Innovations in Body Composition Using Dual X-ray Absorptiometry

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA
BY

Madeline Anne Czeck

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Donald R. Dengel, Ph.D.

May 2023

ACKNOWLEDGMENTS

I am thankful to those who have contributed their time, efforts, and support toward the completion of this dissertation. First, I am grateful to have the opportunity to prepare this dissertation under the advisement of Dr. Donald Dengel and my committee members, Dr. Aaron Kelly, Dr. Sarah Greising, and Dr. Christopher Lundstrom. They have provided invaluable expertise, encouragement, and mentorship throughout the entirety of my graduate career. Next, I wish to thank William Juckett for his contribution to the data collection, help in manuscript production, and feedback throughout this dissertation project. Also, I would like to thank Nicholas Evanoff for his help and support during the past several years. Lastly, a special thanks to my family and friends for their endless encouragement and support throughout my graduate career.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

BIA: Bioelectrical Impedance Analysis

BIS: Bioelectrical Impedance Spectroscopy

BM: Body Mass

BMI: Body Mass Index

BV: Body Volume

BMC: Bone Mineral Content

BMD: Bone Mineral Density

DXA: Dual X-ray Absorptiometry

HDL: High-Density Lipoprotein

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

LDL: Low-Density Lipoprotein

MBR: Muscle-to-Bone Ratio

Mo: Bone Mineral

ROI: Region of Interest

SBR: Soft Tissue-to-Bone Ratio

TBW: Total Body Water

UWW: Underwater Weighing

VLDL: Very Low-Density Lipoprotein

VAT: Visceral Adipose Tissue

CHAPTER 1: INTRODUCTION

The body and its tissues can be measured using a variety of devices. Typical body composition measurement devices separate the body into multiple compartments (i.e., 2-compartment, 3-compartment, and 4-compartment). A 2-compartment body composition measurement device separates the body into fat mass and fat-free mass and the most common devices used are skinfolds, air displacement plethysmography, hydrostatic weighing, and bioelectrical impedance analysis (BIA) (Roubenoff & Kehayias, 1991). A 3-compartment body composition measurement device further separates the fat-free mass into lean and bone mass (Heymsfield et al., 2015). Therefore, the tissues measured by a 3-compartment device are lean, bone, and fat. The most widely used 3-compartment device is dual X-ray absorptiometry (DXA). Lastly, a 4-compartment device separates the body tissues into lean, fat, bone, and water. Each addition of a body tissue allows for more accurate the assessment of body composition due to fewer assumptions being made (Roubenoff & Kehayias, 1991; Wang et al., 2005). An in vivo 4-compartment model requires the use of multiple body composition devices – DXA for bone, lean, and fat; bioelectrical impedance spectroscopy for body water; air displacement plethysmography or hydrostatic weighing for volume.

To date, researchers have explored the use of DXA to measure only total body volume, thus only requiring the use of two body composition devices (Blue et al., 2018; McLester et al., 2018; Ng et al., 2018; Nickerson et al., 2017; Smith-Ryan et al., 2017; Sullivan et al., 2022; Tinsley, 2018; Wang et al., 2002; Wilson et al., 2012, 2013). It should be noted that DXA does allow for the measurement of regional body composition. However, few researchers have explored regional DXA derived volume (Brorson et al., 2009; Fuller, Laskey, et al., 1992; Gjorup et al., 2010, 2017; Wilson et al., 2013). In the

previous studies that have assessed regional DXA derived volumes, the primary population used were females with breast cancer related lymphedema. Therefore, assessing the reliability of DXA derived regional volume in a larger population of both males and females is warranted.

It is also noteworthy that even fewer researchers have examined the use of a regional DXA derived 4-compartment model. The use of a regional DXA derived 4-compartment model would allow for the accurate assessment of body composition in the arms and legs. It would also allow for any assessment of contralateral differences between right and left due to training, injury, or treatment. The inclusion of volume and body water in the regional 4-compartment model may be of use for injuries or disease where volume of specific areas is impacted most, for example, volumetric muscle loss or lymphedema.

Using DXA to assess body composition can provide information on the relationship between the different body tissues and examination of how those relationships affect overall health. Muscle places mechanical loading on the bone; this physiological stress then results in adaptations of the bone, ultimately making them stronger (Anliker & Toigo, 2012; Schoenau et al., 2002). One way to assess the relationship between muscle and bone is to divide the amount of muscle on a particular bone or combination of bones (i.e., muscle-to-bone ratio). To date, most researchers have primarily explored the muscle-to-bone ratio (MBR) in athletes (Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Gomez-Bruton et al., 2019; Holway & Garavaglia, 2009), with even fewer studies using DXA to determine bone mass (Gomez-Bruton et al., 2019). To the best of our knowledge, no studies have explored the MBR in children with obesity. This is noteworthy as children with obesity have higher bone mass and bone density compared to their normal

weight peers (Dimitri, 2019; Litwic et al., 2021; Rinonapoli et al., 2021; Rokoff et al., 2019), however, are at a greater risk of fractures (Compston, 2013; Dimitri et al., 2010). Therefore, the MBR may provide insight into these findings. In addition to the MBR, the soft tissue (lean mass and fat mass)-to-bone ratio (SBR) may provide even further insight into the impact excess mass has on bone health. Thus, this dissertation's purpose was to: (a) expand on DXA derived total volume by exploring DXA derived regional volumes and to use DXA derived regional volumes in a 4-compartment model; and (b) to use DXA to explore the MBR and SBR in children with obesity.

This dissertation's specific aims were as follows:

1. Explore total and regional volumes derived from DXA and further compare the DXA derived volumes to volumes measured from a traditional method.
    a. *Hypothesis:* Total and regional DXA derived volumes will not significantly be different from the traditional measure of volume – underwater weighing for total body and water displacement for regional).
2. Compare a total and regional 4-compartment model with DXA derived volume to a 4-compartment model with volume measured from underwater weighing (total) and water displacement (regional).
    a. *Hypothesis:* The total and regional 4-compartment model with DXA derived volumes will not significantly differ from the four-compartment model with volumes measured.
3. Examine the relationship between muscle and bone in children with obesity and compare the MBR to their normal weight and overweight peers.

a. *Hypothesis:* The MBR will be significantly different between the BMI percentile groups of normal weight, overweight, and obesity; with the obesity group having higher MBR.

This dissertation's second chapter provides a review of the current literature related to body composition compartments and the use of DXA to measure body composition. Additionally, the relationship between muscle and bone will be explored.

The third chapter discusses a study exploring the use of DXA in measuring regional volumes and comparing this DXA derived regional volumes to volumes traditionally measured using water displacement.

The fourth chapter outlines an investigation evaluating the DXA derived regional volume from chapter three in a regional 4-compartment model. Further, this study compares the DXA derived total and regional 4-compartment model to a traditional total and regional 4-compartment model where volume is measured with hydrodensitometry (total) or water displacement (regional).

The fifth chapter reviews a study exploring differences in body composition measures between body mass index (BMI) percentile groupings in children and adolescents. Also, this study explores the MBR and SBR in children with obesity.

Finally, the sixth chapter is a summary of each study and pertinent observations. Future research about the use to DXA for regional volumes and 4-compartment model and the MBR and SBR is proposed.

CHAPTER 2: LITERATURE REVIEW

BODY COMPOSITION

The assessment of body composition is important as it can be a marker for disease, such as cardiovascular disease, and it can also monitor changes related to aging (i.e., sarcopenia and osteoporosis). Notably, lean body mass decreases with age and assessing body composition can monitor the degree of lean mass loss (Peiffer et al., 2010; Roubenoff & Kehayias, 1991). Lastly, the assessment of body composition provides information on the risk of injury, sport performance, and the response of an individual to exercise and nutritional interventions (Roubenoff & Kehayias, 1991). The assessment of body composition breaks the body down into soft tissue and bone, with soft tissue being further divided into lean mass and fat mass. From there, devices that assess body composition can use the different measured tissues and can determine other variables such as percent fat.

Body Composition Compartments

When assessing body composition, the body's contents are split up into compartments (i.e., 2-compartment, 3-compartment, 4-compartment, etc.). The number of these compartments depends on the device(s) and what they are measuring. Direct chemical analysis of cadavers is the most accurate way to measure body composition. This approach analyzes the lipid (via ether extract), water, protein (via nitrogen), and bone mineral (via phosphorous and calcium) of the body. However, direct chemical analysis is not practical for wide scale use and cannot be done in vivo (Heymsfield et al., 2015). Therefore, multiple methods have been developed to measure body composition in vivo, and they range in level of complexity.

2-Compartment Model

The 2-compartment body composition model separates the body into two compartments: fat mass and fat-free mass. With this model, only one compartment is measured and then it is subtracted from the total mass to determine the other compartment (Roubenoff & Kehayias, 1991). Examples of a 2-compartment model include anthropometric measures (i.e., skinfold thickness, bone dimension, and circumference measures), hydrodensitometry, air displacement plethysmography, and bioelectrical impedance (BIA) (Lohman et al., 1997). Anthropometric measurements use an equation or series of equations to predict percent fat (Lohman et al., 1997). The accuracy of anthropometric measures depends on the equation used, technician's skill, and the location of the measurement (Lohman et al., 1997). Hydrodensitometry uses equations to estimate body density, which is then used to estimate percent fat. BIA and bioelectrical impedance spectroscopy (BIS) uses a weak electrical current at multiple frequencies sent throughout the whole body and can be used to measures total body water (TBW), fat mass, fat-free mass, and percent fat (Cannon & Choi, 2019; Lohman et al., 1997). TBW is the sum of the extracellular water and intracellular water and is a valid measure of TBW compared to deuterium oxide dilution (Jaffrin & Morel, 2008; Moon et al., 2009). The 2-compartment model assumes that the composition and density of fat-free mass is constant throughout the body (Heymsfield et al., 2015).

3-Compartment Model

The 3-compartment model for body composition further breaks down the 2-compartment model by separating fat-free mass into lean mass and bone mass (Heymsfield

et al., 2015). An example of a 3-compartment includes DXA, which measures total and regional lean mass, fat mass, and bone mass. Although DXA was originally developed for clinical settings it is now used in laboratory as well as sports performance settings.

