THE VALIDITY OF ULTRASOUND TO ASSESS BODY COMPOSITION IN ADOLESCENT AND YOUNG ADULT BALLET DANCERS

By

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ABSTRACT OF THE THESIS

The Validity of Ultrasound to Asses Body Composition in Adolescent and Young Adult Ballet Dancers

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Body composition (BC) assessments are used to monitor athletes' overall health and track changes over time. These assessments are preferable compared to body weight alone or body mass index (BMI) because they provide additional information in regards to specific tissue quantities. However, the most accurate tools, such as air displacement plethysmography (ADP), are expensive and not feasible for assessing large groups of athletes, and field tools, such as bioelectrical impedance analysis (BIA) lack adequate validity and reliability needed to accurately track changes over time. Ultrasound (US) is proposed as a valid laboratory method that can also be used in field settings, as it is relatively inexpensive and portable. **PURPOSE:** To assess the validity of B-mode US (B-US) compared to ADP to determine BC in high-level adolescent and young-adult ballet dancers. A second study (Study 2) was conducted to further assess validity of different devices by comparing B-US, A-mode US (A-US), and BIA to ADP, as well as to a 3-compartment (3C) model. **METHODS:** Adolescent and young-adult vocational ballet dancers (N=48; M_{age}= 16.6 ± 1.6; M_{BMI}=19.3 ±1.9) had their BC assessed by ADP and B-US. A subset of subjects (n=22) participated in Study 2, where BC was assessed via ADP, B-US, A-US, and BIA. Additionally, body density via ADP and total body

water via BIA were used to calculate BC from Siri's 3C equation for a reference measure. Pearson's correlations were used to determine relationships between reference and experimental methods. Dependent t-tests were used to determine significant differences between methods for %BF, FFM, and FM. Bland-Altman plots were used to assess mean differences and identify the 95% LOA. Significance was set at $P<0.05$ for all measures. **RESULTS:** The primary study showed significant, strong correlations between B-US and ADP for %BF (females r=0.941; males r=0.773), FM (females r=0.943; males r=0.726), and FFM (females r=0.954; males r=0.985). Despite strong correlations among females, B-US significantly overestimated %BF and FM, and significantly underestimated FFM ($P<0.05$) compared to ADP. However, there were no differences between devices for any measure in males. In Study 2, there were no significant differences between devices for all measures in both males and females. There were strong correlations between ADP and both US devices, but lower correlations between ADP and BIA compared to US. Additionally, there were higher correlations between ADP and B-US than with A-US for %BF and FM in both males and females. As seen in the first study, FFM correlations were similarly high for both sexes across all devices. When comparing B-US and A-US to the 3C model, there were higher correlations for B-US than A-US for %BF in both sexes, and for FM in females only. Additionally, there were significant differences between A-US and 3C %BF, FM, and FFM results in males, but not in females. As seen in the previous analyses, there were higher correlations for FFM than there were for both %BF and FM for all methods in both males and females. **CONCLUSION:** B-US and A-US are valid BC assessment tools for adolescent and young-adult ballet dancers. It is possible that the combination of small errors in both FM

and FFM measures resulted in larger %BF error, indicting these tools may be better suited for determining absolute FFM and FM rather than %BF. Overall, there was better agreement between the 3C model and both US devices in females compared to males, but there were higher correlations between 3C and B-US than 3C and A-US for both sexes. Future research should explore using a 4C reference method, as well as the use of different density models and prediction equations to improve %BF accuracy when using US.

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Chapter 1

Using Body Composition Assessments: Validity, Reliability, and Practicality

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Introduction

Body composition (BC) assessments are used to monitor overall health, nutritional status, and track changes over time. These assessments help ensure healthy dieting and lean mass maintenance are occurring, but the tests employed need to be accurate and reliable. Monitoring and assessing BC is especially important for athletes participating in sports that emphasize body weight and aesthetics, such as dance and gymnastics, who are prone to disordered eating and body dysmorphia (Wilmerding et al., 2005; Hidayah & Syahrul Bariah, 2011). Dancers are more likely to show signs of disordered eating, such as amenorrhea, decreased bone mineral density, and musculoskeletal problems, compared to the general population (Wilmerding et al., 2005). These athletes attempt to decrease body weight to gain performance advantages, as lower body fat is positively associated with improved strength, sprint time, and jump heights (Misigoj-Durakovic et al., 2001; DiCagno et al., 2008).

However, it is imperative for athletes and coaches to understand that extreme dieting and weight-loss behavior may impair physical performance and physiological function (Wilmerding et al., 2005; Hidayah and Syahrul Bariah, 2011; Ackland et al., 2011; Pineau et al., 2009; Müller et al., 2016; DiCagno et al., 2008). Athletes with either excess or extremely low body fat levels, as well as unfavorable lean mass changes, may see decreased performance and are at higher injury and illness risk (Ackland et al., 2011). BC assessments may be used to guide training programs and to monitor athletes by determining overall body fat percentage (%BF), fat mass (FM), and fat-free mass (FFM) (Prado & Heymsfield, 2014; Wagner, 2013).

BC assessments are preferable compared to body weight alone or body mass index (BMI) because they provide additional information with regard to specific tissues, such as adipose, skeletal muscle, and bone (Prado and Heymsfield, 2014; Wagner, 2013). However, the most accurate tools are expensive and not feasible for assessing large groups of athletes. In contrast, common inexpensive and portable field methods may not possess ideal levels of validity and reliability needed to accurately track changes over time (Meyer et al., 2013; Prado & Heymsfield, 2014).

There are many different types of BC assessments that determine different body components based on 5 different models of increasing complexity: atomic, molecular (chemical), cellular, tissue-system (anatomical), and whole-body (Heymsfield et al., 1997; Prado & Heymsfield, 2014; Ackland et al., 2011). Different assessment tools analyze different components based on the model used. For example, molecular-based tools assess lipid (FM) and FFM content while anatomical-based methods analyze adipose tissue and lean body mass (LBM) (Heymsfield et al., 1997; Prado & Heymsfield, 2014). Although the terms fat mass (FM) and adipose tissue and the terms FFM and LBM are often used interchangeably, they are referring to different specific components and are assessed using different methods (Prado & Heymsfield et al., 2014).

Adipose tissue specifically refers to connective tissue from adipocytes, collagenous and elastic fibers, fibroblasts, and capillaries, while fat mass (FM) encompasses additional lipid-containing components (Prado & Heymsfield, 2014; Wang et al., 1992). Anatomical approaches are more sophisticated and can differentiate between

subcutaneous, visceral, and intramuscular adipose tissues, which is important in clinical settings and disease-risk screenings (Prado & Heymsfield, 2014; Shuster et al., 2012).

While the differences between FM and adipose tissue are well understood, there is less clarity regarding FFM versus LBM (Prado & Heymsfield, 2014). LBM, sometimes more accurately referred to as lean soft tissue (LST) is defined as body water, proteins, carbohydrates, non-fat lipids, and soft tissue mineral (Prado & Heymsfield, 2014; Shen et al., 2005). Both fat and bone mineral compartments are excluded in this definition and are therefore not part of LBM/LST. However, FFM includes both LBM/LST and bone mineral, as this component is defined as skeletal and non-skeletal muscle, organs, connective tissue, and bone (Prado & Heymsfield, 2014)

Devices that assess LBM as opposed to FFM are more technologically advanced and are often referred to as reference methods (Ackland et al., 2011). Reference methods are most often used in research settings to validate more accessible laboratory and field devices used to assess body composition (Ackland et al., 2011). Different techniques are grouped into three categories: reference, laboratory, and field techniques, based on their technical properties and practical applications (Ackland et al., 2011).

Reference Techniques

Cadaver analysis is the only direct BC assessment method, but computerized tomography (CT), magnetic resonance imaging (MRI), and multi-component models (MCM) provide comprehensive, indirect analyses and can serve as reference methods. While reference methods are the most accurate assessment tools, they are not feasible, practical, or ethical to use for the sole purpose of assessing body composition due to high

equipment costs and time-consuming, invasive procedures (Ackland et al., 2011). However, these methods provide the standards that laboratory and field techniques are validated against (Ackland et al., 2011).

Cadaver Analysis

Cadaver dissection is the most accurate and only direct BC assessment method (Heymsfield et al., 2015). Using cadavers allows researchers to directly measure different body components from either a molecular or an anatomical approach, (Heymsfield et al., 2015; Ackland et al., 2011). Since direct analysis is not feasible for routine assessments, indirect reference BC assessment methods that use advanced imaging techniques have been developed for human assessments (Ackland et al., 2011; Heymsfield et al., 2015). Many of these indirect techniques have been validated against cadaver analyses in order to establish their validity (Ackland et al., 2011).

Computerized Tomography

Computerized tomography (CT) scans are based on the anatomical model, as they determine the amount of adipose and skeletal muscle tissue (Heymsfield et al., 1997). These exams use x-ray to create a two-dimensional (2D) image based on tissue attenuation to in order to quantify different components (Goodpaster et al., 2000). These exams can be used for regional analyses, as well as differentiating between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), which is clinically important when screening for metabolic and cardiovascular disease risks (Andreoli et al., 2016). Previous research found CT scans are reliable and are strongly correlated with cadaver

adipose measurements (r=0.77-0.94) (Rossner et al., 1990; Heymsfield et al., 1997). Despite these findings, high costs, specialized equipment, and unnecessary radiation exposure make this an impractical tool to routinely assess BC (Ross et al., 1991; Prado & Heymsfield, 2014; Brenner et al., 2007).

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) uses radio frequency signals and generates a magnetic field in order to create images that represent skeletal muscle and adipose tissue spatial distributions (Prado & Heymsfield, 2014; Ackland et al., 2011). This technique was first introduced to BC research in 1984, when researchers showed that MRI could differentiate between adipose tissue and skeletal muscle in human cadavers (Foster et al., 1984).

These imaging techniques were first used to measure adipose and lean tissue distributions without exposing subjects to unnecessary radiation, as CT scans do (Heymsfield et al., 1997; Ross et al., 1991). MRI scans are reliable with repeated measures ranging from 1%-10% for SAT and between 0.3%-2.3% for skeletal muscle (Heymsfield et al., 1997). There is high agreement between MRI and cadaver results in both humans and animals, with human tissue weights ranging ±6% between methods (Heymsfield et al., 1997; Ross et al., 1991; Abate et al., 1994). However, since MRI is designed for clinical diagnostic purposes rather than measuring tissue dimensions, current pixel sizes limit the MRI accuracy in extremely lean athletes (Ackland et al., 2011). Additional limitations of MRI for whole-body imaging include high costs and exam length, as images need to be taken individually and later integrated to analyze tissue area,

slice thickness, distance between consecutive images, and total images taken (Ackland et al., 2011; Prado & Heymsfield, 2014).

Multi-Component Models

Multi-component models (MCM) are considered the most practical of the reference methods and can be used in place of cadaver analysis as an indirect criterion measure (Wells & Fewtrell, 2006; Heymsfield et al., 1997). These models combine measurements from multiple techniques in order to simultaneously assess multiple body components. The first MCM expanded the common two-component (2C) model, which separates the body into FM and FFM, by incorporating total body water (TBW) to create a three-component (3C) model (Siri, 1991). Measuring TBW increases measurement accuracy because FFM hydration levels are not always constant, as assumed by the 2C model, and are known to change based on age or health status (Fosbøl & Zerahn, 2015). However, more accurate MCM equations have been developed, including a four- (4C), five- (5C) and six-component (6C) models (Clasey et al., 1999; Fosbøl & Zerahn, 2015).

The 4C model assesses bone mineral in addition to TBW, which increases measurement accuracy (Fosbøl & Zerahn, 2015; Heymsfield et al., 1990; Wang et al., 2005). The 4C model is often used as a reference criteria when evaluating more common laboratory and field BC devices. While the 4C models do not account for soft-tissue mineral content, which is known to have a high density (3.317 g cm 3), this can be estimated from TBW (Fosbøl & Zerahn, 2015; Wang et al., 2002). Additionally, many 4C equations have been validated against 5C and 6C methods (Fosbøl & Zerahn, 2015).

While measuring more body compartments yields more accurate and detailed analyses, MCM exams are often expensive, time-consuming, and more invasive compared to the two-compartment models, as they require multiple assessments to determine different body compartments (Ackland et al., 2011; Prado & Heymsfield, 2014). Therefore, MCM are not applicable or feasible in clinical and athletic settings due the expensive equipment required and the extensive time required to complete exams. However, MCM serve as the reference method in many research settings for validating more accessible techniques (Fosbøl & Zerahn, 2015).

Laboratory Techniques

Laboratory tools are most commonly used in research facilities, clinical settings, and high-level athletic performance centers (Ackland et al., 2011). These tools are often expensive, ranging from $2,000-35,000 for the devices and between $50-200 for individual exams. The accuracy and reliability of laboratory techniques depends on the specific device being used, although they are generally accepted as more accurate tools compared to field methods (Ackland et al., 2011). Laboratory techniques include dual energy x-ray absorptiometry (DXA) scans, hydrostatic weighing (HW), air displacement plethysmography (ADP), body water measurement, and ultrasound (US).

Dual Energy X-ray Absorptiometry

Dual energy x-ray absorptiometry (DXA) scans are most commonly used for osteoporosis screenings but can also be used for both whole-body and regional BC analyses (Bradella et al., 2010; Bazzocchi et al., 2014). DXA scans emit low-dose x-rays

at two different photon energy levels through the body and records the amount of energy absorbed by soft tissue content (Prado & Heymsfield, 2014; Andreoli et al., 2016; Heymsfield et al., 1997). This assessment uses a 3C model that divides the body into FM, non-bone lean mass, and bone mineral (Bazzocchi et al., 2013).

DXA scans are often referred to as a "gold-standard" BC measurement tool, as results strongly correlate with CT scans, MRI scans, and cadaver analyses in healthy adult populations (Santos et al., 2010; Fuller et al., 1999; Kim et al., 2002; Prado & Heymsfield, 2014; Pritchard et al., 1993; Ackland et al., 2011; Bredella et al., 2010). DXA scans also produce %BF results that are strongly correlated with a 4-compartment model (r=0.94, SEE=2.8%), with no significant differences between the two methods (Prior et al., 1997). However, DXA appears to be a less reliable measurement tool in both extremely lean and obese individuals, as prior studies found significant trends for FM overestimation in lean subject and underestimation in obese subjects, indicating practitioners should be cautious when using this as a gold-standard measure for these populations (Prior et al., 1997; Santos et al., 2010; Van der Ploeg et al., 2003).

DXA scans also appear to overestimate lean mass compared to CT and MRI in extremely lean populations including pre-pubertal children, elderly populations, and high-level athletes, likely due to tissue density variations compared to general population adults (Levine et al., 2000; Bredella et al., 2010; Kim et al., 2006; Gallagher et al., 2000). It is imperative that DXA scans be conducted in the fasted state and that hydration status is accounted for (Tinsley et al., 2017; Tomcik et al., 2018). Tinsley et al. (2017) reported that DXA overestimated LBM by 2.8% and underestimated FM by 2.2% when subjects ingested a meal prior to testing. Additionally, acute body water fluctuations influence

lean tissue mass results and alter %BF by up to two percent (Horber et al., 1992; Rodriguez-Sanchez & Galloway, 2014; St-Onge et al., 2004). However, Cutrufello et al. (2017) concluded that hydration status did not influence DXA results in a sample of college-aged males, but there are very few studies that support this finding.

Concerns regarding hydration should be considered, especially when working with weight-class athletes who often lose extreme amounts of body water to compete in a specific weight-class and may limit this method's accuracy in these populations (Prior et al., 1997; Ackland et al., 2011). DXA scans are also limited to either laboratory or medical settings due the large, expensive equipment (Andreoli et al., 2016; Meyer et al., 2013; Heymsfield et al., 1997).

Densitometry

Densitometry, most notably hydrostatic weighing (HW) and air displacement plethysmography (ADP), uses a two-compartment model to determine FM and FFM. These techniques measure body volume, which in conjunction with body mass, is used to determine body density (D_b) based on assumed constant tissue densities for FFM (1.095 g/cm^3) and FM (0.900 g/cm^3) (Andreoli et al., 2016; Tobia et al., 1956; Heymsfield et al., 2015; Behnke et al., 1942). Body density is then used to calculate %BF using previously validated population-specific equations based on age, gender, and ethnicity to account for differences in FFM density (Wells & Fewtrell, 2006; Jackson et al., 2002).

