared to the sedentary group for both younger (10.0% vs. 17.3%) and older (17.1% vs. 28.6%) men. Similar results were seen in comparing trained and sedentary subjects in the younger (16.6% vs. 24.0%) and older (25.3% vs. 38.3%) female subjects. Although %BF levels were lower in the trained group when compared to sedentary subjects, there was little overlap in %BF between younger and older subjects with one older man and one older woman having a %BF level below the means of the young, trained men and women respectively. This demonstrates the impact that physical activity has on %BF regardless of age.

Nutrition

A 2012 randomized control study observing 25 healthy adults (16 men and 9 women) sought to evaluate the effects of consumption of low, normal, and high protein diets on weight gain and body composition (48). After being subjected to a weight stabilizing diet for 13 to 25 days, individuals were randomized into one of three diet groups; 5% of energy from protein (low, n=8), 15% of energy from protein (normal, n=9), and 25% of energy from protein (high, n=8). During the last eight weeks of their 10 to 12 week stay, the patients were overfed so that their energy intake was 39.4% (954 kcal/day) greater than the energy intake during the weight stabilizing period. Body composition was measured at baseline and then biweekly during overfeeding using DXA (Hologic QDR 4500A whole-body scanner, Software version 11.1). At baseline measurements, body weight, LBM and fat mass were not significantly different between the three diet groups. After the overeating period it was found that all groups gained weight from baseline but those in the low protein group gained significantly less weight ($p < .001$) compared to the normal protein and high protein groups. The overall increase in fat mass for all three groups was 3.51 kg (95% CI, 3.06-3.96 kg) from baseline and was not significantly different between the three groups. However, significant changes were seen in LBM between the three groups. LBM

decreased during the overeating period by -0.7 kg in the low protein group whereas LBM was increased in both the normal protein (2.87 kg) and high protein (3.18 kg) groups.

This previous study demonstrates the impact of increase energy intake on fat mass as well as importance of protein in the development of LBM in a healthy young (18-35 years) population. However the importance of protein on an individual's diet has also been demonstrated in the elderly population. Aging has been associated with a decline in food intake, often called the anorexia of aging (26). Castandena et al. (25) demonstrated that eating half the recommended dietary allowance of protein (0.8 g/kg/day) has been shown to lead to significant declines in strength, body cell mass, and insulin growth factor-1 in postmenopausal women. What may be more concerning is that while participating in a 14-week study precisely controlled for diet, 10 men and women were provided a eucaloric diet that contained 0.8 g/kg/day of protein yet still experienced reduced thigh muscle CSA (23). This study demonstrates that the recommended dietary allowance may not be adequate to completely meet the needs of older adults. Compounded with this study, it has been reported that 15% of those over the age of 60 eat less than 75% of the recommended dietary allowance, further increasing their risk for decreased muscle mass (110).

Genetics

Although it is not fully understood, genetics play a large role in the amount of LBM and %BF an individual may have. It has been reported that 70% of a child's body composition is determined through genetics (94). A study by Carey et al. (24) noted that Notch genes, which have been shown to be essential for development of skeletal muscle, were expressed at a significantly lower level in older males (60-75 years old) who tend to have lower amounts of muscle when compared to younger males (18-25 years old). While most studies involving gene expression in relation to aging skeletal muscle included few subjects and limited number of

genes, Welle et al. (133) sought to produce a more comprehensive gene expression profile. In this study, RNA was taken from biopsies of the vastus lateralis obtained from eight younger (21-27 years) and eight older (67-75 years) men. Analysis found that in older muscle, genes that aid proteins involved in energy metabolism and mitochondrial protein synthesis were expressed at a lower rate when compared to the younger group. The authors concluded that gene expression in human skeletal muscle tends to decrease between the third and seventh decades.

While genetic factors also contribute to an individual's LBM, a study by Stunkard et al. (119) found that genetic makeup also plays a contributing factor in an individual's BMI. Using the Danish Adoption Register, information on height and weight from 540 adoptees whose average age at the time of the study was 42 was obtained. Height and weight measurements were also collected from both biological parents and adoptive parents and the relationship between biological parents and adoptees indicated a genetic influence. Results showed that when compared to the adoptive parents, the adoptees weight class was more strongly related to the BMI of their biological parents ($p < .001$ for mothers; $p < .02$ for fathers), demonstrating the presence of a genetic influence on body composition.

Methods to Determine Body Composition

HW is one of the oldest methods of measuring body composition in vivo and has traditionally been considered the gold standard and criterion method for body composition analysis (2, 19, 28). HW determines body density by dividing an individual's weight on land by an individual's weight when fully submerged in water. When an individual's body is immersed in water it is subjected to a buoyant force resulting in a loss of weight equal to the weight of the displaced water. Body fat is less dense than water, thus the lower an individual's body density, the greater the amount of body fat. By knowing the individual's body density, %BF can be estimated through the Siri (117) or Brozek (22) body density equations. These equations are two-

compartment models which are based on the concept that the body is composed of two distinct compartments: fat mass and fat free mass. In using these models it is assumed that water and mineral contents of the body as well as the density of fat and free mass remain constant in all individuals.

ADP reflects the principle of Boyle's Law that, with temperature held constant; a change in pressure equals a change in volume within a closed, two-compartment chamber. A diaphragm (which separates the two internal chambers) oscillates back and forth to create volume changes that produce pressure changes in the two chambers (2). Body volume is calculated by subtracting the volume of air in a closed chamber with a subject inside from the volume of air in an empty chamber. Density is then calculated by dividing an individual's weight by the volume of displaced air in the chamber. Density can then be inserted into the two-compartment model equations (22, 117) to predict %BF in the same manner as HW.

SF thickness measurements are one of the most commonly used methods of determining %BF due to its relative low cost and simplicity (28, 132). SF are calculated on the assumption that subcutaneous fat is directly proportional to total body fat. SF do not directly measure body density or body volume like HW or ADP. Instead, numerous regression equations have been developed to predict body density from SF measurements based on age and gender (1). These body density measurements are then converted into %BF using two compartment model equations (22, 117).

BIA measures the impedance of a small electrical current through the body. This body composition method takes advantage of the principle that tissues conduct electricity based on their water and dissolved electrolyte content (28). Electrodes are applied to the extremities of an individual's body and the impedance of the current is measured between points of contact. An estimate of total body water is acquired, from which total body fat-free mass is calculated. Lean

tissue is largely composed of water (73%) (80) and electrolytes and thus is a good conductor of the signal (low impedance), while fat tissue contains less water and electrolytes and thus is a poor conductor of the electrical current (high impedance).

Potential Errors in Body Composition Methods

While traditionally HW was considered the gold standard for body composition measurements (19, 28, 132), there are three main potential errors that can arise using this method. First, while submerged in water, the subject is required to exhale maximally to help eliminate the effect that residual air in the lungs has on buoyancy of the body. Residual air in the lungs which is not expelled will increase buoyancy which would decrease the volume of water displaced. When the water volume is decreased, the calculation of body density is increased. This increase in body density will lead to an inaccurate decrease in an individual's %BF, making them appear to have less %BF than they in fact have. Even if the subject is able to rid his/her lungs of air through exhaling there is still a small amount of reserve volume of gas in the lungs and the gastrointestinal tract that must be estimated. Second, submersion in water may not be feasible for all individuals. Third, the processes of performing HW measurements are very labor intensive. A large space, intricate plumbing, and specialized equipment are required at a costly expense. Due to these limitations HW is not used by many fitness or medical centers, and is typically used and seen at universities or research facilities.

While accessibility is an issue for ADP as well, it is not as labor intensive as HW and requires minimal effort on the part of the individual. Similar to HW, certain variables can affect ADP measurements, leading to inaccurate results. For the most accurate ADP measurements, subjects should wear minimal, form-fitting clothing such as spandex-style shorts. Loose fitting clothing traps air, which creates increased pressure within the chamber (41). According to Boyle's law, this increased pressure equates to increased body volume. The increase in body

volume results in an underestimated body density and in turn, an over estimation of %BF. Scalp and facial hair have also been shown to influence measurements of %BF (56). Twenty five male subjects (31.4 ± 8 years) were asked to grow a beard for three weeks and then four measurements were taken using different conditions: facial hair and swim cap worn, facial hair without wearing a swim cap, no facial hair with swim cap, and no facial hair without a swim cap. Results demonstrated significant underestimation of %BF vs. the criterion method (no facial hair and swim cap worn) in all other three conditions (16.2% facial hair and swim cap, 14.8% facial hair and no swim cap, 14.8% no facial hair and no swim cap vs. 17.1% criterion method). The authors concluded that excess facial hair should be kept to a minimum and a swim cap should be worn at all times to ensure accurate measurements. Body temperature and moisture have also shown to play a role in the estimation of %BF in ADP measurements. A 2004 study by Fields et al. (41) examined 32 healthy females (age 33 ± 11 years) and the effect of excess heat and moisture from HW on ADP. Body weight and temperature were recorded and the first ADP measurement was taken. Next, the subject's body composition was measured using HW. After measurements were collected, subjects thoroughly dried off and body temperature was re-taken before a second ADP measurement was performed. The moisture trapped in the body hair and the fabric of the swimsuit was defined as the difference in body weight prior to hydrostatic weighing. Excess heat was defined as the difference in body temperature from pre to post HW. Results demonstrated that the presence of excess heat (36.3 °C vs. 36.9 °C; $P < 0.001$) and moisture (63.58 vs. 63.66 kg; $P < 0.05$) in the ADP testing chamber leads to a small but significant underestimation in %BF ($28.91\% \pm 10.3\%$ vs. $27.12\% \pm 10.3\%$). The authors concluded that ADP measurement of an individual should be done before a bout of exercise or afterwards, when the body returns to an at-rest state. If not, the excess heat and moisture could lead to potential measurement error.

Unlike HW and ADP, SF measurements only require the use of small handheld calipers which makes this method more accessible to a wider range of the population. While SF are more

accessible to use, wide variation has been reported when between technicians, using different calipers, technique, and predictions equations to estimate %BF. A study by Lohman et al. 1984 (88) sought to examine the variation in these factors in 16 female athletes aged 18.1-21.3 years (mean 20.0 years). Four investigators used four different SF calipers (Lange, Harpenden, Holtain and Adipometer) and five prediction equations to examine the variation in %BF. A total of 80 different combinations were observed producing variability in estimates of mean fat content, ranging from 14.1 to 28.1%. The results demonstrate the need to standardize the use of SF techniques in order to properly compare measurements between studies. Additional difficulties in utilizing SF measurements include making the measurements on the elderly, whose skin tends to be more elastic than younger individuals, as well as making measurements on those subjects who are obese (21).

Like SF measurements, BIA utilizes non-invasive, portable technology which makes it more appealing and accessible to both patients and clinicians. While potential error in SF measurements are due primarily to technician procedure variation, potential error in BIA is primarily due to the vast amount of pre-test requirements that need to be followed by the subject to ensure that the most accurate measurements are achieved. In order to obtain the most accurate measurement, subjects are not recommended to have exercised within 12 hours of the test or eaten or consumed a beverage within 4 hours of the test (29). The subjects must also be instructed to avoid alcohol and diuretic agents such as caffeine or chocolate. Previous studies have suggested that BIA not be used for large epidemiological studies with a diverse population because BIA equations are not appropriately developed based on age, gender, or ethnicity (29).

Due to its precision as a measurement for body composition, hydrostatic weighing has been traditionally known as the gold standard and most other methods of body composition are compared to it. Theoretically, the ADP and HW should give identical values for body density and

%BF because both methods are based on the principles of densitometry. Any differences between the 2 methods can be attributed to differences in either measured body mass (different scale used for each method) or body volume. Fields et al. in 2002 (40) conducted a review which examined studies published between December 1995 and August of 2001 that compared ADP with HW. A majority of the studies were conducted in young to middle aged subjects (age range 20-56 years), except for one study which included subject's up to age 86 years. The review found mean group difference between ADP and HW measurements ranged from -4.0 to 1.9 %BF. Five of the 12 studies showed no significant differences between the two methods while seven studies showed significant mean difference, with the direction of the differences being inconsistent. Five studies showed a lower %BF with the ADP than with HW (-4.0 to -2.0%BF) and two demonstrated higher %BF with ADP than with HW (1.2 and 1.9 %BF). Bland-Altman limits of agreement indicated wide variations between ADP and HW (9-16% BF) for individuals, even when group mean differences were small. These wide variations among the studies means were attributed to differences in laboratory equipment, study design, and subject characteristics and in some cases to failure to follow the manufacturer's recommended protocol, suggesting a need for more standardization in ADP measurements.

