EVALUATION OF PERCENT BODY FAT ESTIMATES USING DIFFERENT BODY COMPOSITION METHODS IN RECREATIONALLY ACTIVE ADULTS ACROSS BOTH SEXES

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Abstract

This thesis compared percent body fat (%BF) values derived from different body composition (BC) modalities: air displacement plethysmography (Bod Pod), skinfold (SKF) prediction equations (Durnin and Womersley (DW), Jackson and Pollock (JP7), (JP3) and Yuhasz) with dual-energy x-ray absorptiometry (DXA) as the reference and compared whether they differed by sex. Utilizing data from 38 active individuals (20 females, aged 26.3±4.0 years; 18 males, aged 30.5±7.4 years), females demonstrated significant differences between all modalities with DXA ($P<0.05$), except for DW equations. Males demonstrated no significant differences with DXA ($P<0.05$), except for the Yuhasz equation. Bland-Altman (B-A) analyses found significant systematic biases ($P<0.05$) in females and males with magnitudes of difference ranging from -2.0% to -10% and -1.4% to -4.0%, respectively vs DXA. Significant proportional biases between different modalities and DXA were also evident in both sexes. These results highlight the importance of using the most appropriate BC approach to estimate %BF.

Dedication

To my late father and brother, and the many people who have helped me on this journey. Your support and confidence in me will never be forgotten.

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List of Abbreviations

%BF	Percent body fat
2C	Two-compartment
3C	Three-compartment
4C	Four-compartment
6C	Six-compartment
ADP	Air-displacement plethysmography
ANOVA	Analysis of variance
BC	Body composition
B-A	Bland-Altman
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BMC	Bone mineral content
BV	Body volume
Db	Body density
DW	Durnin and Womersley
DXA	Dual-Energy X-ray absorptiometry
FBFM	Fat-and bone-free lean mass
FFDM	Fat free dry mass
FFM	Fat free mass
FM	Fat mass
ISAK	International society for the advancement of kinanthropometry
JP	Jackson and Pollock
JP3	3 site equation by Jackson and Pollock
JP7	7 site equation by Jackson and Pollock
NAA	Neutron activation analysis
UWW	Under water weighing
SKF	Skinfold
TBW	Total body water

CHAPTER 1: INTRODUCTION

1.1 Background

A multitude of methods exist to indirectly measure body fat and other tissue compartments (e.g., skeletal muscle) that are of considerable interest and importance to exercise scientists, coaches, athletes, and individuals seeking general health and fitness. With respect to lifestyle related diseases, the importance of monitoring body fat specifically, is multifactorial as increased or excess levels of body fat are commonly associated with impairments in glucose and lipid metabolism often linked to diabetes and cardiovascular disease (1). Conversely, decreased or reduced levels of body fat have been associated with bone mineral deficits and reduced lean mass (2,3). It is well documented in the literature (4–6), that there is a relationship between body weight, health, and longevity, such that body weight has been associated with all-cause mortality. This relationship has provided the basis for the utility to indirectly evaluate the quantitative and qualitative aspects of the body. Independent of age, race, and sex, body mass index (BMI) uses an individual's weight and height to classify individuals as underweight, normal, overweight, and various classes of obesity (Class, I, Class II, Class III) (7). In population level studies, BMI is the most commonly used measure of overall body fat and associated health risks as BMI classifications are well correlated with percent body fat (%BF) (8). This well-established correlate of health risk is commonly observed as a U- or J-shaped relationship between BMI and mortality risk in a large majority of studies (9,10). Thus, BMI may not be the best assessment for associated health risks for all cohorts, especially individuals that have their total body weight augmented by habitual physical training. As previously stated, the relationship between BMI and disease is explained in part by the ability of BMI to be a surrogate of total body fat (11). Despite this, there is considerable individual variability that often leads to misclassifications of obesity, overweight, healthy weight, and underweight since BMI does not distinguish between tissue types — muscle, fat, bone, among others (12). These inter-individual variations can partially be explained by race, sex, age, and physical activity level (13,14). Thus, on an individual basis the utility of BMI is limited for predicting total body fat (8). Hence, there is a need for alternative measures that can better discern these physical characteristics. Namely, these alternative measures provide a means to indirectly evaluate different tissues of interest, i.e., the composition of the body and tissues that are differentially associated with disease risk.

As a branch of modern science, the study of human body composition (BC) is most recognized as an adjunct of human biology (15). As a distinct scientific discipline, BC attempts to quantify and partition body weight or mass into its basic compartments, such as fat mass (FM) and fat-free mass (FFM) (16). Prior to in vivo methods for estimating the various compartments of the body, cadaver autopsy was the only way to obtain quantitative data on BC (15). Subsequently, cadaver analysis represents the true gold standard for the assessment of BC, as these early studies provided the necessary knowledge for in vivo BC analysis today (15). However, it has been observed that most often, these in vivo estimations are based on assumptions regarding the physical and chemical properties of tissues not directly measured (17). These estimations are commonly attributed to two main compartments of the body: FM and FFM, respectively. However, therein lies one of many systematic shortcomings with estimating BC – such that if the assumptions of FM (0.90 g/cm^3) and FFM (1.1000 g/cm^3) are not met, the BC estimate will be inaccurate (17). These assumptions include that FFM comprises 73.8% water, 19.4% protein, and 6.8% mineral (18). However, the variability among individuals in the water, protein, and mineral fractions of FFM can result in considerable errors if these assumptions are not met in the BC estimate (18). Despite this, several studies have shown that the methods for estimating BC based on these assumptions are reasonably accurate in most people (19,20). Indirect methods such as skinfold (SKF) thicknesses, and air displacement plethysmography (ADP)/Bod Pod, have been developed as a more accessible and practical means for predicting BC in both the field and laboratory (21). Both BC methods, Bod Pod and most SKF prediction equations initially provide an estimate of body density (Db), of which the resultant Db is then used in a generalized or population-specific equation (Brozek or Siri) to estimate %BF (22). However, the principles that underlie BC methods that derived Db sometimes make the erroneous assumption that there is a constant density of FM (0.90 g/cm^3) and FFM (1.1000 g/cm^3) for everyone. This is the basis of the two-compartment (2C) model of BC which does not take into account individual differences in the mineral, water, and protein content of the FFM (22). These assumed FFM density values could be affected by both sex and physiological changes attributed to habitually active individuals. Several studies have found mixed results when comparing %BF estimates from the laboratory method Bod Pod with other established BC methods in different populations (23–25). Levenhagen et al. noted significant sex differences with %BF being overestimated in males and underestimated in females, than that determined from underwater weighing (UWW), also known as hydrodensitometry or hydrostatic weighing (23). Lowry et al. observed biased results in %BF across relative extremes of BMI compared to dual-energy X-ray absorptiometry (DXA) in a mixed group of adults from the general population (24). Such that, the Bod Pod overestimated %BF in individuals classified as normal and underweight as opposed to an underestimation in individuals classified as overweight/obesity (24). Ballard et al. compared %BF estimates between the Bod pod and DXA in female collegiate athletes and nonathletes (25), but the authors did not find significant differences in %BF between the methods. As a field method, SKFs are highly portable and require very little operating costs, but require expertise (26). SKF measurements indirectly facilitate the assessment of BC, due to the relationship between subcutaneous fat and total body fat (27). Studies that have compared the accuracy of several SKF prediction equations with DXA in different population subgroups, have found mixed results (19,28–30). Studies that have evaluated SKF thickness equations in multiracial samples and clinical populations, have found a general trend towards an overestimation of %BF as compared to DXA (19,30). Conversely, other studies have found an underestimation in %BF as compared to values derived from DXA in adolescents and older adults (28,29). Other studies that specifically examined correlations have found strong correlations with existing SKF prediction equations and values derived by DXA in male athletes (31). It is evident that SKF prediction equations may work well on samples from which they were derived, however there are many aspects of study cohorts that may influence the accuracy of those estimations, such as race, activity level, age, sex, and obesity (30). Therefore, there is a need to evaluate the accuracy and agreement of %BF estimations from SKF prediction equations in different cohorts including the current study cohort. Due to several logistical constraints such as cost and accessibility, accurate BC methods that are practical and more financially feasible are a primary focus for professionals in the field and individuals seeking to alter their BC by engaging in exercise interventions.

1.2 Rationale for Investigating Recreationally Active Healthy Adults

My thesis compared %BF estimates obtained from three commonly used BC methods, namely DXA, Bod Pod and SKFs, in recreationally active healthy adults. More specifically, the primary point of comparison was the accuracy of %BF estimates from Bod Pod and SKFs compared with estimates obtained from DXA. DXA is a well-established laboratory BC method that has gained the widest acceptance as the gold standard (32). With some authors using the term practical gold standard, since it is quick, reasonably accurate and requires minimal subject involvement (33). DXA estimates three compartments of the body, namely FM, bone mineral content (BMC), and soft tissue lean mass (16). With the additional estimate of a third compartment, BC estimations by way of DXA are theoretically more accurate as they reduce assumptions governed by other more commonly used laboratory and field methods that only evaluate two compartments of the body (34). DXA has shown a high level of precision and accuracy (35,36), validating its use as a reference method in comparative studies. As previously stated, studies have documented a U-or J-shaped association between BMI, morbidity, and mortality, irrespective of age, race, and sex (9,10). Such that, low and high BMI values have an increased health risk as they are deviations from the optimal range of 18.5-25.0 kg/m^2 (11). Compared with the general population, habitual physical activity alters the proportions of body minerals, water, and protein (37,38), such that, the influence of lean/muscle mass on BMI in recreationally active adults may misclassify this subgroup as overweight or having obesity (14). This justifies the rationale for investigating different BC methods that indirectly estimate %BF in this specific population subgroup. To this end, %BF estimations may be more effective than BMI in assessing health risk in physically active adults (14). Relative to BMI, it has been shown in the literature that a more accurate measure of adiposity can be obtained by indirect measures of body fat (39,40).

Previous studies that have compared %BF estimates from different BC methods have primarily focused on collegiate and professional athletes (41–43), older adults (44), and clinical populations (30). Therefore, there is a paucity of studies specifically assessing the use of these BC methods in recreationally active individuals, which is the subgroup of interest for this thesis. Moreover, the sex comparisons across these specific modalities have not been well-studied.

Therefore, it is important for more accessible BC methods, especially field methods (which are more available) to be compared to practical gold standard methods in different populations to assess their utility, particularly as a measure of health and to track progress with wellness initiatives. This needs to be further evaluated.

1.3 Aim

The aim of this thesis was to examine three different BC methods of measuring %BF in recreationally active adults. This inquiry extends to data across both sexes to determine which methods were most in agreement with a practical gold standard.

1.4 Objectives and Hypotheses

Primary Objective:

To assess whether there were statistical differences in %BF estimates among various BC methods namely Bod Pod, SKF thicknesses, and DXA across both sexes in healthy recreationally active adults.

Primary Hypothesis:

I hypothesized that each of the different BC methods will show a statistical difference between each other as compared to DXA with significant differences between the sexes for each of the respective modalities and their prediction equations.

Secondary Objective:

To evaluate the agreement between the different BC methods with DXA across both sexes.

Secondary Hypothesis:

I hypothesize that the estimated %BF from the Bod Pod and SKF methods will not show agreement with the values derived from DXA across both sexes.

CHAPTER 2: LITERATURE REVIEW

2.1 Brief Introduction to Body Composition Models

There are a multitude of BC methods that exist for which certain and different assumptions underlie their theoretical models. The various theoretical models can be viewed from the framework of Wang et al (45). This model is regarded as the five-level model in the literature (45). According to the schema devised by Wang et al, there are five distinct levels by which the human body can be viewed from: atomic (level I), molecular (level II), cellular (level III), tissue-system (level IV), and whole body (level V) (45). The different levels partition the body mass into multiple compartments for separate *in vivo* BC estimations. Therefore, the various methods available for assessing BC are based on 2C, three-compartment (3C), four-compartment (4C), or multi-compartment models.

2.2 Two-Compartment Model

The simplest approach in BC analysis is the 2C model in which the body mass is assumed to be partitioned into two compartments (FM and FFM) respectively (18,34). The 2C model is represented by the following equation: Body Mass = FM + FFM. Under the assumptions of this model, the FM compartment includes all extractable lipids in the body, whereas all other tissues are assigned to the FFM compartments (18). The term lean body mass is occasionally used interchangeably with FFM in the literature (46), despite there being a distinction between the two terms. LBM includes a small portion of essential lipids, which are essential amounts of fat necessary for health (47), whereas FFM is all lipid-free tissues in the body. Due to its simplicity, and its ability to estimate FM, the 2C model has the widest application in the study of BC, as many methods for estimating BC have been established based on the 2C model. For example the 2C method UWW was once considered the gold standard method for indirectly estimating BC

(23). Consequently, many BC field methods prevalent today, such as SKFs are still utilizing predictive equations validated against UWW (48). Under the assumptions of this model, fat is assumed to have a density of 0.9007 g/cm^3, whereas FFM is assumed to have a density of 1.1000 g/cm^3 (18). The relative percent composition of the FFM comprises 73.8% water, 19.4% protein, and 6.8% mineral (18). Therefore, the primary limitation of the 2C model is the assumption that the FFM compartments is always constant in everyone. The 2C model is based on assumptions made from the analysis of three male cadavers, ages 23, 35, and 46. However, race was not specified (49). The work of Forbes et al (50) and Widdowson et al (51) provided a basis for analysis of the three male cadavers. The technical errors of estimates found within this model are less likely attributed to technical accuracy of the measurements, but the validity of the assumptions (34). Generally, these 2C model equations provide reasonable estimates of %BF as long as the assumptions of the model are met as previously stated (FFM density = 1.1000 g/cm^3, density of fat = 0.9007 g/cm^3, FFM water content = 73.72%) (18). However, there is no guarantee that the FFM composition will match the assumed values for FFM of an individual or specific population subgroup, thereby producing systematic prediction errors (52). For example, research has shown that African American males and females have a higher average FFM density (1.106 g/cm^3) than the assumed 1.1000 g/cm^3; due to their higher relative body protein and mineral content (53,54). Therefore, when 2C model equations are used to estimate %BF in African Americans, there is a trend towards a systematic underestimation (55). Some of the most used 2C model methods are Bod Pod and field methods such as SKF thicknesses.

2.2.1 Air Displacement Plethysmography (Bod Pod)

ADP uses densitometry or Db measurements to predict BC. The term densitometry refers to measurement procedures that use total body density (56). Body density measurements, using

ADP to estimate %BF, are based on the 2C model, where the mass of the body is divided into FM and FFM compartments, respectively. As previously stated, this model assumes a constant density for FM of 0.9007 g/cm^3 and 1.1000 g/cm^3 for FFM.

To determine the density of the body, mass can be readily obtained by measuring the body weight. Body volume is indirectly calculated by the volume of air remaining with the subject inside the chamber from the volume of air in the chamber when its empty (56). Currently, the commercially available modality that uses ADP technology is the Bod Pod (Cosmed Inc.) (56).

Early attempts to use ADP for the assessment of BC proved ineffective. Problems accounting for variability in temperature, pressure, and humidity caused significant volume errors subsequently causing errors in the estimate of body fat; (57,58). The work of Dempster, Aitkens (59) and McCrory et al. (60) represented breakthroughs in providing more accurate assessments of BV by ADP. They introduced a new state-of-the-art system called the Bod Pod which overcame some of the limitations of earlier ADP technology. The Bod Pod is a large, egg-shaped fiberglass chamber that uses air displacement and pressure volume relationships to determine an individual's BV (61). Using Poisson's Law, the volume of the participant in the measuring chamber is determined by the pressure-volume relationship at a fixed temperature (62). Prior to the early 2000s, most studies investigating the accuracy of ADP have compared it with UWW (63,64). UWW was long considered the gold standard for the assessment of BC, prior to newer techniques such as DXA (23).