Hydrostatic weighing was developed based on Archimedes' principle, which states the volume of water displaced by a submerged object is equal to the volume of the object (Behnke et al., 1942; Wagner et al., 1999). Subjects are weighed after expelling as

much air as possible from the lungs while completely submerged underwater (Utter et al., 2008). This procedure has been considered the "gold-standard" BC method and has been used as the criterion measure since the 1940s (Clasey et al., 1999; Gibby et al., 2017; Demerath et al., 2002). Hydrostatic weighing results are strongly correlated with those from CT scans, 4-compartment models, DXA, and ADP in adults, although ADP may be both preferential and a more suitable alternative for some populations (Clasey et al., 1999; Gibby et al., 2017; Demerath et al., 2002). However, the procedures are difficult for some people to perform, especially children, leading to measurement errors (Gibby et al., 2017).

The subject must remain completely still while underwater in order to obtain accurate weight measurements. Additionally, residual lung volume (RLV) must be accounted for, as it is impossible to expel all air from the lungs. However, there will be significant measurement errors if subjects fail to exhale completely due to inaccurate RLV estimates (Utter & Hager, 2008; Wagner et al., 1999). Body fat is significantly overestimated compared to 4-C and DXA and underestimated compared to CT when errors occur from either inaccurate RLV estimates or inaccurate underwater weight measurements (Gibby et al., 2017; Clasey et al., 1999; Claros et al., 2005; Lockner et al., 2000; Wagner et al., 1999).

Air displacement plethysmography (ADP) is often preferential to HW due to faster, automated, and less invasive exam procedures (Vescovi et al., 2001; Fields et al., 2002). Overall, there appears to be good agreement between $\%BF_{ADP}$ and HW, with results ranging from 1-2% of each other, indicating ADP may be a valid alternative procedure (Vescovi et al., 2002; Fields et al., 2002; Vicente-Rodriguez et al., 2009). ADP

uses a sealed chamber with known air volume and small pressure changes to indirectly measure body volume based on air displacement (Fields et al., 2002). The first ADP systems measured air inside the chamber using Boyle's Law, which states that pressure and volume are inversely related at a constant temperature (Fields et al., 2002). There is currently only one commercially available ADP device, known as the BOD POD® (COSMED, Concord, CA) used for these assessments. Rather than depend on constant temperature, as required to apply Boyle's law, this system relies on Poisson's Law, which describes volume and pressure relationships when temperature is not held constant (Fields et al., 2002).

The BOD POD® has strong inter-rater, intra-rater, and inter-day reliability for assessing both body volume and %BF, as well as strong correlations to DXA and MCM in healthy adults (Fields et al., 2002; Biaggi et al., 1999; Levenhagen et al., 1998). Despite strong correlations between methods, the BOD POD® appears to overestimate FFM and underestimate %BF compared to DXA and MCM, except in a sample of black males, where %BF was significantly overestimated compared to DXA (Fields et al., 2002; Vicente-Rodriguez et al., 2009; Delisle-Houde et al., 2017; Lockner et al., 2000; Wagner et al., 2000). %BF underestimations appear to be more common in lean individuals and muscular athletes, likely due to FFM density variations. Higher water content and lower mineral and protein proportions lowers FFM density, leading to inaccurate predictions, as the BodPod assumes a constant FFM density (Fosbøl & Zerahn, 2015)

Different population-specific equations based on age, sex, and ethnicity attempt to account for FFM density variations but are still limited by constant density assumptions

for any given age and sex (Wells & Fewtrell, 2006; Fields et al., 2002; McCrory et al., 1995; Ball et al., 2005; Ginde et al., 2005; Anderson et al., 2007). Additional limitations include high costs, lack of portability, consistent hydration, and normal BMD assumptions (Collins et al., 1999; Vescovi et al., 2002; Wells & Fewtrell, 2006). However, overall, researchers concluded that ADP is a valid and reliable BC method that requires minimal subject involvement and is often preferable to HW (Wells & Fewtrell, 2006).

Hydrometry/Isotope Dilution (Body Water)

Total body water (TBW) is measured using a stable isotope tracer, usually deuterium oxide, to estimate FM and FFM under the assumption of constant FFM hydration levels between 72-73% (Ackland et al., 2011). This process requires minimal subject involvement and the procedures are simple, making it beneficial in clinical and laboratory settings, especially when working with children (Wells & Fewtrell, 2006). While this method is safe and practical for field use, it requires expensive equipment and is limited by assumed constant FFM hydration levels between individuals (Ackland et al., 2011; Prado & Heymsfield, 2014; Ward, 2019).

Ultrasound

Ultrasound (US) has been used in clinical settings for BC purposes for many years because of its ability to quantify tissue thicknesses, but recent technological advances have resulted in smaller and more portable devices, making them accessible for both laboratory and field settings (Booth et al., 1966; Prado & Heymsfield, 2014). US

uses pulse-echoes to calculate the distance between different tissues with varying levels of acoustic impedances, such as skin, adipose, muscle, and bone (Ackland et al., 2011; Prado & Heymsfield, 2014). The most common US devices used in BC research are amplitude- (A) mode and brightness- (B) mode (Wagner, 2013). A-mode US (A-US) devices determine tissue thickness by calculating the time it takes for a single US pulse-echo beam to travel through tissues and be reflected back to the transducer. These devices display signal amplitudes, with peaks representing tissue boundaries such as adipose-muscle and muscle-bone interfaces (Wagner, 2013; Prado & Heymsfield, 2014). B-mode US (B-US) devices use multiple pulse echo signals that create a 2D image when reflected back to the transducer (Prado & Heymsfield, 2014; Wagner et al., 2013). Adipose-muscle and muscle-bone tissue boundaries are identified on the 2D image and SAT thickness can be measured (Prado & Heymsfield, 2014; Wagner, 2013).

Using US to assess body composition follows the same principles of skinfold calipers (SFC) as SAT is assessed and used to calculate D_b, which is used to estimate %BF. However, the ability to provide regional and whole-body assessments makes US a more advanced tool than other methods. This is especially true when regional assessments are more important than whole-body, such as in children who are still growing and when determining disease risk based on differentiating between SAT and VAT. B-mode devices are more expensive than A-US as they are more technologically advanced and can be used to track regional muscle thickness and cross-sectional area changes over the course of a training program (Moreno et al., 1997; Goran et al., 1998; Bazzocchi et al., 2011; Schlecht et al., 2014; O'Neill et al., 2016). While US appears to provide accurate SAT measurements, more research is needed to establish standardized

measurement protocols (Ackland et al., 2011). Details regarding ultrasound validity will be discussed in the next section.

Field Techniques

Field methods are the most accessible ways to measure body composition, as they are inexpensive, portable, and simple to use. However, many factors, including technician experience level, device pre-set equations, and subject's hydration and electrolyte status, influence both accuracy and reliability of these methods.

Bioelectrical Impedance Analysis (BIA)

Bioelectrical impedance analysis (BIA) devices use small, electrical currents at different frequencies to measure impedance to electrical flow. This method is based on the principle that FFM is a better electrical conductor than FM due to greater water and electrolyte content (Heymsfield et al., 1997; Siervo et al., 2010; Lee et al., 2008; Prado & Heymsfield, 2014; Ackland et al., 2011; Wells & Fewtrell, 2006). The first BIA devices were introduced in 1985 and are known as single-frequency (SF) because they measure impedance at a single current frequency of 50 kHz (Ward, 2019; Lukaski et al., 1985). Technological advances led to multi-frequency BIA (MF-BIA) devices that measure impedance at multiple fixed frequencies, as well as over a range of frequencies, improving TBW predictions used to estimate FFM (Ward, 2019). There are both whole-body and segmental MF-BIA devices, and devices differ by type of electrodes used.

Impedance devices may not be the most accurate assessment tools, as they have been found to significantly underestimate FFM and overestimate FM in obese

populations and overestimate FFM and underestimate %BF and FM in healthy adults compared to DXA scans (Hofsteenge et al., 2015; Anderson et al., 2012; Antonio et al., 2019). Additionally, Duz et al. (2009) reported that A-US was a better tool than BIA to determine %BF in healthy adults ages 18-26 years old. However, Volpe et al. (2010) reported no differences between %BF assessed by BIA and HW in a sample of male bodybuilders, and Ling et al. (2011) reported strong correlations between MF-BIA and DXA in healthy adults, suggesting that BIA is valid BC assessment technique in these populations. However, Ling et al. (2011) found higher correlations for FFM, as opposed to %BF, which is consistent with other findings indicating that impedance devices can accurately estimate FFM from TBW in healthy adults (Price & Earthman, 2018; Utter & Lambeth, 2010).

Most BIA devices use proprietary algorithms and equations not made public by companies, meaning researchers do not know how %BF and FFM are calculated. This is a major limitation when using these devices because the user cannot control which equations are used and cannot know if the preset algorithms are appropriate for the populations being tested. While proprietary equations appear to be accurate in various adult populations, including healthy, overweight, obese, and elderly subjects, they may not be accurate for other groups (Sartorio et al., 2005). However, using population-specific equations can improve BIA measurement accuracy, as Moon et al. (2008) found strong correlations (r=0.91) to a 3C-criterion model when using Lohman equations based on the BIA's reactance measures (Lohman, 1992).

One of the reasons population-specific equations improves BIA accuracy is because these devices assume constant FFM hydration levels and constant resistance in

any given tissue in all individuals (Prado & Heymsfield, 2014; Ackland et al., 2011; Siervo et al., 2010; Lee et al., 2008; Ward, 2019). Acute fluid intake, dehydration, food intake, prior exercise status causing fluid and electrolyte shifts, and electrolyte imbalances all cause significant measurement error when using BIA due to the changes in FFM tissue hydration (Duz et al., 2009; Antonio et al., 2019; Ackland et al., 2011). One study found acute food and fluid intakes prior to BIA testing resulted in decreased impedance values by 4.4%, leading to 1.8% lower FM, 2.5% lower %BF, and 2% greater TBW values (Tinsley et al., 2017). However, these values all returned to near-baseline levels following an overnight fast, but only TBW was not significantly different between the two tests in the fasted condition, indicating weak test-retest reliability using BIA (Tinsley et al., 2017).

While decreased measurement accuracy is a major limitation of BIA, MF-BIA devices can be used to reliably track body composition changes over time and estimate TBW for MCM equations (Antonio et al., 2019; Wabel et al., 2009; Cornish et al., 1992). Effectively tracking changes over time, such as during an athletic season or exercise program, may be more valuable than accurate measurements, making these often inexpensive, portable, and simple devices useful in field settings (Heymsfield et al., 1997; Antonio et al., 2019).

Anthropometry

Anthropometry includes various physical body measurements such as height, weight, breadth, circumferences, and skinfold thickness in order to estimate D_b, FM, and FFM (Siervo et al., 2010; Garn et al., 1962). While these methods are inexpensive and

portable, the accuracy and validity of results are limited by technician technique, anatomical locations measured, and equations selected for calculations (Prado & Heymsfield, 2014; Ackland et al., 2011; Hume et al., 2008).

The simplest anthropometric measure is body mass index (BMI), calculated as weight (kg) divided by height squared (m^2). Various equations exist to estimate %BF from BMI (Lee et al., 1981; Benn et al., 1971). While the BMI measurements themselves may be accurate, the extrapolation to body fat levels is limited, as it is well known that amount of adipose tissue varies between individuals with the same BMI (Ackland et al., 2011; Nevill et al., 2010). BMI to %BF equations assume that weight change is due to change in adipose and do not account for changes in FFM, such as muscle (Ackland et al., 2011; Nevill et al., 2010; Norgen et al., 1994). This method is best suited for large-scale data collection in the general population due to minimal subject involvement and relatively quick calculations. However, this method is not applicable to athletic populations due to the lack of accountability for muscle mass changes (Ackland et al., 2011; Nevill et al., 2010).

Circumference measures, especially waist circumference, are used to evaluate obesity and disease-risk. Research suggests that waist circumference and waist-to-hip ratio measurements may be more adequate than BMI when evaluating for obesity-related disease. Additionally, circumferences can be used in conjunction with regional subcutaneous adipose tissue measurements to provide additional regional information (Lopez-Taylor et al., 2018; Zuti & Golding, 1973; Garcia et al., 2005).

Skinfold calipers (SFC) are the most widely reported BC analysis method, as calipers are inexpensive, portable, and easy to use (Meyer et al., 2013). Most practitioners

follow either Jackson-Pollock protocols or the standardized procedures described by the International Society for the Advancement of Kinanthropometry (ISAK) (Meyer et al., 2013). While SFC can be useful to assess regional SAT and track changes over time, the equations to estimate %BF based on these measurements are not highly accurate, with the best standard estimate of error (SEE) reported to be ±3.3%, but some studies report limits of agreement as wide as ±9% BF (Ackland et al., 2011; Lohman, 1981; Ball et al., 2006; Wells & Fewtrell, 2006). However, population-specific equations to calculate D_b and %BF can improve SFC accuracy (Ackland et al., 2011; Wells & Fewtrell, 2006). Lopez-Taylor et al. (2018) found no significant differences between 12 of 31 SFC equations and $\%BF_{DXA}$ in professional male soccer players, with the most accurate SFC equations being those described by Oliver et al. (2012), Ball et al. (2004), and Wilmore & Behnke (1969). These findings indicate SFC can be a useful alternative to DXA scans when technicians are adequately trained and proper prediction equations are selected (Lopez-Taylor et al., 2018). Limitations to using SFC to assess BC include both inter- and intra-rater reliability, invalid assumption that SAT at measured sites adequately represents total body adipose tissue, inaccurate population-specific equations, and the exclusion of measured body mass in prediction equations (Wells & Fewtrell, 2006; Ball et al., 2006). However, intra-rater reliability can be strong with proper training and exam practice in normal-weight individuals (Müller et al., 2013; Wells & Fewtrell, 2006). Additionally, Ball et al. (2006) recommended that practitioners use SFC to track changes, but record the raw measurement data, rather than use measurements to calculate %BF, in order to more accurately assess weight changes.

Summary of Methods

Reference methods, although extremely accurate, are not feasible to use in most cases due to high costs and extensive subject involvement. However, these tools are important, as they are used to validate more accessible laboratory and field techniques. Laboratory methods, such as DXA and densitometry, are more accurate than field methods but often limited to specialized facilities. Additionally, these methods may not be accurate in certain populations, as they use tissue density equations based on population means. While field-based tools, such as SFC and BIA, are the least expensive and easily portable, their validity and reliability are not always strong enough to yield useful results. However, with recent technological advances, US is proposed as a relatively inexpensive laboratory tool that is portable and may be applicable to field settings.

Ultrasound in Body Composition

Ultrasound has been used for BC and adipose tissue measurements for many years (Booth et al., 1966; Bazzocchi et al., 2016). Ultrasound uses sound waves to produce pulses in order to send signals from the transducer through tissues (e.g., skin, adipose, muscle, bone, etc.), which are reflected back to the transducer based on tissue acoustic impedance levels (Wagner et al., 2013). Tissue density determines the amount of acoustic impedance, allowing the differentiation between fat-muscle and muscle-bone interfaces (Wagner, 2013). While various US devices exist, the types most often used for BC assessments are A-US and B-US.

A-mode US transducers produce a waveform with peaks at the interface of two different tissues, such as the border of SAT and muscle tissue, rather than a physical image of tissues (Wagner et al., 2016). A-mode US devices are less expensive than B-US devices and require less technical expertise, as no image needs to be evaluated. Additionally, there are A-US transducers that were specifically designed for measuring BC, such as the BodyMetrix BX-2000 (IntelaMetrix, Inc., Livermore, CA, USA).

B-mode US combines various A-mode signals to produce a 2D image of the tissues, allowing practitioners to measure SAT rather than assessing tissue discontinuity alone (Wagner, 2013; Noce, 1990). Subcutaneous fat is measured as the perpendicular distance between the upper border of the dermal-adipose interface and the upper border of the adipose-muscle interface at specific anatomical sites (Toomey et al., 2011; Bazzocchi et al., 2016).

There are currently no standardized guidelines regarding the anatomical sites that should be measured to most accurately predict %BF via US. However, it is common to use either the ISAK or the Jackson-Pollock skinfold locations for measurement sites (Bazzocchi et al., 2016). These protocols are selected due to the validated D_b and %BF equations from SFC measurements, which can be modified for US measures.

Ultrasound and Reference Techniques

While cadaver studies are limited due to inherent limitations, one study found high correlations between both A-US and B-US and cadaver measurements, indicating both methods are equally able to accurately assess %BF (Wagner et al., 2018). Mean differences in fat-thicknesses between A-US and B-US were <0.7 mm at all sites except

for the calf (Wagner et al., 2018). Additionally, Pearson correlation coefficients were all >0.90 between the three methods (Wagner et al., 2018). However, only six cadavers were evaluated, and the mean age was 80.8 years old, which limits the application to athletic populations (Wagner et al., 2018). Due to the small sample size and limited generalizability to younger populations, US must be compared to other measurement tools in order to establish validity of the technique.