Many prediction equations for determining %BF through SF and BIA measurements have been developed through previous research. A meta-analysis in 1997 by Fogelholm and Lichtenbelth, reviewed 54 papers published from 1985-1996 and compared the means of %BF measured by multiple BIA and SF methods to the %BF means measured by HW (44). The purpose of the study was to quantify the difference and the error (standard deviation of individual differences) of the estimated body fat content between HW and the alternative method. Statistical analyses were done separately for different techniques and instruments. The study examined four methods of BIA: Lukaski et al (89, 90), Valhalla Scientific (San Diego, CA), RJL (RJL Systems Inc., Detroit MI), and Segal et al. (114). Three equations for SF were examined in this study:

Durnin & Womersley (33), Jackson & Pollock (60), and Jackson et al. (61). The authors found that BIA by Lukaski et al. significantly overestimates %BF (+2.0 %BF, 95% CI: 0.2 to 3.8) while BIA by Valhalla Scientific and SF equations by Jackson et al. underestimates %BF (-1.2 %BF, 95% CI: -2.3 to -0.1; -2.6 %BF, 95% CI: -4.5 to -0.6 respectively) when compared to HW. Due to the lack of accuracy and wide possibilities of error, many researchers have turned to DXA for measuring body composition.

Dual Energy X-Ray Absorptiometry (DXA)

DXA technology is based on a three component model in which fat mass, lean tissue mass and bone mineral density are derived. X-rays are delivered at two energies from a low current x-ray located beneath the DXA scanning bed. While scanning, the energy is passed through the supine-lying subject, posterior to anterior, and collected in the detector arm above. The ratio of attenuation at lower energy relative to higher energy distinguishes fat from fat-free mass. The scan duration can range from 5 to 25 minutes depending on the model of DXA and the thickness of the subject. Each scan delivers low amounts of radiation, which are comparable to a chest x-rays and lower than imaging CT scan. Because of its ease of use and low radiation dosage, DXA has been deemed as an appropriate method for repeated use in clinical settings for measuring body composition (28, 96).

Although previous studies demonstrated the improved nature of DXA to measure bone mineral density (BMD) there is still some concern over the assumptions made when measuring soft tissue. The first assumption is that fat content in soft tissue can be estimated from the assumed constant attenuation of pure fat to bone-free lean tissue. Because the DXA estimates the proportion of fat tissue and lean tissue in each pixel rather than directly measuring it (as in CT), it is assumed that the ratio between fat tissue and lean tissue attenuation is constant. In 1996 Pietrobelli et al. (100) reported pure fat attenuation through correlation measurements of

triglycerides to be 1.235 and lean tissue to have an attenuation of 1.345. They further reported that these values were near constant from subject to subject which allows the proportion of each pixel to be calculated based on these attenuations.

A second assumption of soft tissue measurement is that the thickness of the anteroposterior portion of the body does not affect measurements. A study by van der Ploeg et al. (127) examined 152 healthy adults aged 18-59 years (mean 30.0 ± 11.1 years). Authors found that DXA (Lunar DPX-L total body scanner operated in medium scan mode using software versions 1.3z or 1.34; Lunar, Madison, WI) %BF estimates tended to be underestimated in subjects with a lower anterior-posterior thickness when compared to a four compartment model which measured water, bone mineral mass, fat and residual fat. This idea was further verified in a study by Lukaski et al. measuring 20 pigs weighing 52-113 kg using both DXA and chemical analysis. Authors found no significant differences between the two composition methods when the anterior-posterior thickness was < 24 cm. However DXA %BF accuracy was significantly different compared to chemical analysis in pigs with an anterior-posterior thickness >24 cm (91).

A third assumption is that soft tissue quantities can only be accurately determined from where no bone is located. In the regions with bone, soft tissue measurements are assumed to have an equal amount of fat surrounding the bone as the neighboring bone-free tissue. In an average whole body scan, approximately 40 to 45% of pixels are classified as containing bone (35). The remaining pixels are used to estimate the body's fat-to-lean tissue ratio. This value is then applied to the soft tissue component in the adjacent bone pixels. The effect of this assumption has not been studied because the DXA software is proprietary information. Therefore, it is difficult to evaluate how well tissue composition is estimated in these areas.

Despite these three assumptions, DXA scans have shown great reliability in total body and regional BMD, % fat, as well as lean tissue mass measurements. In 1990 Mazess et al. (95)

scanned six males and six females five times over the span of one week using a DPX model total body scanner (Lunar Radiation Crop, Madison, WI). Minimum differences for total BMD, % fat, fat mass and lean tissue mass were found to be $< 0.01 \text{ g/cm}^2$, 1.4%, 1.0 kg and 0.8 kg (1 standard deviation) respectively after the five scans. These precision errors are comparable or smaller than those achieved with other types of noninvasive methods for measuring %fat and fat mass (55).

Oates et al. (98) in 2006 compared %BF measured by a Lunar Prodigy DXA (GE Healthcare, Madison, WI) in 268 people (105 males, 163 females) to measurements done by ADP, BIA and two SF methods. SF method 1 (SF1) was comprised of the chest, abdominal, and thigh measurements for men, and triceps, suprailiac, and thigh measurements for women. SF method 2 (SF2) was comprised of chest, biceps, and subscapular measurements for men, and triceps, suprailiac and abdominal measurements for women. Male mean DXA %BF measurements (18.0%) were reported higher than mean measurements for ADP (16.6%) and SF1 (16.4%). However, it was lower in comparison to mean measurements for BIA (19.8%) and SF2 (19.1%). Females mean DXA %BF measurement (31.6%) was higher than ADP (28.6%), BIA (30.2%) and SF1 (28.2%) but lower than SF2 (31.0%). Regression results indicated that when compared to DXA, other methods reported lower estimates of %BF. DXA measurements have also been shown to correlate with hydrostatic weighing in a variety of ages in both men and women (75, 97, 128). A 1998 study by Kohrt et al. (75) aimed to compare %BF from DXA to HW in 110 men and 225 women ranging in age from 21-80 years. Results demonstrated that estimates of %BF between the two methods were highly correlated ($r=0.95$) but DXA yielded significantly ($p < .001$) higher values than HW ($32.1\% \pm 12.0$ vs. $31.25\% \pm 10.1$). When compared to HW, men in the study experienced significantly lower ($p < .05$) %BF when using DXA ($21.1\% \pm 9.3$ vs. $22.7\% \pm 8.2$), while women saw significantly higher ($p < .05$) %BF when using DXA ($37.5\% \pm 9.3$ vs. $35.3\% \pm 8.1$). Overall the standard error of estimate for DXA technology has been widely accepted at $\pm 1.8\%$ (2).

DXA Manufacturers

Most error in DXA-derived measurements can be attributed to the differences in DXA manufacturers and software technology. DXA scanners are primarily manufactured under two main companies: General Electric (GE) Lunar Densitometry (Madison, WI) and Hologic (Waltham, MA). Each manufacturer has developed several DXA models and has updated their software multiple times since first being released. The variety in models and software, have led to discrepancies in inter-model studies.

Aasen et al. (9) used DXA to compare body composition analysis in vivo and in vitro between three different machines: Lunar Expert (software version 1.92), Lunar Prodigy (version Encore) and Hologic Delphi W (version 11.1). During the study, eighteen measurements were taken of a Hologic whole body phantom (WB 164) on each DXA device. Results showed significant differences ($p < .05$) between the three models with Prodigy estimating a higher bone mineral content (BMC) ($2.79 \text{ kg} \pm .53$) than both Delphi ($2.45 \text{ kg} \pm .48$) and Expert ($2.65 \text{ kg} \pm .52$). Expert measurements were significantly lower ($p < .05$) in lean mass ($47.8 \text{ kg} \pm 9.9$) when compared to Delphi and Prodigy ($50.5 \text{ kg} \pm 9.8$ and $48.7 \text{ kg} \pm 9.8$ respectively) and significantly higher ($p < .05$) in fat mass ($31.7 \text{ kg} \pm 12.0$ vs. $28.6 \text{ kg} \pm 9.3$ and $30.8 \text{ kg} \pm 9.7$). Expert measurements were also significantly higher ($p < .05$) in percentage of fat ($38.0\% \pm 10.7$) when compared to Delphi ($34.9\% \pm 8.6$) and Prodigy ($37.2\% \pm 8.9$).

In addition to scanning the Hologic whole body phantom, 21 healthy subjects aged 30-84 with a mean age of 61.5 ± 12.5 years were scanned. Selection of subjects was based on the need to have a wide range of body weights, while at the same time ensuring that all subjects could be placed completely within the field of measurement of all three DXA machines. Although measurements of fat mass and lean mass did not differ significantly among DXA devices ($p=0.613$ and $p=0.66$ respectively), Bland-Altman plot demonstrated significant differences

between the DXA devices in means of differences fat mass Prodigy and Delphi $2.24 \text{ kg} \pm 1.08$ (mean \pm SD), Prodigy and Expert $-0.85 \text{ kg} \pm 3.30$, and Delphi and Expert $-3.09 \text{ kg} \pm 3.49$. Significant differences were also seen in lean mass, Prodigy and Delphi $-1.76 \text{ kg} \pm 1.56$, Prodigy and Expert $0.98 \text{ kg} \pm 2.73$ and Delphi and Expert $2.74 \text{ kg} \pm 3.07$. Also found were negative correlations between Expert-measured fat mass and fat mass by both Prodigy and Delphi ($r = -0.69$; $p < 0.001$ and $r = -0.79$; $p < 0.001$ respectively), suggesting that Expert DXA estimates of fat mass are significantly higher than those of Prodigy and Delphi.

In a 2012 multinational study by Shepherd et al. (116), 199 adult and pediatric subjects were scanned using either a GE Healthcare Lunar, Hologic Discovery or Hologic Delphi system. All scans were centrally analyzed by a single technologist using GE Healthcare Lunar Encore (version 14.0) and Hologic Apex (version 3.0). When comparing bone and soft tissue measures between the two manufactures the authors found high correlation with r values of 0.98 and 0.96 respectively. However, significant higher %BF, BMC and bone mineral density (BMD) values for GE Healthcare Lunar were found when compared to Hologic, with absolute differences of 1.4%, 176.8 g, and 0.013 g/cm^2 respectively. After adjusting for any calibration bias between the two machines through cross-calibration, no significant differences remained between GE Healthcare Lunar measured results and the results converted from Hologic. The authors concluded that cross-calibration by way of scanning whole body phantoms is suggested when comparing two different manufactures or when changing system hardware.

While inter-model variation has been well-documented, there has also been concern regarding intra-model variation. Tataranni et al. (122) used two DXA machines from the same manufacturer (DPX-L; Lunar Co, Madison, WI) and scanned five males and five females, aged 31-58, once on each machine. The machines were first calibrated against separate phantoms according to the standard Lunar procedures and results were analyzed using the same software

program (Lunar, version 1.3z). They found significant differences in the mean values between machine A and machine B in BMC (68 g), total soft tissue (304 g), %BF (-1.7%), fat tissue (-1146 g), and lean tissue (1450 g). Upon cross calibrating both machines against the same phantom, results of all subjects (n=5) with the exception of one were consistent with those of the 10 subjects scanned when the machines were calibrated against their individual phantoms.

In a 2011 study comparing the GE Lunar Prodigy (Madison, WI) and GE Lunar iDXA (Madison, WI), Hind et al. (57) in found coefficients of variation of LBM and %BF of 0.5% and 0.86% respectively when evaluating 52 men and women age 20-50 years (mean age 34.8 ± 8.4 years). There was also good agreement between consecutive measurements for all measurements ($r^2 = 0.99$). Similar results were seen in Rothney et al. (109) with coefficients of variation equal in LBM but higher in %BF (1.0% vs. 0.86%). A study by Williams et al. (134) scanned a total of 317 individuals three times with either Lunar Prodigy or Lunar iDXA (269 with Lunar Prodigy and 48 with Lunar iDXA). Results showed that all three scans were highly correlated ($p < .01$) with no significant differences between the means ($p > .05$). Intra class coefficients ($p < .05$) of the three measures total body scan to estimate total, android, gynoid, arms, legs, and trunk body fat percentage support that DXA measures are reliable (0.998, 0.998, 0.999, 0.997, 0.999 and 0.952 respectively). In 2011, Kirshner et al. (73) compared subjects that were scanned by both the Lunar Prodigy and Lunar iDXA and found that inter-model body composition scans were suitable for intra-subject comparisons for both total region body fat as well as total lean mass.