For example, the investigation by Vescovi et al. found that ADP overestimated %BF in female athletes compared with both UWW and SKFs (63). In contrast, Collins et al. found that ADP underestimated %BF in male Division I football players as compared to UWW with DXA as a reference (65). Similar trends have been found by Levenhagen et al. with respect to an overestimation of %BF in females and an underestimation of %BF in males when Bod Pod was compared with UWW (23). There may be a growing trend in the literature suggesting a consistent underestimation of %BF in males and overestimation in females (63). The assertion is that there must be some type of sex bias to account for an underestimation of %BF in males and an overestimation in females when ADP is utilized. Further, the investigation by Vescovi et al. notes that most of the significant differences in estimations of %BF when ADP is compared with either UWW or DXA, were found at the lean end of range regardless of sex (63). In another study by the same primary investigator, Vescovi et al. trisected a heterogenous sample into three different subsets consisting of lean, average, and overweight. The estimations of %BF were found to be significantly greater for the lean subset by 14%, whereas the average and overweight groups did not show statistically significant differences when ADP was compared to UWW (66). Additionally, 81% of the lean subset consisted of females in that investigation, further indicating a potential sex bias by ADP (66). However, it is worth noting that the apparent sex bias was not apparent in the average and overweight groups, even though 80% and 52% of the average and overweight subsets consisted of females, respectfully. Therefore, this apparent sex bias may better reflect a bias towards lower levels of body fatness. Nonetheless, these observations lend some support to the acknowledgement that BC estimates may differ between males and females. Such that, males have more lean mass, and females have more fat mass at a given BMI (67).

2.2.2 Skinfold Thicknesses

In field settings BC is commonly measured by SKFs (27). SKFs are a measure of the thickness of two layers of skin and the underlying subcutaneous fat (68). Therefore, SKFs are an indirect measure of the thickness of subcutaneous adipose tissue, and this method is principally governed by the relationship between subcutaneous body fat (SKF thickness) and total body fat

(68)**.** Through systematically measuring several skinfold thicknesses, the site-specific millimetre measurements can be used with their respective equations for predicting Db and %BF (27).

Like Bod Pod, the SKF method is considered a 2C model, in which the body is partitioned into FM and FFM compartments, respectively. As stated, the fundamental problem with this approach is the assumption that the FFM compartment, which consists of BMC, total body water (TBW) and other non-fat tissues; remains the same for all individuals (18). It is highly likely that the densities of the different FFM compartments will vary from the assumed 2C densities in different individuals. For example, research suggests that the average fat free body density of African Americans exceeds 1.1000 g/cm^3 (68). Research has shown that this difference is mainly due to higher mineral content (54).

Research conducted by Hayes et al and Orphanidou et al, demonstrated that assessed subcutaneous fat measurements at selected sites (ex. Biceps, Triceps, Subscapular, and Suprailiac) are similar to values obtained from MRI and CT scans (69,70). Skinfold measurements at these selected sites are typically used to predict Db, from which %BF can be calculated using prediction equations such as the Siri and Brozek equations, respectively (68). The predictive accuracy of the SKF method is governed by the assumption that the sum of several SKFs represented by a fold of skin and the double layer of subcutaneous fat included in that fold at different body sites is representative of overall body fatness (27). As a percent of total fat, subcutaneously fat has been estimated to range from 20-70%, depending on biological factors such as age, sex, and measurement technique (71). This estimation has been shown to be reasonable using a combination of selected skinfold measurements (71). The accuracy and precision of SKF measurements are affected by the technician's skill, type of caliper, client factors, and the prediction equation used to estimate body fatness (27,68). To provide a guide for researchers and clinicians, the Anthropometric Standardization Reference Manual was published in 1988 to provide a standardized set of anthropometric dimensions (72). Prior to the development of this guide, there were no standardized protocols, despite the prevalence of use of SKF measures in the literature (27). Established in 1986, the International Society for the Advancement of Kinanthropometry (ISAK), published its International Standards for Anthropometric Assessment in 2001 on a variety of anthropometric dimensions (circumferences and skeletal breadths) and SKFs (73). ISAK established a certification process with the requisite training for anthropometrists. Specific to the ISAK approach, 8 SKF sites (Triceps, Subscapular, Biceps, Iliac Crest, Supraspinale, Abdominal, Front Thigh, and Medial Calf) are recognized as part of their standard ISAK full profile (73)**.** Unique to the ISAK protocol, their approach places emphasis on the raw skinfold data over Db and %BF conversions through a variety of equations (73). For predicting Db and %BF, there are currently a multitude of equations of which many have not been cross-validated or found to be less effective in different populations (27). Population-specific equations are valid only for individuals with similar characteristics such as age, sex, ethnicity, or physical activity level (68). Population-specific equations are based on a linear relationship between SKF and Db by way of a linear regression model (68). However, across a large range of body fatness there is a curvilinear relationship between SKFs and Db (68). Therefore, population-specific equations tend to underestimate %BF in individuals with more proportional fat mass, and overestimate it in leaner individuals (68). Counter to this, the Durnin and Womersley (DW) equations are based on the log of the sum of four SKFs (Biceps, Triceps, Subscapular, and Suprailiac). These equations were established in four different age groups of adult males and females using a 2C model criterion method (74). In nonathletic adults, one of the most popular equations for estimating Db are the Jackson-Pollock (JP) equations which use the sum of three, four, or seven SKFs (27). The protocols for measuring the sum of three, four, or seven SKFs are well described in the Jackson and Pollock studies (75,76). JP found a curvilinear relationship between body fatness and the sum of several SKFs (27). The JP equations were developed on a heterogenous sample of adult males and females between the ages of 18-61 years that varied considerably in BC and exercise habits (75,76). However, the JP equations have been cross-validated against different reference methods in different adult populations with varied results (77–79). While some studies show an underestimation in estimated %BF in primarily Caucasian males and females (77,78). Similarly, other studies have demonstrated an underestimation in a diverse group (Caucasian, Hispanic, and Black) of males and females (79). Conversely, one of those same studies found an overestimation in %BF in females (77). Another SKF prediction equation that is prevalent in the literature, is the equation by Yuhasz which is typically used in studies with young active adults (80). The Yuhasz equation has been cross validated in young and adult active males between the ages of 21-33 years (80). A 2002 study by Dimitrijevic et al. examined the correlations between anthropometric equations, Bioelectrical Impedance Analysis (BIA), and DXA measurements in male athletes from combat sports, including wrestling, judo, and kickboxing (31). They found that 16 out of 17 anthropometric equations showed strong positive correlations with DXA measurements, with the highest correlation observed using the equation by Yuhasz (31). The presence of a strong positive correlation between %BF estimated by DXA and the Yuhasz equation was also reported in an investigation by Suarez-Arrones et al. in a group of 18 international-level, elite male soccer players (81).

Principally, like most other methods, SKFs suffer from two types of error: methodological error when collecting raw data, and the assumptions that underlie the process of converting the raw data to final values (82). To address this issue, many authors suggest using equations and formulas designed specifically for the population of study (68). For example, a SKF equation developed in active college males will be less accurate in a sample of heterogenous adults. This approach helps to reduce systematic bias. Despite, its perceived simplicity, substantial intra-observer and inter-observer variability has been reported (83). This variability has been attributed in the literature to the use of different calipers, location of anatomical sites, and user technique with grasping the fold. Moreover, there are practical limitations that can prevent obtaining the most accurate and reliable measurements; Such as the presence of edema, difficulties with raising SKFs at certain locations and excess adiposity preventing accurate caliper application (84,85).

Several studies have investigated the agreement between SKF prediction equations and DXA for estimating %BF in different populations (19,30,44). Chambers et al. compared several SKF regression equations to DXA in Caucasian older adults with and without obesity in the United States (44). Among the SKF prediction equations evaluated, mixed results were found in both males and females when the results were compared to the DXA derived %BF values (44). Among the equations evaluated in this study, only the equations by DW in females and JP in males had reasonably good agreement with DXA in older Caucasians, respectively (44). These were the only equations that were not significantly different from DXA, regardless of obesity (44). With specific attention to the SKF prediction equations used in the present thesis, the specific findings from that study found that the JP equation significantly underestimated %BF compared to DXA by 7.6% and 7.12% in females with and without obesity respectively, with no significant differences for males with and without obesity (44). Conversely, the DW equation significant overestimated %BF compared to DXA by 3.32% and 3.61% in males with and without obesity respectively, with no significant differences found in females regardless of obesity (44). Bacchi et al. compared %BF estimated by anthropometric equations with DXA in adults with obesity and type-2 diabetes (30). Among the equations tested, only the DW equation showed close agreement with DXA in both females and males (30). Cedillo et al. assessed the agreement between SKF thickness equations and DXA measured in African American and Caucasian American females (19). The findings from that study that are most relevant to this thesis, and found that the DW equation overestimated %BF as compared with DXA in both African American and Caucasian American females (19). In African American females there was an overestimation by 2.5% and 4.31% using the DW-Siri and DW-Brozek equations (19). In Caucasian American females there was an overestimation of 2.74% and 4.20% using the DW-Siri and DW-Brozek equations (19). Overall, the %BF-Siri conversion equations showed better agreement and lower mean differences with DXA compared to the %BF-Brozek conversion equations (19). Overall, these studies highlight the inconsistencies of SKF equations in accurately estimating %BF compared to DXA, emphasizing the need for caution when using SKF equations as a substitute for DXA in body composition assessment.

2.3 Three-Compartment Model

Like the 2C model, the 3C model includes FM with an additional estimate to partition the FFM compartment even further. One variation of this model was developed by Siri (1961) who partitioned the FFM into TBW and fat-free dry mass (FFDM) which consists of protein and minerals (86). This model is represented by the equation: Body mass = TBW + FFDM + FM. Further, Siri developed a 3C model equation that assumes a constant mineral-to-protein ratio of 0.35, and measurements of Db and TBW (86).

$$\%BF = [(2.118/Db) - 0.78W - 1.354] \times 100$$

where Db = totally body density (g/cc), W = total body water (TBW).

The 3C model better controls for inter-individual variation in FFM hydration as the FFM is divided into water TBW and the remaining solids (protein and minerals, and FFDM) (34). As a result, this 3C model may yield more accurate estimations of %BF as compared to the 2C model (34). When TBW is used, specific attention to hydration status is important when using the 3C model. Specific to this, the BIA method involves a low-level electrical current passed through the body of which the impedance, or opposition to the flow of current is measured (27). The BIA method is governed by the differences in conductivity between tissues (27), as tissues containing larger amount of fluids and electrolytes such as lean tissue is a better conductor of electrical current than adipose tissue which is anhydrous (27,87). The BIA method can use either a single-frequency BIA or a multifrequency BIA. The multifrequency BIA method is considered a 3C model because TBW, FM, and FFM can be determined (27).

2.3.1 Dual-Energy X-Ray Absorptiometry

Other 3C models exist that do not specifically assess TBW, but other compartments of FFM are measured indirectly. This model measures FM, BMC, and fat-and bone-free lean mass. It is represented by the following equation: Body mass = FM + BMC + Fat-and bone-free lean mass (FBFM). DXA method adopts the aforementioned paradigm and is considered another 3C model that provides rapid, non-invasive regional and whole body BC measurements through the transmission of X-rays through the body (56).

Before the development of DXA, single photon absorptiometry and dual photon absorptiometry were used to estimate bone density (88). Dual photon absorptiometry was developed using radionucleotides to provide the dual energy photon to determine bone mass for the diagnosis of osteoporosis (88). The replacement of the radionucleotides with X-ray led to DXA. The DXA method measures FM, BMC, and FBFM utilizing an X-ray beam consisting of two X-ray energies that pass through the body (34,56). The principle of DXA is that the X-rays with high and low photon energies pass through soft tissue and bone at different rates (34,56). The attenuation, or weakening of the X-rays through FM, FBFM, and BMC, are caused by differences in the density and chemical properties of these tissues (34,56,61). The body area scanned consists of thousands of pixels identified by a computer software as containing bone or soft tissue with no bone (56). Bone is a dense material (shows up brightest on the scan) that attenuates the x-ray to a greater degree than both FM, and FBFM; whose densities are lower than bone (56). Therefore, the bone containing pixels are clearly discerned from nonbone-containing pixels (based on the brightness). In nonbone pixels, the different attenuation of the X-ray can also distinguish between FM and FBFM compartments which attenuate to different degrees (56). Thus, DXA is a 3C model. DXA has many advantages over other reference and laboratory methods, due to its assessment speed and because the measurement is minimally influenced by hydration/water fluctuations (62). Despite this, early studies suggested that an individual's hydration status might affect DXA estimates of %BF (89). However, other research has indicated the effect of hydration is minimal (90). Consequently, a best practice recommendation is to have clients avoid exercise (~12h) prior to being scanned to avoid a potential source of measurement error from acute exercise (56). To validate the reliability and accuracy of DXA to assess BC in humans, a 4C model is often utilized. The 4C model is often regarded as the gold standard for BC appraisal, as the body is divided into four compartments respectively (FM, BMC, TBW, and other/residual (i.e., protein, nonbone minerals, and glycogen) (91). Principally, the 4C model eliminates assumptions about the relative proportions of FFM inherent to 2C (Bod Pod, SKF) and 3C DXA model methods providing a more comprehensive picture of BC (91). As a result, providing an effective tool to assess the validity of DXA. Several studies have compared %BF estimates between DXA and the 4C model, with the majority of these studies showing an underestimation of %BF (92–94). In a 1998 study, Withers et al. compared %BF estimates derived from DXA to a 4C model reference method in a sample of highly trained males (n = 12), sedentary males (n = 12), highly trained females (n = 12), and sedentary females (n = 12) (94). The findings from that study found that DXA underestimated %BF in all groups, with the largest discrepancy found in trained males for whom %BF was found to be 3.5% lower than the 4C model estimates (94). A similar study in a cohort of male and female distance runners found that DXA underestimated %BF by 3-4% when compared to the 4C model estimates (92). Further, a number of studies have observed that this underestimation is accentuated in leaner individuals (92,94,95). Conversely, other investigations have observed an overestimation when comparing DXA derived BC variables with a 4C model. Williams et al. reported a significant overestimation of FM by DXA among all categories of adults in their study (males without obesity, females without obesity, and females with obesity) when a Bland-Altman (B-A) analysis of mean biases was performed (96). Even though the 4C model better accounts for inter-individual variations in the FFM compartments, providing the basis to be regarded as the gold standard method for %BF estimations. This approach is costly, laborious and generally inconvenient to administer (95). For these reasons, DXA is still regarded as the preferred reference method for its unique ability to simultaneously measure three compartments of the body, both whole body and regional, from a total body scan, thus it is considered the practical gold standard (96).

2.4 Four-Compartment Model

The 4C model of BC builds on the 3C model by combining multiple methods to further partition body mass. Measurable quantities are combined through multiple methods to evaluate body mass, TBW, BV, and mineral. Body mass is measured on a scale. TBW is measured using tritium or deuterium-labeled water dilution. BV is measured through a densitometry method such as UWW, or Bod Pod. Bone mineral is derived via DXA. The 4C model is represented by the following equation: *Body mass = TBW + BMC + FM + residual (protein).* Measuring each compartment separately as they are now measurable quantities, eliminates the need to make assumptions about the relative proportion of these constituents in the body (52). The 4C model is theoretically more valid than the 3C model, since it controls for biological variability in both bone mineral and TBW (34). To this effect, the 4C model is commonly regarded as the gold standard for assessing the accuracy of BC estimates, albeit impractical (97,98). Consequently, some authors have suggested that the 4C model should be used mainly for the validation of BC methods and prediction equations (18,34). Further, despite the improved accuracy of the 4C model, each primary measurement will have an inherent measurement error that reflects the precision of the method used to assess it (52). It has been suggested in the literature that the cumulative small errors associated with measuring many variables may offset the improved accuracy of 4C model estimates of %BF (34). Despite its purported accuracy benefits, the 4C method is seldom used because it requires several different measurements which tend to be time consuming, costly, and generally not feasible.