MRI is a time-consuming, expensive procedure but does not expose the subject to high radiation as CT scans do (Ackland et al., 2011). Benefits of MRI include the ability to measure skeletal muscle mass, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT), which can have clinical relevance for obesity-related diseases (Sanada et al., 2006; Schlecht et al., 2014). When individual anatomical site measurements were compared, MRI measured skeletal muscle was highly correlated (R^2=0.94) with B-mode US in healthy adults at nine locations (Sanada et al., 2006). B-mode US measures of VAT and SAT thickness at the abdomen are also highly correlated with MRI measures (ICC_{VAT} = 0.990; ICC_{SAT} = 0.976) (Schlecht et al., 2014). The mean differences (MD) in measured thickness between MRI and B-US were small (MD_{VAT} = -0.124mm ±3.21mm and MD_{SAT} = 0.392mm ±1.96mm). However, when subjects were evaluated by BMI, the correlations between methods were higher among non-obese compared to obese individuals (Schlecht et al., 2014).

Ultrasound may not be a valid tool in overweight or obese individuals, as it appears to largely underestimate %BF compared to a 3C model in overweight and obese adults (Smith-Ryan et al., 2014). In this study, D_b measured via ADP and TBW via BIA were used to calculate %BF, which was then compared to results from A-US using the 7-

site Jackson-Pollock protocol and modified equations (Smith-Ryan et al., 2014; Jackson and Pollock, 1978; Gallagher et al., 2000). However, when the same methods were applied to collegiate male football players, researchers found significant positive correlations between $\%BF_{AUS}$ and $\%BF_{3C}$ (Hyde et al., 2016). Although ADP measures were conducted in both of these studies, the researchers did not report $\%BF_{ADP}$, which would be a useful addition in terms of validating US (Hyde et al., 2016; Smith-Ryan et al., 2014).

Ultrasound and Laboratory Techniques

Many factors may influence the accuracy of BC assessments via US, such as the device, scanning protocol, or prediction equations selected. Pineau et al. (2007) found no significant differences in %BF between A-US (BOX US; Lecoeur Electronique Company, Chuelles, France) and DXA using a two-site US scanning protocol, while other studies reported the A-US device BodyMetrix BX2000 (IntelaMetrix, Livermore, CA, USA) did not produce valid results compared to DXA (Johnson et al., 2014; Loenneke et al., 2014). Pineau et al. (2007) reported strong correlations between A-US and DXA for all subjects ($r=0.98$) using a scanning protocol that incorporated both sides of body at the lower back and thigh, while Johnson et al. (2014) found results from a seven-site Jackson-Pollock protocol significantly underestimated %BF compared to DXA. However, %BF differences were no longer significant when males and females were analyzed separately (Johnson et al., 2014).

Conflicting results between A-US and DXA are also reported among athletes, as Pineau et al. (2009) reported strong agreement between A-US and DXA, but Loenneke et

al. (2014) found both one-site and three-site A-US protocols significantly overestimated %BF compared to DXA in collegiate gymnasts. While there were moderate correlations between results for 1-site (r=0.786) and 3-site (r=0.753) A-US protocols and DXA, the average deviations from the line of identity were 6.7% and 4.9% for the 1-site and 3-site protocols, respectively (Loenneke et al., 2014). However, there are higher correlations between A-US and DXA when newly developed regression equations are used to calculate %BF rather than modifying existing SFC-derived equations (Loenneke et al., 2014; Johnson et al., 2014; Bielemann et al., 2016; Pineau et al., 2007; Pineau et al., 2009; Pineau et al., 2010; Pineau et al., 2013).

There is limited research comparing US to densitometry, but what studies have been done suggest that both new prediction equations and modified SFC equations produce valid BC results using A-US (Bielemann et al., 2016; Pineau et al., 2007; Wagner et al., 2016; Utter & Hager, 2008). Multiple studies found strong correlations between $\%BF_{AUS}$ and $\%BF_{ADP}$ using modified Jackson-Pollock equations in both athletic and non-athletic adults (Schoenfeld et al., 2016; Wagner et al., 2016; Bielemann et al., 2016; Pineau et al., 2007). Schoenfeld et al. (2016) reported no differences between %BF and FFM from A-US using a four-site Jackson-Pollock protocol and ADP in females. However, $TE_{\%BF}$ ranged from 3.77-4.17, while TE_{FFM} was lower, ranging from 2.31-2.68 (Schoenfeld et al., 2016). In contrast, Wagner et al. (2016) found A-US significantly overestimated %BF compared to ADP in female, but not male, athletes when using a three-site Jackson-Pollock protocol. It is possible that B-US is a more valid tool than A-US compared to ADP, but the relationship between these devices is yet to be investigated.

While A-US may not accurately determine BC using SFC-derived equations, B-US may be a more valid alternative. Previous studies reported strong correlations between both site-matched B-US and DXA SAT measures and %BF using population-specific regression equations (Midorikawa et al., 2011; Leahy et al., 2012; O'Neill et al., 2016; Duz et al., 2009). However, to our knowledge, no study has applied SFC-derived equations to B-US to compare %BF to either ADP or DXA, so the utility of these equations is still unknown.

Ultrasound and Field Techniques

The most common BC assessment tools used in field settings are SFC and BIA due to portability and relatively low costs (Meyer et al., 2013). However, US produces more accurate and reliable results than both SFC and BIA compared to laboratory methods including ADP, HW, and DXA (Utter and Hager, 2008; Wagner et al., 2016; Duz et al., 2009; Pineau et al., 2007). While both $\%BF_{SFC}$ and $\%BF_{US}$ were strongly correlated to $\%BF_{ADP}$, Utter & Hager (2008) found a higher relationship between FFM from A-mode US and HW compared to FFM_{SFC} and FFM_{HW} results. Other researchers found strong agreement between $\%BF_{US}$ and $\%BF_{DXA}$ but found significant differences between SFC and BIA compared to DXA in the same subjects (Pineau et al., 2007; Duz et al., 2009).

The accuracy and reliability of SFC is largely dependent on the technician performing the exam, leading many to question if the same is true for US. While %BF measured by US and SFC are strongly correlated with each other, there is less variation between technician measurements using US compared to SFC (Wagner et al., 2016;

Müller et al., 2013; Hyde et al., 2016; Fanelli & Kuczmarski, 1984; Selkow et al., 2011; Ulbricht et al., 2012). In addition to less inter-rater variability with US compared to SFC, there are additional benefits to US imaging, such as determining fat patterning and muscle thickness, as well as less measurement variations compared to SFC (Müller et al., 2013; Wagner et al., 2016).

Summary

There are many BC assessment techniques available, but costs, subject involvement, degree of technician expertise required, and logistics should all be considered when selecting a method (Ackland et al., 2011). Reference methods are often not suitable for athletes due to the high costs and length of time, and laboratory techniques are generally limited to those with access to facilities with the appropriate equipment. These barriers lead most coaches and practitioners to select easily affordable methods, such as BIA and SFC, limiting the accuracy and reliability of the results (Meyer et al., 2013). However, US is proposed as a relatively inexpensive laboratory tool that can be used in field settings and offers advantages over traditional field tools.

Ultrasound is a versatile tool that can be used for both total-body BC assessment, as well as regional analyses. These devices can be used to assess regional fat patterning and FFM changes, as well as differentiate between VAT and SAT. Ultrasound appears to provide accurate %BF results compared to traditional tools including DXA, BIA, and SFC, but more research is needed on the accuracy of these devices, especially in athletic populations.

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Chapter 2

Validity of Ultrasound to Assess Body Composition in Adolescent Ballet Dancers

By

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Introduction

Body composition (BC) measurements are valuable to athletes and coaches, but there is a need for easy to use, portable, and accurate tools for these populations. The most accurate reference and laboratory devices are unavailable to most coaches and athletes, while field methods typically have a large degree of error. Although skinfold calipers (SFC) are the most common BC assessment tool used in field-settings, there is a high measurement error rate of up to ±9%, and weak reliability compared to dual energy x-ray absorptiometry (DXA) (Meyer et al., 2013; Ackland et al., 2011). Bioelectrical impedance analysis (BIA) is another low-cost, easy to use technique, but devices are highly sensitive to hydration and electrolyte status, which often leads to inaccurate results (Ackland et al., 2011). Athletes and coaches may prefer using laboratory methods rather than field methods due to the increased accuracy and reliability compared to SFC and BIA. However, laboratory-based techniques, such as air displacement plethysmography (ADP) and DXA, are expensive, require technical expertise, and cannot be used in the field. Ultrasound (US), though, is proposed as a valid laboratory method that can also be used in field settings, as it is now relatively inexpensive and portable.

While US devices have been used in BC research for many years, recent technological advances have led to more affordable and portable tools that can be used in clinical and field settings (Booth et al., 1966; Ackland et al., 2011). US is proposed as a more accurate and reliable BC assessment method compared to SFC and BIA due to reduced inter-rater variability and higher agreement with laboratory techniques (Selkow et al., 2011; Müller et al., 2013). While studies show higher correlations between laboratory techniques and US compared to BIA or SFC, the validity in athletic

populations needs to be further studied, as using US in field settings is still a relatively new BC assessment (Booth et al., 1966; Ackland et al., 2011; Wagner et al., 2016; Duz et al., 2009; Müller et al., 2013).

US is strongly correlated to both site-matched DXA and whole-body DXA measurements in various populations (Leahy et al., 2012; O'Neill et al., 2016). Additionally, the correlations between US and DXA are higher than those between both SFC or BIA and DXA scans, indicating US is possibly a more accurate alternative to these techniques (Duz et al., 2009; Wagner et al., 2016; Pineau et al., 2007). The type of US used may impact validity, as B-mode provides a more in-depth assessment due to the multiple beams and 2D image, as opposed to the single scanning beam produced by A-mode devices.

While the literature on US and ADP is conflicting, it is important to note that all of these studies used A-mode US (Pineau et al., 2007; Wagner et al. 2016; Schoenfeld et al., 2016; Bielemann et al., 2016). A-mode US results are correlated to ADP results in both collegiate athletes and healthy, non-athlete adults, but A-US was not shown to be a valid tool to predict %BF in a sample of collegiate gymnasts when using a 3-site scanning protocol (Pineau et al., 2007; Schoenfeld et al., 2016; Wagner et al., 2016; Bielemann et al., 2016; Loenneke et al., 2014a). It is possible that different US protocols can increase A-mode US device accuracy, and that B-mode US may be a valid alternative to A-mode US in similar populations. However, no study has investigated the relationship between ADP and B-mode US to assess %BF in an athletic population, and there are limited data comparing both B-US and A-US to a more accurate three-component (3C) model. Therefore, this study aimed to examine both the validity of B-US compared to

ADP, as well as to investigate the validity of various BC assessment tools compared to a 3C model in a subset of the sample.

The primary purpose of this study was to determine the validity of B-mode ultrasound compared to ADP to assess body composition in high-level, adolescent male and female ballet dancers. A secondary aim of this study was to further assess the validity of additional field-based BC assessment devices, A-US and BIA. Additionally, this study aimed to compare these laboratory and field methods to a 3C-criterion reference method. We hypothesized that using B-US will produce accurate %BF, FM, and FFM measurements compared to ADP in this population of male and female adolescent and young adult ballet dancers. Additionally, we hypothesized that B-US would be a more accurate tool than both A-US and BIA, but that it would produce similar results to ADP when compared to a 3C reference model.

Methodology

Study Design

This study was designed to assess the validity of using US to measure BC compared to both ADP and a 3C criterion reference model in adolescent and young adult ballet dancers. Prior to data collection, the Institutional Review Board (IRB) at Rutgers University approved all study methods and materials. Subjects had their body composition assessing using multiple devices in order to compare %BF, FFM, and FM results between both laboratory and field tools.

Subjects

Forty-eight vocational ballet dancers (male: n=21; female: n=27) were recruited to participate in this study. All subjects were between ages 12-20 years old and deemed to be in good physical health by a physician. Participants self-reported their date of birth, provided written informed consent prior to data collection, and parental assent was obtained when necessary. Subjects were informed of the research protocol's risks and benefits prior to giving informed consent during the first study visit. Subjects were excluded if they met any of the following criteria: pregnant or lactating, musculoskeletal injuries that would interfere with testing protocols, history of metabolic disease or disorders. Subject characteristics for Study 1 are described in Table 1. A second study was conducted using a subset of subjects (n=22) who participated in the first study to further assess BC tools. Characteristics for subjects in the Study 2 are described in Table 2.

Procedures

Study 1: Subjects reported to the Rutgers University Center for Health and Human Performance for performance testing normally hydrated, at least 2 hours fasted, and without having exercised for 24 hours before testing. Body composition was first assessed using ADP (BOD POD®; LMI, Concord, CA) (Fields et al. 2002). The BOD POD® was calibrated at the beginning of each testing day, as well as before each individual test, as specified by the manufacturer guidelines. Subjects were tested wearing non-padded compression shorts and sports bra and a swim cap. Total body mass was measured, and FM, FFM, and %BF were all determined using the Siri density equation in

the BOD POD® system (Siri, 1961). The error of body volume reading is roughly 0.02%, which allows for calculation of %BF with only 0.01% error (Dempster & Aitkens, 1995).

Next, a trained technician conducted BC assessments using B-US with the Philips Lumify L12-4 broadband linear array transducer (Philips Lumify®, Koninklijke Philips B.V., Netherlands) and associated tablet (Galaxy, Samsung Electronics Co., LTD., Seoul, South Korea). The technician scanned each subject at seven anatomical locations, which corresponded to the Jackson-Pollock SFC locations, in the following order: pectoralis, subscapular, triceps, midaxillary, suprailiac, abdomen, and anterior thigh. The technician conducted two exams on each subject before uploading images to a cloud-based software system (MuscleSound®, Denver, CO, USA) to measure SAT in millimeters (mm). MuscleSound® software used modified Jackson-Pollock equations to calculate body density (D_b), from which %BF was calculated using the Siri equation to determine %BF, FFM, and FM for each subject (Jackson & Pollock, 1985; Siri, 1961). The modified Jackson-Pollock equation for males is: $D_b = 1.112 – (0.00043499*(\Sigma SAT*2)) + (0.00000055*(\Sigma SAT*2)^2) –(0.0002882*age)$ and females is: $D_b = 1.112– (0.00046971*(\Sigma SAT*2)) + (0.00000056*(\Sigma SAT*2)^2) –(0.00012828*age)$. The Siri equation used to calculate %BF is: %BF= (4.95/Db-4.50)*100. Body mass determined by the BOD POD® system was used for US calculations.

Study 2: A subset of subjects (n=22) returned for additional BC testing on a separate day. Subjects first completed ADP testing as described above, followed by MF-BIA assessments using the InBody 770 (InBody, Cerritos, CA, USA), which is a direct segmental MF-BIA device that measures impedance at six different frequencies. The InBody electrodes were wiped down with wipes provided by the manufacturer prior to

teach test to ensure optimal electrical conductance. Then technicians assessed each subject using B-US, as previously described, and A-US using BodyMetrix (IntelaMetrix, Livermore, CA, USA). The technician scanned subjects using A-US at the same seven anatomical locations used for B-US assessments and used the same modified Jackson-Pollock equations that are preset in the software to determine %BF, FM, and FFM. Body density determined by BOD POD® and TBW calculated by MF-BIA were used to determine %BF, FM, and FFM by Siri's 3C equation, defined as $\%BF = [(2.118/D_b - (0.78*TBW/BM)) - 1.354]*100$, where TBW is total body water in liters, and BM is body mass in kilograms (Siri, 1993). The 3C-criterion equation results were used as the reference standard for the sub-study, as previous studies use as a reference measure (Smith-Ryan et al., 2014). Body mass determined by the BOD POD® system was used for 3C, B-US, and A-US calculations, while the body mass determined by the InBody 770 was used for BIA calculations.

Data Analysis

The data were analyzed using IBM SPSS 25.0 software (Armonk, NY, USA). The mean, standard deviation (SD), and standard error of the mean (SEM) were calculated for %BF, FM, and FFM results for all devices. Constant error (CE=estimated-actual), standard estimate of error ($SEE = SD*\sqrt{1-r^2}$) and total error ($TE = \sqrt{\Sigma[\text{estimated-actual}]^2/n}$) were calculated for all experimental devices in order to assess validity against the criterion methods. Statistical significance was set at $P<0.05$ for all analyses.

Pearson product moment correlation analyses were used to determine strength of the relationship between %BF from ADP ($\%BF_{ADP}$) and B-US ($\%BF_{BUS}$), FFM from ADP (FFM_{ADP}) and B-US (FFM_{BUS}), and FM from ADP (FM_{ADP}) and B-US (FM_{BUS}).

Paired sample t-tests were used to examine differences between ADP and B-US for the entire sample and with sex as a covariate. Linear regression analyses were performed to assess %BF, FFM, and FM agreement between ADP and B-US. Bland-Altman analyses were conducted to assess the 95% limits of agreement (LOA) between B-US and ADP in determining %BF, FM, and FFM.