DXA Technology

DXA models use two typical forms of imaging geometry to determine body composition; pencil beam and fan beam. Older DXA models used pencil beam imaging while newer DXA models use fan beam imaging. Fan beams are coupled with a multidetector linear array that distributes the x-rays across a wider area for a whole body scan whereas the pencil beam is

coupled with only a single detector (11). The fan beam allows for a quicker scan time as well as improved geometrical resolution (58). Tothill et al. (124) compared a pencil beam Hologic QDR 1000W (software V5.55; Bedford, MA) and a fan beam Hologic QDR 4500A (software V8.24a:3; Bedford, MA) in both phantom scans as well as with 41 subjects. Differences were seen in calibration against recognized standards for fat proportion between both scanners in which the Hologic QDR 1000W underestimated low fat proportions and the Hologic QDR 4500A underestimated high fat proportions. Measurements also showed that the Hologic QDR 1000W reported a 4% higher BMD and 7% higher BMC than the Hologic 4500A. In comparing measurements of %BF it was found that both models had a strong correlation ($r = .98$) but the Hologic QDR 1000W consistently underestimated percent by fat, on average by 2.8%.

A previous limitation for using DXA was the inability to scan subjects who were too large to fit within the field of scan. However, a study in 1995 showed that half-body scans could be used to predict whole body composition. The study consisted of 183 subjects with a wide variation of body sizes (BMI 17.7-52.8 kg/m²) that were scanned using a DPX-1 (Lunar Radiation Corp, Madison, WI) (123). When subjects were too large to fit within the field of the scan their right side and left side were scanned independently. The least-squares-regression analysis of the right on left side of the body gave the following results: percent body fat $r^2 = 0.99$ (SEE 0.5%), nonbone fat-free soft tissue $r^2 = 0.97$ (SEE= 0.86 kg), fat soft tissue $r^2 = 0.99$ (SEE = 0.6 kg), and bone mineral $r^2 = 0.89$ (SEE = 0.08kg). Due to the high association between values from the two sides, for wider subjects, scans can be made on the right half of the body and total body composition can be estimated assuming bilateral symmetry. The latest software on the GE Lunar iDXA (Madison, WI) currently has the capability of using this information in software called MIRROR IMAGE™.

Another reason that there has been an increase use in DXA technology for body composition measurements is attributed to the additional information that it provides when compared to alternative methods. Unlike most other body composition methods DXA provides added valuable clinical information related to body composition such as lean mass measurements and distribution of body fat.

DXA provides estimates of lean body mass, which is the amount of mass in the body minus the portion of the body that is fat and bone. A 1991 study conducted by Haarbo (52) sought to examine the validity of DXA-measured %BF and LBM versus HW and total body water. Precision in this study was determined by scanning six healthy subjects twice, six months apart. Precision error for LBM reported as standard deviation (CV%) was 1.4 kg (3.1%). To measure the accuracy of DXA measurements, 25 healthy subjects (15 women and 10 men) aged 23-41 were measured using DXA, total body water, and HW. DXA showed strong correlations with both methods for LBM, with $r = 0.91$ for total body water and $r = 0.97$ for HW.

The accuracy of DXA to measure LBM was further demonstrated in 1993, when Svendsen et al. (121) Because chemical analysis is done in vitro, it is the most accurate measurement of body composition. Seven pigs (35-95 kg) were measured twice by a DPX DXA (software version 3.2; Lunar Radiation Corp., Madison, WI), once before and once after being euthanized by intravenous injection. After being euthanized the pigs were frozen for two days before being homogenized with a total body grinder and total body pelletizer. Upon performing the chemical analysis, the authors found that the mean LBM (\pm SEM) difference between the DXA measurements and the results of the chemical fat extraction were insignificant ($p > .05$, 0.4 ± 1.2 kg). The regression line for LBM was not significantly different from the line of identity with an r value equal to 0.98. This demonstrates that LBM measured by DXA and the real value (determined by chemical fat extraction) were not significantly different. Based on these findings

the authors concluded that DXA is an accurate and precise method of measuring LBM in cross-sectional and longitudinal studies.

Distribution of body fat can have large implications for one's metabolic health. Distribution of fat is typically divided into two terms: android fat and gynoid fat - both coined by Vague in 1947 (126). Android fat, often referred to as abdominal fat or "apple shape", is most often found in men, while gynoid fat, often referred to as "pear shape", is most frequently found in premenopausal women. Android fat is associated with a higher risk of CVD risk factors when compared to gynoid fat as first demonstrated by Kissebah (74). This association has been further examined in a review by Votruba et al. which demonstrated that individuals who had a BMI ≥ 30 kg/m² with distribution of body fat concentrated in the upper body had an increased risk for health complications such as hypertension, type II diabetes and dyslipidemia, compared to those individuals with the distribution of body fat concentrated in the lower body (131).

With fat distribution playing a large role in the increase risk of cardiovascular disease it is important to be able to correctly measure regional body fat. Glickman et al. (51) studied the validity of DXA (model DPX-IQ; Lunar Radiation Corp., Madison, WI) to measure regional body fat. To measure the validity of the DXA, 27 subjects, 15 men and 12 women, were measured by both multislice computed tomography (CT) and DXA on the same day from their L1 vertebrae to their L4 vertebrae as a measure of abdominal fat. Subjects were randomized into either multislice CT or DXA scan, with the other test immediately following the first. The results showed that there was a strong correlation between CT measured and DXA measured abdominal tissue mass ($r=0.858$, $p < 0.0001$) and abdominal fat mass ($r=0.967$, $p < 0.0001$). DXA measurements were significantly lower in total abdominal mass (2.22 kg vs. 2.99 kg) and total abdominal fat mass (2.22 kg vs. 2.99 kg) when compared to measurements by multislice CT. Bland-Altman analysis demonstrated moderate agreement between DXA and CT for both total

abdominal tissue mass and abdominal fat mass (95% limits of agreement -1.56 – 2.54 kg and -0.41 – 1.94 kg respectively).

Glickman et al. (51) also examined the reliability of measuring regional body fat by DXA. Twenty-eight subjects, eight men and 20 women were scanned before and after packets of porcine lard of uniform thickness (2.5 cm, 0.5-1.0 kg) were placed over the subject's lumbar spine (L1 to L4) region of interest. DXA estimates with the added fat was corrected for the fat packet mass, the L1 to L4 fat mass was significantly less ($p < .05$) than that measured in the original scan (2.12 ± 0.24 vs. 2.26 ± 0.23 kg respectively). The difference between the mean values demonstrates that DXA is a reliable and reproducible method in the estimation of abdominal adipose tissue.

Interpretation of Body Composition

Interpreting results of %BF testing is problematic because there are no widely accepted reference standards for %BF. As there are many unique methods of measuring %BF as well as significant differences in age, gender and ethnicity, a universal criterion has yet to be established and it is clinically inappropriate to interpret %BF results across these various methods and across different populations. The absence of %BF reference standards and their association to mortality and cardiovascular risk factors have led some practitioners to create a %BF range that supports their idea of good health. In 1982, Lohman et al. (86) recommended a satisfactory %BF range to be 10-22% for men and 20-32% for women, which is similar to the recommendations of 12-20% for men and 20-30% for women by Abernathy and Black in 1996 (10). Recently, the American Society of Bariatric Physicians, an American Medical Association specialty board, has defined obesity as a percent body fat of $\geq 25\%$ for men and $\geq 30\%$ for women (5), comparable to conclusions drawn by Lohman et al. 1997 (87) for men and women aged 34-55 ($>25\%$ and $>38\%$ respectively). The NIH states that individuals are generally classified as obese when body fat

content exceed 30% in women and 25% in men (3). However, the NIH continues to state that the operational definition of obesity is still classified by a BMI of ≥ 30 kg/m².

Perhaps the most widely used criterion for %BF is published by the ACSM from research conducted at the Cooper Institute in Dallas, Texas (1). The published reference ranges are divided by gender and age with reference categories of “Very Lean” (≤ 5 th percentile), “Excellent” (80th – 94th percentile), “Good” (60th – 79th percentile), “Fair” (40th – 59th percentile), “Poor” (20th – 39th percentile), and “Very Poor” (1st- 19th percentile). Although widely used, these reference values were established from SF measures based on a homogeneous group of middle to upper-class, non-Hispanic white males and females. Thus this may be inappropriate as a reference for direct comparisons across different population subgroups.

While the need for a universal set of reference values based on %BF is high, an additional problem is that %BF does not have specific cut points that are directly linked to cardiovascular disease or cardiovascular disease risk factors, unlike the BMI reference values. In an effort to rectify this issue, there has been an attempt to assign a %BF value to correspond with the well-established BMI standards. In 2000, Gallaher et al. (49) examined a total of 1,626 subjects (155 women and 99 men) from three unique ethnic backgrounds: African American, Asian and White. Subjects underwent a variety of measurements including a DXA examination to determine body fat and bone mineral mass, tritium or deuterium dilution to determine total body water, and hydrostatic weighing to determine body density and volume. The measured bone mineral mass, total body water, and body volume values were then used to calculate total body fat by using a four-compartment model. The authors found a curvilinear relationship between percentage body fat and BMI within all ethnic groups. This relationship then became linear when BMI was replaced with 1/BMI. Additionally, regression models with 1/BMI provided a higher r^2 and standard estimating error (SEE) value than those with BMI. Overall, Asians were found to

have a higher %BF at lower BMIs, particularly at younger ages than did African American and White participants for both men and women. Small differences were also observed between African American and White subjects, with African American individuals having a lower %BF. However, the magnitude of this effect was rather small (1-2%). The authors concluded that there is a need for a direct association between morbidity and mortality and %BF. Their study was an attempt to link %BF to the current healthy weight BMI guidelines. This effort has been made several more times in diverse population settings in an attempt to specify these cut points (54, 62).

While BMI classifications have been well-established in association to CVD and CVD risk factors, an attempt to assign a %BF to a specific BMI has limitations. Ellis 2001 (36) states that while %BF and BMI are correlated ($r=0.8$; $P < 0.001$) BMI was not a precise predictor of %BF. For instance, when BMI was 20 kg/m^2 the corresponding fat mass could range from 5-40% of body weight. Conversely, if the fat mass was 20% of body weight, the BMI value could be anywhere from $15\text{-}30 \text{ kg/m}^2$. Due to the variation of %BF for each BMI and BMI at each %BF, it is important to keep these two variables separate in an attempt to establish cut points of %BF for CVD and CVD risk factors.

In 2011, Kim et al. (69) sought to determine optimal percentage body fat cut points in Korean adults for predicting obesity-related cardiovascular disease risk factors. Blood pressure measurements along with a 12 hour fasting blood sample were taken to determine measurements of total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol as well as measurements of triglycerides, fasting blood glucose and hemoglobin A1c (HbA1c%). %BF was measured by BIA (Inbody versions 3.0; Biospace, Norwalk, CA) then converted to %BF through previously established regression equations (70). Cut points of %BF for overweight and obese classifications were determined using the minimum p value approach. Max chi-square statistics

for the overweight classification were 1706.2 ($p < .0001$) and 1407.5 ($p < .0001$) for men and women respectively, which were converted to a %BF of 17% and 32% respectively. Max chi-square statistics for the obese classification were 371.6 ($p < .0001$) and 186.6 ($p < .0001$) for men and women respectively, which were converted to a %BF of 32% and 37% respectively. Based on these cut points, 32.3% of men and 30.3% of women were overweight and 41.8% of men and 15.9% of women were obese. Odds ratio for having at least one CVD risk factor for overweight and obese men was 2.2 (95% CI: 2.07-2.38) and 4.05 (95% CI: 3.78-4.33) respectively and 1.95 (95% CI: 1.79-2.12) and 3.21 (95% CI: 2.87-3.57) for women. This study identified an increase in CVD risk factors related to %BF suggesting that the prevalence of each risk factor increases as the amount of body fat increases.

Farajian et al. 2008 (38) also examined the relationship between %BF measured by DXA (Lunar DPX-MD software version 4.6, GE Healthcare, Madison, WI) and risk factors of cardiovascular disease; specially, total, HDL, and LDL cholesterol as well as fasting glucose. The study was comprised of a total of 220 Greek female undergraduate students 20.1 years \pm 1.2. When obesity was defined by a BMI of ≥ 30 kg/m² a total of 13.3% of the subjects were overweight and 2.7% of the subjects were obese. When obesity was defined by a %BF of $\geq 30\%$ the percent of subjects classified as obese rose significantly when compared to BMI to 48.6%. Results of the blood analysis found that 27.5%, 28% and 40% of all subjects had abnormalities in total cholesterol, LDL cholesterol, and HDL cholesterol respectively, with at least 60.4% of all subjects having at least one cardiovascular risk factor. When examining risk factors between the normal group (%BF $< 30\%$) and those in the obese group (%BF $\geq 30\%$) no significant differences ($p < .05$) were found in fasting glucose (92.4 vs. 96.4 mg/dL), total cholesterol (186.4 vs. 182.0 mg/dL), HDL cholesterol (53.7 vs. 51.6 mg/dL) and LDL cholesterol (118.9 vs. 116.3 mg/dL) suggesting that %BF does not have an effect on risk factors of cardiovascular disease. One limitation to this study is very homogenous sample. Additional studies using these methods

should consider using an older more diverse population as well as adding men which would allow for a better representation of the overall population.