2.5 Other Multicompartment Models

The direct analysis of the chemical composition of the body is evaluated through atomic level models. Within this model, the total body content of the major elements (calcium, sodium, chloride, phosphorus, nitrogen, hydrogen, oxygen, and carbon) is measured through neutron activation analysis (NAA) (18,52). A six compartment (6C) atomic model developed by Wang et al. partitions the body by the following equation: *Body mass = water + nitrogen + calcium + potassium + sodium + chloride* (99). In living humans, the 6C model represents a more refined and definitive reference method (18). However, the lack of NAA facilities, high costs, and participant exposure to radiation limit their use (34). Total body nitrogen, calcium, chloride, sodium, and carbon, are measured through neutron activation methods, with whole body potassium-40 counting used to measure total body potassium, and tritium dilution is used to measure TBW (18). With the direct measurements of each of the compartments in this model, this accounts for over 97.5% of body weight (18). The 6C model appears to be the most accurate method in living humans for measuring BC.

2.6 Field and Laboratory Methods

BC can be measured in several different ways. Of most relevance to this thesis is the difference between methods used in the laboratory versus those used in the field. BC field methods tend to be more cost-effective as well as provide a level of portability that is advantageous in the field for the assessment of many individuals within a short period of time. BIA and SKF measurements fall into this field category. Laboratory methods not only provide stand-alone assessments for estimating BC, but they also provide reference measures for evaluating BC field methods and prediction equations (56) . For example, DXA is commonly utilized as the practical gold standard reference method when evaluating estimates from SKF measurements (32). In terms of laboratory methods, they can be used in isolation or in combination with other laboratory methods like 4C model estimates. Nevertheless, laboratory methods are still subject to prediction errors in terms of accuracy for %BF estimates (56).

In this regard, a true gold-standard for in vivo BC assessment does not exist. Despite a higher degree of accuracy which is usually necessary for research, laboratory methods are typically more expensive, and less convenient than field methods. Laboratory methods are also typically not as practical for mass and multiple repeat testing, and require considerable non-portable equipment, space, and trained technicians (in some cases, with additional regulatory certifications). Of note, technical skills and certifications are also recommended for some field methods like SKFs. Two of the most used laboratory methods, which are of interest in this thesis are DXA and Bod Pod.

CHAPTER 3: METHODS

3.1 Participants

Thirty-eight recreationally active individuals voluntarily participated in this cross-sectional study. Participant characteristics are shown in Table 1.

Table 1: Characteristics of Study Participants

Demographic characteristic	Total sample (n = 38)	Females (n = 20)	Males (n = 18)
Age (years)	28.3 ± 6.1	26.3 ± 4.0	30.5 ± 7.4
Height (cm)	172.6 ± 10.5	165.5 ± 6.4	180.6 ± 8.2
Weight (kg)	72.0 ± 13.2	63.1 ± 6.6	81.9 ± 11.4
BMI (kg/m^2)	24.0 ± 2.6	23.1 ± 2.4	25.0 ± 2.4
Ethnicity			
Caucasian	31 (81.6%)	17 (85%)	14 (77.8%)
Other	5 (13.1%)	3 (15%)	4 (22.2%)
Black	2 (5.3%)		

Reported values are Mean ± SD or Frequency (%)

Each participant self-reported a minimum of four exercise sessions per week for at least 60 minutes, however, none reported to be athletes. As such, the participants have been operationally defined as ‘recreationally active’ for the purposes of this thesis, despite their higher activity levels. All participants provided written informed consent to their involvement in the study. All procedures were approved by the Ontario Tech University ethics committee. Dr. H. Logan-Sprenger (assistant professor at Ontario Tech University) was the principal investigator and initially collected the data. Participants were given autonomy to stop participating in the study at any time with no consequence. Participants were excluded from participating based on the following four criteria: 1. Diagnosed with a medical illness and/or disease that could affect body composition. 2. Taking medications that affect body composition 3. Pregnant 4. Ingested contrast radiation material within the last 2 weeks. 5. Had an ‘athlete’ designation or self-identified as an athlete. The testing protocol, risks, and benefits were discussed during an in-person information session where all participants were given an information sheet and consent form outlining the details. Testing began once informed consent was obtained. Use of secondary data for this thesis was approved by York University's Office of Research Ethics certificate #2023-019. For use of secondary data, all identifying information about the participants was removed, and deidentified data were provided to the researchers in a spreadsheet separated by subject identification number.

3.2 Data Collection

Cross sectional data was collected for each participant between the months of May and August 2015. On each testing day, the participants arrived at Dr. Logan-Sprenger's research lab at the Canadian Sport Institute Ontario (CSIO) between 06:00 – 08:00 hrs after a 14-hour fast from food and fluids. All forms of exercise were also omitted during the 14-hour period. Prior to testing, the participants voided their bladder and changed into standardized clothing (spandex sportswear) for testing. Participants had their height and weight measured using a calibrated digital scale (SECA 869 USA), then completed, in randomized order, DXA scan (GE Lunar, USA), Bod Pod (GS model, Cosmed, USA) assessment, and repeated SKF thickness measurements. For all testing procedures every effort was made to ensure the same skilled examiner collected the data to avoid inter-observer variability and preserve intra-observer reliability. Some of the participants were unable to complete all aspects of the data collection, (i.e., Bod Pod) because of claustrophobia.

3.3 Skinfold Thickness Anthropometric Measurements

A single measurement of body weight and height was taken to the nearest 0.1 kilogram (kg) and 0.1 centimeter (cm) using a calibrated digital scale (SECA 869 USA). SKF thicknesses were measured to the nearest 0.2 mm using Harpenden Skinfold Calipers. A trained SKF technician took repeated skinfold thickness measures on the right side of the body (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, medial calf, chest, axilla). Measurements were taken for each site twice. If variability between the two measurements was greater than 2%, a third measurement was taken. After the individual SKF thickness measurements were taken for each participant, %BF was either calculated directly or Db was calculated first and further converted into %BF, thereafter. All the SKF prediction equations (except Yuhasz) in males and females provided an initial estimate of Db using the DW, JP3, and JP7 equations. Once Db was calculated, further arithmetic requires the Db calculation to be converted to %BF with both Brozek (%BF-Brozek) and Siri (%BF-Siri) equations.

$$Brozek\ \%BF = (4.57/Db - 4.142) \times 100 \qquad Siri\ \%BF = (4.95/Db - 4.50) \times 100$$

The SKF equations used in this thesis are shown in Tables 2 and 3.

Table 2: Skinfold Equations for Males

Durnin and Womersley (1974) (biceps + triceps + subscapular + supraspinale)	Body density (Db) = 1.1620-0.0630 log (biceps + triceps + Subscapular +Supraspinale) for 17-19yrs
	Body density (Db) = 1.1631-0.0632 log (biceps + triceps + Subscapular +Supraspinale) for 20-29yrs
	Body density (Db) = 1.1422-0.0544 log (biceps + triceps + Subscapular +Supraspinale) for 30-39 yrs
	Body density (Db) = 1.1620-0.0700 log (biceps + triceps + Subscapular +Supraspinale) for 40-49yrs
Jackson and Pollock (1978) JP3 = (chest, abdomen, thigh) JP7 = (chest, midaxillary, triceps, subscapular, abdomen, anterior suprailiac, thigh)	JP3 = Body density (Db) = $1.09380 - (0.0008267 \times \Sigma 3SF) + (0.0000016 \times \Sigma 3SF)^2 - (0.0002574 \times age)$
	JP7 = Body density (Db) = $1.112 - (0.00043499 \times \Sigma 7SF) + (0.00000055 \times \Sigma 7SF)^2 - (0.00028826 \times age)$
Yuhasz (1974) (triceps, subscapular, supraspinale, abdominal, thigh, calf)	%BF = $(0.1051 \times \Sigma 6SKF) + 2.585$

Σ3SKF = sum of three skinfold sites; Σ6SKF = sum of six skinfold sites; Σ7SKF = sum of seven skinfold sites; Body density conversion formulas to %BF = (4.95/Db – 4.50) x 100 (Siri 1956); %BF = (4.57/Db – 4.142) x 100 (Brozek 1963); JP3 = 3 site equation by Jackson and Pollock, JP7 = 7 site equation by Jackson and Pollock.

Table 3: Skinfold Equations for Females

Durnin and Womersley (1974) (biceps + triceps + subscapular + supraspinale)	Body density (Db) = 1.1549-0.0678 log (biceps + triceps + Subscapular +Supraspinale) for 16-19yrs
	Body density (Db) = 1.1599-0.0717 log (biceps + triceps + Subscapular +Supraspinale) for 20-29yrs
	Body density (Db) = 1.1423-0.0632 log biceps + triceps + Subscapular +Supraspinale) for 30-39 yrs
	Body density (Db) = 1.133-0.0612 log (biceps + triceps + Subscapular +Supraspinale) for 40-49yrs
Jackson and Pollock (1978) JP3 = (tricep, suprailiac, thigh) JP7 = (chest, midaxillary, triceps, subscapular, abdomen, anterior suprailiac, thigh)	JP3 = Body density (Db) = 1.0994921 – (0.0009929 x Σ3SF) + (0.0000023 x Σ3SF)^{2} – (0.00013924 x age)
	JP7 = Body density (Db) = 1.097 – (0.00046971 x Σ7SF) + (0.00000056 x Σ7SF)^{2} – (0.00012828 x age)
Yuhasz (1974) (tricep, subscapular, supraspinale, abdominal, thigh, calf)	%BF = (0.1548 x Σ6SKF) + 3.580

Σ3SKF = sum of three skinfold sites; Σ4SKF = sum of four skinfold sites; Σ7SKF = sum of seven skinfold sites; Body density conversion formulas to %BF = (4.95/Db – 4.50) x 100 (Siri 1956); %BF = (4.57/Db – 4.142) x 100 (Brozek 1963);
JP3 = 3 site equation by Jackson and Pollock, JP7 = 7 site equation by Jackson and Pollock

3.4 DXA Measurement

GE Healthcare Lunar iDXA was used. A standard DXA operational protocol as described elsewhere (100), was used by the primary investigators while performing whole body scans in a routine clinical manner. The same certified technician performed all the scans for each participant.

3.5 ADP (Bod Pod) Measurement

Densitometry method was done using Bod Pod (GS model, Cosmed USA). Before entering the Bod Pod the participants wore the provided spandex sportswear which included a swim cap over their hair. When sitting inside the Bod Pod chamber, the participants were prompted to remain still and breathe normally. Once all measurements were completed, the results were displayed on the screen.

3.6 Statistical Analysis

All statistical analyses were conducted utilizing IBM SPSS Statistics (version 28; IBM Corp., Armonk, N.Y., USA). Significance level was set to $P < 0.05$. Prior to statistical analysis, data were examined, and normality was verified using the Shapiro-Wilk test. A parametric two-way analysis of variance (ANOVA) was conducted with sex and modality as the two main independent variables of interest and %BF as the dependent variable.

Agreement between %BF prediction equations (Bod Pod, SKFs) with that of DXA in males and females were evaluated using linear regression and B-A analyses (101). The difference between each %BF estimation for each participant is represented by a data point on the plots. The difference of the two measurements is represented on the vertical axis, whereas the average of the two measurements is represented on the horizontal axis. If the modalities are comparable, more data points will be closer to zero (on y-axis) meaning small differences exist. This signifies the mean bias (101). A 95% confidence interval was calculated and plotted around the line-of-best-fit. We subtracted the %BF value obtained from the prediction equations (Bod Pod, SKFs) from DXA (e.g., other modality - DXA), therefore positive mean difference values represent an overestimation and negative values represent underestimation for that respective equation. P values ($P<0.05$) for the T-Tests and regression analyses were used to assess systematic and proportional bias between each method and DXA, respectively.

CHAPTER 4: RESULTS

The body composition methods of Bod Pod and SKF thicknesses were used to measure %BF in recreationally active males and females. The results of these analyses were evaluated and compared to results derived using DXA as the reference method. This chapter presents an overall analysis of the %BF data and then looks for agreement of results between DXA derived BC data with those from Bod Pod and SKF equations in male and female participants separately.

4.1 Verifying Normality

All P values from the Shapiro-Wilk test were above 0.05 except for the Yuhasz data in males, and the JP3 prediction equations (Brozek and Siri) in females. In males, the Shapiro-Wilk tests indicated that %BF values within the Yuhasz prediction equation are not normally distributed. Histogram and skewness (Figure 1) values indicated that the data has slight positive skewness with a few outliers on the upper end. Considering this affects only one out of ten %BF estimations, parametric inferential statistics was still used. In females, the Shapiro-Wilk tests indicated that %BF values within the JP3 prediction methods (Brozek and Siri) are not normally distributed. Histogram and skewness (Figure 2) values indicated that the data has slight positive skewness with a few outliers on the upper end. The histograms depict bell-shaped distributions. Considering this affects only two out of ten %BF estimations, parametric inferential statistics were still used. Thus, running an ANOVA was still appropriate. See Tables 4-5 and Figures 1-2.

Table 4: Statistical Test for Normality for Female Participants

Modality	Females	Skewness	kurtosis
	Sig.		
DXA	.633	0.526	2.666
Bod Pod – Brozek	.941	0.058	2.269
Bod Pod – Siri	.942	0.058	2.270
DW – Brozek	.889	0.079	2.967
DW – Siri	.886	0.079	2.962
JP3 – Brozek	**.015**	1.188	3.758
JP3 – Siri	**.015**	1.189	3.759
JP7 – Brozek	.254	0.634	2.782
JP7 – Siri	.252	0.634	2.783
Yuhasz	.165	0.871	3.450

Table 5: Statistical Test for Normality for Male Participants

Modality	Males	Skewness	kurtosis
	Sig.		
DXA	.055	0.663	2.353
Bod Pod – Brozek	.072	0.572	1.828
Bod Pod – Siri	.072	0.571	1.828
DW – Brozek	.088	-0.227	1.504
DW – Siri	.087	-0.227	1.503
JP3 – Brozek	.110	0.737	2.482
JP3 – Siri	.110	0.737	2.482
JP7 – Brozek	.087	0.707	2.312
JP7 – Siri	.087	0.708	2.312
Yuhasz	**.030**	0.787	2.360

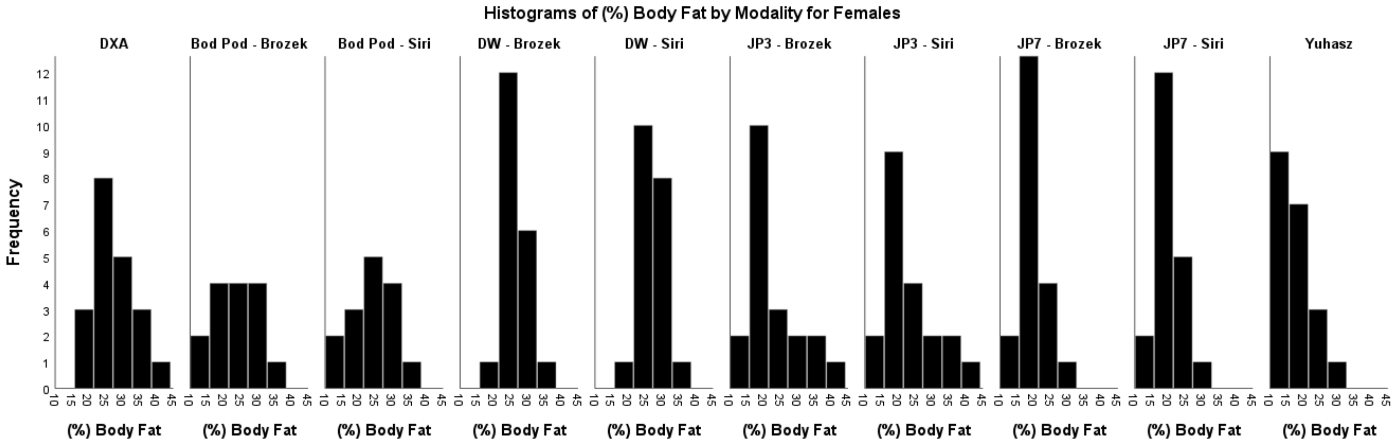


Figure 1: Histogram Representation for Normality for Female Participants

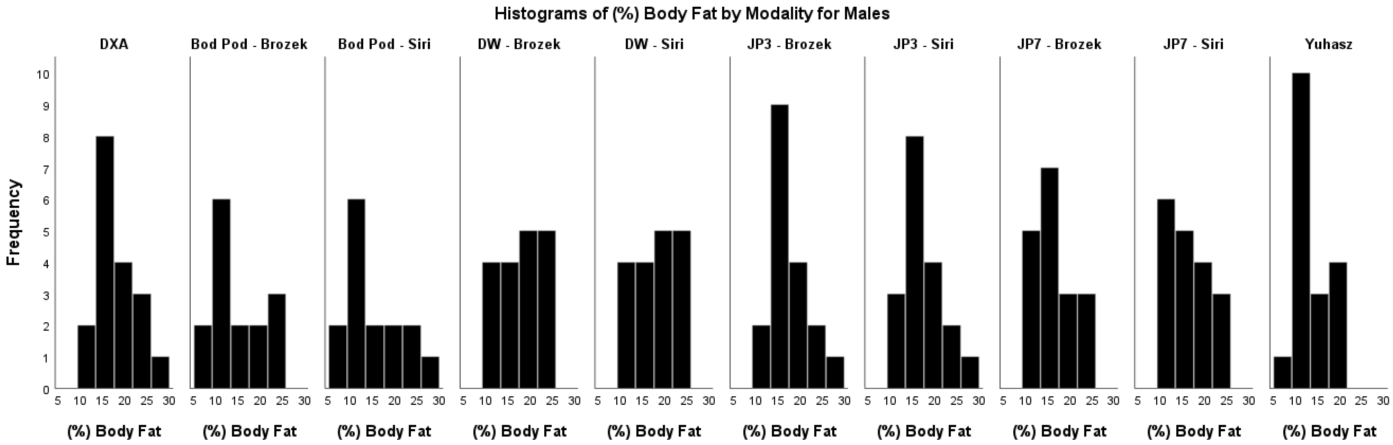


Figure 2: Histogram Representation for Normality for Male Participants

4.2 Analysis of Variance

Results from the two-way ANOVA on the %BF data for each of the four modalities and their respective equations are displayed in Tables 6 and 7. Here, data from DXA, Bod Pod, and the SKF methods DW, JP3, JP7 and Yuhasz are presented, with Bod Pod, DW, JP3 and JP7 data calculated using both Brozek and Siri %BF conversion formulas.