In Study 2, Pearson product moment correlation analyses were used to determine the strength of the relationship for %BF, FM, and FFM between the 3C-criterion model and ADP, B-US, A-US, and BIA. Correlation analyses were also used to determine the relationship between ADP and B-US, A-US, and BIA, as ADP is often considered a laboratory reference device (Gibby et al., 2017). Dependent t-tests were used to compare mean differences in %BF, FFM, and FM between the 3C-criterion model and each device, as well as between ADP and B-US, A-US, and BIA. Linear regression analyses were performed to assess the agreement between devices and Bland-Altman plots were used to identify the 95% LOA between the 3C-criterion measurements and predicted measurements from ADP, B-US, A-US, and BIA ADP in determining %BF, FM, and FFM. Linear regression analyses and Bland-Altman plots were also used to assess the agreement of B-US, A-US, and BIA with ADP in determining %BF, FM, and FFM.

Results

Study 1

The primary study resulted in significant, high correlations between B-US and ADP for %BF (males: r=0.773; females: r=0.941; P<0.01), FFM (males: r=0.985; females: r=0.954; P<0.01), and FM (males: r=0.726; females: r=0.943 P<0.01). FFM correlations were the highest for both sexes, but FM and %BF correlations were higher

among females than males. Additionally, while CE, SEE, and TE were lower for both FM and FFM compared to %BF in both sexes, %BF-TE was lower in males than females (TE_{males}=2.69; $TE_{females}$=3.13) (Table 4).

There were narrower 95% LOA for FFM and FM compared to %BF in both sexes, although they were slightly narrower in females compared to males (Table 3). However, 95% LOA for %BF were similar between sexes (Table 3). Additionally, Bland-Altman plots indicated that B-US overestimated %BF and FM in subjects with lower %BF, and underestimated %BF and FM in subjects with higher %BF levels (Figures 3A-C). However, paired sample t-tests revealed no significant differences between B-US and ADP for any measures in males, but B-US significantly overestimated %BF (+1.6%) and FM (+0.7kg) and significantly underestimated FFM (-0.8kg) among female subjects ($P<0.01$; Table 3; Figure 1A-C).

Study 2A

Results from B-US, A-US, and BIA were compared to ADP in a separate analysis. B-US overestimated %BF and FM compared to ADP, but these differences did not reach statistical significance in females. There were significant, high correlations between ADP and all other devices in females, although correlations between ADP-%BF and B-US %BF were higher than those between ADP-%BF and A-US %BF (Table 6). However, when analyzing %BF, there was a lower CE, but higher TE, for A-US compared to B-US for %BF in females. The lowest correlations for %BF and FM were between ADP and BIA, although these correlations were still statistically significant. However, all FFM correlations, including BIA, were significant and high for both sexes (Table 6). Additionally, there was lower TE for FM and FFM compared to %BF between

ADP and all other devices. Bland-Altman plots revealed narrower 95% LOA for FM and FFM compared to %BF for all devices in females. Both LOA and TE were similar between FM and FFM, but were larger when analyzing %BF results for all devices (Table 5; Table 6). Additionally, there were narrower LOA between ADP and B-US compared to ADP and A-US for all three measures (Table 5; Figures 5A-C).

While there were no significant differences between ADP and any other device for %BF, FM, and FFM in females, B-US and BIA significantly overestimated %BF, and A-US significantly underestimated %BF compared to ADP in males. Additionally, there were significant differences between ADP and both B-US and BIA for FM and FFM, and the differences between ADP and A-US were approaching significance in males (Table 5). In addition to significant differences between devices, only $\%BF_{BUS}$ was moderately significantly correlated with ADP in males (r=0.783, P=0.01). Correlations between $\%BF_{AUS}$ and $\%BF_{ADP}$ only approached significance (r=0.607; P=0.08), and $\%BF_{BIA}$ correlations were weak and non-significant (r=0.484; P=0.19). Correlations for FM were higher between ADP and B-US than with A-US, and the correlation with BIA did not reach significance. When looking at FFM, all correlations were strong and significant, however BIA still produced the greatest TE values and widest LOA compared to both B-US and A-US (Table 5; Table 6).

Study 2B

A secondary analysis was conducted to compare the results from Study 2A to Siri's 3C model, which incorporated D_b via ADP and TBW from BIA (Siri, 1961). There were significantly strong correlations between the 3C-criterion model and each device for both males and females (Table 8). Overall, there were higher correlations between each

device and the 3C-criterion model for females compared to males and higher correlations between FFM measures than %BF and FM, as seen in the primary study. There were higher correlations between 3C and both ADP and BIA than 3C and both A-US and B-US, but TE was similar across devices (Table 8). In males, B-US produced lower TE than ADP and A-US for %BF, FM, and FFM, but in females, ADP-TE was lower than that for B-US for all measures (Table 8).

There were significant differences between devices when analyzing males but not females. In males, ADP and A-US significantly underestimated %BF ($\%BF_{ADP}$: -1.8%; $\%BF_{AUS}$: -2.3%; $P<0.05$), and FM (FM_{ADP}: -1.2kg; FM_{AUS}: -1.4kg; $P<0.05$) and overestimated FFM compared to the 3C model in males. ADP and A-US underestimated FM and overestimated FFM (FFM_{ADP}: +1.2kg; FFM_{AUS}: +1.4kg; $P<0.05$) compared to the 3C model in males. B-US also significantly underestimated %BF (-1.6%, $P=0.04$), and underestimations for FM (-1.0kg) and overestimations of FFM (+0.9kg) compared to 3C only approached significance ($P=0.08$). There were no significant differences between the 3C-criterion model and any devices among females, but there was a trend for BIA to overestimate FFM compared to the 3C-criterion model ($P=0.06$). Bland-Altman analyses showed narrower 95% LOA for FM and FFM compared to %BF (Figures 5A-D & 6A-D). Additionally, there were narrower 95% LOA for %BF for B-US than A-US in both sexes (Table 7). While ADP and BIA produced the smallest error compared to the 3C model overall, B-US error was smaller than A-US error in both males and females (Table 8).

Discussion

The purpose of Study 1 was to assess the validity of B-US compared to ADP to determine body composition in adolescent and young adult vocational ballet dancers. In this study, we found strong correlations between B-US and ADP for %BF, FFM, and FM measurements. There were higher correlations, but larger TE, among females for %BF and FM, but FFM correlations were strong for both sexes. Dependent t-tests revealed B-US significantly overestimated %BF compared to ADP in females, which is similar to previous reports that A-US significantly overestimates %BF in female gymnasts (Loenneke et al., 2014a). However, there were no significant differences between devices in males, as well as smaller TE compared to females, which may indicate that B-US produced more accurate results in males compared to females. This finding supports those from Wagner et al. (2016), who reported better agreement between ADP and A-US in male collegiate athletes compared to female collegiate athletes when determining %BF. Interestingly, in this study, there were higher correlations and lower TE for FFM compared to %BF, regardless of sex, indicating B-US may be a more accurate predictor of absolute FFM, rather than %BF, in younger and mostly lean athletes.

Based on the findings from Study 1, a follow-up study (Study 2A) was conducted to assess the validity of additional field methods including A-US and BIA, as well as B-US. Similar to Study 1, these three devices were compared to ADP, which revealed weaker correlations and larger TE for BIA than for B-US or A-US. There were higher correlations for %BF, FFM, and FM between ADP and B-US than A-US, indicating B-US provides more accurate results than A-US. As previously shown in Study 1, FFM

correlations were higher than both %BF and FM for both males and females, indicating FFM outputs may be more accurate than %BF. While there were no significant differences between any of the measures and ADP in either sex, B-US non-significantly overestimated %BF in females ($\%BF_{ADP}$=16.3 ±6.5; $\%BF_{BUS}$=17.7 ±3.9). However, ADP is known to underestimate %BF in lean individuals, making it unclear if B-US was overestimating %BF or ADP was underestimating %BF in the current study (Fields et al., 2002). Based on this knowledge and the findings from Study 1, we conducted an additional data analysis to compare the findings in Study 2A to a 3C-criterion reference model (Study 2B).

Two-component models, such as ADP, assume constant FFM hydration, limiting their validity, as FFM hydration levels vary by factors including age, sex, and training status (Fosbøl & Zerahn, 2015). The 3C model accounts for TBW, increasing its validity compared to ADP, and is seen as a more accurate assessment method (Fosbøl & Zerahn, 2015). Study 2B showed high correlations between 3C and all other devices for %BF, FM, and FFM, but as previously seen, FFM correlations were highest. There were higher correlations and lower TE between 3C and B-US compared to A-US for %BF, FM, and FFM, regardless of sex (Table 8). Similar to Study 1, %BF correlations were higher among females, but TE was also smaller among females than males. BIA resulted in the strongest correlations and lowest TE compared with the 3C model. This finding was expected as the 3C model relied on the TBW estimate from the whole-body BIA to calculate %BF, FFM, and FM.

Despite strong correlations between methods, ADP and A-US significantly underestimated %BF and FM and significantly overestimated FFM compared to 3C, but

there were no significant differences between 3C and B-US. When analyzing males and females separately, differences 3C and ADP were only significant in males. However, ADP and A-US non-significantly underestimated %BF compared to 3C and B-US in females (Table 5). This supports previous findings that ADP underestimates %BF and overestimates FFM in lean individuals, which may have been the case in this study (Lockner et al., 2000; Vicente-Rodriguez et al., 2009, Delisle-Houde et al., 2017). The males in this study had an average %BF of 9.1±3.0, and females had an average %BF of 17.2 ±5.7, both of which are in the "Excellent" category defined by the American College of Sports Medicine (ACSM, 2018). However, the males in this study are on the lower end of this range, as below 7.1% is considered essential fat, while females are on the upper end of the range, as essential fat ranges from 10-13% (ACSM, 2018). Therefore, it is more likely that the BOD POD® underestimated %BF for more male subjects than females, indicating B-US may be a more accurate assessment tool than ADP in this population. However, weaker correlations and higher TE among males compared to females may indicate that there was higher technician-error in leaner subjects, possibly due to difficulty identifying SAT borders. This may also partially explain findings from Study 1, as technicians may have inaccurately underestimated SAT thicknesses, and therefore underestimated %BF in males, leading to similar mean %BF values as ADP, which also underestimated %BF. The technicians in the current study had approximately one year of previous US experience, which may not be adequate in order to be proficient at analyzing images, as previous findings reported that novice technicians struggled to clearly image and interpret sites with more fascia, such as suprailiac and abdomen (Müller et al., 2013).

Overall, Study 2A and Study 2B both showed higher correlations between the reference method (ADP and 3C, respectively) and B-US than A-US regardless of sex. This finding partially supports that from Loenneke et al. (2014a) who concluded A-US does not produce valid %BF results compared to DXA in female collegiate gymnasts, a similar artistic-athlete population to ballet dancers. In the current study, B-US was a better predictor of %BF and FM than A-US, although A-US still yielded strong correlations. However, this study used a 7-site scanning protocol, while Loenneke et al. (2014a) used 1-site and 3-site protocols. It is possible that increasing anatomical sites scanned may improve accuracy when using A-US to determine %BF, however more research is needed to compare various equations. It is also possible that A-US is not able to accurately determine SAT borders in individuals with extremely low levels of SAT, such as gymnasts and dancers, but manual measurements using two-dimensional images produced by B-US may increase measurement accuracy, if technicians are well-trained.

While B-US may be more accurate than A-US, technician-error and skill level need to be considered. The manual imaging interpretation required by B-US may be difficult for untrained technicians, as they need to identify physical tissue borders, as opposed to changes in impedance shown on a graph by A-US. Interestingly, B-US showed higher TE than A-US in Study 2B, but TE was higher for A-US in Study 2A. One speculation is that 3C was a more accurate reference method, but TE for B-US was greater than for A-US due to technician errors. The US technicians in the current study had only one year of prior US experience, which may lead to image-interpretation errors using B-US.

Both B-US and A-US appeared to be better predictors of %BF and FM than BIA in Study 2A. While correlations between $\%BF_{BIA}$ and $\%BF_{ADP}$ were not as strong as those between $\%BF_{ADP}$ and both B-US and A-US in males (BIA: r=0.484; B-US: r=0.783; A-US: r=0.607) and females (BIA: r=0.876; B-US: r=0.931; A-US: r=0.917), there were strong, significant correlations between FFM_{ADP} ($r > 0.881$) and all other devices (Table 4). These findings indicate that both B-US and A-US may be better than BIA to assess %BF, especially in males who yielded a TE of 22.4% compared to ADP, but all three devices can be used to determine FFM. This conflicts with findings from Volpe et al. (2009) who concluded that BIA is a valid tool to assess %BF in male bodybuilders compared to HW, although similar to the current investigation, other studies concluded BIA is not an accurate assessment device (Duz et al., 2009; Hofsteenge et al., 2015; Eisenkolbl et al., 2001). Duz et al. (2009) reported higher correlations between $\%BF_{DXA}$ and US compared to both BIA and SFC, indicating US may be a better alternative to these common field techniques when DXA is not available.

BIA, as well as US, may be best suited to assess absolute FFM and FM rather than %BF (Rech et al., 2008; Utter & Hager, 2008; Midorikawa et al., 2009). Overall, FFM correlations were higher than %BF and FM, and TE was lower for FM and FFM compared to %BF in all three analyses. Our findings are consistent with those from Ling et al. (2010), who reported higher correlations for LBM and FM than %BF between DXA and MF-BIA, and Schoenfeld et al. (2016) who found smaller SEE and TE for FFM values compared to %BF.

Accurately quantifying FFM, even if %BF is inaccurate, is still a relevant measure since lean mass may be a more important factor than %BF for these athletes. One study

found that ballerinas with more FFM, despite higher total body mass, had significantly greater grip strength compared to those with less FFM (Misigoj-Durakovic et al., 2001). In that study, all ballerinas had similar flexibility and VO_{2max} scores, indicating no difference in fitness based on FFM, except in terms of muscular strength (Misigoj-Durakovic et al., 2001). Additionally, many studies only report either %BF or FFM, making it difficult to compare our results to previous findings (Utter and Hager, 2008; Duz et al., 2009; Loenneke et al., 2014a; Loenneke et al., 2014b; Johnson et al., 2014; Johnson et al., 2016; Hyde et al., 2016; Wagner et al., 2016; Rech et al., 2008).

Further, our results showed no significant differences between either B-US or A-US %BF, FM, and FFM measures compared to ADP. To our knowledge, this is the first study to compare B-US to ADP, but our findings are consistent with previous research comparing A-US and ADP (Wagner et al., 2016; Schoenfeld et al., 2016). Wagner et al. (2016) reported strong correlations for %BF from A-US with ADP in collegiate athletes, and Schoenfeld et al. (2016) found no significant mean differences between A-US and ADP pre- and post-weight-loss intervention in college-aged females. Additionally, previous research suggests B-US can accurately estimate %BF compared to DXA, and our results indicate that this is also true for ADP. The current study found no significant differences between %BF from ADP and B-US in Study 2B, as well as strong correlations, despite significant differences in females, in Study 1.

These conflicting findings between Study 2A and Study 2B may be explained by the different reference methods used in these studies. For instance, ADP is known to underestimate %BF and overestimate FFM in lean individuals (Lockner et al., 2000; Vicente-Rodriguez et al., 2009, Delisle-Houde et al., 2017). Lockner et al. (2000)

reported body volume miscalculations by ADP for children, due to body surface area measurement errors, however, using child-specific equations may help account for this error (Lohman, 1986). Errors for ADP measures in the smaller participants may also be attributed to the equations used in this study. The current study used the Siri equations to convert D_b to %BF for ADP in order to maintain consistency across devices, as the US software used in this study employs the modified Jackson-Pollock and Siri equations (Siri, 1961; Jackson & Pollock, 1978). However, Brozek equations have been found to be more accurate in collegiate athletes, and Lohman equations attempt to control for different water and mineral proportions of FFM found in children compared to adults (Fields et al., 2002; Brozek et al., 1963; Slaughter et al., 1988; Lohman et al., 1984). Future analyses should use the Brozek or Lohman equations, when age-appropriate, to determine the most accurate %BF equation based on D_b for ADP. Therefore, it is unknown whether underestimated %BF or if B-US overestimated %BF in the current study.

ADP also underestimated %BF compared to the 3C model, a widely used reference method in BC research (Fields et al., 2002; Fosbøl & Zerahn, 2015). The 3C equations require TBW measurement, which was obtained from BIA in this study. Previous research indicates that MF-BIA accurately measures TBW compared to deuterium oxide and doubly-labeled water methods in adults, and these devices have been used in previous 4C-MCM validation studies (Matthie et al., 1998; Moon et al., 2008; Van Loan & Mayelin, 1987; Minderico et al., 2007; Moon et al. 2007; Sardinha et al., 2003; Sartorio et al., 2005). While the BIA proprietary equations appear suitable for varying adult populations, they may have not been accurate for the adolescent athletes in

the current study (Sartorio et al., 2005). More research is needed to investigate the validity of the Inbody 770 to determine TBW in both adolescent and athletic populations.