A limitation to both these studies is that they are cross sectional studies therefore causality cannot be established. Mixed results between these studies demonstrate the need for long term prospective studies to determine the validity of these measurements as well as measurements of morbidity and mortality. However, long term prospective studies utilizing direct measures of %BF are costly.

While the need for prospective studies for %BF are still needed, some studies with large sample sizes have been conducted in order to provide a reference value %BF for specific populations. In 2010, Borrud et al. (20) published reference values for DXA measurements gathered from the National Health and Nutrition Examination Survey (NHANES). NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States in which samples are selected through a complex, multistage probability design. The NHANES program began in the early 1960s and has been conducted as a series of surveys focusing on different population groups or health topics. In 1999, the survey became a continuous program that has a changing focus on a variety of health and nutrition measurements to meet emerging needs. DXA was included for the first time in NHANES during NHANES III: 1988-94 to assess femoral bone mineral density. In 1999 DXA was included as a measurement of whole body composition.

The data reported in Borrud et al. (20) was gathered from 1999-2004 using a Hologic QDR 4500A fan-beam bone densitometer (Hologic, Inc., Bedford, MA). Subjects with an age of 8 years or older were eligible to participate in the scan. Exclusions for the DXA scan included pregnant females, those who reported radiographic contrast material use in the past seven days or nuclear medicine studies in the past three days. Individuals who weighed over 300 pounds or

were taller than 6'5" were also excluded from the scan due to physical limitations of the DXA table. The subject population was composed of 22,010 individuals with oversampling of low-income persons, adolescents aged 12-19 years, persons 60 years of age or older, African Americans, and Mexican Americans in order to improve the precision of the statistical estimates for these groups. The percentage of DXA participants with 100% valid data (regions that were able to be analyzed correctly) was found to decrease with age primarily due to an increase in implants such as pacemakers, stents, and hip replacements. To address the problem of bias due to nonrandom invalid and missing data multiple imputation of the data was performed.

Measurements were broken down categories based on gender, ethnicity, and age with age groups of 8-11, 12-15, 16-19, 20-39, 40-59, 60-79, and 80 years and above. These categories were then broken down into the 5th, 10th, 15th, 25th, 50th, 75th, 85th, 90th, and 95th percentiles. Results showed that in adult's ≥ 20 years of age, women had a significant higher mean %BF than men (39.9% vs. 28.0%). Mean differences in %BF were also seen between ethnicities in men 20 years or older with non-Hispanic blacks have significantly lower %BF than Mexican Americans and non-Hispanic Whites (25.5% vs. 28% and 28.5% respectively). Differences in mean %BF of women 20 years or older were also seen between ethnicities with non-Hispanic white women having lower %BF than Mexican Americans and non-Hispanic blacks (39.7% vs. 40.9% and 40.6% respectively). Men and women saw an increase in mean %BF from the lowest age group until a drop in the ≥ 80 year old age group.

This study also provides reference values for LBM. Significant differences were seen in LBM between men and women ≥ 20 years old with men having a significantly higher amount of LBM than women (59.5 kg vs. 41.8 kg). Mean differences in LBM were also seen between ethnicities in men and women ≥ 20 years or old. Non-Hispanic blacks had significantly higher LBM than non-Hispanic Whites and Mexican Americans (men: 61.7 kg vs. 60.2 kg and 55.4 kg;

women: 46.3 kg vs. 41.6 kg and 40.3 kg) respectively. Men and women both saw an increase in mean LBM from the youngest age group until the 60-79 year old age group, where LBM continually declined into the ≥ 80 year old age group. This is the only study that provides reference values or recommendations for LBM which further demonstrates the need for additional reference ranges for LBM.

Chapter III

Methodology

While DXA technology has been a valid method for determining body composition, there is a need to develop normative standards to better interpret DXA-derived body composition data. Therefore, the primary purpose of this study was to develop a set of reference standards for %BF and LBM based on DXA derived data. The secondary purpose of the study was to characterize the degree of agreement of obesity based on BMI using %BF from DXA derived data.

Data Source

A request was made to the Clinical Exercise Physiology (CEP) Program at Ball State University (Muncie, Indiana) to provide a de-identified dataset with demographic and clinical variables. Demographic variables include age, gender and ethnicity, while clinical variables include body weight and height, BMI, DXA measures including region fat percentage, lean tissue mass, android fat tissue mass, and gynoid fat tissue mass. Scans were administered by trained research technicians and the data was only provided to key research personnel in the form of excel spreadsheet and was without individual identifiers.

Individual scans were performed from July 2003 until February 2013 through numerous research studies as well as through the Adult Physical Fitness Program (APFP). The APFP is one of the service programs that are available to the local community through Ball State University. The APFP provides a comprehensive exercise program that assists individuals in improving

and/or maintaining their health and functional ability. The APFP was started in 1971 and has operated continuously since its inception.

Procedures

Height and Weight

Height and weight measurements were collected using standard CEP procedures. Height was measured in inches by a wall mounted stadiometer (SECA model #222, SECA Corporations, Medical Scales and Measuring Systems, Hanover, MD) to the nearest 0.25 inch. Subjects were required to remove their shoes and stand with their heels together, keeping their back flat against the wall. Subjects were instructed to look forward and take and hold a deep breath at which time the measurement was collected. Body weight was measured by a calibrated electronic weight scale (Heathometer, Inc., Bridgeville, IL) to the nearest 0.1 lbs. Subjects removed their shoes, coats and additional clothing so that they were dressed in a t-shirt and athletic shorts when weighed. Subjects were also instructed to empty their pockets before the measurement was taken.

DXA Measurements

Subjects were required to remove their shoes, all objects from their pockets and any jewelry or metal before being scanned. The subjects were required to be still in the supine position on the DXA platform throughout the duration of the scan ranging from 5 to 25 minutes depending on the model of DXA and the thickness of the subject. The subject's body was positioned in the center of the DXA platform with their entire body within the field of the scan as outlined on the DXA platform. X rays were then delivered at two energies from a low current x ray located beneath the DXA machine. The two x-ray energies were then passed through the subject, posterior to anterior, and collected in the detector arm above.

Two total body DXA models were utilized in the creation of this data set. The first model was a GE Medical Systems Lunar Prodigy DXA (Madison, WI) which was used from 2000 to 2010. The second DXA model was a GE Medical Systems Lunar iDXA (Madison, WI) which has been used since 2010. Before participation, subjects were required to meet specific criteria. They were asked to disclose information about weight and pregnancy status, both which could exclude them from having the scan performed due to safety and health reasons. Subjects were also instructed to disclose any information about implanted metal that could affect the analysis of the scan. Both systems have been shown to be valid and reliable in measurements (57, 73, 109, 134).

Inclusion criteria for the Lunar Prodigy model stated that subjects must have weighed less than 300 pounds and fit within the field of the scan which measured 196 cm long by 66 cm wide. If the subject's width was outside of the criteria they were instructed to place their arms across their chest in order to fit within the scan. The Lunar iDXA model which is currently being used has two major advantages over the Lunar Prodigy model. First the Lunar iDXA model was able to support 450 pounds compared to the 300 pounds of the Lunar Prodigy. Second, the Lunar iDXA model had an added software feature of MIRROR IMAGE™ which doubles half of the body for those subjects who are too wide to fit within the scanner field in order to predict total body measurements.

Total body measurements that were assessed include: %BF, LBM and android and gynoid fat mass. %BF which was measured as the total body fat mass as a percentage of the total mass of bone mineral content, fat mass, and lean mass. Lean body mass was measured as the amount of tissue in the body that is neither fat nor bone. Android fat mass is the amount of mass in the lowest 20% of the region from the top of an individual's iliac crest to the bottom of their head. Gynoid fat mass is the sum of fat mass in the region that extends twice the distance of the android region downward from the top of an individual's greater trochanter.

Multiple scans of the same individual were included in the analysis providing that at least three months elapsed between scans. Including the three month provisional period between scans eliminates multiple scans of the same individual that were performed for previous precision studies. Individual scans were excluded if: the subject was < 20 years old, key data such as %BF or LBM were missing due to incomplete scans, or if an individual was scanned within the three month provisional waiting period.

Statistical Analysis

The descriptive statistics were used to report the results as frequencies or mean \pm SD. Gender- and age-specific body composition measurements were analyzed and subjects were classified into 1 of 4 age groups; Group A: 20-39 years old (n=879), Group B: 40-59 years old (n=1003), Group C: 60-79 years old (n=801) and Group D: \geq 80 years of age (n=78). All statistical procedures were performed using Statistical Package for the Social Sciences (SPSS) software (Version 20.0, Chicago, IL). Independent t-tests were completed to determine differences between genders and Analysis of variance (ANOVA) were performed to determine differences between age groups in %BF, LBM, and android and gynoid fat between genders. Kappa statistic was performed to determine the consistency among identifying classifications of obesity between %BF and BMI. An alpha level of 0.05 was set as statistical significance.

Chapter IV

Research Manuscript

Journal Format: Medicine and Science in Sport and Exercise

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Abstract

DXA Reference Standards for Percent Body Fat and Lean Body Mass in Adults

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Purpose: Dual energy x-ray absorptiometry (DXA) provides accurate measurements of percent body fat (%BF) and lean body mass (LBM). No universal references of body composition exist using DXA measurements. The purpose of this study was to develop a set of reference data for DXA-derived %BF and LBM and to characterize the agreement between classifications of obesity based on the BMI (≥ 30 kg/m²) and %BF ($\geq 25\%$ for men and $\geq 30\%$ for women). **Methods:** De-identified sample of 2,761 subjects was obtained through Ball State University's Clinical Exercise Physiology Program (Muncie, Indiana). Subjects were aged 20-94 years and were scanned from July 2003 until February 2013 using either the GE Medical Systems Lunar Prodigy DXA or the GE Medical Systems Lunar iDXA. **Results:** Normative reference tables displaying select percentiles and mean values were created for %BF and LBM across defined age groups for both males and females. Mean %BF and LBM closely resembled the means of the National Health and Nutrition Examination Survey (NHANES) in both men and women. Agreements between BMI and %BF were 97% with identified as obese and 33% when identified as non-obese. Kappa statistics showed fair agreement (0.236) in men and slight agreement (0.185) in women. Forty-seven percent were misclassified as non-obese BMI while meeting %BF standards. **Conclusion:** Future research should consider creating a national registry for DXA derived measurements. This national registry would allow clinicians to identify those who are at an increased risk to health problems associated with an increase in %BF and decrease in LBM.

Key Words: Obesity, Sarcopenia, BMI, Body Composition, Reference Standards

Introduction

Obesity has been linked to numerous chronic diseases and comorbidities including increased risk in developing diabetes, hypertension, heart disease, stroke, cancer, dyslipidemia, sleep apnea and/or respiratory problems(17, 19, 32, 35). Sarcopenia has been associated with negative health factors such as a decrease in functional capacity, quality of life, resting metabolic rate, insulin sensitivity, and an increased risk of injury (22, 48). Rates of obesity in adults aged 20-74 years have increased from 45% in 1960 to 65% in 2000 (35), while sarcopenia currently affects over 20% of the 60-70 years old population and nears 50% in those over 75 years of age (11).

Most studies that have shown strong association between obesity and chronic diseases have utilized the body mass index (BMI) to classify obesity. BMI is a simple calculation of an individual's weight in kilograms divided by their height in meters squared (kg/m^2). The current BMI classifications set by the National Institute of Health (NIH), the Center for Disease Control (CDC), and the World Health Organization (WHO) are: underweight ($\text{BMI} < 18.5 \text{ kg}/\text{m}^2$), healthy weight ($\text{BMI} 18.5\text{-}24.9 \text{ kg}/\text{m}^2$), overweight ($\text{BMI} 25\text{-}29.9 \text{ kg}/\text{m}^2$) and obese ($\text{BMI} > 30 \text{ kg}/\text{m}^2$).

Although BMI is readily used to identify an individual's weight classification it has several limitations. BMI assumes that after adjusting an individual's body weight for stature, all individuals have the same relative fatness regardless of their age, sex, or ethnicity, race (13). However, more recent studies have provided evidence that demonstrates that BMI is not independent of age, gender, or ethnicity and these factors should be taken into account when using BMI measurements as a main study variable (20, 21, 36, 38). More importantly, the numerator in the BMI calculation takes into account total body weight and does not distinguish between fat and lean mass. Thus, individuals with a normal weight but excess body fat may not be classified as overweight or obese, whereas individuals with large amounts of lean body mass (LBM) may be misclassified as overweight or obese. Misclassifications when comparing BMI and excess fat has been demonstrated in a cross sectional study by Shah and Braverman (39). The authors found that when comparing BMI to percent body fat (%BF) 39% of the population was misclassified as

non-obese based on BMI standards while meeting the obese standards for %BF ($\geq 25\%$ for men and $\geq 30\%$ for women). Due to BMI being a measure of excess weight rather than excess fat or lean mass, alternative methods for measuring %BF should be used instead. One newer and encouraging method for measuring %BF is dual energy x-ray absorptiometry (DXA).