Table 6: Descriptive Statistics for %BF Measurements for Female Participants

Modality	Females	
	n	Mean ± SD
DXA	20	27.46 ± 5.94
Bod Pod – Brozek	15	23.24 ± 5.41
Bod Pod – Siri	15	23.81 ± 5.86
DW – Brozek	20	25.85 ± 3.54
DW – Siri	20	26.64 ± 3.83
JP3 – Brozek	20	22.94 ± 6.99
JP3 – Siri	20	23.48 ± 7.57
JP7 – Brozek	20	19.07 ± 3.97
JP7 – Siri	20	19.30 ± 4.30
Yuhasz	20	17.52 ± 4.27

Table 7: Descriptive Statistics for %BF Measurements for Male Participants

Modality	Males	
	n	Mean ± SD
DXA	18	17.85 ± 4.68
Bod Pod – Brozek	15	15.43 ± 5.91
Bod Pod – Siri	15	15.35 ± 6.40
DW – Brozek	18	17.80 ± 4.29
DW – Siri	18	17.92 ± 4.64
JP3 – Brozek	18	17.52 ± 4.29
JP3 – Siri	18	17.62 ± 4.65
JP7 – Brozek	18	16.41 ± 4.10
JP7 – Siri	18	16.42 ± 4.44
Yuhasz	18	13.81 ± 3.51

Regarding these findings, it must be noted that in the tests of the female participants' %BF, the sample size for the DXA, DW, JP3, JP7, and Yuhasz analyses was 20, while the Bod Pod trial was 15; and in the tests of the male participants, the DXA, DW, JP3, JP7, and Yuhasz sample size was 18 but 15 for the Bod Pod trials. Less participants completed the Bod Pod due to claustrophobia.

The above table shows that the highest mean %BF estimated for female participants was derived from DXA, at 27.5% ± 5.9%, while the lowest estimates observed was seen in SKF equations, specifically the Yuhasz prediction equation, at 17.5% ± 4.3%. Similarly, in male participants, the highest mean %BF estimated was noted using the DW equation with the Siri %BF conversion formula at 17.9% ± 4.6%, and the lowest mean %BF estimated was the Yuhasz prediction equation, at 13.8% ± 3.5%. The methods that were significantly different from the DXA derived %BF estimates in females were the following: Bod Pod-Brozek (23.24% ± 5.41), Bod Pod-Siri (23.81% ± 5.86), JP3-Brozek (22.94% ± 6.99), JP3-Siri (23.48% ± 7.57), JP7-Brozek (19.07% ± 3.97), JP7-Siri (19.30% ± 4.30), and Yuhasz (17.52% ± 4.27). The method that was significantly different from the DXA derived %BF estimate in males was the Yuhasz equation (13.81% ± 3.51).

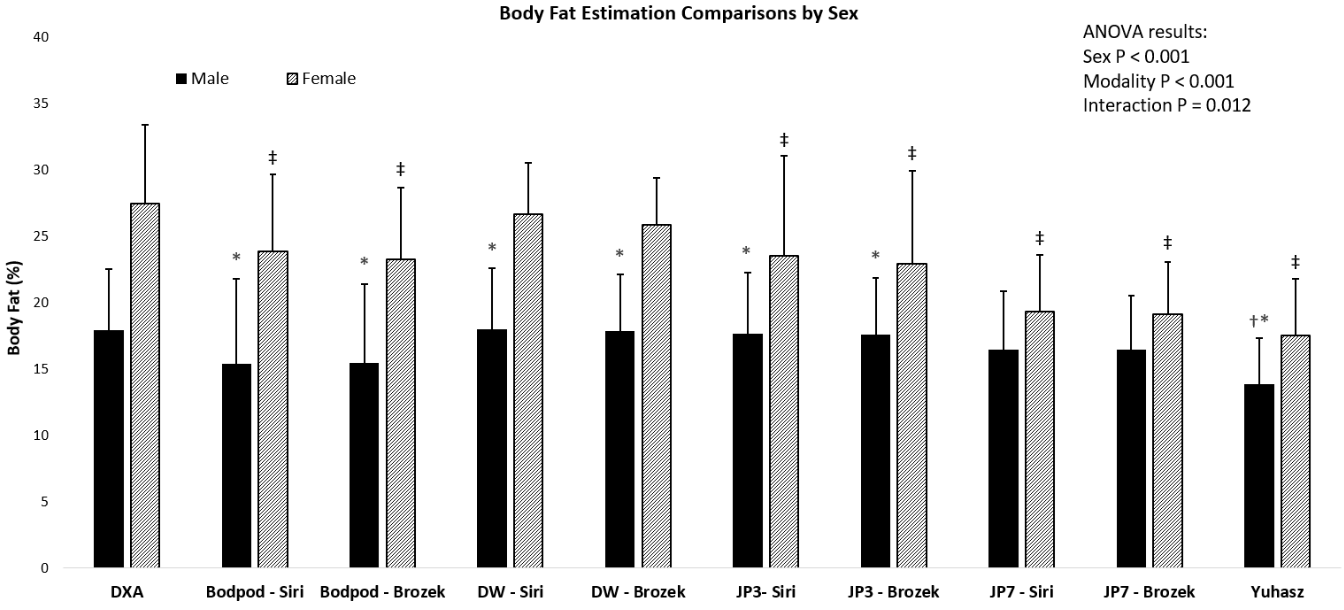
4.2.1 Effect of Sex and Modality

Parametric analysis was conducted as a two-way factorial ANOVA with sex and modality as the independent variables of interest. This showed a significant difference for the main effects of sex and modality, and a significant interaction effect between sex and modality (Figure 3).

The two-way ANOVA showed a statistically significant main effect of modality, F (9, 344) = 7.39, $P < 0.001$ meaning that amongst the different modalities evaluated with their respective %BF formulas; significant differences were found in the sample of males and females combined compared to the DXA derived %BF. This analysis also showed a significant main effect of sex, F (1, 344) = 141.90, $P < 0.001$ meaning that significant differences were found between the sexes for the various %BF calculations. Lastly, a significant interaction effect between modality and sex was found, F (9, 344) = 2.41, $P = 0.012$ meaning that the predictive accuracy of %BF estimations are dependent on the sex of the individual and the respective modality and predictive equation selected. With the presence of a significant interaction, the differences between modalities were interpreted separately for male and female participants.

4.2.2 Least Significant Difference

Post-hoc analyses were run using the least significant difference test to determine which independent variables (sex and modality) were different (see Figure 3). This analysis found significant differences among the independent variables (sex and modality) analyzed for both male and female participants. Significant differences were found between the sexes (denoted by the * in Figure 3) for all modalities and their respective prediction equations except for the JP7 equations (Brozek and Siri). Additionally, significant post-hoc differences were found for the Yuhasz equation (denoted by the † in Figure 3) as compared to DXA in males.


Body Fat Estimation Comparisons by Sex

ANOVA results:
Sex $P < 0.001$
Modality $P < 0.001$
Interaction $P = 0.012$

* denotes post-hoc difference between males and females at $P < 0.05$
‡ denotes post-hoc difference from DXA for females at $P < 0.05$
† denotes post-hoc difference from DXA for males at $P < 0.05$

Figure 3: Percent Body Fat Comparisons Between the Sexes by Modality

Conversely, significant post-hoc differences were found for all modalities (denoted by ‡ in Figure 3) and their respective equations as compared to DXA in females, except the DW equations (Brozek and Siri). Moreover, significant underestimations of %BF as compared to

DXA were found for all predictive equations in females except DW (Brozek and Siri) equations. Similar significant underestimation of %BF as compared to DXA was only found in the Yuhasz prediction equation in males. In addition, within each modality and prediction method, males and females were compared and %BF was always significantly higher for females except the JP7 (Brozek and Siri) equations (see Figure 3), which were not significantly different between the sexes.

4.3 Assessing Agreement Between Modalities

B-A analyses with T-Tests and regression analyses were performed for each %BF prediction equation compared with DXA. The results of these analyses are presented in Figures 4 to 7 for female participants and Figures 8 to 11 for male participants.

4.3.1 Bland-Altman Analyses: Assessing Bias Using T-Tests and Regression

T-Tests and regression analyses were used to assess systematic and proportional bias between each method and DXA, respectively. In this study, females demonstrated larger significant systematic biases ($P<0.05$) with magnitudes of difference ranging from -2.0% to -10.0% (DXA vs. Bod Pod-Siri [-2.03% ± 2.47]; Bod Pod-Brozek [-2.60% ± 2.25]; JP3-Siri [-3.98% ± 4.30]; JP3-Brozek [-4.53% ±3.96]; JP7-Siri [-8.16% ± 3.24]; JP7-Brozek [-8.39% ± 3.33]; and Yuhasz [-9.95% ± 3.07]). Conversely, males demonstrated smaller significant systematic biases ($P<0.05$) with magnitudes of difference from -1.4% to -4.0% (DXA vs. JP7-Siri [-1.43% ± 1.36]; JP7-Brozek [-1.44% ± 1.41]; Bod Pod-Brozek [-2.92% ± 2.40]; Bod Pod-Siri [-3.00% ± 2.72]; and Yuhasz [-4.04% ± 1.71].

In the subsequent Figures, namely Figures 4-11 comparisons are presented between different BC methods and their respective equations for calculating %BF separated by sex. Specifically, the equations investigated include, Bod Pod-Brozek, Bod Pod-Siri, and the following SKF prediction equations: DW-Brozek, DW-Siri, JP3-Brozek, JP3-Siri, JP7-Brozek, JP7-Siri and Yuhasz. These equations are compared to the reference method, DXA, separately for females (Figures 4-7) and males (Figures 8-11).

Each data point on the plots corresponds to an individual's %BF estimation. The horizontal axis represents the average %BF value of the two modalities of interest, while the vertical axis indicates the difference between DXA and another BC method with its respective equation (e.g., Bod Pod-Brozek – DXA). If both methods were perfectly accurate and thus in agreement, all data points would align horizontally at the Y-axis value of 0. This represents no systematic bias. However, when the observed data points exhibit vertical dispersion, this indicates a bias or discrepancy between DXA and the other method. Moreover, when a mean discrepancy between the two modalities exists, negative values suggest that the other method underestimates %BF compared to DXA. Hence, any data point below the Y-axis value of zero signifies an underestimation by the equation method relative to DXA. Conversely, positive values indicate an overestimation. To determine whether this systematic bias significantly deviates from zero, a T-test was performed, providing a p-value. If the p-value is less than 0.05, it indicates the presence of a significant systematic bias.

Additionally, to assess proportional bias, which is the results of the regression analysis, a correlation coefficient (r-value) for each individual plot is generated. If the correlation coefficient is zero (or close to it, also meaning the slope of the line is close to 0), this would suggest no proportional bias across all %BF values, irrespective of whether the systematic bias is large or small. This would yield more consistent results when comparing the two methods (DXA and one of the other methods) across a range of %BF levels (i.e., across the x-axis). If the correlation coefficient is significant this signifies proportional bias meaning that the bias observed is not constant across all %BF values. The descriptive statistics for the different BC modalities with their respective equations, all vs. DXA are shown in Tables 8 and 9.

Table 8: Descriptive Statistics from the Bland-Altman Plots vs. DXA for Female Participants

Modality	Females					
	n	Mean Bias ± SD	95% CI	p-value (t-test)	r-value	p-value (correlation)
Bod Pod – Brozek	15	-2.60 ± 2.25	-7.01 to 1.81	< **0.001**	0.22	0.427
Bod Pod – Siri	15	-2.03 ± 2.47	-6.87 to 2.81	**0.007**	0.39	0.152
DW-Brozek	20	-1.61 ± 3.73	-8.92 to 5.70	0.069	-0.67	**0.001**
DW-Siri	20	-0.82 ± 3.65	-7.97 to 6.33	0.327	-0.61	**0.005**
JP3-Brozek	20	-4.53 ± 3.96	-12.29 to 3.23	< **0.001**	0.28	0.238
JP3-Siri	20	-3.98 ± 4.30	-12.41 to 4.45	< **0.001**	0.40	0.083
JP7 – Brozek	20	-8.39 ± 3.33	-14.92 to -1.86	< **0.001**	-0.61	**0.004**
JP7 – Siri	20	-8.16 ± 3.24	-14.51 to -1.81	< **0.001**	-0.53	**0.018**
Yuhasz	20	-9.95 ± 3.07	-15.97 to -3.93	< **0.001**	-0.56	**0.010**

4.3.1.1 Bland-Altman Analysis of Female Participants' Data

Figures 4a and 4b both indicate a significant difference between DXA and the Bod Pod-Brozek (Figure 4a) and Bod Pod-Siri (figure 4b) equations in estimating %BF. The mean difference between the modalities is -2.60% ($p < 0.001$) and -2.03% ($p = 0.007$), indicating a significant disparity. Both equations (Bod Pod-Brozek and Bod Pod-Siri) underestimate %BF compared to DXA in females. The correlation ($r = 0.22$) between DXA and Bod Pod-Brozek equation is not statistically significant ($p = 0.427$), suggesting a non-significant proportional bias. Similarly, the correlation ($r = 0.39$) between DXA and Bod Pod-Siri equation is also not statistically significant ($p = 0.152$), suggesting a non-significant proportional bias. That is, the systematic bias remains relatively constant across different %BF values in females. Thus, the data suggest that the Bod Pod-Brozek and Bod Pod-Siri equations exhibit a significant and consistent systematic underestimation of %BF in females compared to DXA.