To our knowledge, this study is the first to compare B-US to ADP and the first to compare A-US, B-US, and BIA to both a 3C-criterion model and ADP. Finding relatively inexpensive, portable, and accurate devices is important in order to make BC assessments accessible and useful to coaches and athletes (Ackland et al., 2011). There are many tools available for to assess BC, but several factors including cost, subject involvement, population, portability, reliability, and validity, and the tester's experience should all be considered when choosing the appropriate testing device. It appears as though US encompasses these characteristics, while also providing additional benefits, such as the ability to conduct regional assessments. Regional assessments, rather than total %BF, may be more beneficial for youth athletes who are still experiencing changes in body proportions, which may not be accounted for by some assessment devices and equations (Bayer & Bayley, 1959; Midorikawa et al., 2009; Kim et al., 2006). In the current study, B-US showed higher correlations than A-US with both the 3C model and ADP, indicating that B-US may be a more valid %BF measurement tool than A-US.

However, this study is not without limitations, which include both the sample selection and the instrumentation used. The sample size was relatively small, with 47 total subjects (21 males and 27 females), and only 22 subjects (9 males and 13 females) in the secondary study, as we were limited to a convenience sample for both studies. Additionally, hydration status was self-reported by subjects, rather than analyzed using urine-specific gravity testing, meaning we cannot be sure that all subjects arrived in the euhydrated state. Dehydration impacts both total body mass and TBW measurements,

and therefore subsequently impacts %BF and BC estimated by both BIA and 3C (Hofsteenge et al., 2015; Tinsley et al., 2017; Gallagher et al., 1998) Further, the equations utilized to determine BC assume normal bone mineral density, which may have been altered in this sample due to menstrual disruptions (Keay et al., 1997). Health history questionnaires revealed that 80% of female subjects self-reported irregular menstruation, defined by cessation of regular menses for at least two months, indicating potentially decreased bone mineral density levels (Christo et al., 2008; Keay et al., 1997). This study used the Siri density model in order to remain consistent across devices, but this may not be the most appropriate equation for youth and adolescents, who are known to have different FFM densities than adults (Lohman et al., 1984). Future validation studies should investigate different population-specific equations in attempt to account for possible altered lean mass composition to improve total BC assessment results.

Despite limitations, this is the first study, to our knowledge, to use the modified 7-site skinfold caliper protocol for B-mode US, as well as to employ both B-mode and A-mode US devices in the same study. Using both US devices allows a direct comparison of the validity and accuracy of these devices, which is important for researchers, coaches, and practitioners, when selecting a BC assessment tool. This study is also the first of its nature to study high-level adolescent and young-adult ballet dancers, which is a very specific athletic population. Future research should employ current population-specific equations, or aim to develop new equations that may better represent different groups, in attempt to increase the accuracy of these devices. Additional comparison and validation studies for these newer, more accessible devices are imperative for finding tools

developing protocols that are affordable and accessible for both clinical and field settings when attempting to optimize athletic performance and overall health.

Conclusions

Ultrasound appears to be a useful tool to assess both whole-body and regional SAT in adolescent and young-adult ballet dancers. Our findings suggest that both B-US and A-US are valid BC assessment tools for this population, but B-US may be more accurate than A-US, BIA, and ADP in lean adolescent athletes, shown by comparisons to the 3C model. However, adequate US-technician training for both scanning technique and image interpretation are essential to ensure accurate results. Sex-differences need to be further investigated, as this study found weaker correlations in males compared to females for %BF with each device, and greater total error compared to the 3C model in males compared to females.

Higher correlations and lower TE for FFM than %BF in both sexes indicates that US may be best suited for estimating absolute FFM and FM when using the devices and equations employed in the current study. However, this study is limited by a small sample size and the equations used to calculate %BF. Future research should investigate different modified SFC equations and different density models to determine more methods for both ADP and US. Additionally, new US-specific equations should be developed, as SFC equations may not be applicable to US.

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Tables and Figures

Table 1. Study 1 Subject Characteristics

	Mean ±SD	Range
Males (n=21)		
Age (yr)	17.2 ±1.7	13.3-19.9
Height (cm)	173.4 ±7.5	158.8-184.2
Weight (kg)	62.2 ±10.0	40.9-76.1
Females (n=27)		
Age (yr)	16.1 ±1.4	14.1-18.2
Height (cm)	162.8 ±6.3	152.4-172.7
Weight (kg)	48.7 ±4.7	38.9-56.0

Table 1. Subject characteristics separated by sex for Study 1.

Table 2. Study 2 Subject Characteristics

	Mean ±SD	Range
Males (n=9)		
Age (yr)	17.4 ±1.8	13.7-19.9
Height (cm)	173.0 ±7.6	161.3-184.2
Weight (kg)	62.5 ±10.6	43.9-76.1
Females (n=13)		
Age (yr)	16.2 ±1.4	14.4-18.2
Height (cm)	162.9 ±6.3	153.7-172.7
Weight (kg)	49.6 ±4.6	40.5-56.0

Table 2. Subject characteristics separated by sex for Study 2.

Table 3. Study 1 Body Composition Results

	Device	Mean	SD	SEM	95% LOA
Males					
%BF	ADP	8.1	± 4.2	0.91	
	B-US	8.2	± 2.4	0.52	-5.5 to 5.3
FFM	ADP	57.2	± 9.7	2.1	
	B-US	57	± 9.0	2	-3.3 to 3.6
FM	ADP	5	± 2.6	0.56	
	B-US	5.2	± 1.8	0.4	-3.6 to 3.3
Females					
%BF	ADP	15.8	± 6.0	1.2	
	BUS	17.4**	± 3.8	0.73	-7.0 to 3.7
FFM	ADP	40.9	± 4.2	0.8	
	B-US	40.1**	± 3.9	0.75	-1.7 to 3.2
FM	ADP	7.8	± 3.1	0.59	
	B-US	8.5**	± 2.1	0.41	-3.3 to 1.7

*Denotes significance at the P<0.05 level; ** denotes significance at the P<0.01 level. Significant mean values indicate significant difference compared to ADP. SEM= standard error of measurement; 95% limits of agreement (LOA) is MD ±1.96*SD.

Table 4. Study 1 Correlations and Error

	R	R^2	Adj. R^2	CE	SEE	TE
Males						
%BF	0.773**	0.598	0.577	0.11	2.70	2.69
FFM	0.985**	0.97	0.968	-0.18	1.72	1.72
FM	0.726**	0.528	0.503	0.18	1.80	1.72
Females						
%BF	0.941**	0.886	0.881	1.6	2.06	3.13
FFM	0.954**	0.91	0.906	-0.78	1.28	1.46
FM	0.943**	0.89	0.886	0.76	1.04	1.46

*Denotes significant correlation at the P<0.05 level; ** Denotes significant correlation at the P<0.01 level.
CE=constant error; SEE= standard estimate of error; TE= total error.

Table 5. Study 2A Body Composition Results

	Device	Mean	SD	SEM	95% LOA
Males					
%BF	ADP	7.3	± 3.3	1.1	
	B-US	7.5*	± 1.7	0.55	-4.6 to 4.2
	A-US	6.8*	± 2.0	0.65	-4.6 to 5.6
	BIA	9.4**	± 3.6	1.2	-9.0 to 4.8
FFM	ADP	57.9	± 9.8	3.3	
	B-US	57.6*	± 9.5	3.2	-2.6 to 3.1
	A-US	58.1#	± 9.3	3.1	-3.3 to 2.8
	BIA	56.6**	± 9.2	3.1	-3.2 to 5.7
FM	ADP	4.6	± 2.2	0.74	
	B-US	4.8*	± 1.4	0.48	-3.1 to 2.6
	A-US	4.4#	± 1.7	0.56	-2.8 to 3.3
	BIA	6.0**	± 2.6	0.88	-5.9 to 3.1
Females					
%BF	ADP	16.3	± 6.5	1.8	
	B-US	17.7	± 3.9	1.1	-7.7 to 5.0
	A-US	16.3	± 2.9	0.79	-7.9 to 7.9
	BIA	16.8	± 5.5	1.5	-6.6 to 5.7
FFM	ADP	41.4	± 4.1	1.1	
	B-US	40.7	± 3.6	0.99	-2.4 to 3.7
	A-US	41.4	± 3.6	1.0	-3.9 to 3.8

	BIA	41.4[#]	± 4.8	1.3	-3.0 to 2.9
FM	ADP	8.2	± 3.4	0.94	
	B-US	8.8	± 2.3	0.83	-3.7 to 2.5
	A-US	8.1	± 1.7	0.48	-3.7 to 3.9
	BIA	8.3	± 2.8	0.78	-2.9 to 2.6

*Denotes significant compared to ADP at the P<0.05 level; ** Denotes significant compared to ADP at the P<0.01 level. [#]Denotes trend towards significance (P<0.10). SEM= standard error of measurement; 95% limits of agreement (LOA) is MD±1.96*SD.

Table 6. Study 2A Correlations and Error

	Device	R	R^2	Adj. R^2	CE	SEE	TE
Males							
%BF	B-US	0.783**	0.612	0.557	0.18	2.18	2.12
	A-US	0.607#	0.369	0.279	-0.48	2.78	2.50
	BIA	0.484	0.234	0.125	2.11	3.07	3.94
FFM	B-US	0.989**	0.979	0.976	-0.25	1.53	1.38
	A-US	0.988**	0.976	0.972	0.25	1.62	1.50
	BIA	0.973**	0.946	0.939	-1.25	2.42	2.49
FM	B-US	0.775**	0.600	0.543	0.25	1.52	1.38
	A-US	0.717*	0.514	0.444	-0.23	1.66	1.49
	BIA	0.562	0.315	0.218	1.39	1.97	2.58
Females							
%BF	B-US	0.931**	0.866	0.854	1.35	2.49	3.38
	A-US	0.917**	0.841	0.827	0.00	2.71	3.87
	BIA	0.876**	0.767	0.746	0.45	3.27	3.05
FFM	B-US	0.928**	0.862	0.849	-0.69	1.6	1.65
	A-US	0.881**	0.776	0.756	0.04	2.04	1.87
	BIA	0.956**	0.914	0.906	0.02	1.27	1.43
FM	B-US	0.919**	0.845	0.831	0.65	1.4	1.65
	A-US	0.919**	0.844	0.830	-0.07	1.4	1.85
	BIA	0.914**	0.835	0.820	0.14	1.44	1.36

*Denotes significant correlation with ADP at the P<0.05 level; ** Denotes significance at the P<0.01 level.
CE=constant error; SEE= standard estimate of error; TE= total error.

Table 7. Study 2B Body Composition Results

	Device	Mean	SD	SEM	95% LOA
Males					
%BF	3C	9.1	± 3.0	1.0	
	ADP	7.3*	± 3.3	1.1	-2.0 to 5.6
	B-US	7.5*	± 1.7	0.55	-2.2 to 5.5
	A-US	6.8**	± 2.0	0.65	-1.8 to 6.4
	BIA	9.4	± 3.6	1.2	-3.4 to 2.8
FFM	3C	56.7	± 9.3	3.1	
	ADP	57.9*	± 9.8	3.3	-3.7 to 1.4
	B-US	57.6#	± 9.5	3.2	-3.6 to 1.4
	A-US	58.1**	± 9.3	3.1	-3.7 to 0.90
	BIA	56.6	± 9.2	3.1	-1.9 to 2.0
FM	3C	5.8	± 2.2	0.74	
	ADP	4.6*	± 2.2	0.74	-1.4 to 3.7
	B-US	4.8#	± 1.4	0.48	-1.8 to 3.6
	A-US	4.4**	± 1.7	0.56	-0.94 to 3.8
	BIA	6.0	± 2.6	0.88	-2.2 to 1.8
Females					
%BF	3C	17.2	± 5.7	1.6	
	ADP	16.3	± 6.5	1.8	-2.8 to 4.5
	B-US	17.7	± 3.9	1.1	-5.7 to 4.7
	A-US	16.3	± 2.9	0.79	-6.0 to 7.6

	BIA	16.8	± 5.5	1.5	-2.1 to 2.9
FFM	3C	41	± 4.4	1.2	
	ADP	41.4	± 4.1	1.1	-2.0 to 1.3
	B-US	40.7	± 3.6	0.99	-2.3 to 2.9
	A-US	41.4	± 3.6	1	-3.9 to 3.0
	BIA	41.4[#]	± 4.8	1.3	-1.7 to 0.92
FM	3C	8.6	± 3.0	0.83	
	ADP	8.2	± 3.4	0.94	-1.3 to 2.0
	B-US	8.8	± 2.3	0.83	-2.9 to 2.3
	A-US	8.1	± 1.7	0.48	-2.9 to 3.8
	BIA	8.3	± 2.8	0.78	-0.94 to 1.4

*Denotes significant compared to 3C at the P<0.05 level; ** Denotes significant compared to 3C at the P<0.01 level. [#]Denotes trend towards significance (P<0.10). SEM= standard error of measurement; 95% limits of agreement (LOA) is MD±1.96*SD.

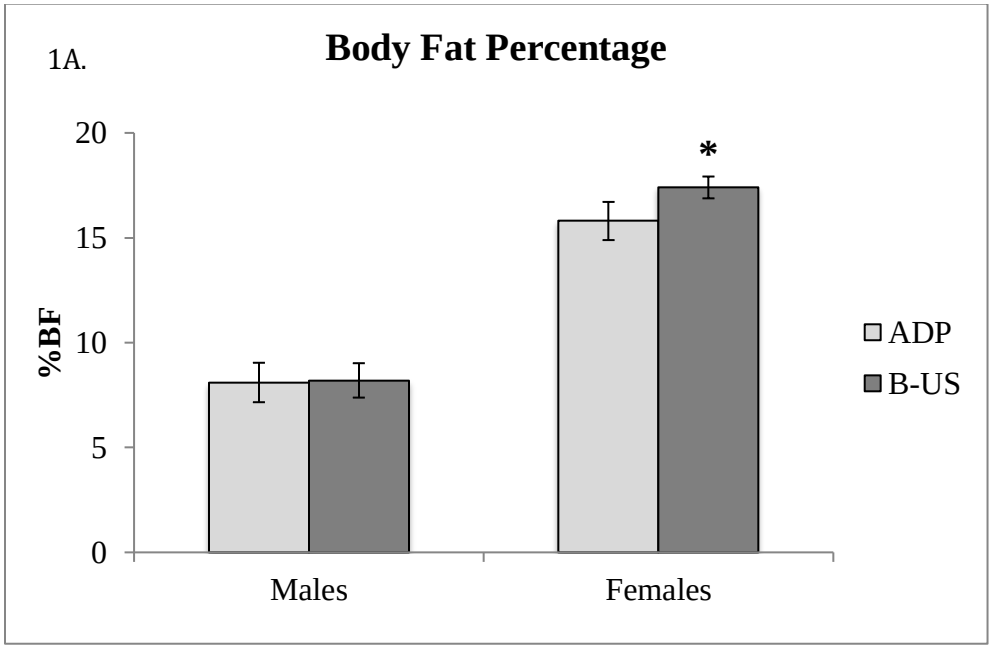
Table 8. Study 2B Correlations and Error