DXA scans were originally designed to measure a body's bone mineral density (BMD) to evaluate the presence of osteoporosis. DXA technology uses low dose x-rays to measure BMD as well as other components of body composition specifically fat and lean mass tissue. DXA and has been shown to be a valid method for measuring body composition (1, 33, 42). In a 1993 study by Svendsen et al. (42) accuracy of DXA in vivo was compared to chemical analysis in 7 pigs (35-95 kg). When comparing results by DXA to chemical analysis regression lines were not significantly different from the line of identity with r values for %BF, fat tissue mass, and LBM of 0.98, 0.99, and .098 respectively. Corresponding SEEs were 2.9%, 1.9 kg, and 2.7 kg for %BF, fat tissue mass, and LBM demonstrating that DXA provides an accurate method of measuring soft tissue body composition measurements.

Unlike other body composition methods, DXA measures LBM, which can be helpful in assessing the presence of sarcopenia, and distribution of fat which has been shown to be a predictor of adverse health complications and disease (27, 45). Although DXA provides an effective way to determine %BF and LBM, one limitation is that there are no widely recognized standards for body composition, specifically %BF and LBM derived from DXA.

Some practitioners have provided a range of %BF for what they consider appropriate for good health (6, 31), while The American Bariatric Society and the NIH have classified obesity using %BF of $\geq 25\%$ for men and $\geq 30\%$ for women (2, 3). A more widely used set of references is data provided by the Cooper Institute (Dallas, TX). This reference set is published in the ACSM's Guidelines for Exercise and Testing Prescription 8th edition (1), and has been derived from skinfold measurement. However, the lack of diversity in socio-demographic characteristics (e.g., education, income, race/ethnicity) in this data may limit its application to other populations (29). Most recently in 2010, a study by Borrud et al. (12) used

data from the third National Health and Nutrition Examination Survey (NHANES III) to develop reference ranges for %BF based on DXA measurements.

There has been a growing interest in research involving DXA to measure body composition, specifically %BF and LBM. While the DXA is an accurate method for measuring body composition, the limitation of DXA derived body composition is that there are currently no universally accepted reference ranges for body composition based on DXA results. The development of these reference values can be used in research, clinical practice, and policy development, allowing for opportunities to evaluate and compare other %BF and LBM data to a variety of clinical indicators of health risk. In the comparison to the created reference standards researchers and clinicians would be able to effectively classify and identify those who are at an increased risk for developing certain negative health risk factors associated to sarcopenia and obesity.

Therefore, the primary purpose of this study was to develop a set of normative standards for %BF and LBM data derived from the Ball State University's Clinical Exercise Physiology (CEP) Program DXA. The secondary purpose of this study was to compare classifications of obesity based on the BMI and %BF measurements.

Methods

Data Source

The CEP program of the Human Performance Laboratory (Ball State University, Muncie, IN) has utilized DXA for body composition measurements for approximately ten years. The CEP program provided a de-identified dataset that included demographic and clinical variables. Demographic variables were age, gender and ethnicity, and clinical variables were body weight and height, BMI, DXA derived region fat percentage, lean tissue mass, android fat tissue mass, and gynoid fat tissue mass. Scans were administered by trained research technicians and the data was only provided to key research personnel in the form of excel spreadsheet and was without individual identifiers. Measurements were made from July

2003 until February 2013 through numerous research studies conducted by the Human Performance Laboratory as well as through the Adult Physical Fitness Program. The original data source contained 4,348 total scans. Multiple scans of the same individual were included in the analysis providing that at least three months elapsed between scans. Three months allows for enough time for an individual to produce body composition changes as seen in previous literature (7), whereas, shorter time periods are likely to not see change. Including the three month provisional period between scans also helps eliminates multiple scans of the same individual that were performed for previous precision studies using these machines (26, 47). Individual scans were excluded if: the subject was < 20 years old, key data such as %BF or LBM were missing due to incomplete scans due to the scan being terminated early, or if an individual was scanned with the three month period. The study was approved by the Institutional Review Board at Ball State University.

Procedures

The data set provided clinical variables of height and weight which was collected using standard CEPP procedures utilizing a wall mounted stadiometer (SECA model #222, SECA Corporations, Medical Scales and Measuring Systems, Hanover, MD) for height assessment measured to the nearest 0.25 inch, as well as a calibrated electronic weight scale (Heathometer, Inc., Bridgeville, IL) to measure body weight to the nearest 0.1 lbs. BMI was calculated as weight (kg) divided by height (m) squared. Classification of obesity using BMI standards ($\geq 30 \text{ kg/m}^2$) were made with recommendations by the World Health Organization, the Center for Disease Control, and the American College of Sports Medicine, while obesity classification using %BF (≥ 25 %BF for men and ≥ 30 %BF for women) were made following those suggested by the National Institute of Health (3) and the American Society of Bariatric Physicians (2).

The data set also provided DXA derived information gathered from one of two DXA models using standard CEPP procedures. The first model was a GE Medical Systems Lunar Prodigy DXA (Madison, WI) which was used from 2000 to 2010. The second DXA model was a GE Medical Systems

Lunar iDXA (Madison, WI) which has been used since 2010. In 2011, Kirshner et al. (22) compared subjects that were scanned by both the Lunar Prodigy and Lunar iDXA and found that inter-model body composition scans were suitable for intra-subject comparisons for both total region body fat as well as total lean mass. Before participation, subjects were required to meet specific criteria. They were asked to disclose information about weight and pregnancy status, both which could exclude them from having the scan performed due to safety and health reasons.

Inclusion criteria for the Lunar Prodigy model stated that subjects must have weighed less than 300 pounds and fit within the field of the scan which measured 196 cm long by 66 cm wide. If the subject's width was outside of the criteria they were instructed to place their arms across their chest in order to fit within the scan. The Lunar iDXA model which is currently being used has two major advantages over the Lunar Prodigy model. First the Lunar iDXA model was able to support 450 pounds compared to the 300 pounds of the Lunar Prodigy. Second, the Lunar iDXA model had an added software feature of MIRROR IMAGE™ which doubles half of the body for those subjects who are too wide to fit within the scanner field in order to predict total body measurements.

Statistical Analysis

The descriptive statistics were used to report the results as frequencies or mean \pm SD. Gender- and age-specific body composition measurements were analyzed and subjects were classified into 1 of 4 age (years) groups; Group A: 20-39 (n=879), Group B: 40-59 (n=1003), Group C: 60-79 (n=801) and Group D: \geq 80 (n=78). All statistical procedures were performed using Statistical Package for the Social Sciences (SPSS) software (Version 20.0, Chicago, IL). Independent t-tests were completed to determine differences between genders and analysis of variance (ANOVA) were performed to determine differences between age groups in %BF, LBM, and android and gynoid fat between genders. Kappa statistic was performed to determine the consistency among identifying classifications of obesity between %BF and BMI. An alpha level of 0.05 was set as statistical significance.

Results

Subject Characteristics

The original data set provided contained 4,347 initial scans. After exclusion the final data set contained 2,761 scans from 1,857 individuals. DXA measurements of %BF and LBM were provided from a total of 2,761 individual scans. A total of 1,961 scans were evaluated using the Lunar Prodigy System, while 800 scans were evaluated using the Lunar iDXA System, with the Lunar iDXA MIRROR IMAGE™ software being utilized in 297 scans. The population consisted of 58% women and 42% men, 96% of which were Caucasian, with a mean age of 48.7 ± 18.3 years (Table 1). Men weighed significantly more, were significantly taller, and had a significantly higher BMI compared to women ($p < .001$). Men had significantly higher ($p < .001$) mean android fat mass when compared to women (2.9 kg vs. 2.6 kg), while women had significantly higher ($p < .001$) mean gynoid fat mass compared to men (5.6 kg vs. 4.2 kg). Differences were seen in distribution of LBM with men having 64.6% of their LBM in their trunk and arms while females had 67.7% of their lean body mass in their lower body.

Percent Body Fat

Table 2 and 3 provide normative values for %BF, with specific reference to age for both men and women, respectively. Men and women experienced a significant increase from the youngest group (20-39 years) to the 40-59 years age group (men: $23.7\% \pm 9.1$ to $31.1\% \pm 7.2$; women: $33.9\% \pm 9.7$ to $41.2\% \pm 8.4$) (Figure 1). After the first initial increase in %BF no other significant changes occurred between means in age groups 40-59 years, 60-79 years and ≥ 80 years. However, mean %BF remained significantly higher ($p < .001$) in age groups 60-79 years (men: $31.6\% \pm 6.7$; women: $42.3\% \pm 7.0$) and ≥ 80 years (men: $30.2\% \pm 6.5$; women: $39.3\% \pm 6.6$) when compared to those in the youngest age group. Differences in %BF were also observed between men and women (Table 1), with women having 34% more ($p < .001$) more %BF than men ($39\% \pm 9.3$ vs. $29\% \pm 8.5$). This significant difference between men and women was

observed in each age group (Figure 1). Largest absolute (10.2%) differences between men and women occurred in the youngest age group.

Lean Body Mass Values

Table 4 and 5 provide normative values for LBM, with specific reference to age for both men and women, respectively. Men experienced a significant decrease ($p < .01$) in LBM between each age group with the largest decrease (8%) of LBM occurring between groups 60-79 years and ≥ 80 years (Figure 2). Unlike men, women did not notice a significant decrease ($p < .01$) in LBM until the 60-79 years ($39.1 \text{ kg} \pm 5.2$ vs. $42.6 \text{ kg} \pm 7.7$ in 40-59 years) (Figure 2). This significant decrease ($p < .01$) continued into the oldest age group ($35.9 \text{ kg} \pm 3.8$). In comparing LBM between genders men were found to have significantly higher ($p < .001$) LBM than women ($59.9 \text{ kg} \pm 9.6$ vs. $41.4 \text{ kg} \pm 6.9$) (Table 1). This significant difference was also observed at each age group with largest differences occurring at in the youngest age group ($63.8 \text{ kg} \pm 12$ vs. $42.3 \text{ kg} \pm 6.9$) (Figure 2). In comparing the decrease in LBM throughout age men experienced larger relative decreases (-18% vs. -15%) as well as larger absolute differences (-11.9 kg vs. -6.5 kg) when compared to women (Figure 2).

Agreement of BMI and %BF

Thirty percent of subjects were classified as obese based on BMI standards, while 76% of the individuals were classified as obese based on %BF standards (%BF $\geq 25\%$ men and $\geq 30\%$ for women) (Table 6 and 7). Men were more likely than woman to be overweight (42% vs. 27%) or obese (32% vs. 27%) when using BMI standards, however when using %BF standards more women were obese when compared to men (81% vs. 70%).

Table 6 and 7 demonstrates the agreement seen between classifications of obesity based on BMI versus %BF standards in men and women respectively. While there was an agreement for 52% of the sample, 47% were misclassified as non-obese based on BMI, while meeting obesity criteria based on %BF (25% for males and 30% for females). Only 1% was classified as obese based on BMI, but non-

obese by percent body fat. Fifty three percent of women were misclassified as non-obese by BMI, but were found to be obese by percent body fat. In contrast 39% of men were misclassified as non-obese by BMI, but were in considered obese by percent body fat. Kappa statistics were performed to determine the consistency among %BF and BMI. The kappa statistic for men were found to be stronger agreement between %BF and BMI with a kappa of .236 (fair agreement) when compared to women .164 (slight agreement) (44). Figure 3 shows the percent of individuals who were classified as non-obese by BMI while meeting the obesity standards for %BF. Results demonstrated that misclassification increased with age in both men and women with women being misclassified more than men in each age group.

Discussion

BMI has been used as the standard measure of obesity in the United States and other countries. However, the limitation to using BMI is that it is a measure of excess weight for height and does not distinguish between fat and lean mass. DXA has been shown to be a valuable method for the use of measuring body composition through the assessing the proportions of fat mass and fat-free mass in the body which provides measures of %BF and LBM (33, 41, 42). However, the limitation to using data derived from DXA measurements is that there is no normative reference data. The lack of representative body composition reference values from DXA-derived data has resulted in limitations for both researchers and clinicians. The current study provides reference values for %BF and LBM for both male and female adults across several different age ranges based on DXA measurements.