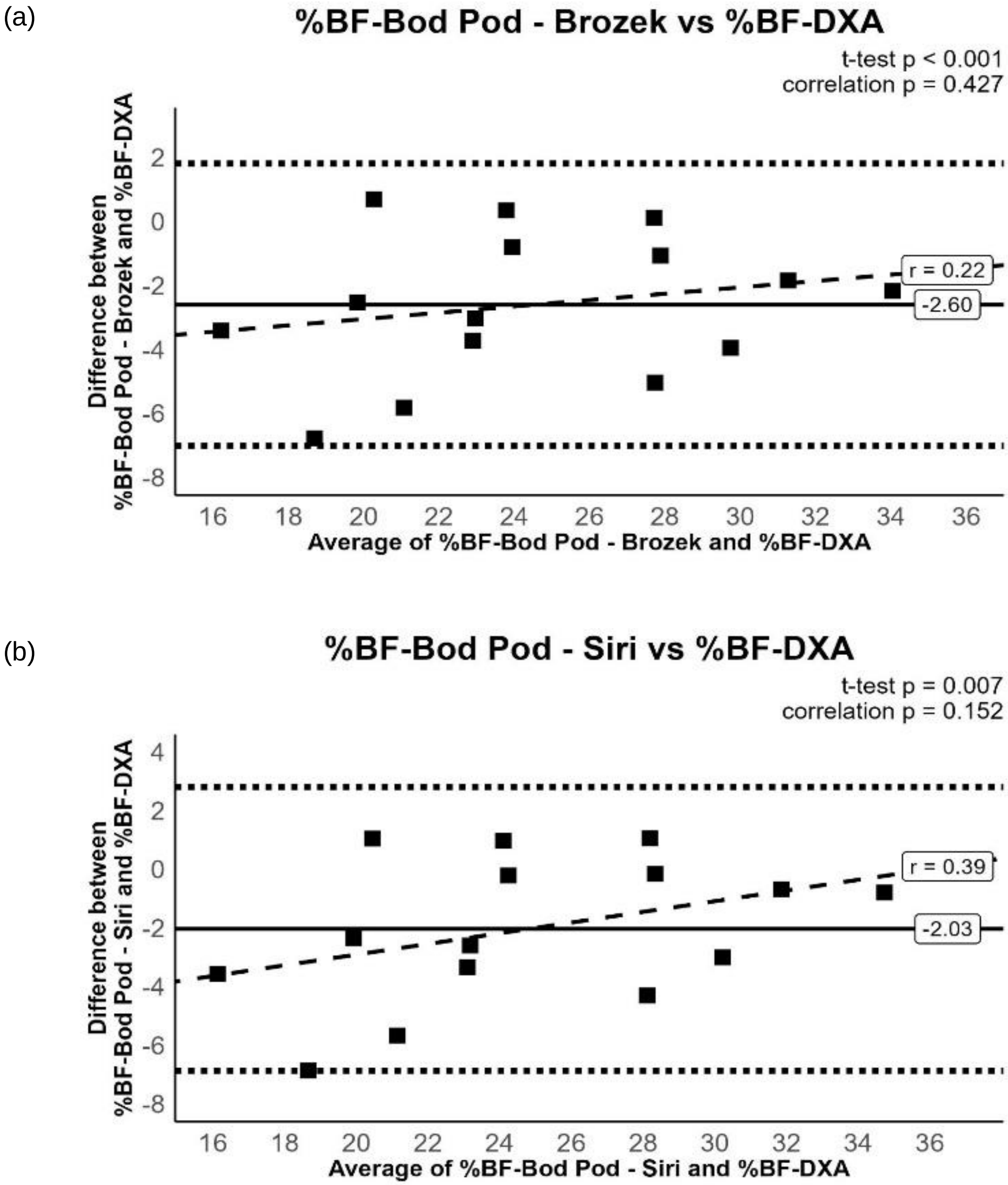


Figure 4: %BF of Female Participants Measured Using DXA Compared with Bod Pod Equations. (a) Bod Pod-Brozek (*n*=15); (b) Bod Pod-Siri (*n*=15).

Figures 5a and 5b both indicate a non-significant difference between DXA and the DW-Brozek (Figure 5a) and DW-Siri (Figure 5b) equations in estimating %BF. The mean difference between the modalities is -1.61% (p = 0.069) and -0.82% (p = 0.327), indicating a non-significant disparity between DXA and the DW equations (Brozek and Siri) in females. Both equations (DW-Brozek and DW-Siri) underestimate %BF compared to DXA in females. The correlation (r = -0.67) between DXA and DW-Brozek equation is statistically significant (p = 0.001), suggesting a significant proportional bias.

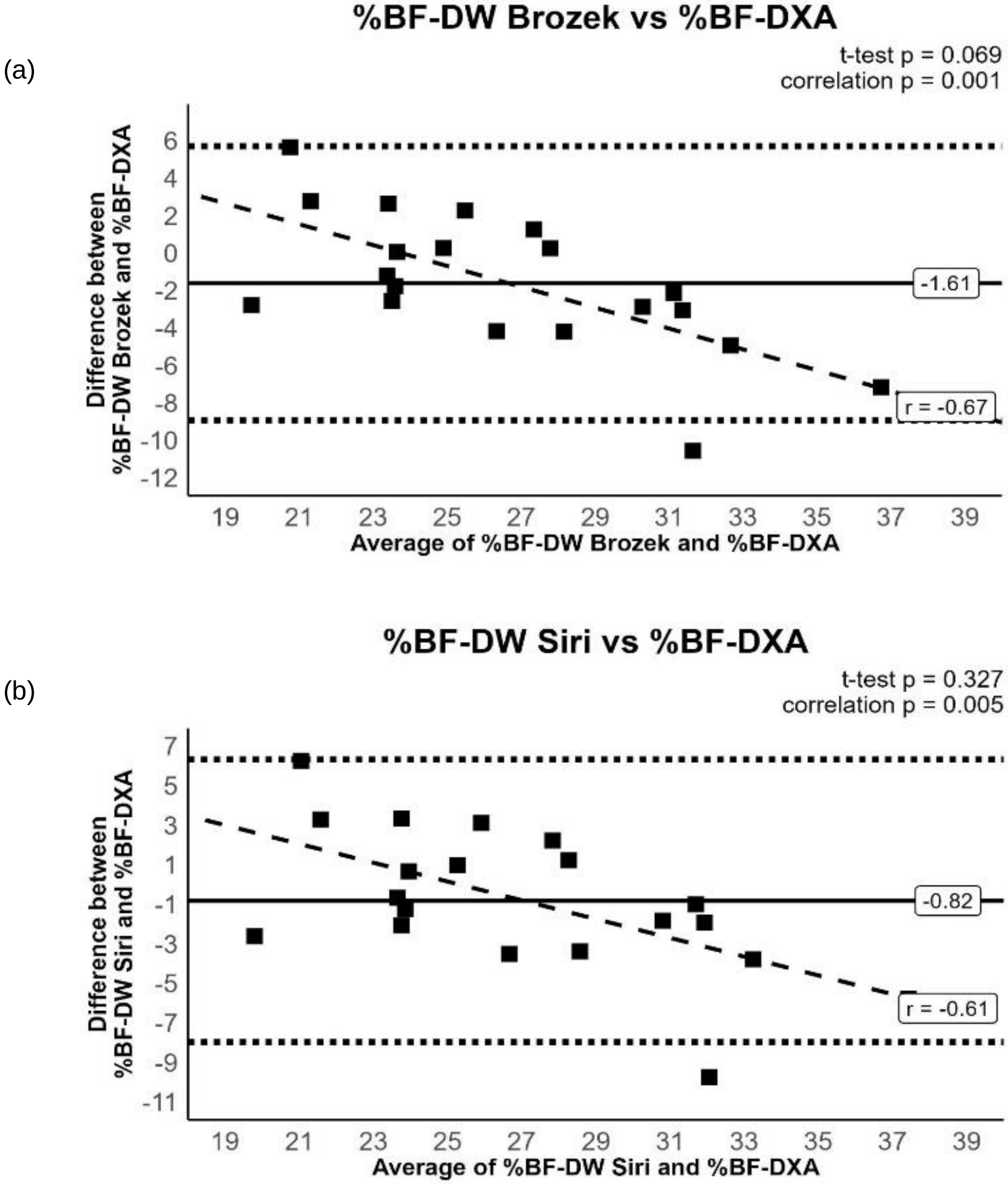
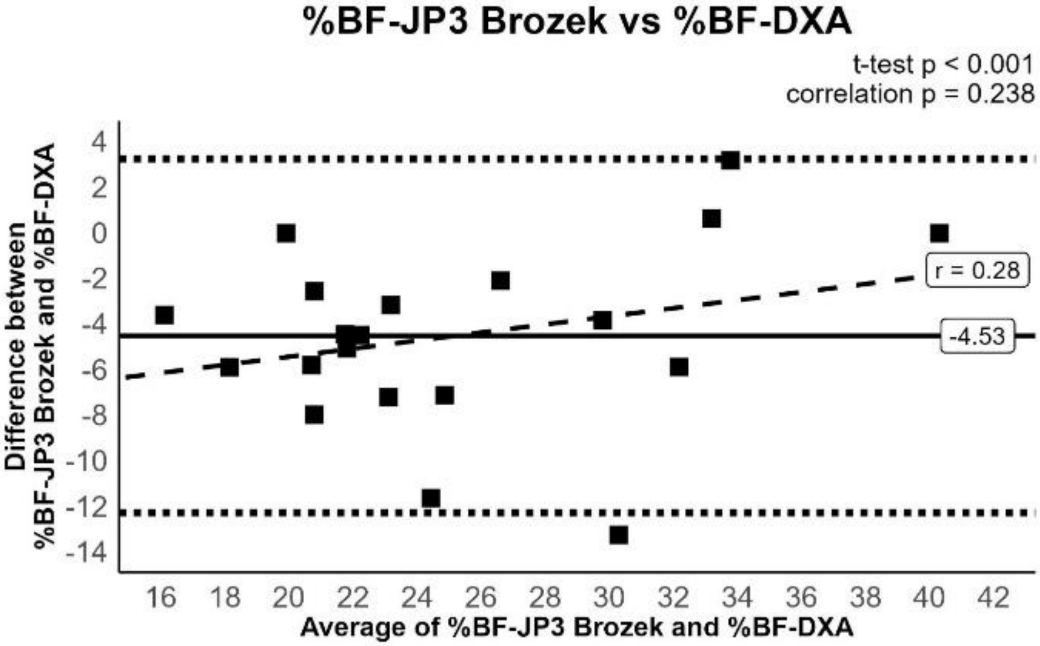


Figure 5: %BF of Female Participants Measured Using DXA Compared with the Durnin and Womersley Equations. (a) DW-Brozek (n=20); (b) DW-Siri (n=20**).**

Similarly, the correlation (r = -0.61) between DXA and DW-Siri equation is statistically significant (p = 0.005), suggesting a significant proportional bias. That is, as %BF values increase, the DW-Brozek and DW-Siri equations tend to underestimate %BF more, hence the bias is non-constant (i.e., proportional) across all values of body fat, with larger amounts of body fat reflecting greater biases. Thus, the data suggest that the DW-Brozek and DW-Siri equations exhibits a non-significant systematic underestimation of %BF in females, with a proportional bias that gets proportionally larger as %BF increases compared to DXA.

Figures 6a, 6b, 6c, and 6d all indicate a significant difference between DXA and the JP3-Brozek (Figure 6a), JP3-Siri (Figure 6b), JP7-Brozek (Figure 6c), and JP7-Siri (Figure 6d) equations in estimating %BF. The mean differences between the modalities were JP3-Brozek -4.53% (p < 0.001), JP3-Siri -3.98% (p < 0.001), JP7-Brozek -8.39% (p < 0.001) and JP7-Siri -8.16% (p < 0.001); indicating significant disparities. Thus, all the Jackson and Pollock equations significantly underestimated %BF compared to DXA in females.

a)



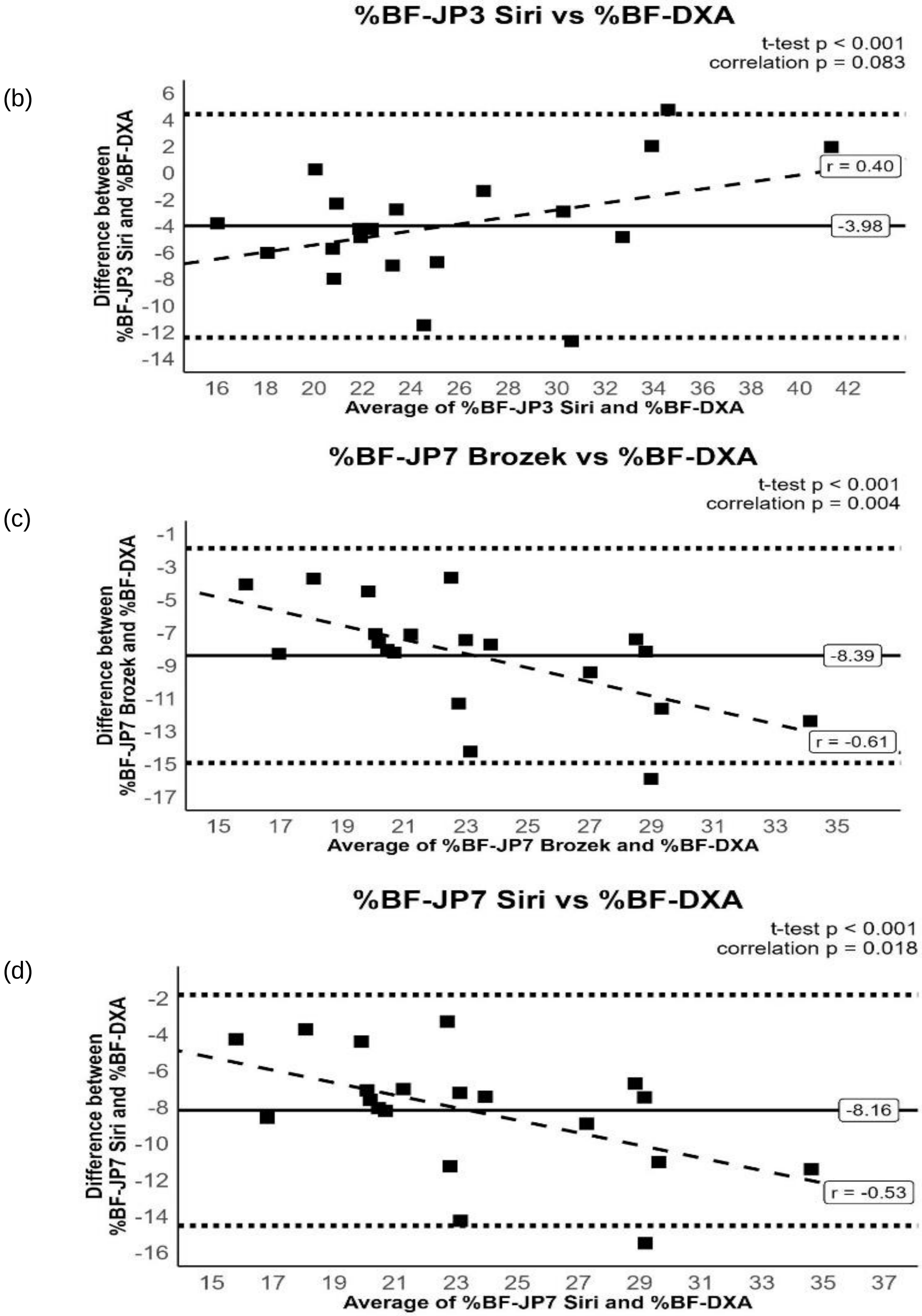


Figure 6: %BF of Female Participants Measured Using DXA Compared with Jackon and Pollock equations. (a) JP3-Brozek (n=20); (b) JP3-Siri (n=20); (c) JP7-Brozek (n=20); (d) JP7-Siri (n=20).