4-Compartment Model

The 4-compartment model further breaks the fat-free mass compartment into protein, water, and mineral (Fuller, Jebb, et al., 1992; Heymsfield et al., 2015). The 4-compartment model was developed based on elemental partitioning and in vivo chemical analysis (Heymsfield et al., 2015). However, these methodologies require cadaver analysis, high cost, and/or a high level of expertise, thus limiting its practical use as a 4-compartment model (Heymsfield et al., 2015). As a result, researchers aimed to develop a 4-compartment model that can be used in vivo (Heymsfield et al., 2015; Lohman & Going, 1993; Wang et al., 2002; Wilson et al., 2012). An in vivo 4-compartment model has been developed by measuring body mass, body volume, bone mineral, and TBW. Bone mineral can be determined using DXA's measurement of bone mineral content. However, most models adjust the DXA bone measurements to reflect the bone ashing process – when bone mineral is heated and labile elements are released (Heymsfield et al., 2015). TBW can be measured by deuterium oxide dilution or BIS. Using BIS to measure TBW further simplifies the 4-compartment model process because it is easy, quick, and inexpensive compared to deuterium oxide (Moon et al., 2009). Notably, the 4-compartment model builds on the 2- and 3-compartment model because it does not assume fixed hydration, and removes assumptions about the proportions of fat-free mass (Fuller, Jebb, et al., 1992). Ultimately,

by measuring multiple parts of fat-free tissue, it reduces measurement error and the amount of assumptions in the model (Roubenoff & Kehayias, 1991; Wang et al., 2005).

Measuring Body Volume

One vital component of the in vivo 4-compartment model of body composition is body volume. Multiple techniques have been created to measure body volume, including air displacement plethysmography, hydrodensitometry, water displacement, and DXA. Each of these methods has certain advantages as well as limitations.

Air Displacement Plethysmography

Air displacement plethysmography uses a pod like device, where the subject sits inside. During this time, the chamber uses a system to create small pressure fluctuations that are analyzed. From these pressure fluctuations, the air volume of the chamber can be derived. The subject's volume is estimated from the difference between the air volume of the empty chamber and the air volume with the subject inside the chamber (Lohman et al., 1997). Notably, the air in the lungs also impacts this measure. Therefore, lung volume is either measured or predicted (Heymsfield et al., 2015).

Hydrodensitometry

Hydrodensitometry or underwater weighing (UWW) is a common laboratory technique for measuring body volume. This method requires the participant to fully submerge into a tank of water, during which their underwater weight is measured. UWW is based on Archimedes' principle that body volume can be estimated by the loss of mass

in water. However, the derived weight needs to be corrected for water density and lung volume (either measured or predicted) (Lohman et al., 1997). Thus, total body volume is obtained by dividing mass by density. Also, measuring body mass underwater and dry body mass, percent fat via the Siri equation can be estimated using a 2-compartment model (Heymsfield et al., 2015).

Water Displacement

Water displacement is similar to UWW because it uses Archimedes' principle of measuring the volume of an object by measuring the water displaced. This method is inexpensive and safe, however, it can have high intra- and inter-rater variation (Gjorup et al., 2010). While this method cannot provide information about tissue composition, it allows for regional (i.e., arms and legs) measurement of volume.

Dual X-ray Absorptiometry

Recently, investigators have explored DXA as a method to estimate body volume. The DXA uses a calibration phantom of known densities that are equivalent to biological material. These calibration equations are used to predict fat and lean mass from the measured pixel attenuation values. By creating calibration equations to attenuate pixel volume for total body estimates, this allows the DXA to be used to estimate body volume (Heymsfield et al., 2015). Notably, this method does not require lung volume measures or estimates, thus eliminating errors related to modeling estimation of this value.

Dual X-Ray Absorptiometry

DXA is a minimally invasive measurement of fat mass, lean mass, and bone mineral content. Each tissue type has a known ratio of mass attenuation (R value) and DXA uses two different X-rays (low and high photon energy) to determine each tissue (Blake & Fogelman, 1997; Pietrobelli et al., 1996). The R value is given to every pixel in the DXA scan, and along with delineation of skeletal boundaries, the DXA system can solve for two components in each pixel (i.e., fat and lean or soft tissue and bone mineral) (Blake & Fogelman, 1997; Mazess et al., 1990; Pietrobelli et al., 1996). DXA software not only allows for total body scan analysis, but also allows for regional (i.e., arms, legs, and trunk) measures of fat mass, lean mass, and bone mass through the use of region of interest (ROI) boxes (Fuller, Laskey, et al., 1992; Heymsfield et al., 2014). DXA has been shown to be valid and reliable for total body as well as regional body measure of lean mass, fat mass, and bone mass (Bilsborough et al., 2014; Haarbo et al., 1991; Mazess et al., 1990; Rothney et al., 2012). Similarly, new advances in DXA technology allow for the quantification of visceral adipose tissue (VAT) mass. DXA has been shown to be a valid and highly reproducible method to measure VAT mass compared to computed tomography (Glickman et al., 2004; Kaul et al., 2012). Notably, previous studies have explored DXA-derived VAT as it relates to health (i.e., insulin resistance, fitness level, etc.) and thresholds for the accumulation of VAT (Bantle et al., 2019; Bosch, Chow, et al., 2015; Bosch, Dengel, et al., 2015; Bosch, Steinberger, et al., 2015; Kelly et al., 2020). The assessment of body composition can assess disease, injury risk, and response to an exercise or nutritional intervention. Measuring regional body composition allows for the assessment of potential

muscle asymmetries and any changes during recovery from disease or injury and how each limb is affected.

Dual X-ray Absorptiometry Derived 4-Compartment Model

A traditional in vivo 4-compartment model requires a minimum of 3 testing procedures including: 1) DXA for measures of bone mineral content (BMC), 2) deuterium dilution or BIS to measure total body water, and 3) air displacement plethysmography or hydrostatic weighing for measures of body volume. Being able to use DXA to measure BMC and body volume would not only decrease the total amount of equipment necessary but would also decrease the total time spent on testing procedures. As a result, previous studies have looked at a way to use DXA in a convenient 4-compartment model (Blue et al., 2018; McLester et al., 2018; Ng et al., 2018; Nickerson et al., 2017; Smith-Ryan et al., 2017; Sullivan et al., 2022; Tinsley, 2018; Wang et al., 2002; Wilson et al., 2012, 2013). Wang et al. (Wang et al., 2002) proposed a new 4-compartment model to determine body fat percent from body volume, total body water, bone mineral, and body mass. Other studies then used the regression equation by Wilson et al. (Wilson et al., 2013) to validate DXA's use in the 4-compartment model (Blue et al., 2018; McLester et al., 2018; Nickerson et al., 2017; Smith-Ryan et al., 2017; Sullivan et al., 2022; Tinsley, 2018). Specifically, Smith-Ryan et al. (Smith-Ryan et al., 2017) created their own linear regression equation based off Wilson et al., (Wilson et al., 2013) and used a larger population to validate total body volume DXA from the traditional method of body volume from air displacement plethysmography. From this study, other researchers used the Smith-Ryan (Smith-Ryan et al., 2017) equation to in a variety of populations to validate total body

volume from DXA (Blue et al., 2018; McLester et al., 2018; Nickerson et al., 2017; Sullivan et al., 2022; Tinsley, 2018). While studies found total body volume from DXA to be reliable using Smith-Ryan (Smith-Ryan et al., 2017) equation, some studies reported significantly different fat mass and percent fat measures from the DXA derived 4-compartment model (Blue et al., 2018; McLester et al., 2018; Tinsley, 2018). Thus, creating a new linear regression equation to determine total body volume from DXA may further expand previous literature.

As previously discussed, DXA allows for total and regional measures of body composition. Therefore, the use to DXA to measure total body volume may be applied to regional measures. Previous studies have explored the use of DXA to derive regional body volume (Brorson et al., 2009; Fuller, Laskey, et al., 1992; Gjorup et al., 2010, 2017; Wilson et al., 2013). Of these previous studies, three compared DXA-derived regional volume to a traditional method of body volume measurement of water displacement (Brorson et al., 2009; Gjorup et al., 2010) and anthropometry (Fuller, Laskey, et al., 1992). Only one study used a population of presumably healthy adults (Fuller, Laskey, et al., 1992), while the other studies examined females with breast cancer-related lymphedema (Brorson et al., 2009; Gjorup et al., 2010). Notably, the equations to determine regional volume were different between the study in the healthy adults and the studies in females with breast cancer. Gjorup et al. (Gjorup et al., 2010) and Brorson et al. (Brorson et al., 2009) used the same equations, however, only Gjorup et al. (Gjorup et al., 2010) reported differences between DXA derived volume compared to volume from water displacement. Similarly, Wilson et al. explored both arm and leg regional volumes, but did not report a different equation from total body volume derived from DXA, and did not compare it to a traditional

method (Wilson et al., 2013). In addition, regional volumes derived from DXA would expand on previous literature as it would allow for a convenient regional 4-compartment model. Thus, more research on regionally derived volume from the DXA and its application in a 4-compartment model is warranted.

MUSCLE-TO-BONE RATIO

While more research on DXA's application in a 4-compartment model is needed, DXA is a valid and reliable way to measure total and regional lean mass, fat mass, and bone mass. Therefore, DXA can be used to measure the relationship between tissues, specifically the relationship between muscle and bone. Muscle and bone work together to create movement and thus the unit is termed the "functional muscle-bone unit" (Anliker & Toigo, 2012; Schoenau et al., 2002). Importantly, the force of muscle during contraction places strain on the bones, which helps to strengthen them (Anliker & Toigo, 2012; Schoenau et al., 2002). This functional muscle-bone unit can be explored in a variety of ways. Some studies explore the functional muscle-bone unit by looking at the cross-sectional area of muscle, total lean mass, or muscle function via single leg jump in relation to bone mass (Anliker & Toigo, 2012; Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Holway & Garavaglia, 2009; Ireland et al., 2013; Schoenau et al., 2002; Withers et al., 1991). Both cross-sectional area of muscle and total lean mass are structural components and not the functional components of muscle. One way to explore the structural relationship between muscle and bone is through the MBR. The MBR explores the physiological adaptations of bone as a result of the mechanical loading of muscle. Previous studies have explored the MBR, specifically in animal science (Hopkins,

1996) and in athletes (Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Holway & Garavaglia, 2009; Ireland et al., 2013; Withers et al., 1991).

Muscle-to-Bone Ratio in Athletes

Studies in athletes exploring the MBR have primarily used the 5-way fractionation method (Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Holway & Garavaglia, 2009). The 5-way fractionation method uses skinfolds, girths, and breadths to estimate adipose, muscle, residual, skeletal, and skin tissues (Holway & Garavaglia, 2009). Notably, the 5-way fractionation method only provides an estimate, rather than a measure, of bone mass. DXA allows for the direct measurement of not only bone mass, but also lean mass and fat mass. Thus, using DXA to explore the MBR may be beneficial as it directly measures the tissues rather than estimating them through skinfolds, girths, and breadths. A previous study in adolescent athletes used DXA to explore the relationship between muscle and bone in swimmers and compared the swimmers' MBR to a control group (Gomez-Bruton et al., 2019). The study by Gomez-Bruton et al. (Gomez-Bruton et al., 2019) observed that swimmers had significantly lower total and regional bone to lean mass ratios compared to controls. This is indicative of either more lean mass to bone mass or less bone mass to lean mass. This relationship is important as is shows the impact that swimming may have on body composition. Since swimming is a non-weight bearing activity, bone may not be receiving the strain that it needs to create more bone mass. Therefore, swimmers have either more lean mass to bone or less bone to lean mass, when compared to controls. This ratio of muscle to bone is especially of importance in children with obesity.