When comparing %BF between genders, the current study results were consistent with those of previous studies (12, 30). The present study found that women's mean %BF was 29% more when compared to men, but differences as large as 46% have been found in previous studies when DXA was used in estimating %BF (30). The current study saw similar results of mean %BF to those presented by Borrud et al. (12) using the data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004 in both men (29% vs. 28%) and women (39 vs. 39.9%). An additional difference noted

between men and women is the area in which the fat mass was located. Previous studies have demonstrated that android fat is highly associated with CVD risk factors and that men tend to have higher amounts of android fat mass when compared to women (27, 45, 46). Wiklund et al. (46) compared the measurements of android and gynoid fat mass in 175 males (45.3 ± 9.9 years old) and 417 females (46.8 ± 9.3 years old). Android and gynoid fat mass was measured using one of two different DXA machines; Lunar DPX-L and Lunar IQ (GE Lunar, Madison, WI). Android fat region was defined by the upper part of the pelvis extending upward 96 mm, while gynoid fat region was defined by the superior part of the trochanter extending downward 96 mm. Results demonstrated that men had a significantly higher ($p < .05$) android fat mass ($1.8 \text{ kg} \pm .48$) when compared to women ($1.5 \text{ kg} \pm .63$), while women had significantly higher ($p < .001$) gynoid fat mass ($2.7 \text{ kg} \pm .80$) when compared to men ($1.9 \text{ kg} \pm .54$). In the current study, men also had a significantly higher ($p < .001$) mean android fat mass when compared to women (2.9 kg vs. 2.6 kg), while women had significantly higher ($p < .001$) mean gynoid fat (5.6 kg vs. 4.2 kg). However, android and gynoid fat measurements were higher in both men and women in the current study when compared to those in Wiklund et al. This is likely attributed to the difference in how each study defines android and gynoid fat regions. Wiklund et al. defined each region with a fixed distance of 96 mm, while the current study defined android fat as the lowest 20% of the region from the top of the iliac crest to the bottom of the individuals head, and the gynoid region as twice the distance of the android region downward from the greater trochanter.

Previous research by Bassocchi et al. (10) aimed to investigate age and gender related differences in DXA-measured body composition. Twenty-five males and 25 females were categorized into one of five age groups: 18-30 years, 31-40 years, 41-50 years, 51-60 years, and 61-70 years, for a total of 250 subjects. Subjects were scanned utilizing the same model DXA (GE Lunar iDXA, Madison, WI) as the current study for estimating %BF, however all subjects fit within the field of measurement so no MIRROR IMAGE™ software was employed. The results demonstrated that fat mass increased between each age group in men, while women saw a significant increase of 13% from the youngest group (18-30

years) to the next group (31-40 years) and then held constant throughout the last age group (61-70 years). While age categorization in the current study is not identical to those by Basocchi et al. a significant increase ($p < .001$) in %BF was observed in the youngest group (20-39 years) to group 40-59 years ($29.8\% \pm 10.7$ to $37.5\% \pm 7.3$) with results remaining constant throughout the next three age groups ($p > .05$) in both men and women. These findings suggests that %BF is more likely to change in younger adults (≤ 59 years) when compared to older adults (≥ 60 years) (Figure 1). Means in the present study at each age group were comparable to those by Borrud et al. (12) who demonstrated that %BF increased from the youngest group (20-39 years) until the group 60-79 years after which they experienced a slight decrease in %BF, in both men (20-39 years 26.1%; 40-59 years 28.6%; 60-79 years 30.8%; ≥ 80 years 30.7%) and women (20-39 years 37.8%; 40-59 years 40.5%; 60-79 years 42.4%; ≥ 80 years 40.4%).

A further study by Kirchengast (25) aimed to analyze gender difference in body composition from childhood to old age. The adult sample was recruited from and around Vienna, Austria. Subject population consisted of 513 women and 412 men between the ages of 19 and 92 years with a mean age of 51.7 ± 15.2 years with body composition analyses performed using a Hologic 4000 DXA scanner (Bedford, MA). Results revealed that while there were no statistically significant weight differences found between genders, women exhibited a significantly higher ($p < .001$) amount of fat mass when compared to relative men at each decade. The largest differences between genders were found at 50-59 years (15.3%) while the smallest difference was found at ≥ 80 years of age (8.5%). The smallest difference seen in the current study was also observed in those subjects' ≥ 80 years of age; with women having 9 percentage points more %BF than men (39.3% vs. 30.3%) (Figure 1). The largest differences between women and men were seen in age group 60-79 years with women having 10.6 percentage points more than men (42.3% vs. 31.7%).

With the ability to measure LBM, DXA has been a well utilized tool in the detection of individuals with sarcopenia. To date, there is not a unique, well-accepted, validated, and/or standardized way to identify the presence of sarcopenia in an older person. Although there is no universally accepted way to

identify sarcopenia one definition is greater than two standard deviations below the mean in young adults of the same sex and ethnic background (9, 37). In the current study, when using the youngest age group (20-39 years) as the reference group to be identified with sarcopenia females would have to have a LBM less than 28.7 kg while men would have to have a LBM less than 39.8 kg. Results showed that only one female (age 58, LBM 28.2) and one male (age 73, LBM 34.0) had a LBM low enough to be considered as having sarcopenia. This finding suggests that only .07% of the study population has sarcopenia. This is dramatically different from other findings suggesting that sarcopenia is prevalent in over 20% of adults aged 60-70 years old while nearly 50% of the population is affected in adults over 75 years of age.

Although only two subjects met the classification of sarcopenia when using greater than two standard deviations of a young control group, the operational definition of sarcopenia is the reduction in muscle mass and strength associated with aging. The current study saw significant differences in LBM between age groups. Men in the current study saw significant decreases ($p < .05$) between the previous age group (Table 4). Largest differences occurred between age groups 60-79 years and ≥ 80 years with a loss of 8% of LBM occurring (4.67 kg). Mean LBM in men of the current study were found to be comparable to those presented by Borrud et al. (12) (20-39 years: 63.8 kg vs. 59.9 kg; 40-59 years: 65.5 vs. 60.9 kg; 60-79 years: 56.6 vs. 57.1 kg; ≥ 80 years: 51.9 vs. 50.0 kg). While men in the current study saw significant decreases between each age group, women in the current study also did not see significant decreases in LBM until age 60 - 79 years (Table 5). LBM in women in the current study did not change significantly ($p > .05$) between 20 - 39 years (42.3 kg \pm 6.8) and 40 - 59 years (42.6 kg \pm 7.7) before experiencing a significant decrease ($p < .01$) in the 60 - 79 years (39.1 kg \pm 5.2) and ≥ 80 years (35.9 kg \pm 3.8) age groups. Like the means in men between the present study and Borrud et al., women in these two studies also saw comparable means at each age group: (20-39 years: 42.3 vs. 42.3 kg; 40-59 years: 42.6 vs. 43kg; 60-79 year: 39.1 vs. 40.3 kg; ≥ 80 years: 35.9 vs. 36.2 kg).

While significant differences in LBM have been shown to occur with age, additional differences have been noted between genders. A study by Janssen et al. (24) compared LBM between healthy men ($n=268$)

and women (n=200) as measured seven regional MRI scans. Subjects varied in age from 18-88 years with a mean age of 40 years (± 14) in men and 43 years (± 16) in women. Sixty-seven percent of the subjects were Caucasian, 17% were African-American, 8% were Asian, and 7% were Hispanic. In comparing results between genders results demonstrated that men had significantly higher ($p < .01$) LBM ($33.5 \text{ kg} \pm 5.3$) when compared to women ($21.0 \text{ kg} \pm 3.8$). This significant difference between genders was also observed in the current study with men having significantly larger ($p < .001$) amounts of LBM when compared to women ($59.9 \text{ kg} \pm 9.6$ vs. $41.4 \text{ kg} \pm 6.9$) (Table 1). The same significant difference was also observed between genders by Borrud et al. (12) with means for men and women closely resembling those in the current study (59.5 vs. 59.9 kg in men, 41.8 vs. 41.4 kg in women).

Janssen et al. (24) also found a significant difference between genders in the distribution of LBM. Distribution was divided into upper and lower body measurements. Upper body measurements were calculated using the images extending from the L4-L5 vertebrae to the hand and lower body measurements were calculated using images extending from one image below the L4-L5 vertebrae to the foot. Men had significantly more LBM in the upper body ($14.1 \text{ kg} \pm 2.6$ vs. $8.4 \text{ kg} \pm 1.8$) and in the lower body ($18.1 \text{ kg} \pm 3.1$ vs. $12.2 \text{ kg} \pm 2.5$) when compared to women. While the current study defined upper body and lower body differently than Janssen et al. significant differences between genders were still observed. Upper body mass was defined by LBM in the trunk and arms while lower body mass was defined as the LBM in the legs. While men had significantly more ($p < .001$) LBM in both their upper and lower body than women, both men and women had significantly ($p < .001$) more LBM in their upper body ($36.1 \text{ kg} \pm 2.7$ in men, $24.9 \text{ kg} \pm 5.9$ in women) when compared to the lower body ($19.8 \text{ kg} \pm 5.9$ in men, $13.4 \text{ kg} \pm 3.9$ in women) (Table 1).

Men in the current study observed larger decreases in LBM between the youngest and oldest group when compared to women (-18.7% vs. -15%) with absolute decreases of -11.9 kg and -6.4 kg respectively (Figure 2). These results are consistent with results seen in Gallagher et al. (18) who examined 148 women (88 African American and 68 Caucasian) with a mean age of 46.0 years (± 18.8)

and 136 men (72 African American and 64 Caucasian) with a mean age of 51.8 years (± 15.7). LBM was determined by a DPX DXA (Lunar Radiation, Madison, WI) software version 3.4. Results showed that men experienced a relative loss of -14.7% and women experienced a loss of -10.8% in LBM from those in their twenties to those in their seventies. The reason for the larger decreases in LBM in men compared to women is not quite understood, however authors have suggested that it could be related to the decrease in hormonal factors related to growth in muscle mass with aging, including growth hormone, insulin like growth factor, and testosterone (23).

A study by Feldman et al.(16) in 2002 measured testosterone levels in 1,709 health men aged 40-70 years at baseline (mean age 55.2 years ± 8.7). Testosterone measurement were measured at baseline then follow up measurements were taken 7-10 years later with 1,156 subjects reporting (mean age 62.7 years ± 8.3). In comparing testosterone levels at baseline to follow up it was noticed that men experienced a decrease in 1.6% per year. When compared to men in the study by Feldman et al. women in a study by Davison et al. (14) noticed smaller rates of decline in testosterone per year. Davison et al. examined testosterone levels in 1,423 women aged 18-75 years (mean age 48.5 years ± 14.2). Results showed a decrease in testosterone levels by 0.9% per year. When comparing the two studies men (Feldman et al.) saw larger decreases in testosterone levels per year when compared to women (Davison et al.) (1.6%/yr vs. 0.9%/yr.) However, in comparing these two studies it is important to keep in mind that Feldman et al. was a longitudinal study showing the change in testosterone levels between the same individual while Davison et al. performed a cross sectional study comparing levels of testosterone between the means of individuals at different ages.

While the DXA is an established measure of estimating %BF and LBM, many clinics and research studies continue to use BMI as a measure of obesity. The results presented in the present study demonstrate how BMI significantly underestimates the prevalence of obesity when compared to the DXA's direct measurement of percent body fat. This misclassification of BMI when compared to %BF could lead misidentifying those individuals who are at risk to developing various diseases that are

associated to an increase in adiposity. The %BF classifications for obesity put forth by the American Society of Bariatric Physicians and the National Institutes of Health are $\geq 25\%$ for men and $\geq 30\%$ for women. However, the limitation to using these classifications is that neither organization specifies which method of measuring %BF should be used. This becomes a serious issue because previous literature has demonstrated that different methods of measuring %BF can produce significant results. A study by Oates et al. (34) in 2006 compared %BF measured by a Lunar Prodigy DXA (GE Healthcare, Madison, WI) in 268 people (105 males, 163 females) to measurements done by ADP (BOD POD, Life Measurement Inc., Concord, CA), BIA (1990B, Valhalla Scientific, San Diego, CA), and two SF methods. SF method 1 (SF1) was comprised of the chest, abdominal, and thigh measurements for men, and triceps, suprailiac, and thigh measurements for women. SF method 2 (SF2) was comprised of chest, biceps, and subscapular measurements for men, and triceps, suprailiac and abdominal measurements for women. Male mean DXA %BF measurements (18.0%) were reported higher than mean measurements for ADP (16.6%) and SF1 (16.4%). However, it was lower in comparison to mean measurements for BIA (19.8%) and SF2 (19.1%). Females mean DXA %BF measurement (31.6%) was higher than ADP (28.6%), BIA (30.2%) and SF1 (28.2%) but lower than SF2 (31.0%).