The correlation (r = 0.28) between DXA and JP3-Brozek equation (Figure 6a) was not statistically significant (p = 0.238), suggesting a non-significant proportional bias. Similarly, the correlation (r = 0.40) between DXA and JP3-Siri equation (Figure 6b) were not statistically significant (p = 0.083), suggesting a non-significant proportional bias. That is, both JP3 equations (Brozek and Siri) demonstrate a systematic bias that remains relatively constant across different %BF values in females. Thus, the data suggest that the JP3-Brozek and JP3-Siri equations exhibit a significant and consistent systematic underestimation of %BF in females compared to DXA.

Additionally, the correlation (r = -0.61) between DXA and JP7-Brozek equation (Figure 6c) was statistically significant (p = 0.004) suggesting a significant proportional bias. Furthermore, the correlation (r = -0.53) between DXA and JP7-Siri equation (Figure 6d) was also statistically significant (p = 0.018) suggesting a significant proportional bias. That is, as %BF values increases, the JP7-Brozek and JP7-Siri equations underestimate %BF more, hence the bias is non-constant (i.e., proportional) across all values of body fat, with larger amounts of body fat reflecting greater biases. Thus, the data suggest that the JP7-Brozek and JP7-Siri equations exhibits a significant systematic underestimation of %BF in females which gets proportionally larger as %BF increases compared to DXA.

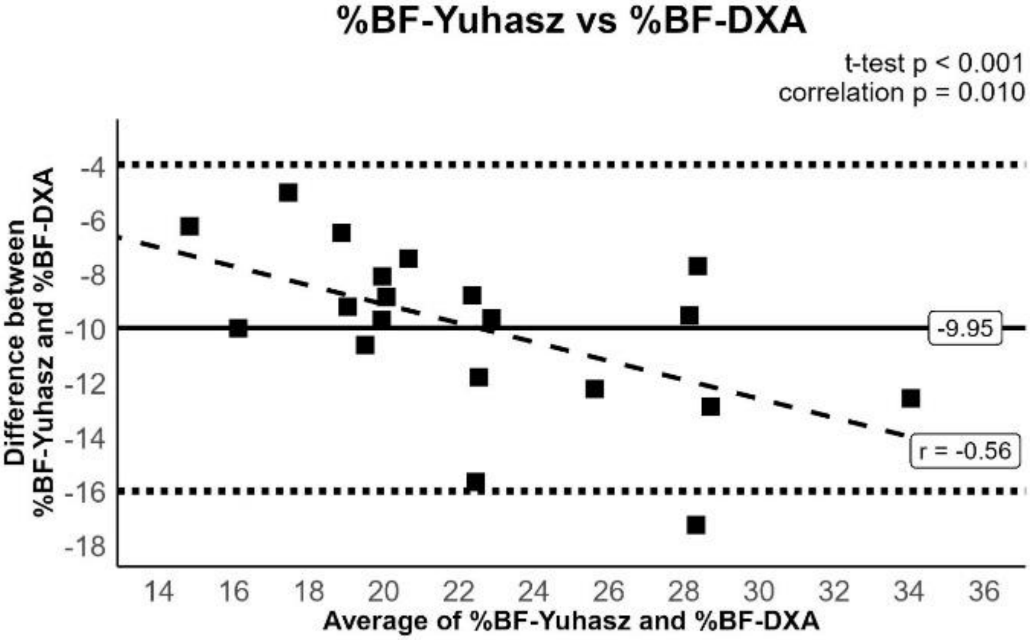


Figure 7: %BF of Female Participants Measured Using DXA Compared with Yuhasz Equation (*n*=20).

Figure 7 indicates a significant difference between DXA and the Yuhasz equation in estimating %BF. The mean difference between the modalities was -9.95% ($p < 0.001$), indicating a significant disparity. The Yuhasz equation underestimated %BF compared to DXA in females across all data points. The correlation ($r = -0.56$) between the two methods was statistically significant ($p = 0.010$), suggesting a significant proportional bias. That is, as %BF values increase, the Yuhasz equation underestimated %BF more, hence the bias is non-constant (i.e., proportional) across all values of body fat, with larger amounts of body fat reflecting greater biases. Thus, the data suggest that the Yuhasz equation exhibits a significant systematic underestimation of %BF in females which gets proportinally larger as %BF increases compared to DXA.

Table 9: Descriptive Statistics from the Bland-Altman Plots vs. DXA for Male Participants

Modality	Males					
	n	Mean Bias ± SD	95% CI	p-value (t-test)	r-value	p-value (correlation)
Bod Pod – Brozek	15	-2.92 ± 2.40	-7.62 to 1.78	**< 0.001**	0.48	0.070
Bod Pod – Siri	15	-3.00 ± 2.72	-8.33 to 2.33	**< 0.001**	0.61	**0.017**
DW-Brozek	18	-0.05 ± 2.36	-4.68 to 4.58	0.927	-0.17	0.490
DW-Siri	18	0.07 ± 2.42	-4.67 to 4.81	0.905	-0.02	0.948
JP3-Brozek	18	-0.33 ± 1.53	-3.33 to 2.67	0.378	-0.26	0.297
JP3-Siri	18	-0.23 ± 1.54	-3.25 to 2.79	0.541	-0.03	0.923
JP7 – Brozek	18	-1.44 ± 1.41	-4.20 to 1.32	**< 0.001**	-0.42	0.086
JP7 – Siri	18	-1.43 ± 1.36	-4.10 to 1.24	**< 0.001**	-0.18	0.481
Yuhasz	18	-4.04 ± 1.71	-7.39 to -0.69	**< 0.001**	-0.69	**0.001**

4.3.1.2 Analysis of Male Participants' Data

Figures 8a and 8b both indicate a significant difference between DXA and the Bod Pod-Brozek (Figure 8a) and Bod Pod-Siri (figure 8b) equations in estimating %BF. The mean difference between the modalities is -2.92% ($p < 0.001$) and -3.00% ($p < 0.001$), indicating a significant disparity. Both equations (Bod Pod-Brozek and Bod Pod-Siri) underestimate %BF compared to DXA in males. The correlation ($r = 0.48$) between DXA and Bod Pod-Brozek equation is not statistically significant ($p = 0.070$), suggesting a non-significant proportional bias. Thus, the data suggest that the Bod Pod-Brozek equation exhibits a significant and consistent systematic underestimation of %BF in males compared to DXA.

Conversely, the correlation ($r = 0.61$) between DXA and Bod Pod-Siri equation is statistically significant ($p = 0.017$) suggesting a significant proportional bias. That is, as %BF values increase, the Bod Pod-Siri equation tends to underestimate more, hence the bias is non-constant (i.e., proportional) across all values of body fat, with larger amounts of body fat reflecting smaller biases. Thus, the data suggest that the Bod Pod-Siri equation exhibits a significant systematic underestimation of %BF in males which gets proportionally smaller as %BF increases compared to DXA

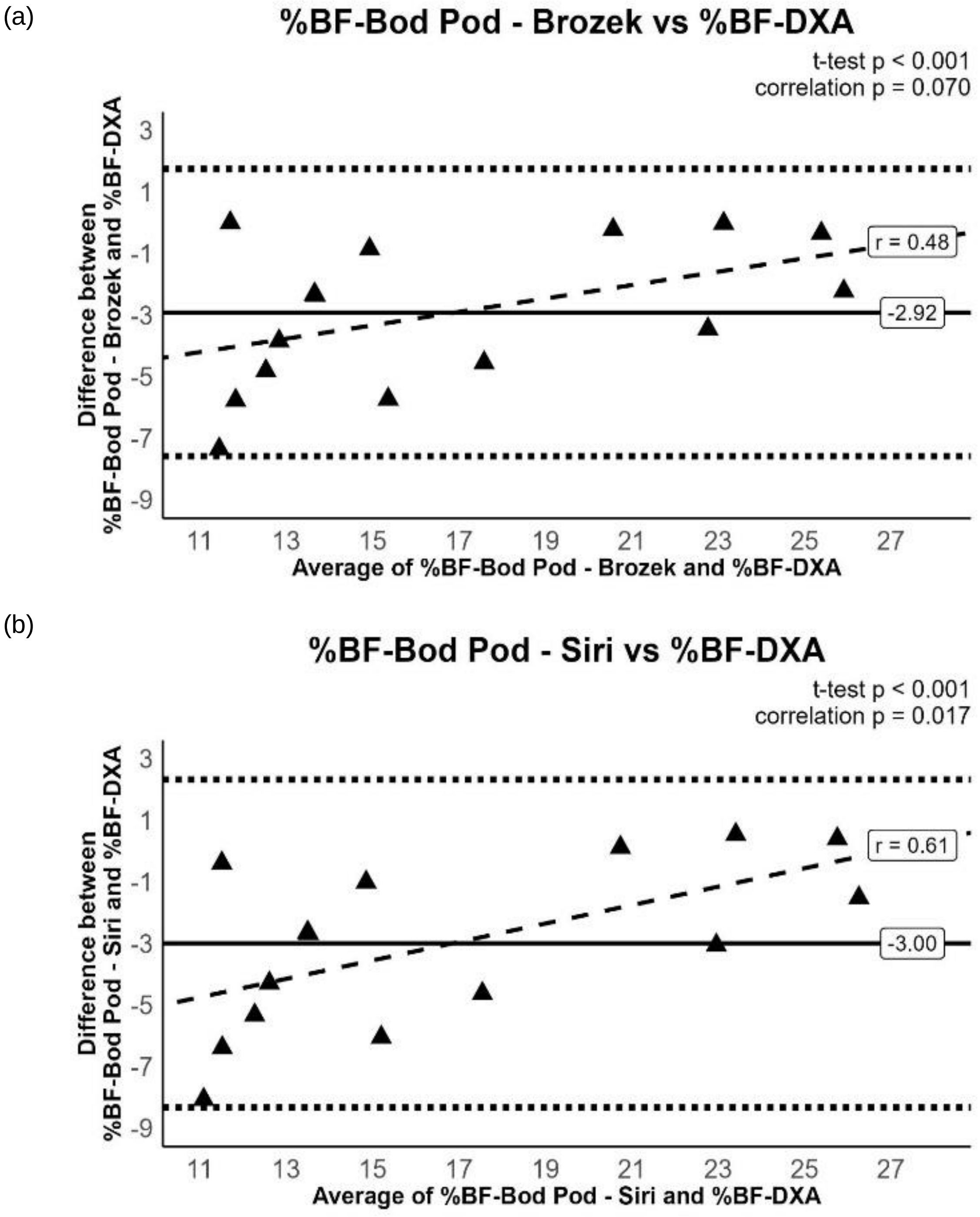


Figure 8: %BF of Male Participants Measured Using DXA Compared with Bod Pod Equations.

(a) Bod Pod-Brozek (n=15); (b) Bod Pod-Siri (n=15).

Figures 9a and 9b both indicate a non-significant difference between DXA and the DW-Brozek (Figure 9a) and DW-Siri (Figure 9b) equations in estimating %BF. The mean difference between the modalities is -0.05% (p = 0.927) and 0.07% (p = 0.905), indicating a non-significant disparity. The DW-Brozek equation underestimated %BF compared to DXA in males. Conversely, the DW-Siri equation overestimated %BF compared to DXA in males.

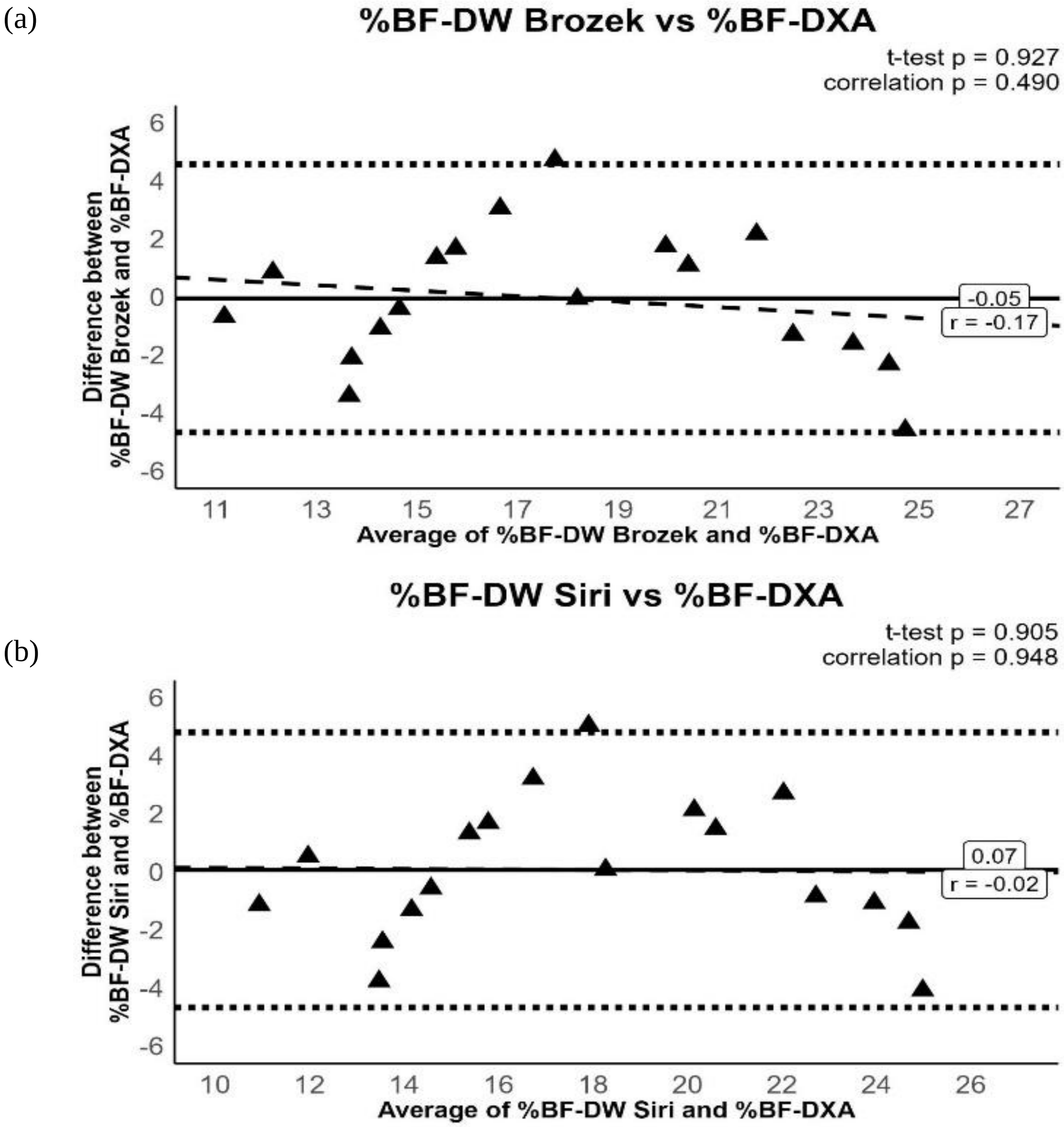
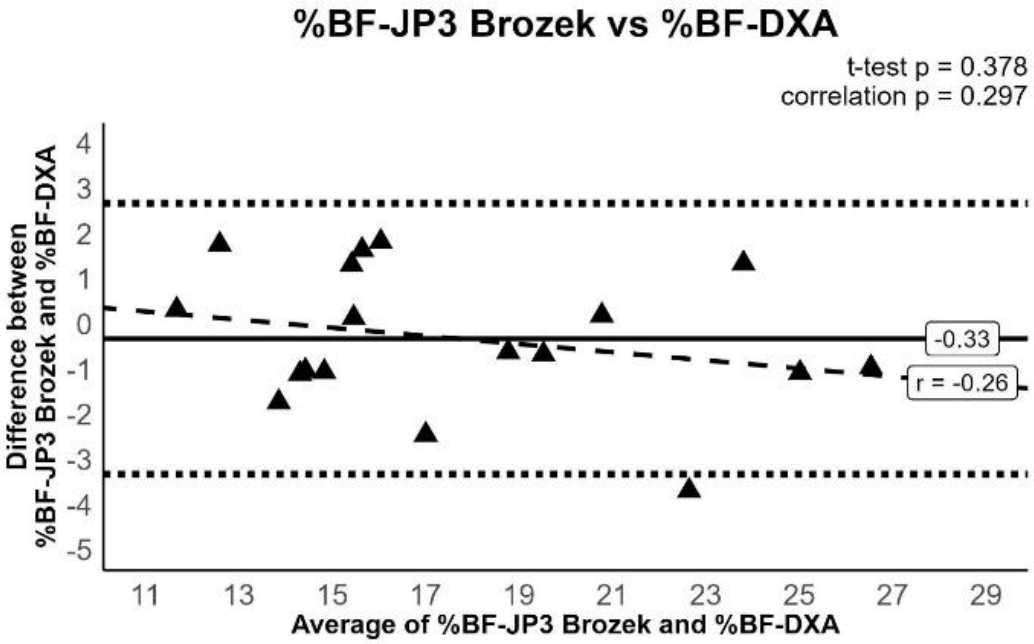


Figure 9: %BF of Male Participants Measured Using DXA Compared with Durnin and Womersley equations. (a) DW-Brozek (n=18); (b) DW-Siri (n=18).