Bone Health in Children with Obesity

Studies exploring fracture risk in children with obesity have reported increased risk of fracture (Compston, 2013; Dimitri et al., 2010). The exact reason for this relationship is still unclear. Researchers have explored BMC and bone mineral density (BMD) in children with obesity. These studies have reported higher BMC and BMD in children with obesity compared to their normal weight peers (Dimitri, 2019; Litwic et al., 2021; Rinonapoli et al., 2021; Rokoff et al., 2019). However, some studies have normalized bone mass to body weight and observed lower values of BMC and BMD in children with obesity (Litwic et al., 2021; L. N. Mosca et al., 2014). These observations are interesting because the excess mass that is associated with obesity may artificially increase bone mass. This artificial increase in bone mass may be through increased strain on bones by the higher amounts of mass that load the bone. However, once normalized for weight, children with obesity may not have more bone mass or denser bones compared to their normal weight peers.

Cardiometabolic Markers and Bone Health in Children with Obesity

There are cardiometabolic disease risk factors (i.e., insulin resistance, dyslipidemia, and hypertension) that may accompany obesity. These risk factors may also have a negative impact on bone health. Studies examining the relationship between DXA bone measures and cardiometabolic risk factors have reported that BMC is negatively associated with insulin, homeostatic model assessment for insulin resistance (HOMA-IR) (do Prado et al., 2009; Hetherington-Rauth et al., 2019; Karimi et al., 2021; Kindler et al., 2019; Lawlor et al., 2012; K. Lee, 2013; Pollock et al., 2011; Shawar et al., 2022), fasting glucose and total

cholesterol (Pollock et al., 2011). There is also a positive association between BMC and high-density lipoprotein (HDL) (Pollock et al., 2011). Notably, previous studies have observed no significant associations between BMC and systolic or diastolic blood pressure, triglycerides, and low-density lipoprotein (LDL) (Hetherington-Rauth et al., 2019; Pollock et al., 2011). Thus, these observations indicate that obesity related cardiometabolic risk factors may negatively impact bone health. Also, exploring these relationships may provide additional insight into the bone health in children with obesity.

Muscle in Children with Obesity

Due to the physiological relationship between muscle and bone strength previously described, exploring muscle function in children with obesity is important. Studies exploring anthropometric aspects of muscle (e.g., muscle thickness and muscle cross-sectional area) and muscular strength (e.g., grip strength, knee extensor and plantar flexor strength, and voluntary contraction) in children with obesity have observed incongruent findings. Depending on the methodology used to explore muscle, either a positive or negative relationship has been observed. For example, previous studies using grip strength, muscle thickness, muscle cross-sectional area, voluntary contraction and activation, and elbow extensors have reported a positive relationship with obesity (Deforche et al., 2003; Garcia-Vicencio et al., 2016; C. K. Lee et al., 2022; Thivel et al., 2016). Whereas, studies that utilized knee extensor strength, and plantar flexor strength have reported negative relationships with obesity (Bonney et al., 2018; Musálek et al., 2020; Singh et al., 2021; Thivel et al., 2016; Tsiros et al., 2016). The relationship between anthropometric aspects of muscle and muscular strength in children with obesity is important because previous

studies have reported increased lean mass in children with obesity (Dimitri et al., 2010). Similarly, previous studies have reported increased lean mass with increased fat mass (L. Mosca et al., 2013; Rinonapoli et al., 2021). Therefore, increased fat mass may indirectly have a positive effect on bone strength. To support this, previous studies have reported positive correlations between bone mineral density and BMC with lean mass and fat mass in females (Maïmoun et al., 2016; L. N. Mosca et al., 2014). Bone variables were positively correlated with only lean mass in males (L. N. Mosca et al., 2014). However, a previous study reported that an increased proportion of fat mass relative to lean mass was negatively associated with bone strength (Ducher et al., 2009). Therefore, these results suggest that lean mass may have a greater impact on bone health compared to fat mass alone. Lean mass directly impacts bone health through placing mechanical strain on the bone which helps strengthen bones. When normalizing BMC to lean mass, a previous study reported lower amounts of bone mass, which increases the risk of fractures (Golec & Chlebna-Sokół, 2014). Bone strength in children with obesity is important to assess as previous studies have reported an increased risk of fractures in children with obesity (Compston, 2013; Dimitri, 2019; Dimitri et al., 2010). Notably, one study reported a 25% increase in fracture risk (Dimitri, 2019). Thus, exploring the relationship between muscle and bone in children with obesity may provide insight into bone adaptations to increased loading from higher amounts of fat and lean mass.

Muscle-to-Bone Ratio in Children with Obesity

One way to explore the relationship between muscle and bone is to use the MBR. The MBR explores the adaptations of bone as a result of mechanical loading from the

muscle. Typically, mechanical loading from the muscle on the bone results in strengthening of the bones. Notably, a previous study has explored the total and regional muscle-to-bone relationship in children and how body fat percent impacts this relationship (Duran et al., 2019). For regional muscle-to-bone, these researchers combined the arms and legs and referred to their grouping as the appendicular muscle to bone unit. This study reported that body fat percentage was negatively correlated with appendicular bone mineral content for lean mass (Duran et al., 2019). These results suggest that as body fat percent increases, the appendicular muscle bone unit decreases. This decreased muscle to bone unit indicates either more lean mass for bone mass or less bone mass for lean mass. Increased lean mass to bone mass may negatively impact bone health as the bones may not be able to handle the strain that the muscles place on the bones. Thus, more research is warranted to explore the impact of pediatric obesity on bone health and how other body composition tissues (i.e., muscle to bone ratio) may affect the physiological bone adaptations.

CHAPTER 3. DUAL X-RAY ABSORPTIOMETRY-DERIVED TOTAL AND REGIONAL BODY VOLUME

Dual X-ray Absorptiometry-Derived Total and Regional Body Volume

Authors: Madeline A. Czeck[1], Erica J. Roelofs[1], William T. Juckett[1], Donald R. Dengel[1]

[1]School of Kinesiology, University of Minnesota, Minneapolis, MN, 55455

Key Words: regional body volume; DXA; underwater weighing; water displacement

Publication Reference: Czeck, MA, Roelofs, EJ, Juckett, WT, Dengel, DR. (2022). Dual X-ray absorptiometry-derived total and regional body volume. *Clinical Nutrition ESPEN, 52,* 100-104.

Disclosures: DRD receives consulting fees from Hologic, Inc. Madeline A. Czeck prepared the first draft of the manuscript without any associated honorarium, grant, or other form of payment to produce the manuscript.

Summary

Purpose: Although dual X-ray absorptiometry (DXA) has been used to determine total body volume, using DXA to determine regional (i.e., arm and leg) volumes needs further assessment. Thus, the aim of the present study is to evaluate the validity of total and regional DXA-derived body volume compared to a traditional method for measuring body volume. **Methods:** A total of 30 males and females (Age: 25.9 ± 4.0 yrs.; Height: 1.75 ± 0.10 m; Weight: 70.98 ± 14.02 kg) underwent one whole body DXA scan, underwater weighing, and regional measures of volume via water displacement. Manually created DXA region of interest boxes were used to determine regional DXA body composition. Total body volume was calculated by taking the participant's dry weight and dividing it by the average density from underwater weighing. Linear regression models with body volume from underwater weighing for total body volume and water displacement for regional volume as the dependent variable and DXA lean mass, fat mass, and bone mass as independent variables created total and regional DXA-derived body volume. T-tests assessed DXA-derived body volume to the traditional method of body volume assessment. Regression models were cross-validated using the Repeated k-fold Cross Validation method. **Results:** DXA-derived total body volume was not significantly ($p=0.999$) different from total body volume measured via total body water displacement. In addition, both arm and leg regional DXA-derived volume was not significantly different ($p=0.999$) compared to regional volume measured by regional water displacement. Cross-validation of each model produced R^2 values of 0.992, 0.923, and 0.932 for total body, arm, and leg, respectively. **Conclusions:** The DXA may be used as valid method for estimating total and regional body volume. Thus, these results expand the DXA's capabilities and potentially

allow for a convenient regional four-compartment model with DXA-derived regional volume.

Introduction

The assessment of body composition has been used to assess disease, injury, or performance and has been used to monitor changes related to aging (i.e., sarcopenia), exercise, or nutritional interventions (S. Y. Lee & Gallagher, 2008; Roubenoff & Kehayias, 1991). There have been many devices created to measure body composition, breaking down the body into compartments (i.e., 2-compartment, 3-compartment, 4-compartment). In particular, the measuring of body volume has been of interest as it allows researchers the ability to use a 4-compartment model (i.e., fat mass, lean mass, bone mass, body water) when assessing body composition (S. Y. Lee & Gallagher, 2008). Currently, there are a variety of ways to measure total body volume, including air displacement plethysmography, hydrodensitometry, water displacement, and more recently dual X-ray absorptiometry (DXA) (Blue et al., 2018; Brorson et al., 2009; Gjorup et al., 2010; Ng et al., 2018; Nickerson et al., 2017; Smith-Ryan et al., 2017; Tinsley, 2018; Wilson et al., 2012, 2013).

DXA is a widely accepted and highly used device for the determination of body composition as it separates the body tissue into three compartments (i.e., lean mass, fat mass, and bone mass) (Ackland et al., 2012; Bilsborough et al., 2014; Blake & Fogelman, 1997; Glickman et al., 2004; Haarbo et al., 1991; Kaul et al., 2012; S. Y. Lee & Gallagher, 2008; Mazess et al., 1990). Notably, previous studies have explored the use of DXA-derived total body volume compared to a traditional method (i.e., air displacement plethysmography and hydrodensitometry) and have reported that DXA-derived total body volume is highly correlated and not significantly different from the traditional methods of air displacement plethysmography (Ng et al., 2018; Smith-Ryan et al., 2017; Wilson et al.,

2012, 2013) and hydrodensitometry (Nickerson et al., 2017; Sullivan et al., 2022). However, DXA allows for total body as well as regional (i.e., arms and legs) assessment of body tissues. In addition, DXA software allows for further segmentation through the use of manually created region of interest (ROI) boxes. Manually created ROIs allow for examination of specific regions, such as the forearm or lower leg. Assessing regional body composition provides important insight into tissue imbalances, deficiencies or excesses (S. Y. Lee & Gallagher, 2008). Therefore, regional body composition can be useful when evaluating disease, interventions, performance, injury risk, and rehabilitation. Previous studies have explored the use of DXA to derive regional body volume (Brorson et al., 2009; Fuller, Laskey, et al., 1992; Gjorup et al., 2010, 2017; Wilson et al., 2013). Of these studies, only three compared DXA-derived regional volume to a traditional method of body volume measurement of water displacement (Brorson et al., 2009; Gjorup et al., 2010) and anthropometry (Fuller, Laskey, et al., 1992). Only one of these studies used a population of presumably healthy adults (Fuller, Laskey, et al., 1992) and the other were in a population of females with breast cancer-related lymphedema (Brorson et al., 2009; Gjorup et al., 2010). Thus, the purpose of the present study is to derive total and regional (i.e., arm and leg) body volume from the DXA and compare it to a traditional method of determining total and regional body volume in a healthy population of males and females.