Although the American Society of Bariatric Physicians and the National Institutes of Health do not specify which method for measuring %BF should be used, these are currently the only classifications that identify obesity in terms of %BF. In comparing these classifications for %BF to those individuals in the present study it was found that 76% of the individuals were obese, while only 23% of the individuals were obese using BMI standards. A major shortcoming of BMI is that it provides a measure of excess weight, and does not distinguish between fat and lean mass. Despite the inability to distinguish between fat and lean mass, when an individual was classified as obese by BMI standards it was in agreement with standards for %BF 97% of the time. This agreement demonstrates that BMI is accurately identifying 97% of the subjects as obese when compared to %BF (Table 6 and 7).

While obesity based off BMI standards is in 97% agreement with %BF standards for obesity, a much smaller agreement occurs when an individual is identified as non-obese. The current study found that agreement between a BMI of < 30 and a %BF less than 25% for men and $< 30\%$ for women was in agreement 33% of the time. As a result, 47% of individuals were misclassified non-obese based on BMI standards while meeting the obesity standards of %BF. Similar results were seen in a 2012 study by Shah and Braverman (39) which utilized DXA measurements from 9,088 subjects between 1998 and 2009. Results demonstrated that while 60% of the subject population was in agreement with both BMI and %BF standards, 39% of the individuals were classified as non-obese based on BMI standards while meeting the obesity classification of %BF standards.

As a result of a large percentage of the subject population not in agreement between %BF and BMI kappa statistics were performed to determine consistency among %BF and BMI classifications. The number of observed agreements between BMI and %BF were 589 (55%) in men and 746 (47%) in women. The number of agreements expected by chance were 422 (42%) in men and 579 (37%) in women. This resulted in a kappa statistic of 0.236 in men and 0.164 in women which demonstrates a fair and slight agreement between %BF and BMI respectively (44) (Table 6 and 7). As a result of the poor agreement the current study found that 53% of women were misclassified as non-obese using BMI standards while meeting the obesity standards for %BF, with 39% of men were misclassified using those same standards. Similar differences were seen between genders by Shah and Braverman (39) in which 48% of their female population was misclassified compared to 22% of the male population being misclassified. The increased misclassification in women when compared to men could partially be attributed to the significantly larger ($p < .001$) LBM in men when compared to women. Forty percent of those subjects who were misclassified as non-obese based on BMI standards while meeting obesity standards of %BF were in the lowest quartile of LBM, while 34%, 18% and 8% of those misclassified were in the lower three quartiles for LBM, respectively.

Misclassification on BMI standards in relation to %BF standards were also seen to increase throughout age by both Shah and Braverman as well as in the current study. Misclassification increased an average of 10 percentage points more per age group in the current study with a total of 40% of those 20-39 years old, 50% of those 40-59 year old, 57% of those 60-79 years old and 72% of those ≥ 80 years old being misclassified. This is most likely attributed to the increase in %BF as previously described, while BMI increases significantly ($p < .05$) from 20-39 years ($26.0 \text{ kg/m}^2 \pm 6.5$) to 40-59 years ($29.3 \text{ kg/m}^2 \pm 6.8$). After the first initial increase in BMI decreases in group 60-79 years ($28.6 \text{ kg/m}^2 \pm 6.8$) and group ≥ 80 years ($26.4 \text{ kg/m}^2 \pm 3.7$).

Limitations

The reference values reported here are only directly compatible with either Lunar Prodigy or Lunar iDXA DXA scanners. Comparisons of estimates using other models should be done with forethought and caution as it has been documented that intra- and inter-machine variation exist (5, 40, 43). Another limitation to the study was the lack of racial diversity in the subjects. A total of 96% of the subject population is Caucasian which is attributed to the fact that East Central Indiana is predominately Caucasian in nature (4). Ethnic variation of %BF and LBM has been well documented (8, 15, 28). Lastly, the study would have benefited from a large sample size in order to develop a better representation of the population. In comparison to Borrud et al. (12) which used data from NHANES consisting of 13,091 subjects (6,559 men and 6,532 women) the present study only used 2,761 subjects (1,164 men and 1,597 women). Lastly, there was a lack of older subjects (≥ 80 years) that were included in the analysis ($n=78$) of the study when compared to the other age groups (20-39 years: 879; 40-59 years: 1003; 60-79 years: 801).

Conclusions

The current study provides references standards of %BF and LBM for men and women aged 20-94 years (men mean age: 50.0 years \pm 19.1; women mean age: 47.8 years \pm 17.7) based on DXA measurements. Women had significantly higher %BF than men (39% vs. 29%) which was seen in each

age group. Men had significantly higher LBM than women (59.9 kg vs. 41.4 kg) which was seen in each age group. Although the sample size was smaller in the present study (n=2,761) when compared to Borrud et al. (12) (n=22,010) who developed DXA reference standards based on data from NHANES means for %BF and LBM were comparable to those in the present study in men (%BF: 29% vs. 28%; LBM: 59.9 kg vs. 59.5 kg) and women (%BF: 39% vs. 39.9%; LBM: 41.4 kg vs. 41.8 kg). Due to the similar findings between the two studies future research should consider the expansion of DXA norms through the combination of these studies as well as with further research.

In addition to providing reference ranges the current study examined the agreement between classifications of obesity based on BMI and %BF standards put forth by the American Bariatric Society (2) and the NIH (3). Overall agreement between BMI and %BF was shown as 55% in men and 47% in women. Results showed that when individuals were identified as obese using BMI it was in agreement with %BF 96% of the time in men and 99% in women. However, when an individual was identified as non-obese using BMI it was only in agreement with %BF 41% of the time in men and 26% in women. Kappa statistics demonstrated a fair agreement between BMI and %BF in men (0.236) and a slight agreement between BMI and %BF in women (.185). As a result 39% of men and 53% of women were misclassified as non-obese by BMI while meeting standards for %BF.

Table 1. Descriptive characteristics of subject participants by gender.

	Men (n=1164)	Women (n=1570)
Age (yrs)	50.0 ± 19.1*	47.8 ± 17.7
Height (in)	69.9 ± 2.9*	64.4 ± 2.8
Weight (lbs)	198.0 ± 40.9*	162.7 ± 42.4
BMI (kg/m ²)	28.5 ± 5.2*	27.6 ± 7.1
Body Fat (%)	29.0 ± 8.5*	39.0 ± 9.3
Lean Body Mass (kg)	59.9 ± 9.6*	41.4 ± 6.9
Android Fat Mass (kg)	2.9 ± 1.6*	2.6 ± 1.6
Gynoid Fat Mass (kg)	5.6 ± 1.7*	4.2 ± 2.2
Upper Body Lean Mass (kg)	36.1 ± 2.7*	24.9 ± 4.6
Lower Body Lean Mass (kg)	19.8 ± 5.9*	13.4 ± 3.9

Data presented are mean ± SD.

* Significantly different ($p < .01$) between men and women.

Table 2. Normative percentiles of body fat (%) for male subjects by 20-year age-groups.

Age (years)	Sample Size	Mean %BF	Std. error	Percentile									
				5 th	10 th	15 th	25 th	50 th	75 th	85 th	90 th	95 th	
Body Fat (%)													
All Ages	1164	29.0	.25	13.4	17.3	19.4	23.6	29.9	35.1	37.6	39.3	41.7	
20-39	356	23.7	.48	9.5	11.9	14.2	17.2	22.7	30.1	34.2	36.5	39.7	
40-59	370	31.0*	.38	19.3	22.2	23.4	26.4	31.6	35.9	38.7	39.9	42.0	
60-79	405	31.7*	.33	19.4	23.3	24.7	27.3	32.0	36.1	38.4	39.8	42.3	
≥ 80	33	30.2*	1.13	19.5	20.6	22.7	26.4	29.4	35.1	37.7	39.8	42.5	

*Significantly different ($p < .001$) from 20-39 years.

Table 3. Normative percentiles of body fat (%) for female subjects by 20-year age-groups.

Age (years)	Sample Size	Mean %BF	Standard error	Percentile								
				5 th	10 th	15 th	25 th	50 th	75 th	85 th	90 th	95 th
Body Fat %												
All Ages	1597	39.0	.23	23.0	25.9	28.2	32.4	39.5	46.4	49.2	50.6	52.9
20-39	523	33.9	.43	21.1	22.4	23.7	26.2	32.6	40.7	45.7	48.4	51.6
40-50	633	41.2*	.34	26.6	30.1	32.3	35.5	41.5	47.9	50.1	51.9	53.6
60-79	396	42.3*	.35	29.9	32.7	34.7	37.9	42.4	47.6	49.5	50.9	52.6
≥ 80	45	39.3*	.99	27.1	28.7	29.9	34.1	40.8	43.9	45.3	45.9	48.9

*Significantly different ($p < .001$) from previous age group.

Table 4. Normative percentiles of lean body mass (kg) for male subjects by 20-year age-groups.

Age (years)	Sample Size	Mean LBM	Standard error	Percentile								
				5 th	10 th	15 th	25 th	50 th	75 th	85 th	90 th	95 th
Lean Body Mass (kg)												
All Ages	1164	59.9	.28	47.2	49.8	51.1	53.8	58.7	64.1	67.7	71.4	78.6
20-39	356	63.8	.64	49.2	51.8	53.8	56.3	61.3	68.3	74.4	83.4	88.9
40-59	370	60.5*	.44	48.1	50.5	52.8	55.3	60.0	64.8	68.7	71.7	76.4
60-79	405	56.6*	.32	47.0	48.3	50.1	51.7	56.7	60.7	62.7	64.8	67.1
≥ 80	33	51.9*	.86	44.1	45.4	45.9	49.1	51.5	54.1	59.8	60.4	61.0

*Significantly different ($p < .01$) from previous age group.

Table 5. Normative percentiles of lean body mass (kg) for female subjects by 20-year age-groups.

Age (years)	Sample Size	Mean LBM	Standard error	Percentile								
				5 th	10 th	15 th	25 th	50 th	75 th	85 th	90 th	95 th
Lean Body Mass (kg)												
All Ages	1597	41.4	.17	32.6	34.2	35.3	36.7	40.1	44.7	47.5	50.2	54.6
20-39	523	42.3	.30	33.5	35.4	36.2	37.7	41.3	45.6	48.4	50.5	55.3
40-59	633	42.5	.30	33.0	34.5	35.6	37.2	41.2	46.1	49.6	52.3	56.6
60-79	396	39.1*	.26	31.6	33.5	34.4	35.8	38.3	42.1	44.2	45.9	48.1
≥ 80	45	35.8*	.56	30.2	31.5	32.1	32.9	35.2	39.1	40.8	41.8	42.5

*Significantly different ($p < .01$) from previous age group.

Table 6. Agreement between percent body fat and BMI in men.

	%BF Obese ($\geq 25\%$)	%BF Non-obese ($< 25\%$)
BMI Obese ($\geq 30 \text{ kg/m}^2$)	358	16
BMI Non-Obese ($< 30 \text{ kg/m}^2$)	459	331

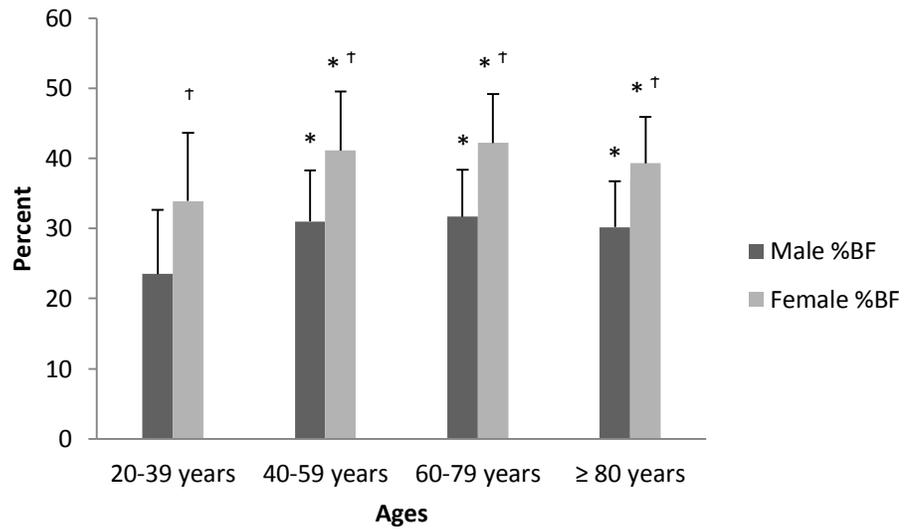
* Kappa Statistic: 0.286 (fair agreement).

Table 7. Agreement between percent body fat and BMI in women.