The correlation (r = -0.17) between DXA and DW-Brozek equation is not statistically significant (p = 0.490), suggesting a non-significant proportional bias. Similarly, the correlation (r = -0.02) between DXA and DW-Siri equation is also not statistically significant (p = 0.948), suggesting a non-significant proportional bias. Thus, the data suggest that the DW-Brozek equation exhibits a non-significant underestimation of %BF in males as compared to DXA, whereas the DW-Siri equations exhibits a non-significant overestimation of %BF in males as compared to DXA.

Figures 10a and 10b both indicate non-significant differences between DXA and JP3-Brozek (Figure 10a) and JP3-Siri (Figure 10b) equations in estimating %BF. The mean differences between the modalities is -0.33% (p = 0.378) and -0.23% (p = 0.541), indicating a non-significant disparity between DXA and the JP3 equations (Brozek and Siri) in males.

(a)



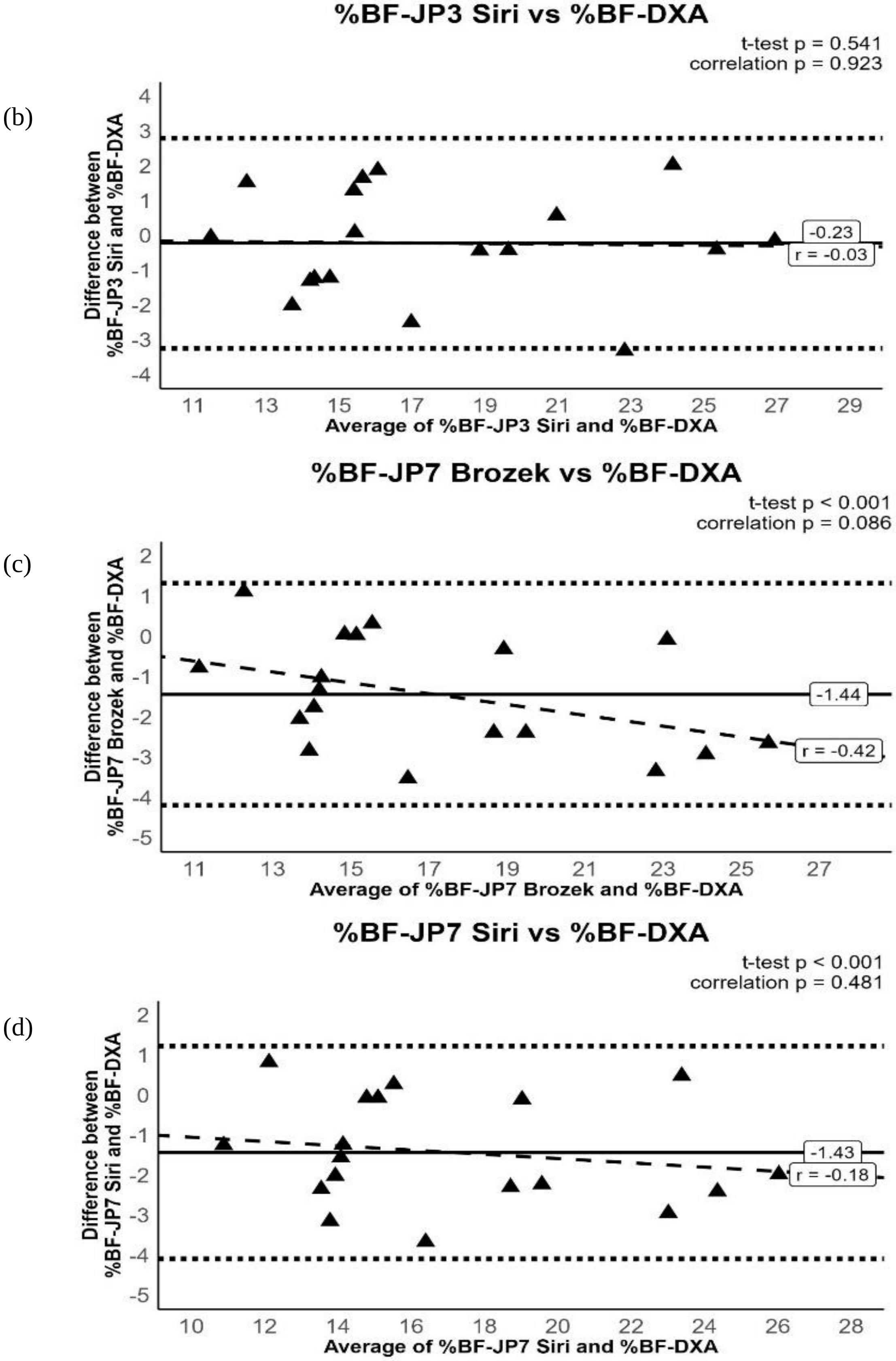


Figure 10: %BF of Male Participants Measured Using DXA Compared with Jackon and Pollock equations. (a) JP3-Brozek (n=18); (b) JP3-Siri (n=18). (c) JP7-Brozek (n=18); (d) JP7-Siri (n=18).

Figures 10c and 10d both indicate a significant difference between DXA and the JP7-Brozek (Figure 10c) and JP7-Siri (Figure 10d) equations in estimating %BF. The mean difference between the modalities is -1.44% ($p < 0.001$) and -1.43% ($p < 0.001$), indicating a significant disparity. Both equations (JP7-Brozek and JP7-Siri) significantly underestimate %BF compared to DXA in males.

The correlation ($r = -0.26$) between DXA and JP3-Brozek equation (Figure 10a) is not statistically significant ($p = 0.297$), suggesting a non-significant proportional bias. Similarly, the correlation ($r = -0.03$) between DXA and JP3-Siri equation (Figure 10b) is also not statistically significant ($p = 0.923$), suggesting a non-significant proportional bias. Thus, the data suggest that the JP3-Brozek and JP3-Siri equations exhibit no significant systematic or proportional biases of %BF in males compared to DXA. Furthermore, The correlation ($r = -0.42$) between DXA and JP7-Brozek equation (Figure 10c) is not statistically significant ($p = 0.086$), suggesting a non-significant proportional bias. Similarly, the correlation ($r = -0.18$) between DXA and JP7-Siri equation (Figure 10d) is also not statistically significant ($p = 0.481$), suggesting a non-significant proportional bias. That is, the systematic bias remains relatively constant across different %BF values in males. Thus, the data suggest that the JP7-Brozek and JP7-Siri equations exhibit a significant and consistent systematic underestimation of %BF in males compared to DXA.

Figure 11 indicates a significant difference between DXA and the Yuhasz equation in estimating %BF. The mean difference between the modalities is -4.04% ($p < 0.001$), indicating a significant disparity. The Yuhasz equation underestimates %BF compared to DXA in males across all data points. The correlation ($r = -0.69$) between the two methods is statistically significant ($p = 0.001$), suggesting a significant proportional bias. That is, as %BF values increase, the Yuhasz equation tends to underestimate %BF more, hence the bias is non-constant (i.e., proportional) across all values of body fat, with larger amounts of body fat reflecting greater biases. Thus, the data suggest that the Yuhasz equation exhibits a significant systematic underestimation of %BF in males which gets proportionally larger as %BF increases compared to DXA.

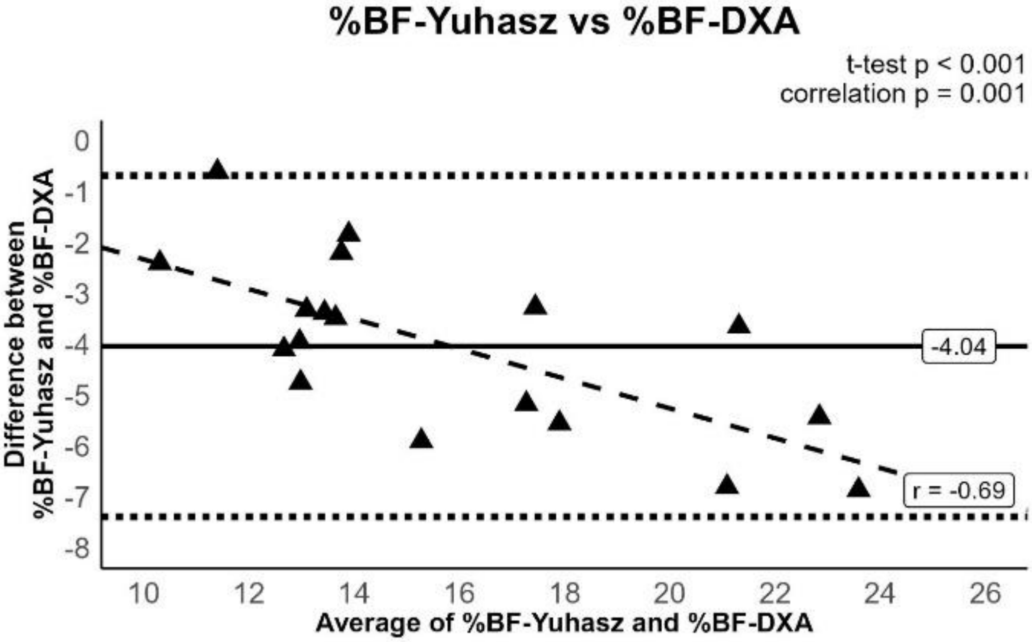


Figure 11: %BF of Male Participants Measured Using DXA Compared with Yuhasz Equation (*n*=18).

CHAPTER 5: DISCUSSION

5.1 General Discussion

This current thesis represents a comprehensive analysis of the accuracy of different %BF methods and their respective prediction equations in comparison to DXA—considered the practical gold standard for assessing BC (32). The main objectives of this thesis were twofold: (1) To evaluate if statistically significant differences were found in %BF estimates amongst different BC methods and their respective prediction equations across both sexes in healthy recreationally active adults. (2) To evaluate agreement between the different BC methods and their respective equations with DXA across both sexes. To assess the accuracy of BC methods in different population samples, it was necessary to evaluate commonly utilized laboratory and field methods for their ability to indirectly assess %BF. In this study, statistically significant differences between the methods and their prediction equations were evaluated using an ANOVA with post-hoc analyses. The main findings of this study demonstrated clear statistically significant differences between most of the prediction methods in estimating %BF as compared to DXA in both sexes. However, our findings revealed a somewhat consistent pattern across sex and prediction methods, such that females displayed a greater number of significant differences vs. males as compared to DXA. Agreement between the methods and their prediction equations were further evaluated using B-A plots with t-tests and regression analyses to assess systematic and proportional biases, respectively.

The combination of these two analyses enabled a thorough evaluation of the nine prediction methods vs. the reference method DXA, separated by sex. Different conclusions were found from the separate analyses, the results from each analysis generally led to different interpretations, such that the main findings of this study were as follows.

(1) All prediction methods significantly ($P<0.05$) underestimated %BF relative to DXA, except DW (Brozek and Siri) equations in females. Additionally, a significant ($P<0.05$) underestimation of %BF was only found for the Yuhasz prediction equation in males, relative to the value derived from DXA.

(2) Among the female participants, significant systematic biases ($P<0.05$) were found when agreement was assessed with magnitudes of difference ranging from -2.0% to -10.0% (DXA vs. Bod Pod-Siri [-2.03% ± 2.47]; Bod Pod-Brozek [-2.60% ± 2.25]; JP3-Siri [-3.98% ± 4.30]; JP3-Brozek [-4.53% ±3.96]; JP7-Siri [-8.16% ± 3.24]; JP7-Brozek [-8.39% ± 3.33]; and Yuhasz [-9.95% ± 3.07]).

(3) Among the female participants, significant proportional biases ($P<0.05$) were found when agreement was assessed between DXA and the prediction methods (DW-Brozek, DW-Siri, JP7-Brozek, JP7-Siri, and Yuhasz).

(4) Among the male participants, significant systematic biases ($P<0.05$) were found when agreement was assessed with magnitudes of difference from -1.4% to -4.0% (DXA vs. JP7-Siri [-1.43% ± 1.36]; JP7-Brozek [-1.44% ± 1.41]; Bod Pod-Brozek [-2.92% ± 2.40]; Bod Pod-Siri [-3.00% ± 2.72]; and Yuhasz [-4.04% ± 1.71].

(5) Among the male participants, significant proportional biases ($P<0.05$) were found when agreement was assessed between DXA and the prediction methods (Bod Pod-Siri, and Yuhasz) among male participants.

In this present study, DXA served as the reference method to provide what is perceived to be the most accurate estimate of %BF, as this 3C method has been previously demonstrated to be valid in different populations (31,42,44). In the current investigation, the majority of the SKF prediction equations (JP3-Brozek, JP3-Siri, JP7-Brozek, JP7-Siri, and Yuhasz) demonstrated an underestimation of %BF estimates as compared to DXA in both sexes. Further, among the SKF prediction equations, only the DW-Siri equation demonstrated an overestimation of %BF as compared to DXA in our male sample. These findings were somewhat in agreement with the literature as mixed results have been found in both males and females when SKFs were compared to DXA. SKF thicknesses are an indirect method of assessing %BF. As such, this method is fundamentally based on the idea that a representative regional measure of subcutaneous adipose tissue provides a reasonable estimate of total body fat (102).

The current study found the majority of SKF prediction equations significantly underestimated %BF relative to DXA in our female sample. These results coincided with a previous investigation by Ball et al. which demonstrated significant underestimates in %BF using SKF prediction equations (-3.2 to -5.6%; $p < 0.01$) as compared to DXA in 150 females (ages 18-55 years; 87% White, 2% Black, 9% Hispanic, 2% Asian) (48). The investigators also noted that the degree of underestimation depended on body fatness for the JP7 and JP3 equations (48). However, a regression analysis was not performed in that study to further ascertain if there was a positive or negative relationship with the proportional bias. Similarly, significant underestimations were found across the same SKF equations (JP3, JP7) evaluated in the present study. This is noteworthy considering the representative sample of female participants, comprising (85% White, 3% other) was similar to that of Ball et al. (48). Despite this, differing results were demonstrated for the proportional bias for the JP3 and JP7 equations. Our results solely demonstrated significant proportional biases for the JP7 equations (Brozek and Siri), such that the underestimation increased with increasing levels of body fatness. Barnas et al. compared four commonly recommended SKF prediction equations with a DXA-derived %BF estimation in 77 normal active females (aged 28.0 ± 10.2 years) (103). The means for age, height, weight, and

BMI of the female participants in the current investigation closely matches those of the female participant characteristics of the Barnas et al. investigation (age 26.3 ± 4.0 vs 28.0 ± 10.2 years; height 165.5 ± 6.4 vs 165.2 ± 5.9 cm; weight 63.1 ± 6.6 vs 63.2 ± 10.2 kg; BMI 23.1 ± 2.4 vs 23.1 ± 3.0) (103). Barnas et al. found that all four SKF prediction equations significantly underestimated %BF, which coincides with the majority our female SKF data. Unlike the study by Ball et al.(48), the authors of the Barnas et al. study did not find that proportional biases existed with the JP3 and JP7 equations (103). Similarly, our results did not show proportional biases for the JP3 equations, however proportional biases were reflected in the JP7 equations as stated. Bacchi et al. compared %BF estimations from several SKF prediction equations to DXA in 12 female adults with obesity and type-2 Diabetes (age 56.2 ± 8.1 years); concluding that five out of six SKF equations overestimated %BF in their female sample (30). It is worth noting that two out of the five SKF equations that showed an overestimation in the Bacchi et al. investigation were the DW equations (Brozek and Siri) (30), of which both prediction equations demonstrated an non-significant underestimation in our investigation (and were most closely matched to DXA). One likely explanation for these under-and overestimation difference could be due to differences in body fat distribution as the investigators of the Bacchi et al. study noted that obesity may affect the accuracy of SKF thickness assessments—as the DW equations were developed in apparently healthy adult subjects (74), whereas the Bacchi et al. investigation used a clinical population, of which excess adiposity was evident (30).