Methods

Participants

A total of 30 males and females (age range: 21.3 – 35.8 yrs.; body fat percent range: 13.4-33.7%) participated in this study. Participants were recruited from the Twin Cities Metro area to complete one visit of body composition measures. Inclusion criteria included

the age range of 18-55 years old, participants were under the weight limit of the scanner table, have not undergone multiple X-rays in the past 12 months or any nuclear medicine, and are not pregnant. All female participants were screened for a negative pregnancy test prior to participation. All procedures were approved by the University's Institutional Review Board and all participants signed a written informed consent prior to testing. Participants were asked to be fasted for at least 6 hours and abstain from caffeine, alcohol, or nicotine for at least 14 hours prior to testing. Participants were also asked to not participate in vigorous exercise 48 hours or moderate exercise 14 hours prior to testing. Testing was conducted in the morning during the same time of day for all participants.

Dual X-Ray Absorptiometry

Height was measured using a wall-stadiometer (Seca, Hamburg, Germany), weight was measured using an electronic scale (Tanita Corporation, Tokyo, Japan), and age was recorded. Participants' limb length was measured using a tape measure. Arm length was measured from the ulnar styloid to acromion-clavicular junction, and leg length was measured from the beginning of the inseam to the medial malleolus of the tibia. Arm and leg length was used to determine 80% of each limb length and the 80% length was marked on the participant for body volume measurements. Participants received one whole body DXA scan (Hologic Horizon A; Hologic Inc., Marlborough, MA, USA) in the supine position. To prevent interference with the scan, participants removed all metal and thick clothing. All DXA scans were analyzed by the same technician using Hologic APEX software (APEX Version 5.6.0.4, Hologic Inc., Marlborough, MA, USA). Prior to the scan, metal markers were placed at 80% of limb length, which allowed for placement of manual ROI boxes post scan. The use of metal markers to create boundaries for post-scan creation

of manual ROI boxes have been previously described (Raymond-Pope et al., 2020). Manual ROI boxes were defined as from the 80% mark of the limb to the middle of the head of the ulna for the arms and the middle of the medial malleolus on the tibia for the legs. Using the 80% mark as the boundary for the regional DXA scan allowed for consistency in comparison of regions between methods of DXA and water displacement for statistical analysis. Body composition variables included total mass, lean mass, fat mass, body fat percent, bone mineral content (BMC) and bone mineral density for total body and regional measurements. To derive body volume from DXA, prediction equations for total-body and each limb followed the prediction equation proposed by Wilson et al. (Wilson et al., 2013)

$$\boldsymbol{DXA\ Volume\ (mL) = v_{fat}\ x\ fat + v_{lean}\ x\ lean + v_{BMC}\ x\ BMC + v_{residual}}$$

Where fat, lean, and BMC correspond to the DXA mass measures. The coefficients correspond to the respective inverse densities for fat (v_{fat}), lean (v_{lean}), and BMC (v_{BMC}), and $v_{residual}$ is the volume unaccounted for by DXA.

Underwater Weighing

Underwater weighing was performed using a specialized underwater weighing system (EXERTECH, La Crosse, WI). Subjects wore tight fitting clothing and a nose clip. Subjects were seated in the apparatus and were instructed to maximally exhale and slowly submerge into the water. Residual volume was estimated based on subject's height, sex, and age. The average density of three trials of underwater weighing was used to derive

total body volume. Total body volume was calculated by taking the participant's dry weight and dividing it by the averaged density.

Water Displacement

Limb volume was measured using water displacement via two custom designed volumetric cylinders, one for the arm and one for the leg. The volumeters had a valve at the top to control for water flow. At the beginning of measurements, the participant's hands and feet were tared. Once the hands and feet were zeroed, the participant slowly immersed the rest of their limb until the 80% mark was in line with the valve on the volumetric cylinder. The water displaced was collected and measured using graduated cylinders and measured to the nearest 10 mL.

Statistical Analysis

Descriptive statistics were calculated for the participant population. Linear regression using the step Akaike Information Criteria method with body volume from underwater weighing for total body volume and water displacement for regional volume as the dependent variable and fat mass, lean mass, and BMC from the DXA as independent factors determined the coefficients for the prediction equations. Paired t-tests compared DXA-derived regional volume to the regional volume measured by underwater weighing and water displacement. The volume measured by underwater weighing and water displacement was compared to the DXA-derived volume using constant error, total error, standard error of the estimates, and Bland-Altman plots determined limits of agreement. Regression models were cross-validated using the Repeated k-fold Cross Validation method. All analyses were conducted using R software (R Foundation for Statistical Computing, Vienna, Austria) with an alpha level of $p \leq 0.05$.

Results

Subject demographics and anthropometrics are reported in Table 1. For total body DXA-derived body volume, the coefficients are 1.21 ($p<0.001$), 0.94 ($p<0.001$), and -1.17 ($p=0.454$) for fat, lean, and BMC, respectively, with a residual volume of -783.70 mL. A total of 60 limbs were used for the leg and arm linear regression equation. Coefficients for the DXA-derived arm volume equation are 0.66 ($p<0.001$), 0.68 ($p<0.001$), and 1.40 ($p=0.539$) for fat, lean, and BMC, respectively, with a residual volume of 311.99 mL. The coefficients for the DXA-derived leg volume equation are 1.13 ($p<0.001$), 0.83 ($p<0.001$), and 1.04 ($p=0.586$) for fat, lean, and BMC, respectively, with a residual volume of -24.96 mL.

$$\boldsymbol{Total\ Body\ Volume\ (mL) = 1.12\ x\ fat + 0.94\ x\ lean - 1.17\ \ x\ BMC - 783.70}$$

$$\boldsymbol{Arm\ Volume\ (mL) = 0.66\ x\ fat + 0.68\ x\ lean + \ 1.40\ x\ BMC + 311.99}$$

$$\boldsymbol{Leg\ Volume\ (mL) = 1.13\ x\ fat + 0.83\ x\ lean + \ 1.04\ x\ BMC - 24.96}$$

DXA-derived total body, arm, and leg volume were not significantly different compared to volume measured by underwater weighing and water displacement (Table 2). Table 3 presents the constant error, total error, standard error of estimate, and limits of agreement between the two models for total body, arm, and leg. Bland-Altman plots for validity are presented in Figure 1 for total body (Panel A), arm (Panel B), and leg (Panel C).

Each model was cross-validated using the repeated k-fold cross-validation approach, specifically, 3 repeats of a 10-fold cross-validation. The R^2 value for each model is 0.992, 0.923, and 0.932 for total body, arm, and leg volumes, respectively.

Discussion

The current study aimed to quantify total and regional body volumes derived from the DXA and compare it to a traditional method of measuring body volume (i.e., underwater weighing and water displacement). Notably, the current study observed no significant differences between DXA-derived total and regional body volumes when compared to the traditional method of measuring body volume. Additionally, the current study cross-validated these regression models and reported high R^2 values, suggesting high validity of the models.

Total Body DXA-Derived Volume

Previous studies that determined total body volume using DXA-derived have reported DXA-derived total body volumes were highly correlated and not significantly different from the traditional methods of determining body volume (i.e., air displacement plethysmography and hydrodensitometry) (Ng et al., 2018; Nickerson et al., 2017; Smith-Ryan et al., 2017; Sullivan et al., 2022; Wilson et al., 2012, 2013). The present study also observed no significant differences between DXA-derived total body volume and total body volume determined by underwater weighing. Notably, the present study observed different linear regression coefficients/densities for the prediction equation proposed by Wilson et al. (Wilson et al., 2013) and from another previous study (Smith-Ryan et al., 2017; Wilson et al., 2013). The present study reports coefficients of 1.21, 0.94, and -1.17 for fat, lean, and BMC, respectively. These coefficients correspond with densities of 0.83,

1.06, and 0.53 g/mL for fat, lean, and BMC, respectively. Studies conducted by Smith-Ryan et al. and Wilson et al. have reported fat, lean, and BMC densities as 0.84 vs. 0.88, 1.03 vs. 1.05, and 11.63 vs. 4.85 kg/L (Smith-Ryan et al., 2017; Wilson et al., 2013). While the densities for fat and lean are similar between studies, the density for BMC are not only different between these studies, but also the present study. Both Wilson et al. (Wilson et al., 2013) and Smith-Ryan et al. (Smith-Ryan et al., 2017) used Hologic Discovery-W DXA scanners whereas the present study used Hologic Horizon A scanner.

Bland-Altman plots demonstrated a slight negative bias (-0.03 mL) between underwater weighing and DXA-derived total body volume. The current study also observed no significant differences between DXA-derived total body volume and total body volume measured from underwater weighing. Thus, along with the high correlation ($R^2 = 0.992$) from the cross-validation models, these results suggest that total body volume can be accurately derived from a single DXA scan.

Regional DXA-Derived Volume

The present study expands on previous literature by exploring regional DXA-derived volumes. Previous studies have explored the use of DXA to derive regional volumes of the arms and legs (Brorson et al., 2009; Fuller, Laskey, et al., 1992; Gjorup et al., 2010, 2017; Wilson et al., 2013), however, only three of these studies compared the DXA-derived regional volume to a traditional method for measuring volume (i.e., water displacement and anthropometry) (Brorson et al., 2009; Fuller, Laskey, et al., 1992; Gjorup et al., 2010). The study conducted by Fuller et al. compared DXA-derived regional volume to volume estimated from circumference and length measures of the upper arm, forearm, thigh, and lower leg (Fuller, Laskey, et al., 1992). Also, this previous study used the

densities of fat mass (0.9 g/cm^3) and fat-free mass (1.1 g/cm^3) to derive regional volume from the DXA, thus combining lean mass and bone mass together. The study by Fuller et al. reported no significant differences between the anthropometric arm volume and the DXA-derived arm volume (Fuller, Laskey, et al., 1992). However, Fuller et al. did report significant differences in leg volume between the two measures (Fuller, Laskey, et al., 1992). Notably, definitions of the regions measured were defined differently between the two methods of DXA and anthropometry, ultimately limiting the comparison between the two methods (Fuller, Laskey, et al., 1992). The authors did not report manual ROI for the regional DXA volume, thus a typical arm region automatically defined by DXA consists of the hand. Whereas anthropometry measures were taken at the upper arm and forearm, suggesting there was no hand in the determination of arm volume. The present study used the same defined arm and leg regions for measures of volume from the DXA and water displacement. Thus, the present study expands on the previous study by using water displacement – the gold standard for volume measures – to measure regional volume instead of estimating volume from anthropometry.