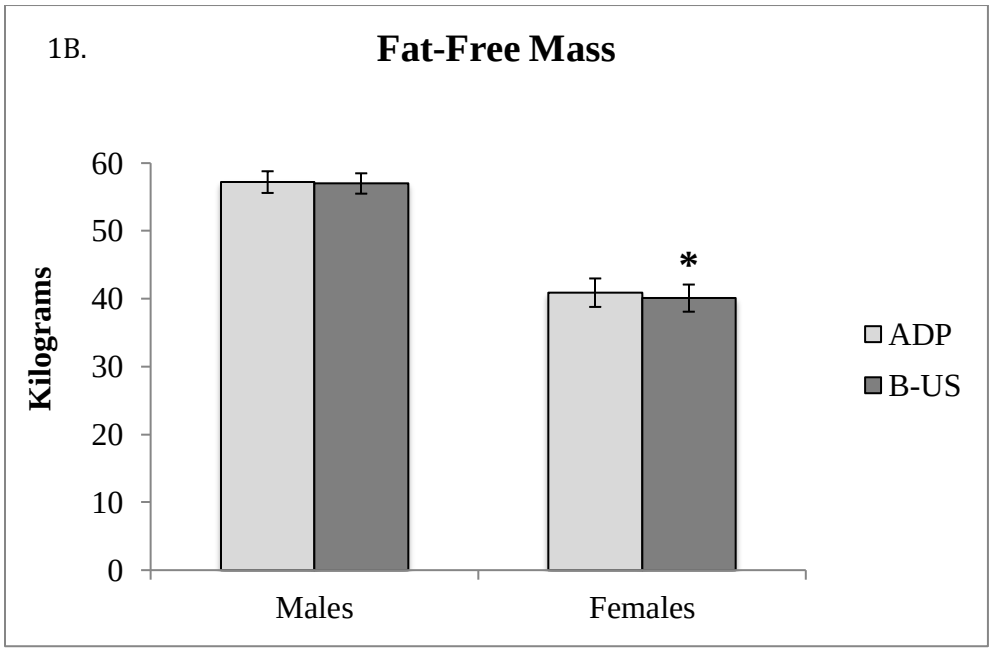
	Device	R	R^2	Adj. R^2	CE	SEE	TE
Males							
%BF	ADP	0.811**	0.658	0.609	-1.81	1.89	2.58
	B-US	0.801**	0.641	0.59	-1.63	1.94	2.47
	A-US	0.733*	0.537	0.471	-2.29	2.2	3.01
	BIA	0.904**	0.817	0.791	0.3	1.38	1.52
FFM	ADP	0.992**	0.984	0.982	1.16	1.26	1.69
	B-US	0.989**	0.979	0.976	0.92	1.45	1.59
	A-US	0.992**	0.984	0.981	1.42	1.26	1.8
	BIA	0.994**	0.989	0.987	-0.09	1.06	0.94
FM	ADP	0.833**	0.695	0.651	-1.17	1.34	1.69
	B-US	0.805**	0.648	0.597	-0.92	1.42	1.59
	A-US	0.850**	0.723	0.683	-1.4	1.26	1.8
	BIA	0.925**	0.855	0.834	0.22	0.91	0.99
Females							
%BF	ADP	0.961**	0.924	0.917	-0.83	1.64	1.98
	B-US	0.918**	0.843	0.829	0.51	2.36	2.6
	A-US	0.880**	0.775	0.754	-0.83	2.83	3.43
	BIA	0.974**	0.949	0.945	-0.38	1.34	3.05
FFM	ADP	0.984**	0.968	0.965	0.36	0.831	0.879
	B-US	0.968**	0.938	0.932	-0.33	1.16	1.32
	A-US	0.924**	0.854	0.841	0.4	1.77	1.74
	BIA	0.993**	0.986	0.984	0.39	0.554	1.43

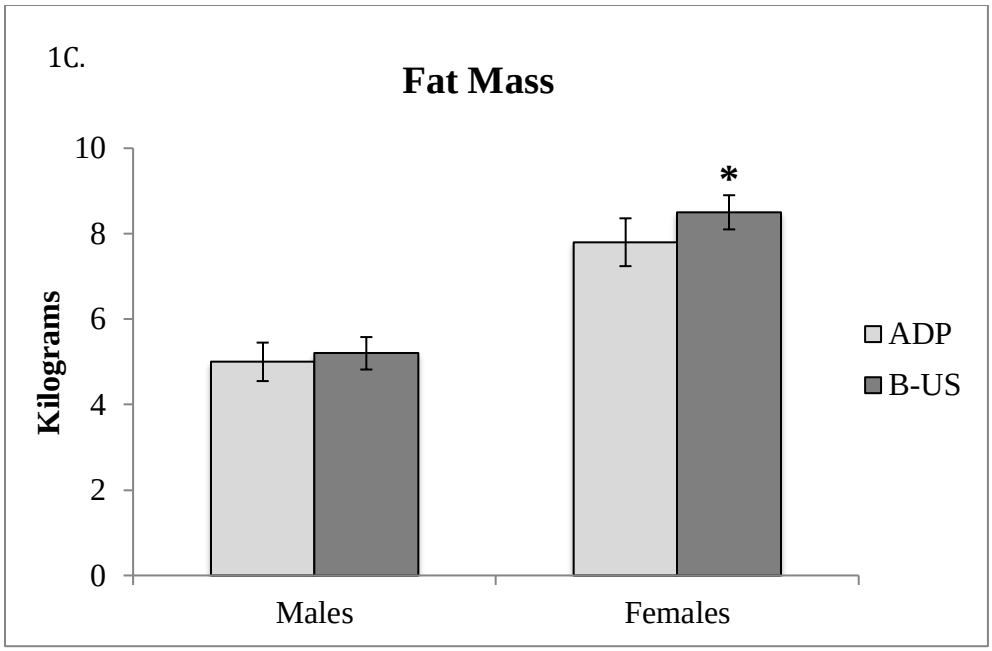
FM	ADP	0.974**	0.949	0.945	-0.36	0.701	0.878
	B-US	0.906**	0.821	0.805	0.29	1.32	1.31
	A-US	0.868**	0.754	0.732	-0.43	1.54	1.69
	BIA	0.981**	0.962	0.958	-0.22	0.61	1.36

Denotes significant correlation with 3C at the P<0.05 level; ** Denotes significant correlation with 3C at the P<0.01 level. CE=constant error; SEE= standard estimate of error; TE= total error.

Figures 1A-C.


1A.
Body Fat Percentage
%BF
20
15
10
5
0
*
Males
Females
ADP
B-US


1B.
Fat-Free Mass
Kilograms
60
50
40
30
20
10
0
*
Males
Females
ADP
B-US



Figures 1A-C. Bar graphs for Study 1 comparing %BF, FFM, and FM from ADP to B-US for total sample, males, and females with standard error of estimate (SEE). *Denotes significantly different than ADP ($P<0.05$).

Figure 2A-C.

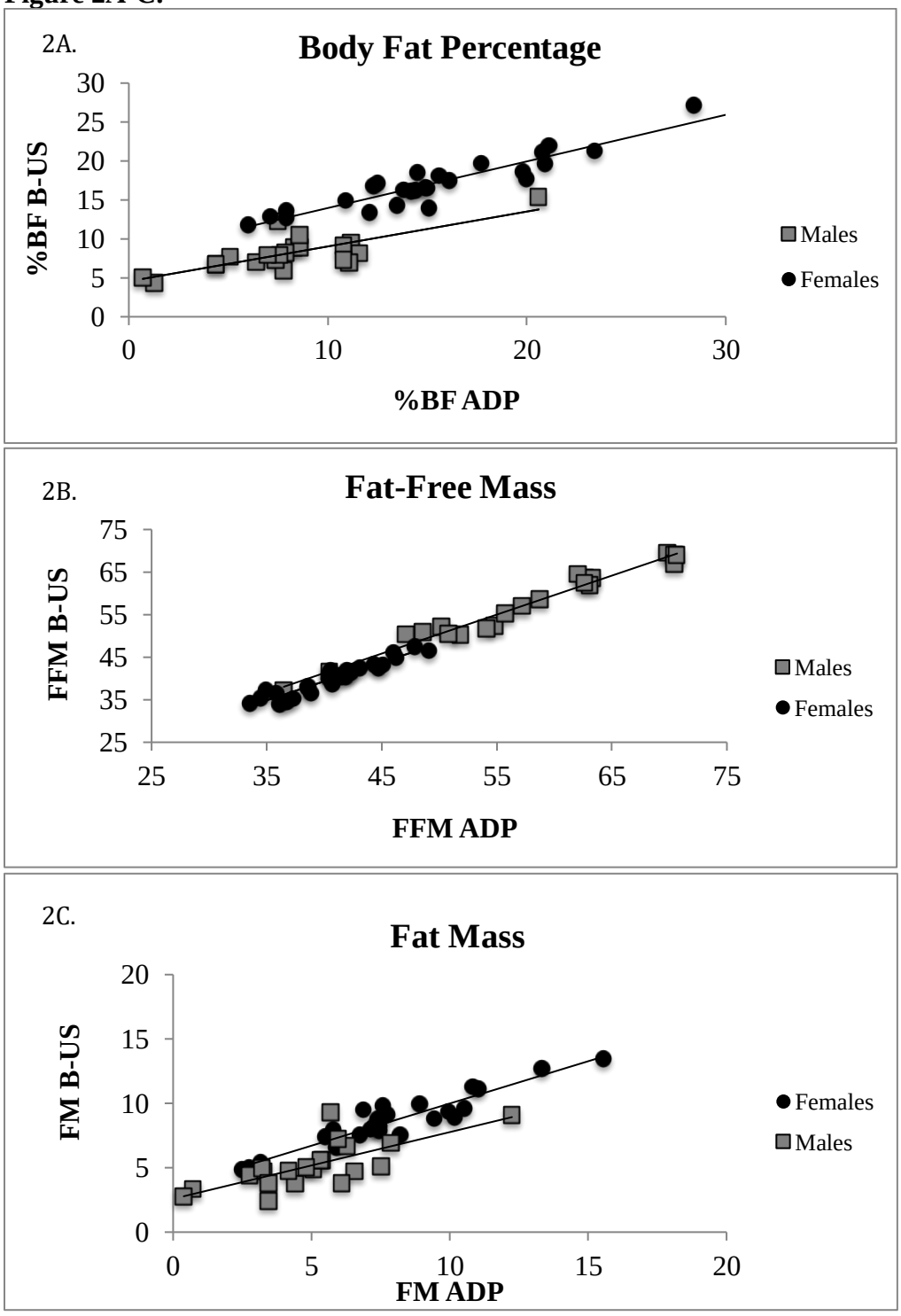
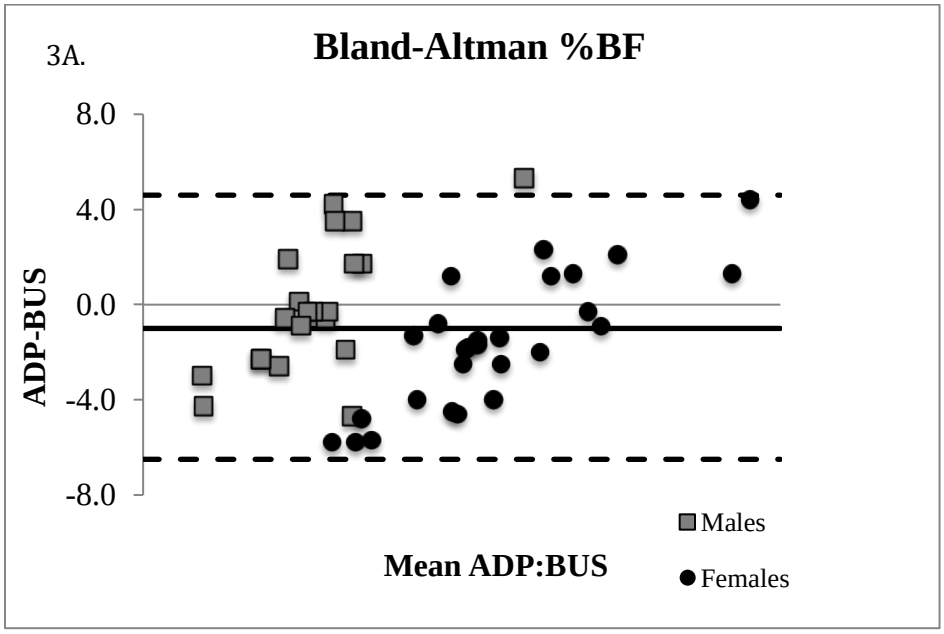
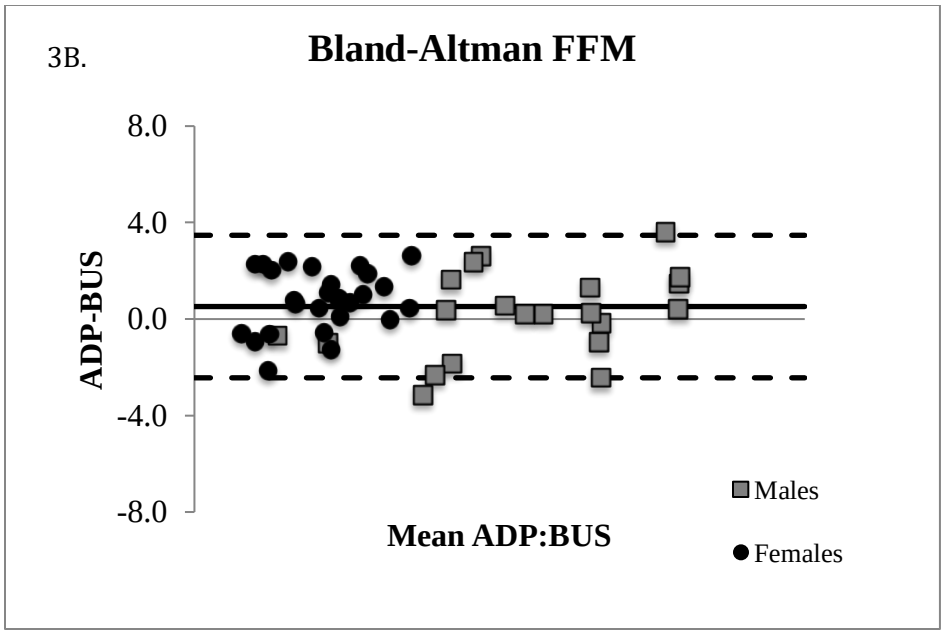


Figure 2A-C. Study 1 Linear Correlations
%BF: Female r^2=0.886; Male r^2=0.598; FFM. Female r^2=0.970; Male r^2=0.910; FM: Female r^2=0.890; Male r^2=0.528.

Figure 3A-C.