It is also worth noting, that the DW prediction equations (Brozek and Siri) were the only two SKF equations that were not significantly different from DXA in our female sample, while Bacchi et al. found that only the DW-Brozek equation in females was not significantly different from DXA (30). In contrast to our female sample, all SKF prediction equations were not significantly different from DXA in our male participants, except for the Yuhasz equation. This was confirmed by the ANOVA post-hoc analyses. B-A plots (Figs 7 and 11) also showed that the Yuhasz equation had the largest significant systemic biases (9.95±3.07% and 4.04 ± 1.71%; $p<0.001$) for females and males, respectively, among the SKF equations evaluated.

The data recorded for the Yuhasz equation in our investigation differed from those reported in the literature in a few noteworthy ways. Several studies have shown the Yuhasz equation to exhibit strong positive correlations with %BF values derived from DXA in male athletes (31,81). The study by Dimitrijevic et al. found among the different SKF equations examined that the strongest correlation was observed using the equation derived by Yuhasz ($r = 0.909$; $p < 0.001$) in male combat sports athletes (31). Similarly, strong positive correlations were found by Suarez-Arrones et al. who assessed %BF using Yuhasz ($r = 0.61$; $p < 0.01$) compared to DXA in international-level elite male soccer players (81). In comparison with the present study, an important statistical distinction should be made, such that, the studies that found a strong positive correlation with the Yuhasz equation in males, examined just the correlations and not ANOVA. Between two measures, a correlation is a measure of association and not accuracy (79,101). Equation accuracy involves analyzing measurement error and determining the level of agreement between the two measures (79,101). Of these two measures, the latter was carried out in the present study. To further validate the strong positive correlation with the Yuhasz equation observed in males in both the Dimitrijevic et al. and Suarez-Arrones et al. investigations, (31,81) we ran a separate correlation from our current statistics to observe if similar results could be found in our data. We similarly found a strong positive correlation of %BF between DXA and the Yuhasz equation in males ($r = 0.953$; $P<0.001$).

When comparing the literature with the results of the DW equations (DW-Brozek and DW-Siri) in our male sample, conflicting results were observed. Several studies have found mixed results in terms of accuracy for the DW equations (DW-Brozek and DW-Siri) when compared to DXA in different populations (29,44,81,104) such as older Caucasian adults in the U.S. with (n=19) and without obesity (n=20) (44), older Brazilian adults (n=52; age 70.5 ± 6.7 years) (29), healthy adult Chinese males (n=33; age 43.1 ± 0.7 years) (104), and international-level elite male soccer players (n=18; age 27.6 ± 3.0 years) (81). In older Caucasian adults with and without obesity, Chambers et al. found that the DW-Siri equation significantly overestimates %BF in males, regardless of obesity (44). Our results contrast with those obtained by Chambers et al. and were not significantly different from DXA. An investigation by Silveira et al. found that the DW-Siri equation demonstrated a significant underestimation of %BF around 2.3% as compared to DXA in 52 older Brazilian adult males (29). Suarez-Arrones et al. found a similar underestimation of around 3.4% with the DW-Siri equation in male international-level elite soccer players as compared to DXA (81). In healthy adult Chinese males, Yao et al. demonstrated that %BF estimated from the equations of DW did not differ significantly from the reference method used in that study, whether the Brozek equation (%BF = 24.7 ± 0.9) or Siri equation (%BF = 25.4 ± 1.0) was used to convert estimated Db into %BF in their male sample (104). It is worth noting that the investigation by Yao et al. used isotope dilution and deuterium oxide as their reference method for measuring body fat and not DXA (104**).** This method is a form of hydrometry, which is a measurement of total body water (TBW) (56). As water is the most abundant component of the human body, accounting for about 60% of the body weight, it is predominantly associated with FFM. Therefore, %BF can be estimated from hydrometry using a 2C model converting TBW to %BF (61). This notable difference between the reference method used in the investigation by Yao et al. and our investigation, made the interpretation of accuracy with the DW equations (Brozek and Siri) not comparable in this regard.

In our male sample, the results of the JP equations (JP3-Brozek, JP3-Siri, JP7-Brozek, JP7-Siri) showed no significant difference vs. DXA and the B-A analyses demonstrated a significant systematic underestimation of %BF for JP7s only by approximately -1.4% as compared to DXA. These results differ with an investigation by Ball et al. of which the participants' characteristics were similar to ours. Moreover, both investigations shared similar participant characteristics with the original JP validation study (75,105). Almost all participants in the original JP validation study were Caucasian and 77.8% of our male sample was Caucasian. Additionally, our male sample was also represented by 5.6% Black, and 5.6% Hispanic. Further, the age, height, and weight of our male sample was 30.5 ± 7.4 years, 180.6 ± 8.2 cm, and 81.9 ± 11.4 kg, whereas the same characteristics in the Ball et al. study were 32.1 ± 11.0 years, 178.8 ± 6.9 cm, and 82.3 ± 14.1 kg (105). The physical characteristics of participants in our investigation and those of the Ball et al. study were very similar to the same characteristics in the original JP study (32.6 ± 10.8 years, 179 ± 6.5 cm, and 74.8 ± 11.8kg, respectively) (75). The non-significant underestimation by each of the JP equations (JP3-Brozek, JP3-Siri, JP7-Brozek, JP7-Siri) in the present study, coincided with similar underestimations (JP3-Siri, JP7-Siri) observed in the Ball et al. study (105). Albeit Ball et al. study found significant differences with DXA derived %BF estimations, whereas we did not (105). A potential explanation for this difference could be attributed to differences in sample sizes in the present study and that of Ball et al. Our male sample comprised 18 male participants in comparison to 160 male participants in the Ball et al. investigation (105).

With respect to Bod Pod, the results of the present study found significant differences for %BF estimates between Bod Pod and DXA in females but not males. Results from the B-A analyses also found significant systematic underestimations of %BF as compared to DXA in both sexes. However, mixed results have been found in several studies in adults that have evaluated the accuracy of Bod Pod using different reference methods (23,24,26,65,106,107). Specifically, studies to date have found both under- and overestimation of %BF by bod pod when compared to either UWW or DXA (23,24,26,106). Demerath et al. found an overestimation of %BF in male and female adults when Bod Pod was compared to UWW (106), such that, with increasing levels of body fat, Bod Pod's overestimations increased. Reflecting a proportional bias that tended to underestimate %BF in adults that are leaner and overestimate it in adults with more body fat (106), a similar pattern was found by Levenhagen et al. in healthy male and female adults (n=20) (23). This investigation suggested that Bod Pod underestimated %BF at lower values of fatness, while overestimating %BF at higher values of fatness (23). More recent studies have found opposing results related to this under-and overestimation bias. Lowry et al. evaluated the accuracy of %BF estimates with Bod Pod as compared to DXA in adults classified as either underweight, normal weight, or with obesity (24). They found that Bod Pod overestimated %BF in individuals classified as underweight and normal weight and an underestimation in %BF in individuals classified with obesity (24). Results from another more recent study by Bi et al. found results consistent with Lowry et al. (26). The authors from the Bi et al. investigation similarly found that the Bod Pod significantly underestimated %BF with increasing levels of fatness as compared to DXA in Singaporean adults (26). In our study, specifically with the Bod Pod-Siri prediction equation, agreement between %BF-DXA vs Bod Pod-Siri reflected a significant proportional bias when compared by B-A analysis, such that, the Bod Pod-Siri equation in males underestimated %BF to a lesser extent with increasing levels of fatness. Hence, approaching an overestimation. This proportional bias is in direct opposition to what was observed in the Bi et al. investigation (26).

While Bod Pod underestimated %BF in both sexes in our investigation, there seems to be a sex bias when %BF estimates from Bod Pod were compared to other reference methods in the literature. The study by Levenhagen et al. revealed that Bod Pod underestimated %BF in males, while it overestimated %BF in females, as compared to UWW (23). These results are corroborated by others (65,107). Collins et al. demonstrated that Bod Pod significantly underestimated %BF compared to the %BF values from both UWW and DXA in male Collegiate football players (65). Our investigation saw a similar underestimation in %BF when compared to DXA derived %BF estimates in males; however, our results were not significant. In a study by Wingfield et al. among females (albeit non-significant) overestimations were observed in %BF estimates between Bod Pod and DXA in adult females with obesity (107). This particular result contrasts with our study, which found a significant underestimation of %BF when Bod Pod was compared to DXA albeit in normal weight active female adults. While the direction and existence of biases were not consistently demonstrated in comparative studies with Bod Pod, overall, %BF tended to be underestimated in males and overestimated in females in the literature. Additionally, the under- and overestimation in adults was dependent on adiposity, hence %BF in adults tends to be underestimated in individuals with less fat and overestimated in individuals with more fat. This observation was evident in the present study with both sexes (normal weight) demonstrating systematic underestimations in %BF as compared to DXA. The results of the current study observed systematic underestimations of %BF compared to DXA across almost all prediction equations. This bias, although consistent, indicates limitations inherent in the prediction equations and method specific methodology suggesting the biases observed may be attributed in the reference method used to develop these equations. As stated, these prediction equations were developed using Db from UWW, and not DXA or some other multicomponent method. Therefore, if the assumptions that govern the 2C model (FM (0.90 g/cm^3); FFM (1.1000 g/cm^3) are not met (18), a greater FFM density will cause systematic underestimations in %BF. This lack of agreement between the reference methodology used to develop these equations, and DXA being a multicomponent method is a likely factor for the observed underestimations in the present study.

5.2 Strengths

The findings from this thesis may provide insights into the accuracy of various BC methods and their respective %BF prediction equations. One of the main strengths of this study was the use of DXA as the reference method. DXA is highly repeatable as a BC method, practically speaking. Despite some methodological short comings inherent in the method, DXA is well accepted in the literature as the practical gold standard (33). Another strength of the current study was that one certified and trained technician was used to obtain BC data for all subjects, as this helped to avoid inter-observer variability and preserve intra-observer reliability. Intra-observer reliability is of the utmost importance with the SKF method. For instance, approximately 3-9% of the variability in SKF measurements between SKF technicians could be attributed to measurement errors with taking the SKF thicknesses themselves (68). Moreover, the inclusion of a wide variety of BC methods (DXA, Bod Pod, SKFs) used to estimate %BF provided an opportunity to evaluate the degree of measurement error among the various BC measurement methods. Lastly, the statistical analyses included an ANOVA, and B-A plots with T-Tests and Regression analyses to assess systematic and proportional biases among the comparisons. These inclusions enhanced the confidence of the study findings.

5.3 Limitations

The present study had some limitations, principally because the data was not collected by myself (due to COVID-19 restrictions), I do not know the accuracy/reliability of its acquisition. Nonetheless, the individual who did all the data collection is a trained and certified clinical densitometrist through the International Society of Clinical Densitometry, which ensures they are qualified as a DXA technician. Moreover, they are ISAK-certified to administer surface anthropometry (SKF thicknesses). Another limitation of our study was attributed to the participant sample not being racially diverse.

5.4 Implications

The current investigation demonstrated results which were congruent with other comparative studies in several areas: (1) Statistically significant differences were observed in %BF for the main effect of sex, with females displaying higher %BF than males for a given modality. These observations have been observed in several comparative studies finding significant differences in %BF between the sexes when different modalities are evaluated (20,26,108). (2) Statistically significant differences were observed in %BF for the main effect of modality. Among female participants Bod Pod underestimated %BF (range = 3.7-4.2%) ($P<0.05$) with DXA. Additionally, SKF prediction equations underestimated %BF (range = 4.0-10.0%) ($P<0.05$) with DXA. Among male participants, a significant underestimation of 4.0% ($P<0.05$) was observed with the SKF prediction equation of Yuhasz with DXA. In females, the only prediction equations that were not significantly different from DXA were the DW (Brozek and Siri) equations. Unlike the JP equations (JP3 and JP7) which include lower limb SKFs, the DW equations only includes four upper body SKFs ($\sum$4SKF, biceps, triceps, subscapular, and iliac crest) to ultimately estimate %BF. This difference may partially explain the validity of the DW prediction equations (Brozek and Siri), especially in females. It has been acknowledged that one of the most difficult and least convenient SKF sites to measure is the anterior thigh (102). Our results provide evidence that the DW prediction equations (Brozek and Siri) may be a better estimate of %BF in normal weight, active males and females. In the present study, both the Bod Pod and SKF modalities demonstrated better agreement with DXA in males compared to females. Both modalities may be considered viable options for estimating %BF in males, based on the results of this study. However, among the specific SKF prediction equations, the equations that demonstrated the most agreement with DXA in estimating %BF were the DW (Brozek and Siri) equations. The B-A plot for agreement between DXA and the DW prediction equations (Brozek and Siri), provided further support for its use in normal weight, active males due to the smallest biases observed (-0.05%, p = 0.927; 0.07%, p = 0.905) in DW-Brozek and DW-Siri, respectively. These notable results can serve as an important practical consideration especially in field settings using SKFs while trying to minimize the potential of measurement error. The DW prediction equations use a four-site SKF measurements to estimate BC. Hence, providing a more time efficient method, while potentially improving accuracy, as fewer sites of measurements may lead to more accurate results. Even though, it has been postulated that a seven-site analysis provides a more accurate representation of overall BC compared to a three-site (109). Others have shown that seven-site and three-site SKF prediction equations are similar or no better in accuracy (48,97,110). Even though the DW predictions equations (Brozek and Siri) performed the best in among the different prediction equations in both sexes, relatively speaking, recommendations for their use cannot be made in all contexts, as these results are highly specific to the homogeneity of the study sample. Further, a significant proportional bias was evident in our female sample, despite the smallest non-significant systematic bias observed. In males, even though a non-significant systematic and proportional biases was demonstrated, the individual data points had a varied dispersion across the confidence intervals in the B-A plots. Suggesting large individual variances albeit non-significant. These results can be used to reinforce the importance of selecting the most appropriate modality and respective prediction equation for the population subgroup of interest.

5.5 Future Directions

The results and subsequent implications of the present study further highlight that it will be useful if future comparative studies focus more on female samples. The current study demonstrated that between the sexes, females had more prediction equations that estimated %BF significantly different from the values derived from the reference method DXA. Additionally, females displayed larger systematic biases ranging from -2.0% to -10.0% body fat, as well as more significant proportional biases, further underscoring potential sex biases.

Further research should be conducted to develop and cross-validate a generalized SKF equation in a larger sample of adult females accounting for age, and race using a 4C model as the reference. To date, two other investigations have notably implemented a 4C model approach with other samples (78,97). Peterson et al. developed and cross-validated a SKF prediction equation using the 4C model in 681 Caucasian adults (78). Evans et al. developed and cross-validated a SKF prediction equation using the 4C model in 132 collegiate athletes (78 males: 28 Black, 50 Caucasian; 54 females: 10 Black, 44 Caucasian) (97). Collectively, both studies provide evidence that further research is needed in developing generalized SKF prediction equations in different population subgroups using a 4C model as the reference method.

5.6 Conclusion

In conclusion, this thesis emphasizes the need for further investigation and future validation of accurate, accessible, and pragmatic BC assessment methods in practical settings. The discrepancies observed in sex, modality, and prediction equation highlight the importance of using the most appropriate BC approach to avoid potential errors while maintaining accuracy.

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