In addition, the studies by Brorson et al. and Gjorup et al. only explored DXA-derived regional volumes of the arm (Brorson et al., 2009; Gjorup et al., 2010). These studies were also only conducted in females with breast cancer lymphedema. Therefore, the current study adds to previous literature by exploring DXA-derived regional volumes of both the arm and the leg and comparing both limbs to the gold standard of measuring volume via water displacement in a population of presumably healthy adult males and females.

Notably, the previous study by Gjorup et al. observed arm volume from water displacement was significantly underestimated compared to DXA-derived arm volume (Gjorup et al., 2010). One possible explanation for this could be the equation they used to estimate DXA-derive volume. Gjorup et al. used densities based on a previous study by Brorson et al. (Brorson et al., 2009; Gjorup et al., 2010). While Gjorup et al. reported the densities for each body composition measure they used, the equation for DXA-derived volume proposed by Wilson et al. suggest a residual volume, which Gjorup et al. did not report. The residual volume is the volume that is unaccounted for in the lean mass, fat mass, and bone mass from the DXA. On the other hand, the study conducted by Wilson et al. explored regional DXA-derived volumes of the trunk and legs, however, they did not compare these volumes to a traditional method of measuring volume (Wilson et al., 2013). They also did not report different densities used for total body and regional DXA-derived volume, thus suggesting the densities they reported were used for both equations. The current study observed different densities and residual volumes for total body, arm, and leg DXA-derived equations. These observed differences in regional coefficients/densities from total body coefficients may suggest total body, arm, and leg volume equations are not interchangeable.

Bland-Altman plots demonstrated a slight negative bias between water displacement and DXA-derived arm (-0.51 mL) and leg (-0.21 mL) volume. The current study also observed no significant differences between regional DXA-derived volume and regional volume measured by water displacement for both the arm and the leg. Thus, along with the high correlation from the cross-validation models for both the arm (R^2=0.923) and

leg (R^2=0.932), these data suggest that regional volume can be accurately derived from a single DXA scan.

Limitations of the present study include the homogeneous sample population; thus, the regional equations may not be generalizable to other populations (i.e., obesity, disease, and older or younger populations). Also, measurement of limb volume via water displacement required participants to stay still for long periods of time, which was challenging for some participants. Lastly, the equations proposed were created only for 80% of limb length due to limitations of measuring the full limb by water displacement. Thus, the full limb length that is defined by DXA automatically created ROI boxes could not be used in the analyses.

Conclusion

The current study observed no significant differences between total and regional DXA-derived volume compared to a traditional method for measuring volume. Therefore, the results of the current study suggest that DXA can derive not only total but also regional volumes. The development of a method to derive regional volumes from DXA can be implemented in future clinical and research settings. DXA provides a quick assessment of total body and regional body composition measures and thus being able to derive body volume further expands DXA's capabilities. Similarly, DXA-derived body volume allows for a "convenient" DXA-derived 4-compartment model. It is more convenient than traditional methods to determine body volume as hydrodensitometry and air displacement plethysmography require specialized equipment, and body water from isotope dilution requires multiple hours to complete (Smith-Ryan et al., 2017; Wang et al., 2002). Thus, DXA-derived volume reduces the time and cost required to obtain body volume. In

addition, the use of DXA, along with bioelectrical impedance spectroscopy, may allow for a regional 4-compartment model. A regional 4-compartment analysis can be used to track regional changes due to site specific cancers, tumors, or trauma (e.g., volumetric muscle loss). Future research should explore DXA-derived regional volume in a variety of populations and its utility in a regional 4-compartment model.

Table Legends

Table 1. Demographics and Anthropometrics of Cohort.

Table 2. Mean ± standard deviation of total and regional volume derived from the DXA and measured by water displacement.

Table 3. Constant error, total error, standard error of estimate, and limits of agreement of body volume between water displacement and DXA-derived volume.

Tables

Table 1. Demographics and Anthropometrics of Cohort.

	Total	Male (n=18)	Female (n=12)
Age (yrs)	25.9 ± 4.0	24.6 ± 3.8	26.4 ± 4.4
Height (m)	1.75 ± 0.10	1.82 ± 0.06	1.65 ± 0.04
Weight (kg)	70.98 ± 14.02	79.33 ± 11.24	58.46 ± 6.15
Total Mass (kg)	71.0 ± 13.99	79.27 ± 11.34	58.59 ± 6.11
Total Fat Mass (kg)	14.16 ± 3.35	13.41 ± 2.80	15.28 ± 3.91
Total Lean Mass (kg)	56.84 ± 13.79	65.86 ± 10.07	43.31 ± 3.36
Total BMC (kg)	2.57 ± 0.50	2.85 ± 0.43	2.15 ± 0.21
Total BMD (kg)	1.18 ± 0.10	1.22 ± 0.11	1.13 ± 0.06
Total Body Fat Percent (%)	20.48 ± 5.67	16.96 ± 2.85	25.78 ± 4.62

Table demographics and anthropometrics are reported at mean ± standard deviation.

Abbreviations: BMC, bone mineral content; BMD, bone mineral density

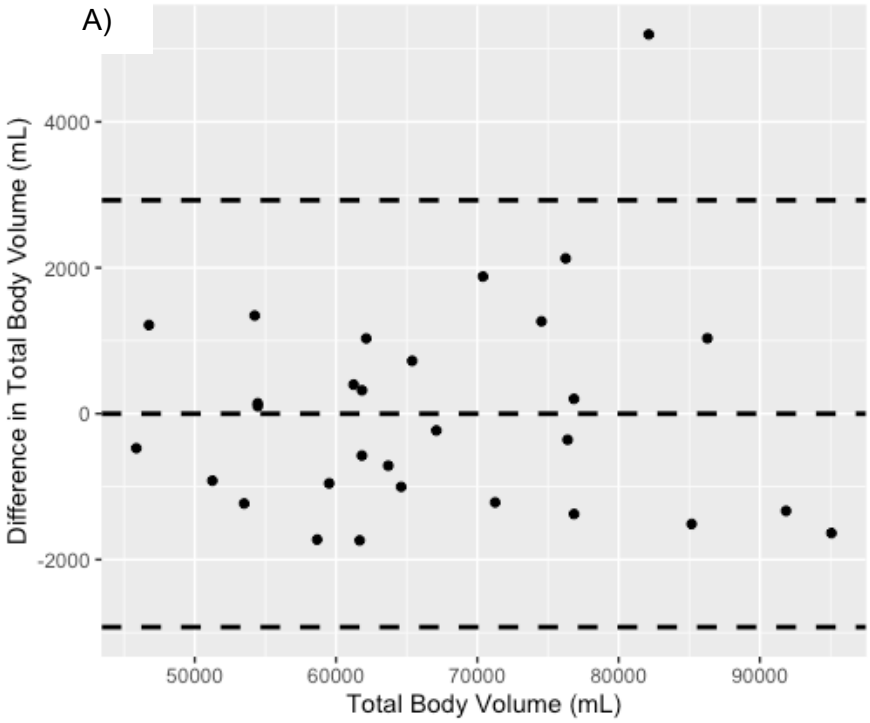
Table 2. Mean ± standard deviation of total and regional volume derived from the DXA and measured by water displacement.

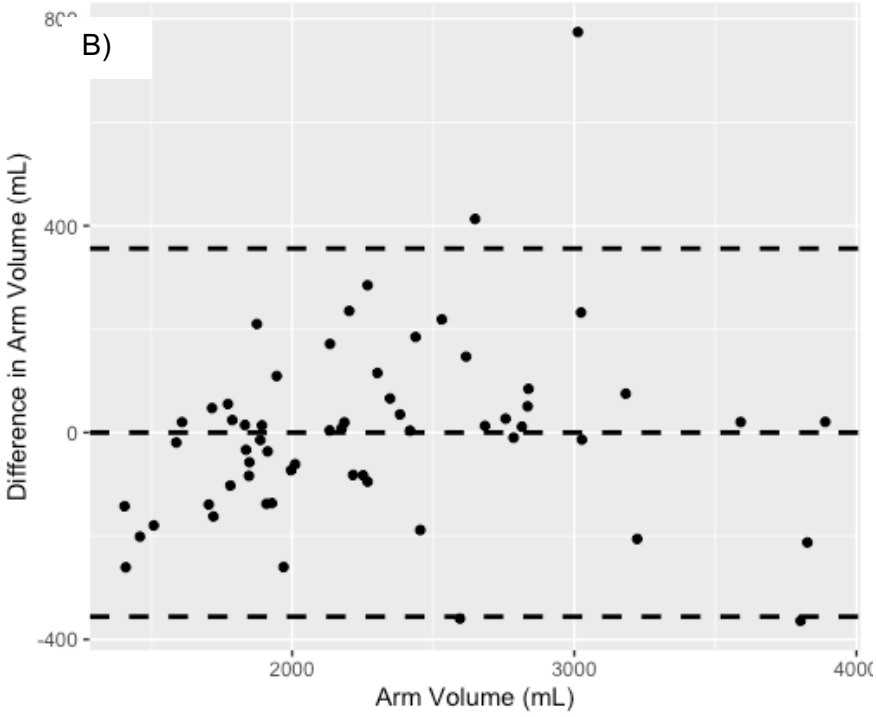
	DXA Volume (mL)	Water Displacement Volume (mL)	p-value	95% Confidence Interval
Total Body	67040.13 ± 12895.87	67039.89 ± 12981.87	0.999	-557.47, 556.99
Arm	2300.26 ± 602.17	2300.25 ± 628.94	0.999	-46.91, 46.90
Leg	6528.73 ± 1299.95	6528.75 ± 1354.64	0.999	-98.40, 98.44

Abbreviations: DXA, dual X-ray absorptiometry

Table 3. Constant error, total error, standard error of estimate, and limits of agreement of body volume between water displacement and DXA-derived volume.