3A.
Bland-Altman %BF
8.0
4.0
0.0
-4.0
-8.0
ADP-BUS
Mean ADP:BUS
Males
Females



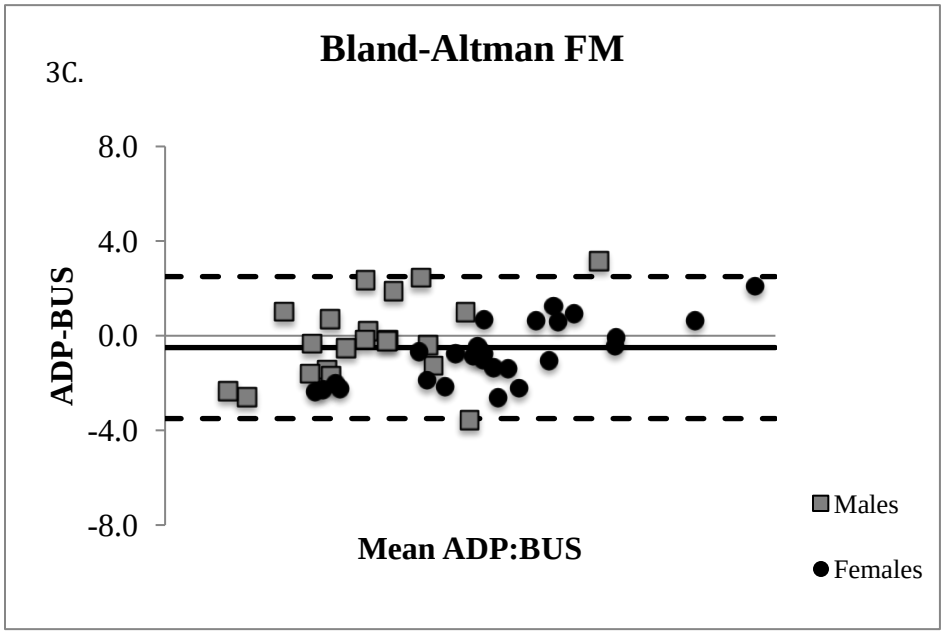
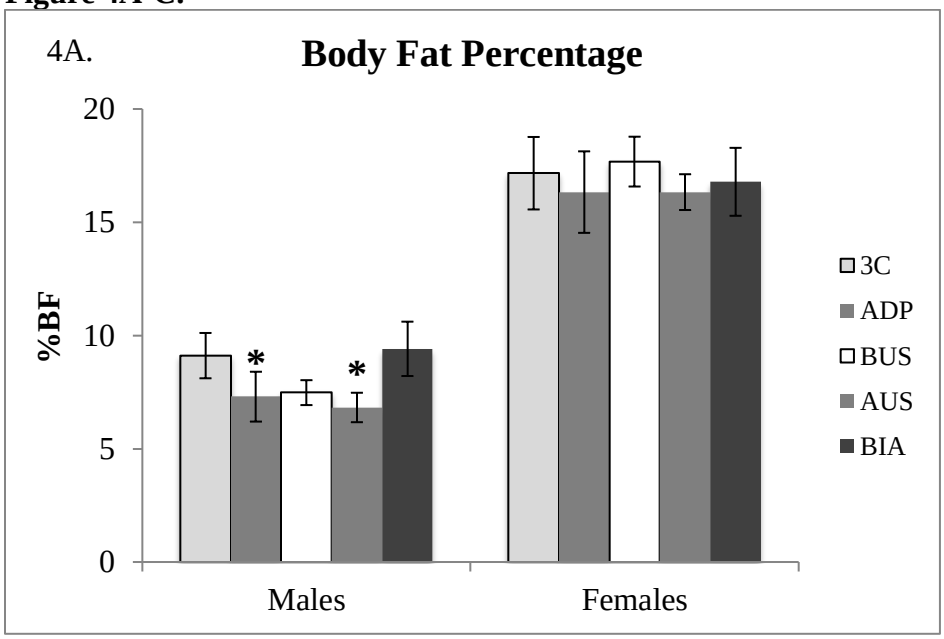
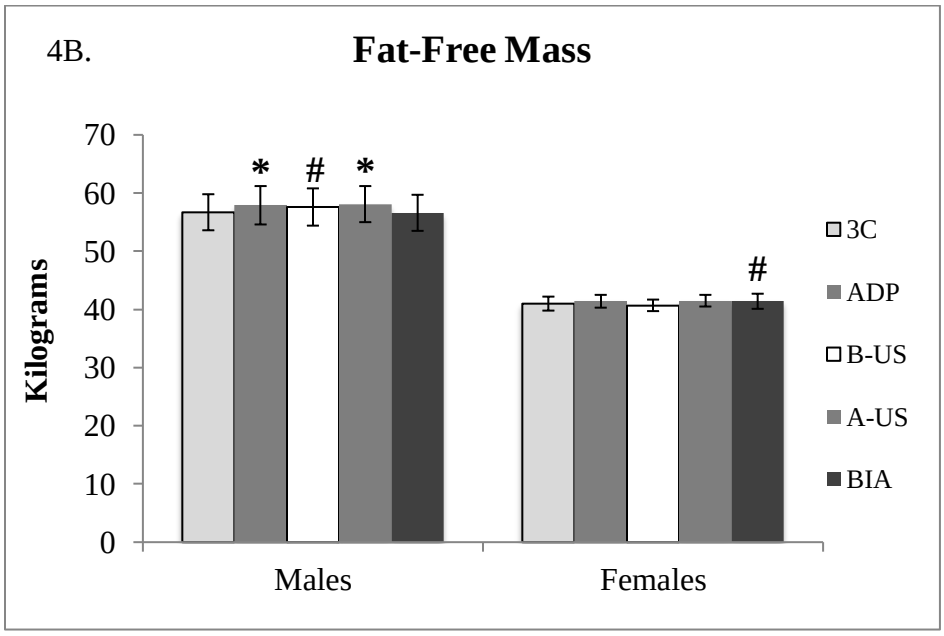
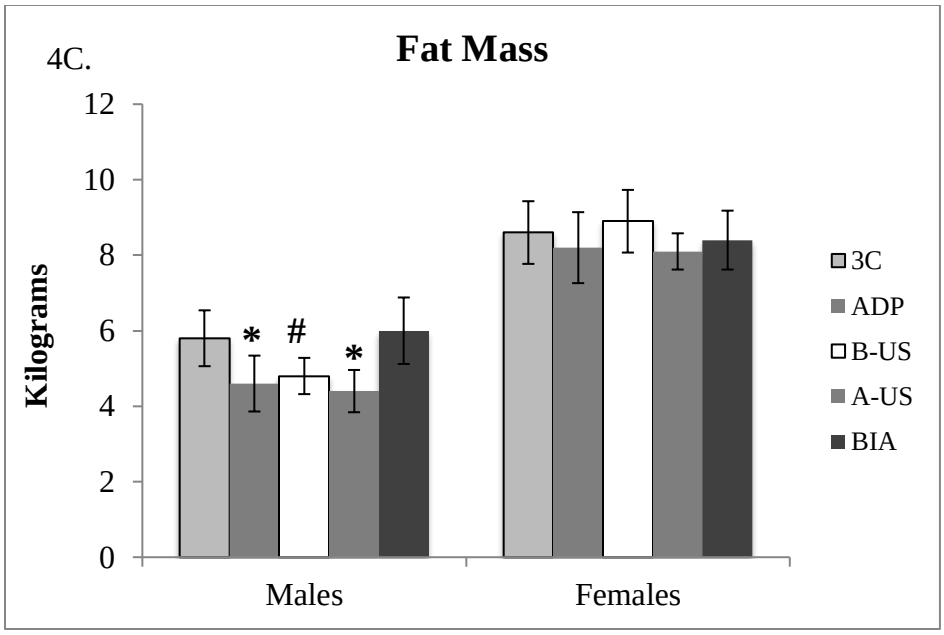


Figure 3A-C. Bland-Altman plots showing 95% LOA for Study 1. Solid line represents mean difference; dashed lines indicate MD±1.96*SD.

Figure 4A-C.

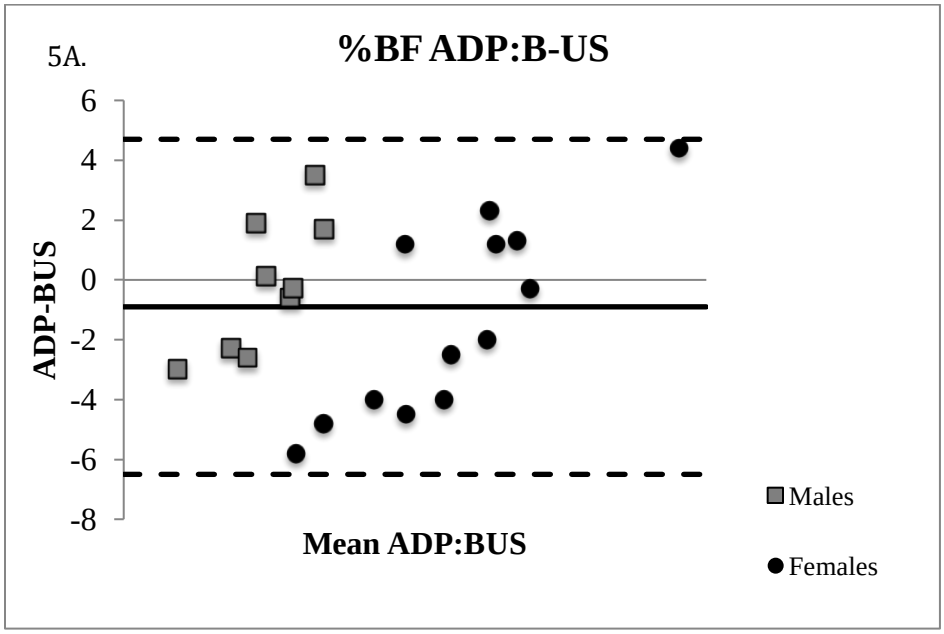

4A.
Body Fat Percentage
%BF
20
15
10
5
0
*
*
Males
Females
3C
ADP
BUS
AUS
BIA

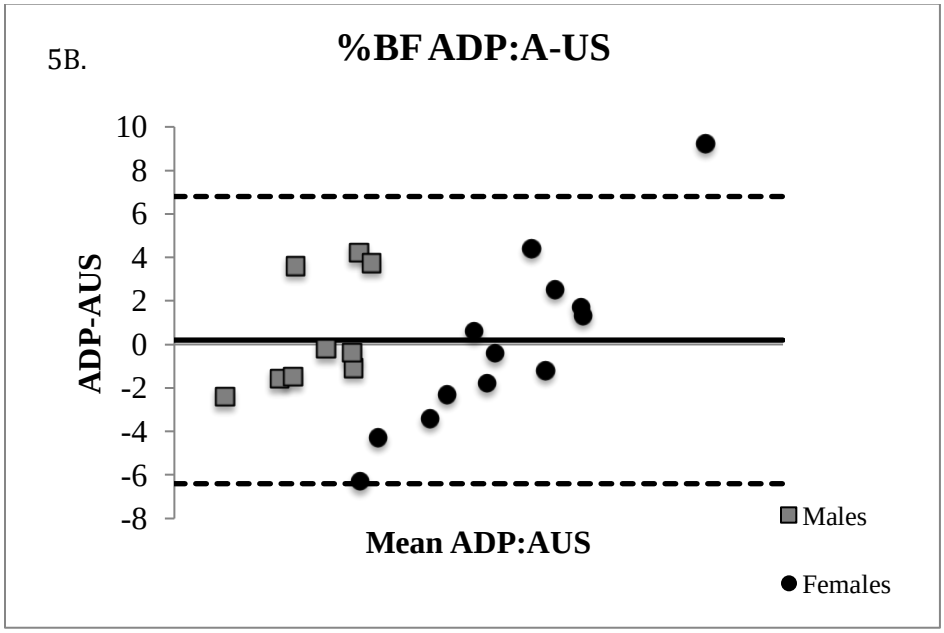

4B.
Fat-Free Mass
Kilograms
70
60
50
40
30
20
10
0
*
#
*
#
Males
Females
3C
ADP
B-US
A-US
BIA



Figures 4A-C. Bar graphs for Study 2 comparing %BF, FFM, and FM from all devices for total sample, males, and females with standard error of estimate (SEE). *denotes significantly different than ADP (P<0.05); # denotes trend towards significance (P<0.1.

Figure 5A-C.


5A.
%BF ADP:B-US
6
4
2
0
-2
-4
-6
-8
ADP-BUS
Mean ADP:BUS
Males
Females


5B.
%BF ADP:A-US
10
8
6
4
2
0
-2
-4
-6
-8
ADP-AUS
Mean ADP:AUS
Males
Females

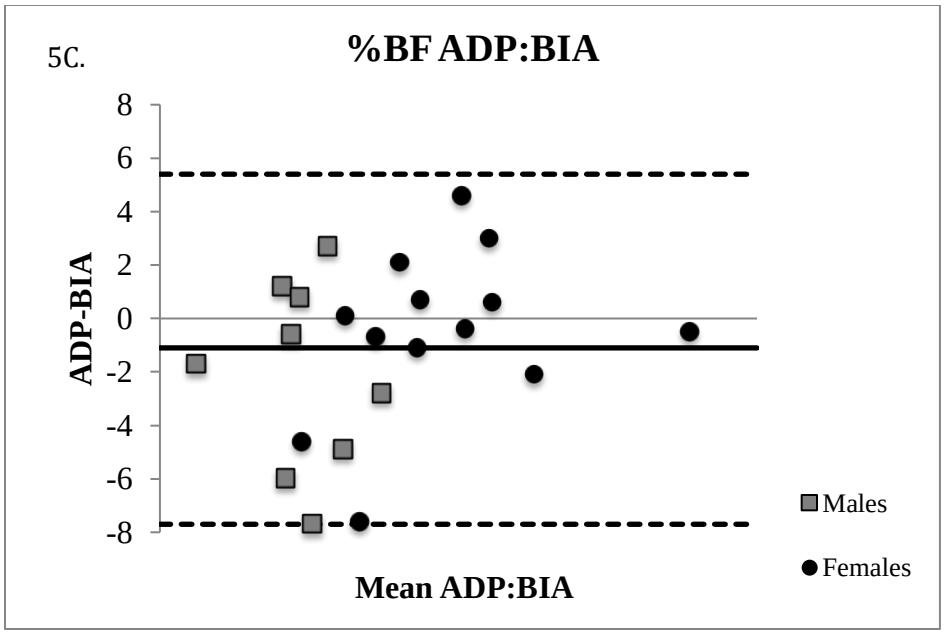
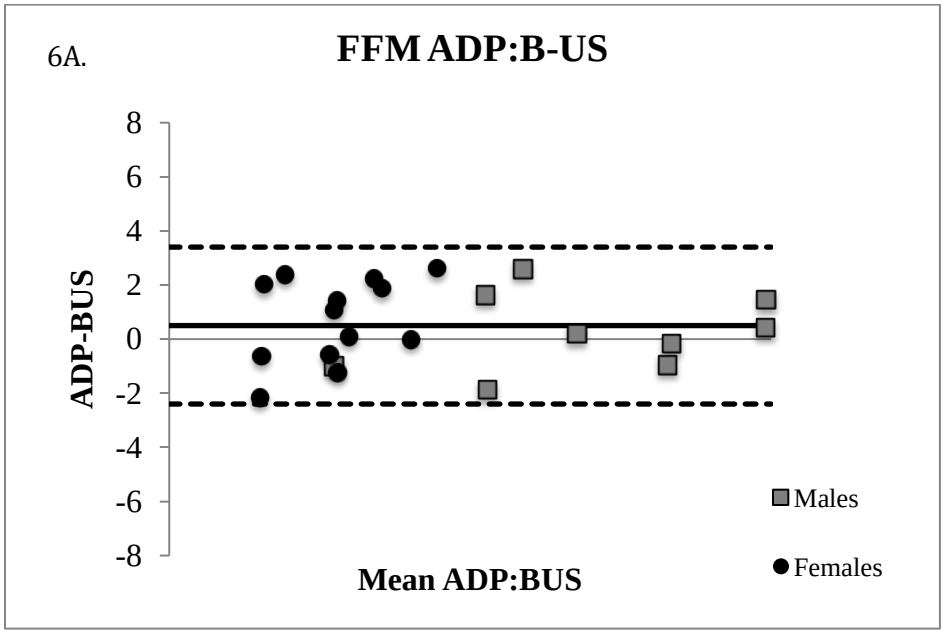
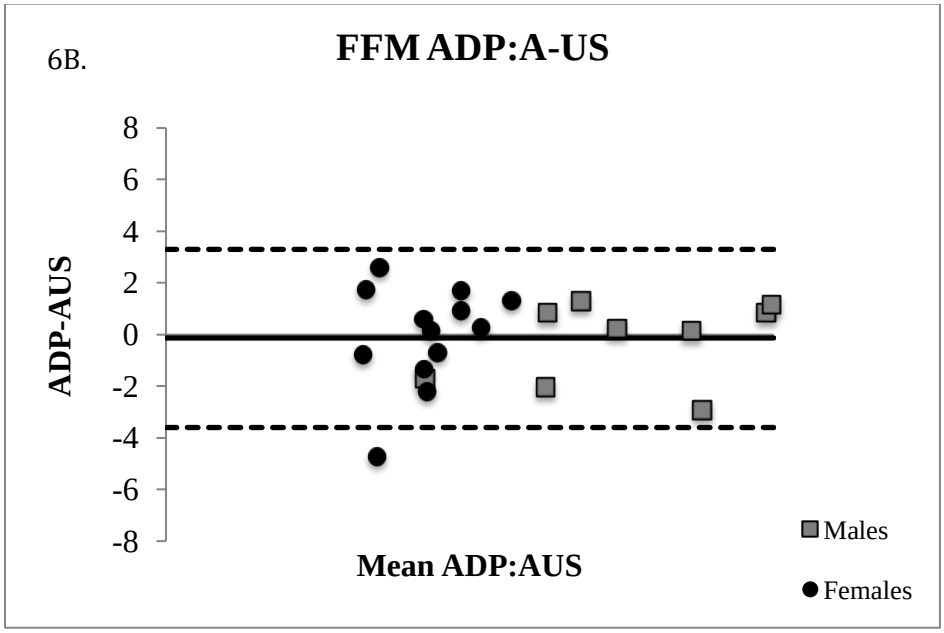


Figure 5A-C. Bland-Altman plots showing 95% LOA for Study 2A-%BF. Solid line represents mean difference; dashed lines indicate MD±1.96*SD.

Figure 6A-C.