	%BF Obese ($\geq 30\%$)	%BF Non-obese ($< 30\%$)
BMI Obese ($\geq 30 \text{ kg/m}^2$)	437	2
BMI Non-Obese ($< 30 \text{ kg/m}^2$)	849	309

*Kappa Statistic: 0.164 (slight agreement).

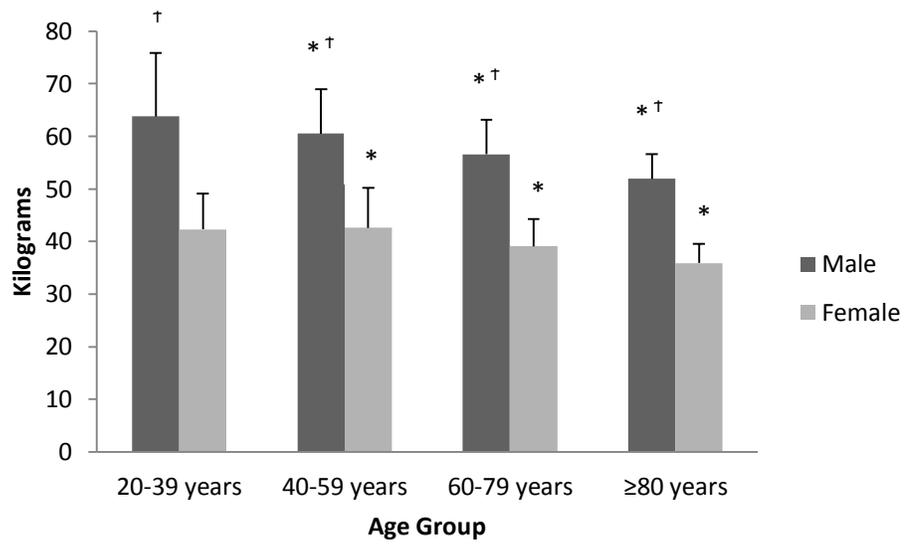
Figure 1. Comparison of percent body fat (mean \pm SD) by age and gender.



*Significantly different from 20-39 years ($p < .001$).

†Significantly different from males ($p < .001$).

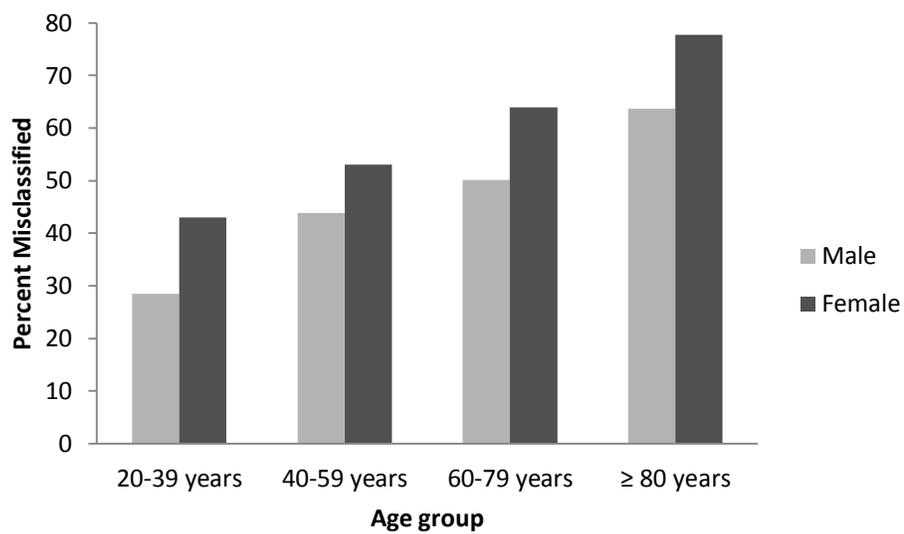
Figure 2. Comparison of lean body mass (mean \pm SD) by age and gender.



*Significantly different from previous age group ($p < .01$).

†Significantly different from females ($p < .001$).

Figure 3. Comparison of percent identified as non-obese by BMI and obese by %BF between age and gender.



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Chapter V

Summary

The primary purpose of this study was to develop a set of reference standards for %BF and LBM based on DXA-derived estimates from data provided by the CEP Program at Ball State University (Muncie, IN). These reference standards are divided by gender and age to provide a better representation of specific demographics. Women had significantly higher ($p < .001$) %BF than men ($39\% \pm 9.3$ vs. $29\% \pm 8.4$) which was seen in each age group. %BF increased significantly ($p < .001$) in both men and women from the youngest age group (20-39 years) to the 40-59 year age group (men: $23.7\% \pm 9.1$ to $31.1\% \pm 7.2$; women: $33.9\% \pm 9.7$ to $41.2\% \pm 8.4$). After the first initial increase in %BF, no other significant changes occurred between means in age groups 40-59 years, 60-79 years and ≥ 80 years. However, mean %BF remained significantly higher ($p < .001$) in age groups 60-79 years (men: $31.6\% \pm 6.7$; women: $42.3\% \pm 7.0$) and ≥ 80 years (men: $30.2\% \pm 6.5$; women: $39.3\% \pm 6.6$) when compared to those in the youngest age group.

An additional benefit of using DXA to assess body composition compared to other methods is that it provides measurements of LBM which have been shown to be helpful in the detection of sarcopenia. Although there is no universally accepted way to identify sarcopenia, one definition is greater than two standard deviations below the mean of young adults of the same sex and ethnic background (8, 35). In the current study, when using the youngest age group (20-39 years) as the reference group, only one female and one male had a LBM value low enough to be

considered as having sarcopenia. Although the percentage of the population in the current study with sarcopenia was vastly different from literature (20% of adults 60-70 years old and 50% in adults over 75 years old) (18), the operational definition of sarcopenia is the reduction in muscle mass and strength associated with aging (16, 31) which was seen in the current study. LBM significantly decreased ($p < .01$) between each age group in men (20-39 years: 63.8 kg \pm 12; 40-59 years: 60.5 kg \pm 8.4; 60-79 years: 56.6 kg \pm 6.5; \geq 80 years: 51.9 kg \pm 5.0). Unlike men, women did not notice a significant decrease ($p < .01$) in LBM until 60-79 years (39.1 kg \pm 5.2 vs. 42.6 kg \pm 7.7 in 40-59 years). This significant decrease ($p < .01$) continued into the oldest age group (35.9 kg \pm 3.8). Results also revealed that men had significantly higher LBM than women (59.9 kg vs. 41.4 kg) which was seen in each age group.

The secondary purpose of this study was to examine the agreement between classifications for obesity based on BMI standards (≥ 30 kg/m²) and %BF ($\geq 25\%$ in men, $\geq 30\%$ in women). Thirty percent of subjects were classified as obese based on BMI standards, while 76% of the individuals were classified as obese based on %BF. Men were more likely than women to be obese (32% vs. 27%) when using BMI standards, however when using %BF standards more women were obese when compared to men (81% vs. 70%). Overall agreement between BMI and %BF was 52% in all subjects (55% in men, 47% in women). In order to determine the inter-rater agreement between %BF and BMI, a kappa statistic was performed. The number of agreements expected by chance in men was 442 (42%) and 579 (37%) in women. As a result, kappa statistics for men and women were 0.236 (fair agreement) and 0.184 (slight agreement) respectively. The majority of the disagreement between BMI and %BF occurred in those identified as non-obese by BMI while meeting %BF standards for obesity. However, only 18 individuals were identified as obese based on BMI standards while being identified as non-obese by %BF. As a result, when BMI identified an individual as obese, it was correct 97% of the time (96% in men, 99% in women). Conversely, when an individual was identified as non-obese

it was in agreement with %BF only 33% of the time (41% in men, 26% in women. As a result 47% of the total population (39% of men, 53% of women) was misclassified as non-obese by BMI while meeting standards for obesity by %BF. Similar differences were seen between genders by Shah and Braverman (115) in which 48% of their female population was misclassified compared to 22% of the male population.

Recommendations for Future Research

Borrud et al. (20) has previously developed reference standards for %BF and LBM derived from DXA data. This study was comprised of 22,010 individuals who were scanned from 1999-2004 as part of NHANES. NHANES is comprised of a series of cross-sectional, nationally representative surveys of the health and nutrition status of the U.S. population. Samples are selected through a complex, multistage probability design. As a result of this design and the population sampled, multiple ethnic groups were included in the study and ethnic specific references were also created. However, one limitation the NHANES data as compared to the current study is the limitation of the DXA model used. NHANES utilized a Hologic Discovery DXA (Hologic, Bedford, MA) which has a weight limit of 300 pounds. The current study utilized two DXA models: Lunar Prodigy and Lunar iDXA (GE Healthcare, Madison, WI). While the Lunar Prodigy also had a weight limit of 300 pounds, weight limit for the Lunar iDXA was 450 pounds, allowing for analysis of %BF and LBM in individuals over 300 pounds. Another limitation to the Hologic Discovery used in the NHANES study was that subjects were required to fit within the field of the scan. However, a 1995 study by Tataranni and Ravussin (123) showed that the right and left side of the bodies had strong association with %BF (0.99), non-bone fat-free soft tissue (0.97), fat soft tissue (0.99), and bone mineral (0.89). Due to the high association between the two sides, scans for subjects who are wider than the field of the scan measurements can be made on one side and total body composition can be estimated assuming

bilateral symmetry. The Lunar iDXA in the current study contains the MIRROR IMAGE™ software, which has the capability of performing such measurements.

Although the sample size was smaller in the present study (2,761 vs. 22,010) and there was a lack of ethnic diversity (96% Caucasian), means for %BF and LBM were comparable to those in Borrud et al. in both men (%BF: 29% vs. 28%; LBM: 59.9 kg vs. 59.5 kg) and women (%BF: 39% vs. 39.9%; LBM: 41.4 kg vs. 41.8 kg). Due to the similar findings between the two studies, future research should consider the expansion of DXA norms through the combination of these studies as well as with further research to create a national registry based on DXA-derived data. This national registry would allow for a more expansive representation of the population. These standards would allow for clinicians and researchers to classify individuals with these references which could lead to the detection and prevention of negative health factors associated with LBM and %BF.

One such negative health factor associated with LBM is sarcopenia. Although sarcopenia is a large concern regarding body composition, no universal standards exist for the identification of sarcopenia. The most commonly used method to measure sarcopenia is when an individual has a LBM greater than two standard deviations below the mean of a same sex and ethnic control. When using the 20-39 year age group as the young control, only one female and one male in the current study were classified as having sarcopenia using this method. These results are widely different than the prevalence presented in the literature (16). As a result of these differences, it is recommended that a universal method and standard be created to avoid such large discrepancies in the prevalence reported between studies. In addition to developing a universal method assessing the quantitative facet of sarcopenia (amount of muscle mass), future research should consider the qualitative facet (muscle strength and function) as rates of decline in muscle mass and strength enormously differ over time, with the rates of decline in muscle mass explaining less than 5% of the variance in the age-related change in strength (27, 130).

In addition to creating this national registry, future researchers should also assess physical activity status, dietary habits, as well as cardiovascular risk factors allowing for further exploration of factors that influence differences in both LBM as well as %BF in individuals. The assessment of cardiovascular risk factors in addition to the registry would allow for the prevalence of each risk factor to be identified at each percentile. For example, the prevalence of hypertension has been previously demonstrated to increase as %BF increases (112). Using the current study as an example, if the prevalence of hypertension significantly increased after the 25th percentile in men aged 40-59 years, if a man in that age group were able to reduce his %BF from 27% to 25% he may not reduce his %BF significantly, however, his risk for hypertension would be significantly reduced. With the future assessment of cardiovascular risk factors as well as the use of the DXA reference standards, clinicians would be able to properly identify those individuals who are more prone to negative health risk factors.

While the American Bariatric Society and the NIH have provided standards for obesity based on %BF, these organizations do not state which method of measuring %BF should be used. The current reference standards provided in this study were made using DXA measurements and future researchers should take caution when comparing measurements between two methods, as significant differences have been shown to occur (98). While DXA provides measurements such as %BF and LBM, the current initial measure recommended to clinicians measuring total body adiposity is BMI (28). BMI is the recommended tool for clinicians and epidemiological research due to the low cost and simplicity and ease of use. Results of the current study demonstrate that clinicians should feel confident (97%) in the results when BMI identifies an individual as obese. However, when BMI identifies an individual as non-obese, future research should take additional steps and measurements should be taken to ensure accuracy, as 47% of the population in the current study was misclassified as non-obese using BMI while meeting standards for obesity using %BF. This large percentage of misclassification demonstrates that obesity, body fat and

increased adiposity maybe more widespread than previously believed. This problem is confounded knowing that an increase in co-morbidities such as hyperlipidemia, coronary artery disease, hypertension and diabetes are strongly associated with an increased in adiposity.

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