	Constant Error	Total Error	Standard Error of Estimate	Limits of Agreement
Total Body (mL)	-0.24	1.31	0.38	-2925.13, 2924.65
Arm (mL)	-0.01	0.05	0.19	-355.90, 355.88
Leg (mL)	0.02	0.18	0.34	-746.73, 746.78


A)
Difference in Total Body Volume (mL)
4000
2000
0
-2000
50000
60000
70000
80000
90000
Total Body Volume (mL)


B)
Difference in Arm Volume (mL)
400
0
-400
2000
3000
Arm Volume (mL)

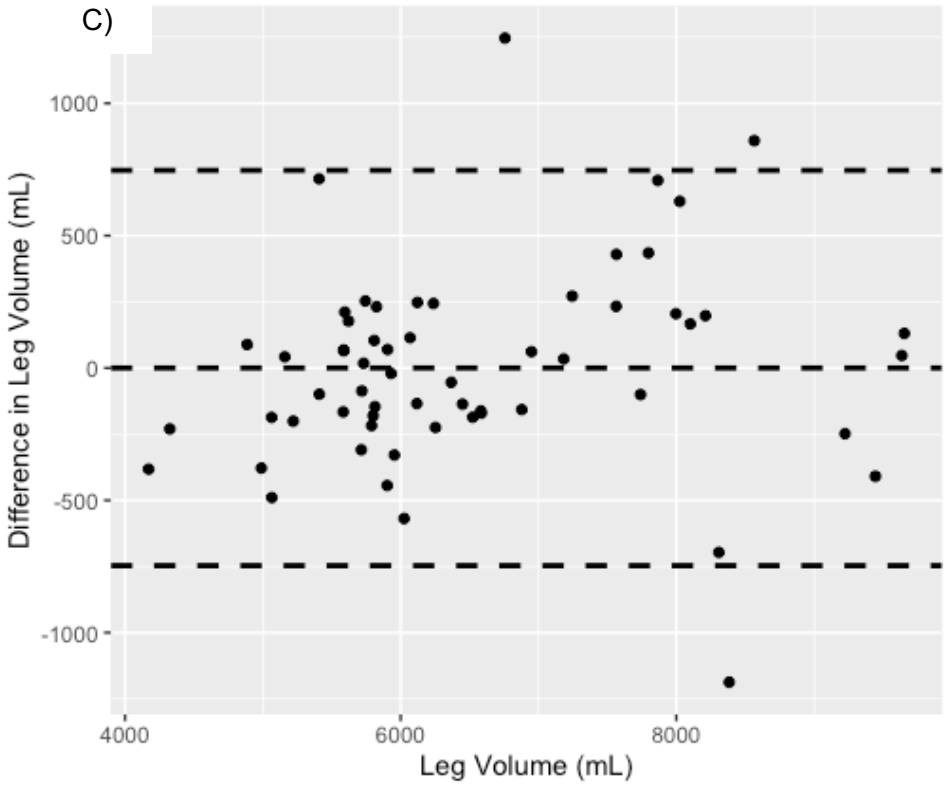


Figure 1. Differences between DXA-derived volume and water displacement for A) total body (mean difference = - 0.03 mL) B) arm (mean difference = -0.51 mL), and C) leg (mean difference = -0.21 mL). Note: x-axis represents mean of volume measures from DXA and water displacement for total body, arm, and leg, respectively.

CHAPTER 4. TOTAL AND REGIONAL DUAL X-RAY ABSORPTIOMETRY DERIVED FOUR-COMPARTMENT MODEL

Total and Regional Dual X-ray Absorptiometry Derived Four-Compartment Model

Madeline A. Czeck[1], William T. Juckett[1], Erica J. Roelofs[1], Donald R. Dengel[1]

[1]School of Kinesiology, University of Minnesota, Minneapolis, MN, 55455

Short Title: Regional DXA derived 4CM

Key Words: regional body volume; DXA; underwater weighing; water displacement; 4-compartment model

Publication Reference: Czeck, MA, Juckett, WT, Roelofs, EJ, Dengel, DR. (2023). Total and regional dual X-ray absorptiometry-derived four-compartment model. *Clinical Nutrition ESPEN*, *55*, 185-190.

Disclosures: DRD receives consulting fees from Hologic, Inc. Madeline A. Czeck prepared the first draft of the manuscript without any associated honorarium, grant, or other form of payment to produce the manuscript.

Summary

Purpose: Dual X-ray absorptiometry (DXA) software allows for total and regional (i.e., arms and legs) assessment of body composition, with recent advancements allowing for DXA derived volume. The use of DXA derived volume allows for the development of a convenient four-compartment model to accurately measure body composition. The purpose of the current study is to evaluate the validity of a regional DXA derived 4-compartment model. **Methods:** A total of 30 males and females underwent one whole body DXA scan, underwater weighing, total and regional bioelectrical impedance spectroscopy, and regional measures of water displacement. Manually created region of interest boxes assessed regional DXA body composition. Linear regression models with fat mass from the DXA as the dependent variable and body volume from water displacement, total body water from bioelectrical impedance spectroscopy, and DXA bone mineral and body mass as independent variables created regional 4-compartment models. Measures of fat-free mass and percent fat were calculated using the 4-compartment derived fat mass. T-tests assessed DXA derived 4-compartment model to the traditional 4-compartment model with volume assessed by water displacement. Regression models were cross-validated using the Repeated k-fold Cross Validation method. **Results:** Arm and leg regional DXA derived 4-compartment model for fat mass ($p=0.999$, both arm and leg), fat-free mass ($p=0.999$, both arm and leg), and percent fat (arm: $p=0.766$; leg: $p=0.938$) were not significantly different from the regional 4-compartment model with regional volume measured via water displacement. Cross-validation of each model produced R^2 values of 0.669 for the arm and 0.783 for the leg. **Conclusions:** The DXA can be used to create 4-compartment model for

estimating total and regional fat mass, fat-free mass, and percent fat. Thus, these results allow for a convenient regional 4-compartment model with DXA derived regional volume.

Introduction

The body is composed of different tissues, or compartments, and there are a variety of ways to measure those tissues (S. Y. Lee & Gallagher, 2008). For example, underwater weighing separates the tissue into fat mass and fat-free mass and is called a 2-compartment model (S. Y. Lee & Gallagher, 2008). Dual X-ray absorptiometry (DXA) further separates fat-free mass into lean mass and bone mass, and is called a 3-compartment model (Bilsborough et al., 2014; Haarbo et al., 1991; Heymsfield et al., 2015; Mazess et al., 1990; Rothney et al., 2012). A 4-compartment model further separates the body tissues by including body water, hence the compartments include fat mass, lean mass, bone mass, and body water (Fuller, Jebb, et al., 1992; Heymsfield et al., 2015). Thus, body composition measures can be classified based on the compartments they measure, typically ranging from a 2-compartment model to a 4-compartment model (S. Y. Lee & Gallagher, 2008). Accuracy of body composition measurement improves when increasing the number of compartments. By increasing the amount of components (i.e., fat, lean, bone, and water) measured, the overall measurement error is reduced and there are fewer assumptions in the model (Roubenoff & Kehayias, 1991; Wang et al., 2005). A 4-compartment model requires several different devices to measure each component. However, recent studies have attempted to use a DXA derived 4-compartment model, ultimately, simplifying the process.

Previous studies have explored the use of DXA to measure total body volume, with these studies reporting DXA derived body volume is highly correlated and not significantly different from the traditional methods of air displacement plethysmography (Ng et al., 2018; Smith-Ryan et al., 2017; Wilson et al., 2012, 2013) and hydrodensitometry (Nickerson et al., 2017; Sullivan et al., 2022). Similarly, percent fat using DXA derived

volume in a 4-compartment model is highly correlated and not significantly different from a traditional 4-compartment model with either air displacement plethysmography or hydrodensitometry as the traditional method for determining total body volume (Ng et al., 2018; Wilson et al., 2012). Ultimately this demonstrates that DXA derived body volume is valid and reliable (Blue et al., 2018; Nickerson et al., 2017; Tinsley, 2018) and that DXA may be used to derive a 4-compartment model.

The DXA region of interest (ROI) box allows for the assessment of regional measures including arm and leg fat, lean, and bone masses. Regional assessment of body composition is important as it allows for assessment of any asymmetries and injury risk. Previous studies have explored the use of DXA to derive regional volume. These studies reported that DXA derived volume is not significantly different from a traditional method of measuring volume (i.e., water displacement) (Brorson et al., 2009; Czeck et al., 2022; Fuller, Laskey, et al., 1992; Gjorup et al., 2010). Therefore, while previous studies have explored a DXA derived total body 4-compartment model, it is to the best of our knowledge that previous studies have not explored a DXA derived regional 4-compartment model.

Thus, the purpose of the present study is to create a DXA derived regional 4-compartment model and compare it to a traditional (i.e., water displacement) regional 4-compartment model for fat mass, fat-free mass, and percent fat.

Methods

Participants

Participants were recruited from the Twin Cities metro area, and a total of 30 males and females (Age: 25.9 ± 4.0 yrs.; Height: 1.75 ± 0.10 m; Weight: 70.98 ± 14.02 kg) volunteered to participate in this study. Exclusion criteria included under 18 years old or

over 55 years old, exceeding weight capacity of the scanner table, having undergone multiple X-rays and/or nuclear medicine in the past 12 months, and being pregnant. Participants completed body composition measures of DXA, bioelectrical impedance spectroscopy (BIS), underwater weighing, and water displacement on the same day. All testing was completed during the same timeframe in the morning. Prior to testing, all participants signed a written informed consent. Participants were asked to arrive fasted for at least 6 hour and not participate in vigorous exercise 48 hours or moderate exercise 14 hours prior to testing. In addition, participants were asked to abstain from caffeine, alcohol, or nicotine for at least 14 hours prior to testing. The University's Institutional Review Board approved all procedures.

Dual X-Ray Absorptiometry

Participants' weight was measured using an electronic scale (Tanita Corporation, Tokyo, Japan), their height was measured using a wall-stadiometer (Seca, Hamburg, Germany), and their age was recorded. Each participant had their limb length measured using a tape measure to determine 80% of each limb as previously described by Czeck et al. (Czeck et al., 2022). For the DXA scan, metal markers were placed at 80% of limb length, which allowed for placement of manual ROI boxes post scan. Participants received one whole body DXA scan (Hologic Horizon A; Hologic Inc., Marlborough, MA, USA) using standard procedures in the supine position. To prevent interference with the scan, participants removed all additional metal and thick clothing. All DXA scans were analyzed by the same technician using Hologic APEX software (APEX Version 5.6.0.4, Hologic Inc., Marlborough, MA, USA). Manual ROI boxes were defined as from the 80% mark of the limb to the middle of the head of the ulna for the arms and the middle of the medial

malleolus on the tibia for the legs. Body composition variables included total mass, lean mass, fat mass, percent fat, bone mineral content (BMC) and bone mineral density (BMD) for total body and regional measurements. To derive body volume from DXA, prediction equations for total body and each limb followed the prediction equation proposed by Czeck et al. (Czeck et al., 2022).

$$(1)\ \boldsymbol{Total\ Body\ Volume\ (mL)} = \mathbf{1.12}(\boldsymbol{fat}) + \mathbf{0.94}(\boldsymbol{lean}) - \mathbf{1.17}(\boldsymbol{BMC}) - \mathbf{783.70}$$

$$(2)\ \boldsymbol{Arm\ Volume\ (mL)} = \mathbf{0.66}(\boldsymbol{fat}) + \mathbf{0.68}(\boldsymbol{lean}) + \mathbf{1.40}(\boldsymbol{BMC}) + \mathbf{311.99}$$

$$(3)\ \boldsymbol{Leg\ Volume\ (mL)} = \mathbf{1.13}(\boldsymbol{fat}) + \mathbf{0.83}(\boldsymbol{lean}) + \mathbf{1.04}(\boldsymbol{BMC}) - \mathbf{24.96}$$

Where fat, lean, and BMC correspond to the DXA mass measures. The coefficients correspond to the respective inverse densities for fat, lean, and BMC, and v_{residual} is the volume unaccounted for by DXA.