6A.
FFM ADP:B-US
8
6
4
2
0
-2
-4
-6
-8
ADP-BUS
Mean ADP:BUS
Males
Females


6B.
FFM ADP:A-US
8
6
4
2
0
-2
-4
-6
-8
ADP-AUS
Mean ADP:AUS
Males
Females

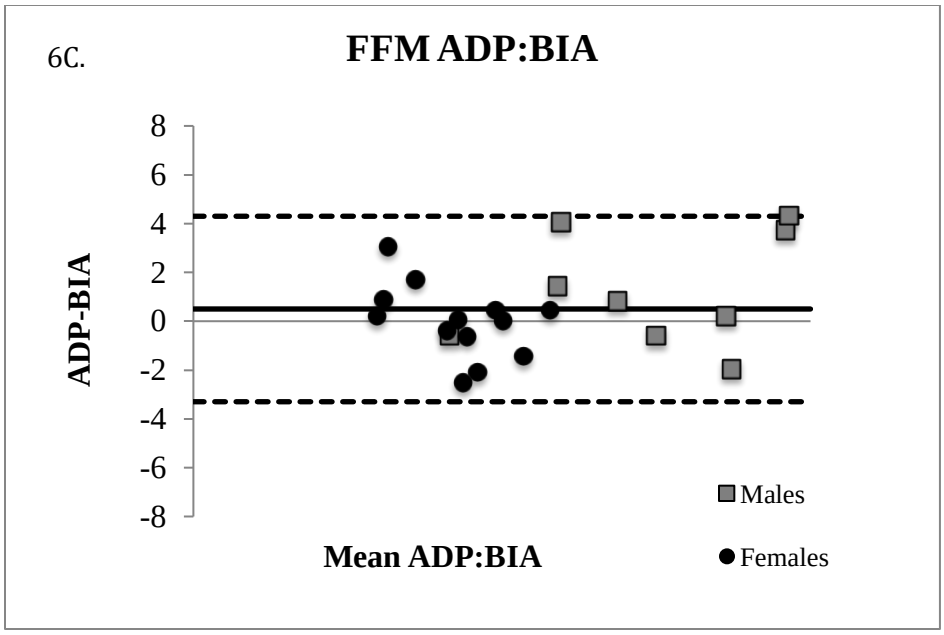
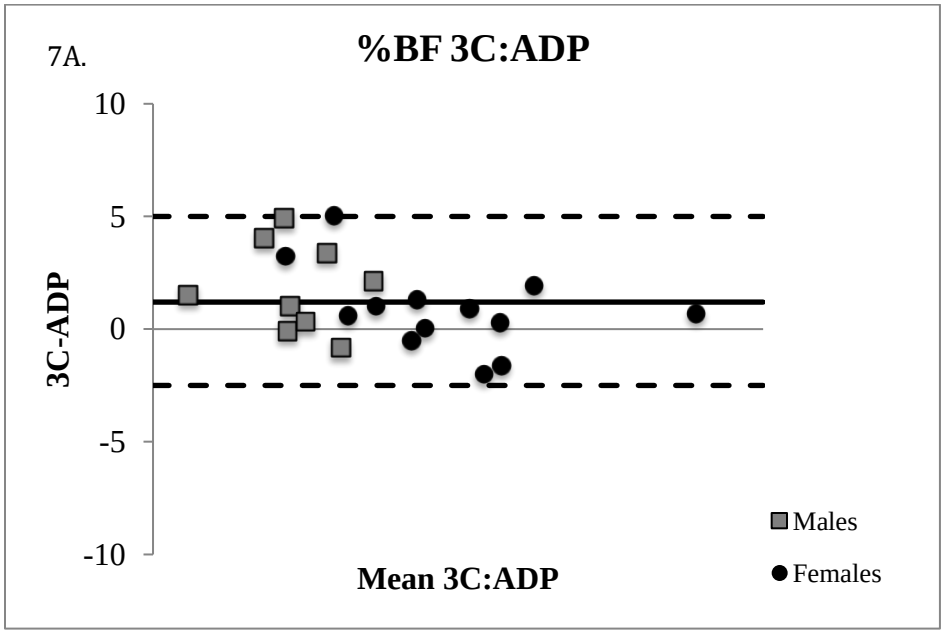
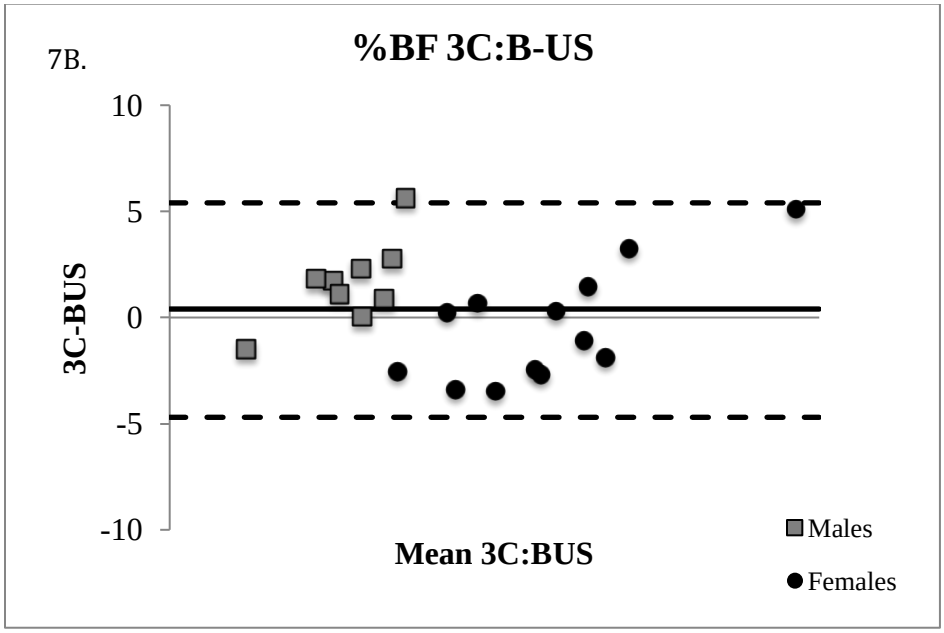


Figure 6A-C. Bland-Altman plots showing 95% LOA for Study 2A-FFM. Solid line represents mean difference; dashed lines indicate MD±1.96*SD.

Figure 7A-D.


7A.
%BF 3C:ADP
10
5
0
-5
-10
3C-ADP
Mean 3C:ADP
Males
Females


7B.
%BF 3C:B-US
10
5
0
-5
-10
3C-BUS
Mean 3C:BUS
Males
Females

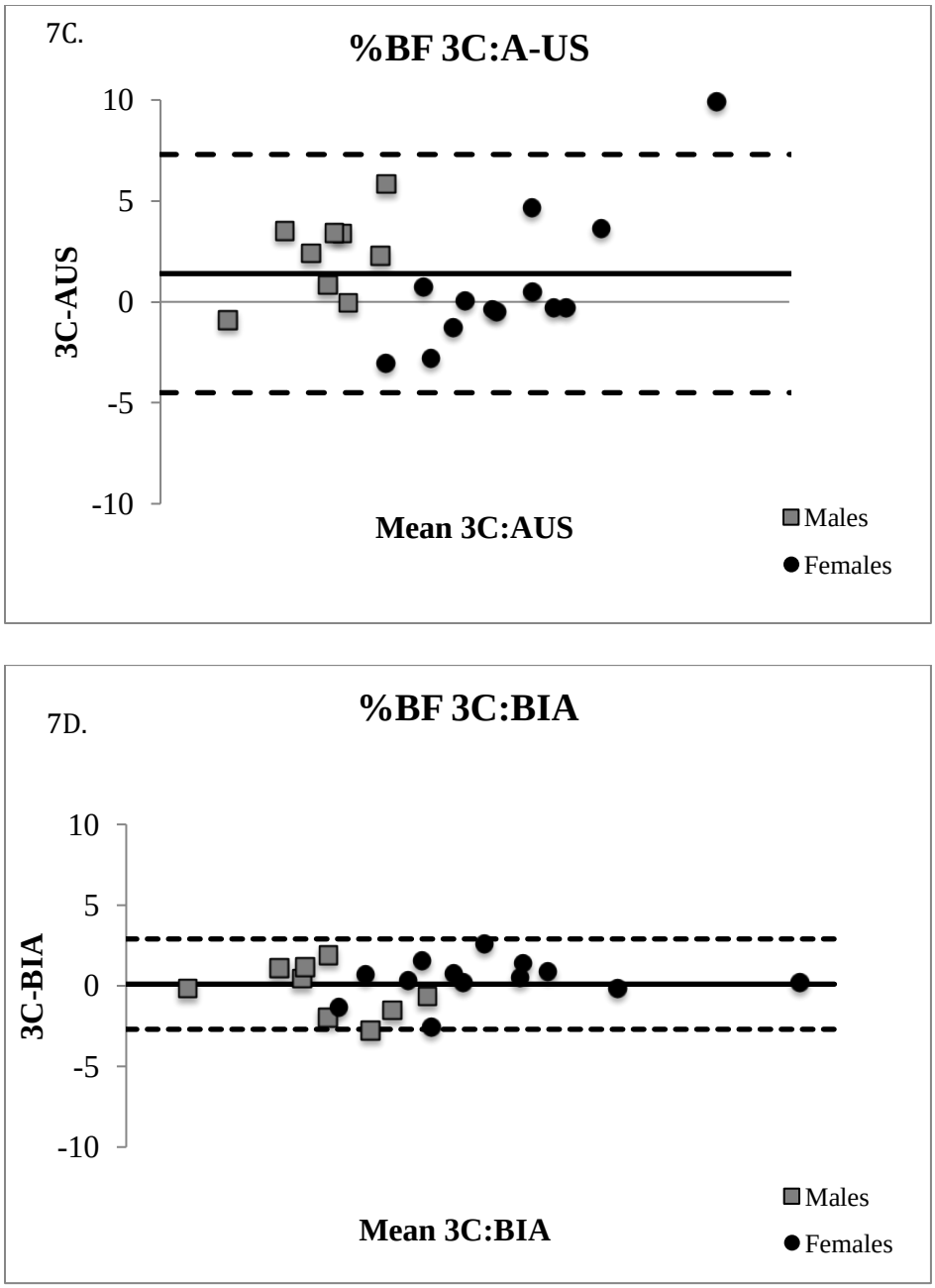
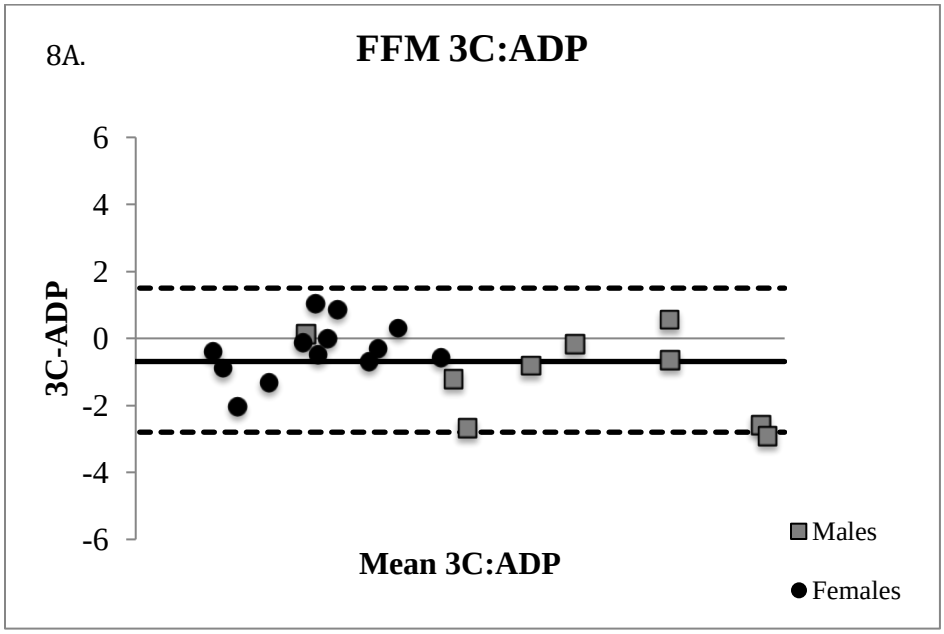
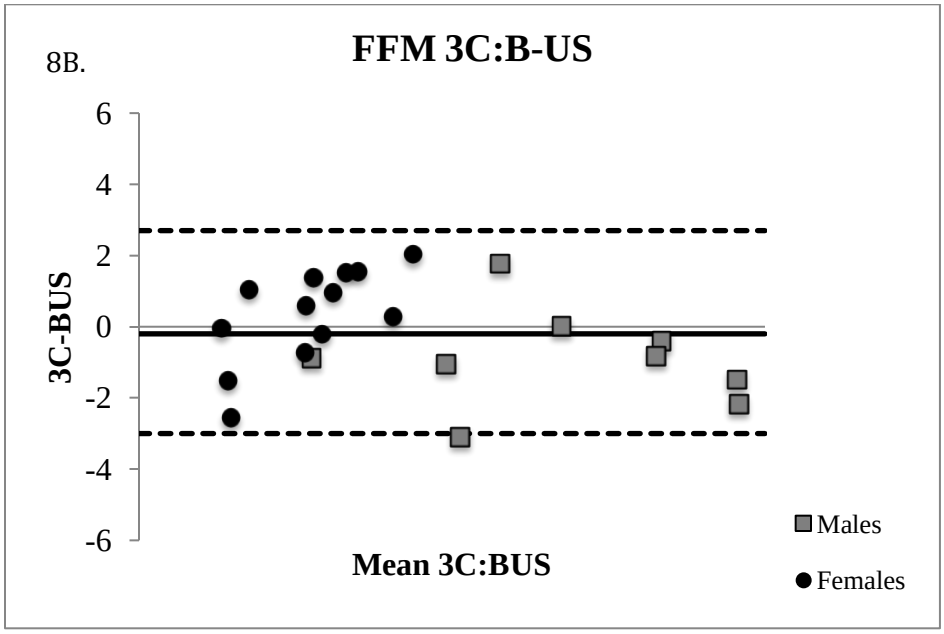
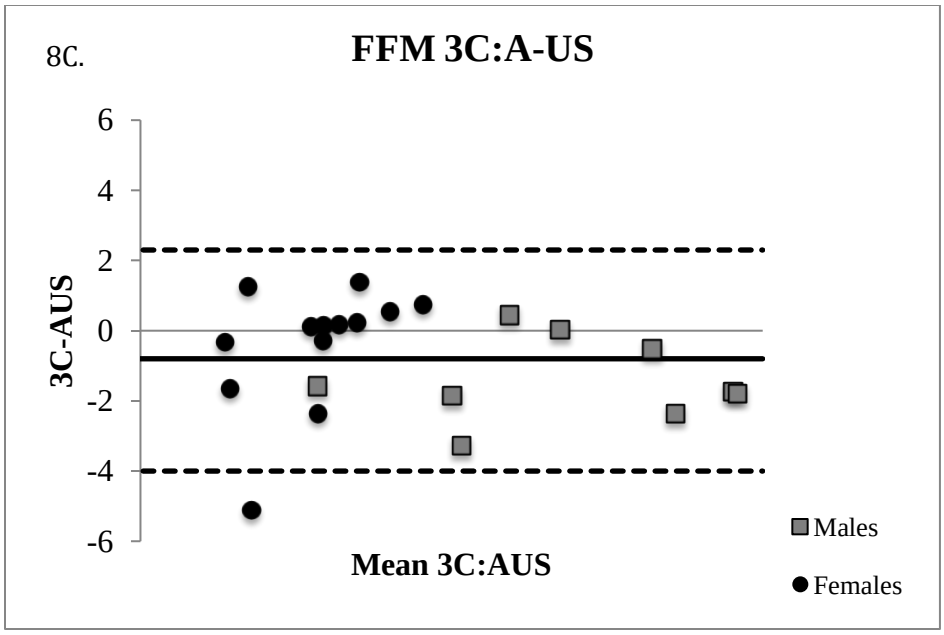


Figure 7A-D. Bland-Altman plots showing 95% LOA for Study 2B-Body fat percentage. Solid line represents mean difference; dashed lines indicate MD±1.96*SD.

Figure 8A-D.


8A.
FFM 3C:ADP
6
4
2
0
-2
-4
-6
3C-ADP
Mean 3C:ADP
Males
Females


8B.
FFM 3C:B-US
6
4
2
0
-2
-4
-6
3C-BUS
Mean 3C:BUS
Males
Females



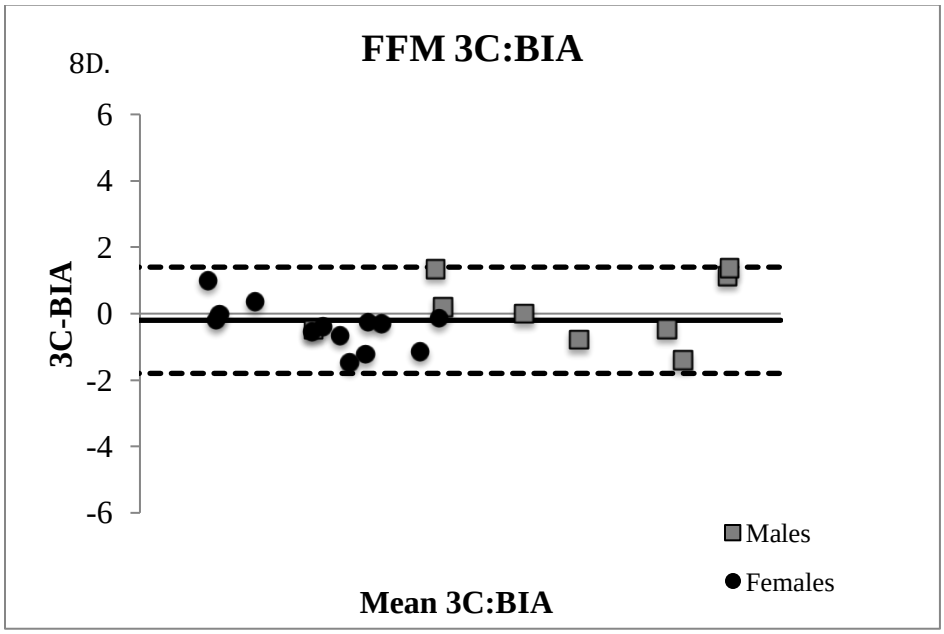


Figure 8A-D. Bland-Altman plots showing 95% LOA for Study 2B-Fat-free mass. Solid line represents mean difference; dashed lines indicate MD±1.96*SD.

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