Bioelectrical Impedance Spectroscopy

BIS was used to measure total and regional body water (SFB7, ImpediMed, Queensland, Australia). BIS to estimate TBW in adults has been observed to be valid (r = 0.98, standard error of estimate = 2.12L, total error = 2.21L) when compared to deuterium oxide (Moon et al., 2008). Participants were in a relaxed supine position with space between their arms and torso and between their legs. Two electrodes (one sensing and one injection with 5 cm between each electrode) were placed at the subject's left wrist/hand and left ankle/foot for total body measures. For regional BIS, electrodes were placed at each of the 80% marks (right arm, left arm, right leg, and left leg) and either the wrist or

ankle for arm and leg regional, respectively. Length between electrodes was measured and marked. In addition, circumferences at each sensing electrode (i.e., wrist and upper arm or ankle and upper leg) were measured with a tape measure and recorded for regional body water analysis. Resistivity coefficients for intracellular water and extracellular water were provided by the software. In addition, the BIS company provided the equations to determine regional body water.

Underwater Weighing

Total body volume was calculated by taking the participant's dry weight and dividing by their density in water. Body density was measured using underwater weighing. Underwater weighing was performed in a specialized apparatus (EXERTECH, LaCrosse, WI). Participants wore tight fitting clothing and a nose clip. Participants were seated in the apparatus and were instructed to maximally exhale and slowly submerge into the water. Residual volume was estimated based on subject's height, age, and sex. The average of three trials of underwater weighing was used to derive total body volume.

Water Displacement

Limb volume was measured using water displacement via two volumetric cylinders, one for the arm and one for the leg, using methods previously described (Czeck et al., 2022). After participant's hands and feet were zeroed, the participant slowly immersed the rest of their limb until the 80% mark was in line with the valve on the volumetric cylinder. The water displaced was collected and measured using graduated cylinders and measured to the nearest 10 mL.

Four-Compartment Model

Total fat mass, fat-free mass, and percent fat was estimated using the 4-compartment model described by Wang et al. (Wang et al., 2002)

$$(4)\ \ Fat\ Mass\ (kg) = 2.748(BV) - 0.699(TBW) + 1.129(Mo) - 2.051(BM)$$

$$(5)\ \ Fat - Free\ Mass\ (kg) = BM - Fat\ Mass$$

$$(6)\ \ Percent\ Fat = \left(\frac{Fat\ Mass}{BM}\right) x100$$

A traditional 4-compartment model was derived using body volume (BV) from underwater weighing, total body water (TBW) from BIS, bone mineral (Mo), and body mass (BM) from DXA. Mo is calculated by dividing BMC from DXA by 0.9582. A convenient DXA derived 4-compartment model was also assessed, where BV, Mo, and BM were derived using DXA and TBW from BIS.

The equation (i.e., equation 4) from Wang et al. (Wang et al., 2002) for total body fat mass resulted in negative fat mass values for regional measures of the arm and leg. Therefore, this study explored new regional 4-compartment equations in the following format

$$(7)\ \ Fat\ Mass\ (kg) = a(BV) + b(TBW) + c(Mo) + d(BM) + e$$

The variable a, b, c, and d correspond to the coefficients for BV, TBW, Mo, and BM. The letter e represents the residual mass unaccounted for by DXA.

Statistical Analysis

Descriptive statistics were calculated for the participant population. An a priori power calculation for the linear regression modeling was performed using a standard effect size of 0.35 with four independent variables, a significance level of 0.05, and a power of 0.9. From this calculation, a sample size of 50 was required. Paired t-tests compared DXA derived total body 4-compartment model to the four-compartment model measured by

underwater weighing using the equation described by Wang et al. (Wang et al., 2002). Regional four-compartment fat mass was determined using linear regression models. Linear regression using the step Akaike Information Criteria method with fat mass from the DXA as the dependent variable and BV from water displacement, TBW from BIS, and Mo and BM from the DXA as independent factors determined the coefficients for the prediction equations. Regression models were cross-validated using the Repeated k-fold Cross Validation method. Paired t-tests compared DXA derived regional four-compartment model to the regional four-compartment model measured by water displacement. Analysis of Variance with Tukey's Honestly Significant Difference assessed differences between DXA, DXA derived, and traditional model measures of fat mass, fat-free mass, and percent fat. Fat-free mass from the DXA was determined by adding lean mass and BMC, whereas fat-free mass from the four-compartment model was determined via equation 5. The four-compartment model calculated by water displacement was compared to the DXA derived four-compartment model using constant error, total error, standard error of the estimates, and Bland-Altman plots determined limits of agreement. All analyses were conducted using R software (R Foundation for Statistical Computing, Vienna, Austria) with an alpha level of $p \leq 0.05$.

Results

Participant demographics and anthropometrics are reported in Table 1. DXA derived total body fat mass ($p = 0.999$, 95% CI [-3.52, 3.51]), fat-free mass ($p = 0.999$, 95% CI [-3.41, 3.52]), and percent fat ($p = 0.947$, 95% CI [-4.23, 4.51]) were not significantly different compared to the 4-compartment model with volume measured by underwater weighing (UWW). There were no significant differences between DXA

derived, traditional model, and DXA results for total body percent fat (17.13 ± 7.72 vs. 17.27 ± 12.43 vs. 20.48 ± 5.67, respectively) (Figure 1). In addition, there were no significant differences between DXA derived, traditional model, and DXA results for total body fat mass and fat-free mass (Table 2).

Linear regression equations were used to create regional 4-compartment models. A total of 60 limbs were used for the linear regression. Coefficients for the arm 4-compartment equation are 0.33 ($p < 0.001$), -0.43 ($p < 0.001$), -3.94 ($p < 0.001$), and 0.47 ($p < 0.001$) for BV, TBW, Mo, and BM, respectively, with a residual mass of 0.32 kg. The coefficients for the leg 4-compartment equation are 0.37 ($p < 0.001$), -0.43 ($p < 0.001$), -3.96 ($p < 0.001$), and 0.39 ($p < 0.001$) for BV, TBW, Mo, and BM, respectively, with a residual mass of 0.63 kg.

(8) $Arm\ Fat\ Mass\ (kg) = 0.33(BV) - 0.43(TBW) - 3.94(Mo) + 0.47(BM) + 0.32$

(9) $Leg\ Fat\ Mass\ (kg) = 0.37(BV) - 0.43(TBW) - 3.96(Mo) + 0.39(BM) + 0.63$

Regional 4-compartment fat mass (arm: $p = 0.999$, 95% CI [-0.02, 0.012]; leg: $p = 0.999$, 95% CI [-0.04, 0.04]), fat-free mass (arm: $p = 0.999$, 95% CI [-0.02, 0.02]; leg: $p = 0.999$, 95% CI [-0.04, 0.04]), and percent fat (arm: $p = 0.766$, 95% CI [-0.66, 0.49]; leg: $p = 0.938$, 95% CI [-0.53, 0.49]) were not significantly different between the DXA derived model and the model with volume measured by water displacement. There were no significant differences between DXA derived, traditional model, and DXA results for percent fat for arm (22.41 ± 7.98 vs. 22.32 ± 7.40 vs. 22.26 ± 8.42, respectively) and leg (22.57 ± 7.26 vs. 22.55 ± 7.09 vs. 22.54 ± 7.93, respectively) (Figure 1). In addition, there were no significant differences between DXA derived, traditional model, and DXA results

for fat mass and fat-free mass for the arm and leg (Table 2). Constant error of body volume between water displacement and DXA derived volume for arm fat mass is -2.18 x 10^{-6} kg and 8.43 x 10^{-6} kg for leg fat mass. The total error for arm fat mass is 1.69 x 10^{-5} kg and leg fat mass is 6.53 x 10^{-5} kg, with the standard error of estimate 0.01 for both the arm and leg.

Bland-Altman plots for validity are presented in Figure 2 for total body (Panel A), arm (Panel B), and leg (Panel C).

Each model was cross-validated using the repeated k-fold cross-validation approach, specifically, 3 repeats of a 10-fold cross-validation. The R^2 value for each model is 0.669 for the arm (equation 8) and 0.783 for the leg (equation 9).

Discussion

The purpose of the present study was to create a regional DXA derived 4-compartment model for the estimation of fat mass, fat-free mass, and percent fat. The present study also aimed to compare the regional DXA derived 4-compartment model to a regional 4-compartment model where body volume was measured using water displacement. A noteworthy observation of the present study includes no significant differences between the regional DXA derived 4-compartment model estimations for fat mass, fat-free mass, and percent fat compared to the traditional 4-compartment model.

Total Body DXA Derived Four-Compartment Model

Notably, previous studies have explored the use of DXA derived total body volume, with these studies reporting DXA derived body volume is highly correlated and not significantly different from the traditional methods of air displacement plethysmography (Ng et al., 2018; Smith-Ryan et al., 2017; Wilson et al., 2012, 2013) and hydrodensitometry

(Czeck et al., 2022; Nickerson et al., 2017; Sullivan et al., 2022). Particularly, one study compared DXA derived total body equations from Smith-Ryan et al. (Smith-Ryan et al., 2017) and Wilson et al. (Wilson et al., 2013). The previous study reported potential biases with the equations based on BMI classification and waist circumference (McLester et al., 2018). Thus, the present study used a DXA derived total body equation from a different study (Czeck et al., 2022). In addition, previous studies have used DXA derived total body volume in a 4-compartment model for determining fat mass and percent fat. They reported that percent fat using DXA derived volume in a 4-compartment model is highly correlated and not significantly different from a traditional 4-compartment model with either air displacement plethysmography or hydrodensitometry as the traditional method for determining total body volume (Ng et al., 2018; Wilson et al., 2012). The present study also observed no significant differences between a DXA derived 4-compartment model and a traditional 4-compartment model with underwater weighing as the source of total body volume for fat mass, fat-free mass, and percent fat.

Regional DXA Derived Four-Compartment Model

In addition to a total DXA derived four-compartment model, the present study explored a regional DXA derived 4-compartment model. The equation used for the total body 4-compartment model was applied to the arm and leg. However, fat mass results for each region provided negative mass values. Therefore, the present study used linear regression to create regional 4-compartment models. Notably, the present study observed no significant differences between the DXA derived 4-compartment model and the traditional 4-compartment model for fat mass, fat-free mass, and percent fat. This present study expands upon previous studies by including the use of DXA derived regional volume

into a 4-compartment model. The present study used DXA derived regional volume equations from Czeck et al. (Czeck et al., 2022) which the previous study showed to be valid. The present study used the same regional definitions for measurements as Czeck et al. (Czeck et al., 2022) to allow for use of their equations in the current study.

The current study observed a slight bias between the traditional 4-compartment and the DXA derived 4-compartment arm (-2.2×10^{-6} kg) and leg (8.4×10^{-6} kg) fat mass as seen by the Bland-Altman plots. In addition, the current study observed no significant differences between regional DXA derived 4-compartment and a regional traditional 4-compartment model for fat mass, fat-free mass, and percent fat for both the arm and the leg. Therefore, the no significant difference between methods and the strong correlation from the cross-validation models for both the arm ($R^2 = 0.669$) and leg ($R^2 = 0.783$), suggest that a Hologic DXA scan can be used to in a regional 4-compartment model.

Limitations of the present study include the sample size and population. The subject demographics were homogenous in nature, which limits the generalizability of the regional 4-compartment equations to other populations. Also, due to limitations of measuring the full limb via water displacement, regional values were for 80% of the limb length. However, the results from the present study provides the scientific community methodology for the use of regional DXA derived 4-compartment model. This is noteworthy as a DXA derived 4-compartment model is more convenient compared to the traditional method. The proposed methodology is more convenient as it requires the use of fewer equipment and considerably less time to complete the measurements.

In conclusion, the current study reported no significant differences between total and regional DXA derived 4-compartment measures compared to a traditional 4-

compartment model for measuring fat mass, fat-free mass, and percent fat. Thus, these observations suggest that Hologic DXA can be used for a convenient DXA derived total and regional 4-compartment model. A regional 4-compartment allows for tracking of regional changes due to site specific tumors, cancers, or trauma (e.g., volumetric muscle loss). Future research should explore Hologic DXA derived regional 4-compartment measures of fat mass, fat-free mass, and percent fat in a variety of populations. Also, the use of GE DXA scan in regional volume and regional 4-compartment measures.

Table Legends

Table 1. Demographics (mean ± standard deviation) of cohort and body composition variables from DXA.

Table 2. Mean ± standard deviation of body composition variables from DXA derived 4-compartment model, traditional method 4-compartment model, and DXA.

Tables

Table 1. Demographics (mean ± standard deviation) of cohort and body composition variables from DXA.

	Total	Male (n=18)	Female (n=12)
Age (yrs)	25.9 ± 4.0	24.6 ± 3.8	26.4 ± 4.4
Height (m)	1.75 ± 0.10	1.82 ± 0.06	1.65 ± 0.04
Weight (kg)	70.98 ± 14.02	79.33 ± 11.24	58.46 ± 6.15
Total BMC (kg)	2.57 ± 0.50	2.85 ± 0.43	2.15 ± 0.21
Total BMD (kg)	1.18 ± 0.10	1.22 ± 0.11	1.13 ± 0.06
Total Body Fat Percent (%)	20.48 ± 5.67	16.96 ± 2.85	25.78 ± 4.62

Abbreviations: DXA, dual X-ray absorptiometry; BMC, bone mineral content; BMD, bone mineral density

Table 2. Mean ± standard deviation of body composition variables from DXA derived 4-compartment model, traditional method 4-compartment model, and DXA.

	DXA Derived	Traditional Method	DXA
Total Fat Mass (kg)	11.83 ± 5.14[a]	11.83 ± 9.14[a]	14.16 ± 3.35[a]
Total Fat-Free Mass (kg)	59.17 ± 14.44[a]	59.17 ± 17.06[a]	59.41 ± 14.25[a]
Arm Fat Mass (kg)	0.56 ± 0.14[a]	0.56 ± 0.12[a]	0.56 ± 0.15[a]
Arm Fat-Free Mass (kg)	2.13 ± 0.81[a]	2.13 ± 0.81[a]	2.13 ± 0.81[a]
Leg Fat Mass (kg)	1.52 ± 0.43[a]	1.52 ± 0.42[a]	1.52 ± 0.47[a]
Leg Fat-Free Mass (kg)	5.45 ± 1.50[a]	5.45 ± 1.50[a]	5.45 ± 1.52[a]

Note: Comparisons are made between groups within each row. Values that share a letter denote no significant difference. Abbreviations: DXA, dual X-ray absorptiometry

Figures

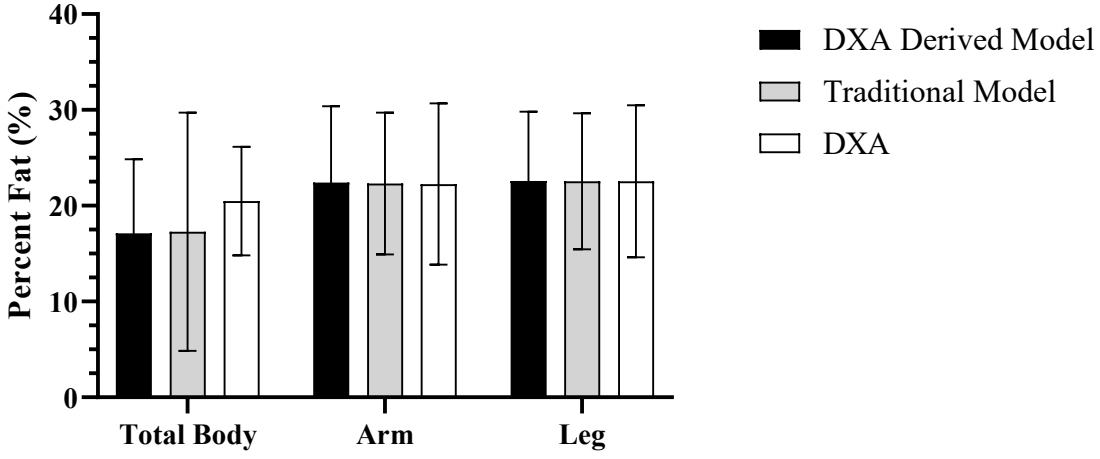
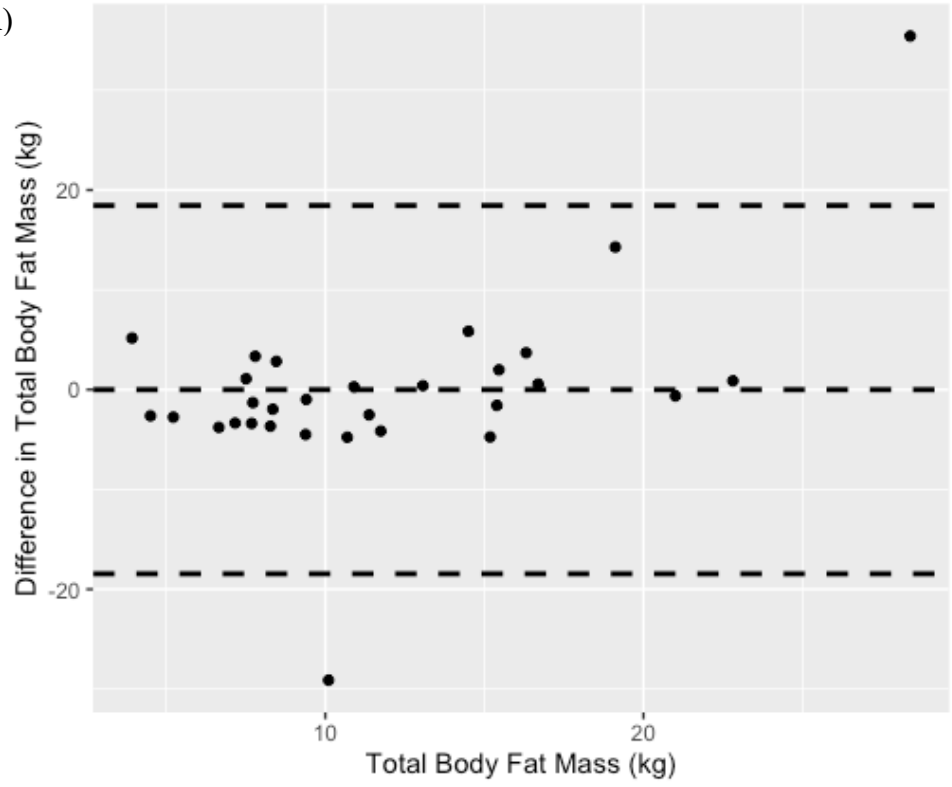
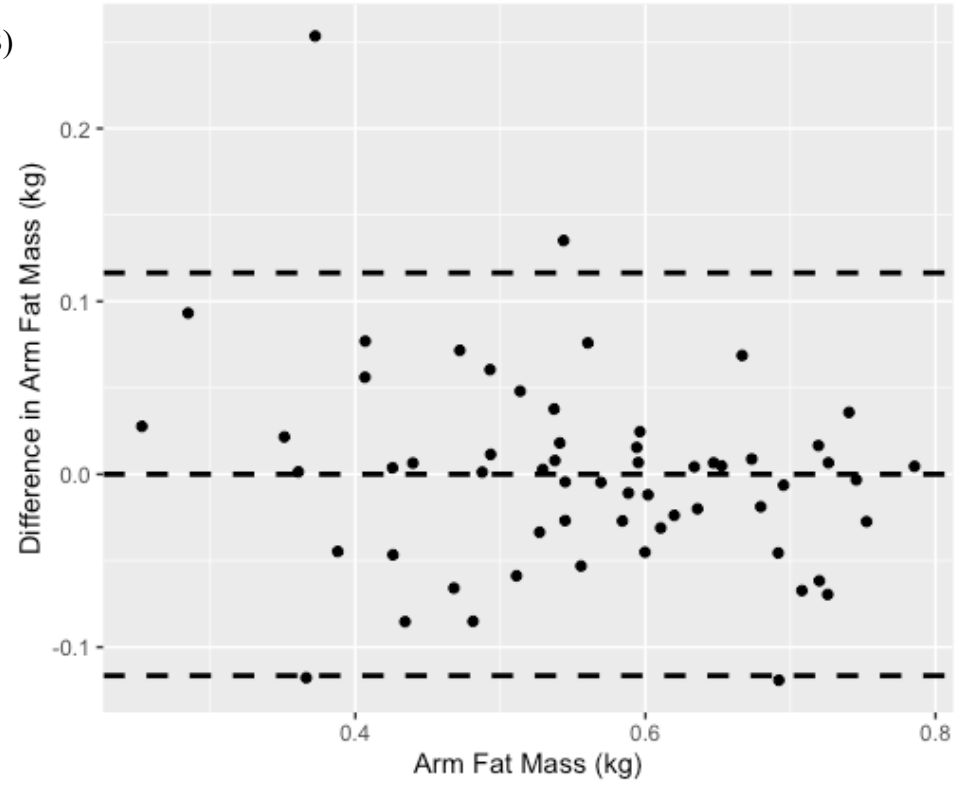


Figure 1. DXA derived, traditional model, and DXA variables for percent fat for total body, arm, and leg.

A)


Difference in Total Body Fat Mass (kg)
20
0
-20
10
20
Total Body Fat Mass (kg)

B)


Difference in Arm Fat Mass (kg)
0.2
0.1
0.0
-0.1
0.4
0.6
0.8
Arm Fat Mass (kg)

C)

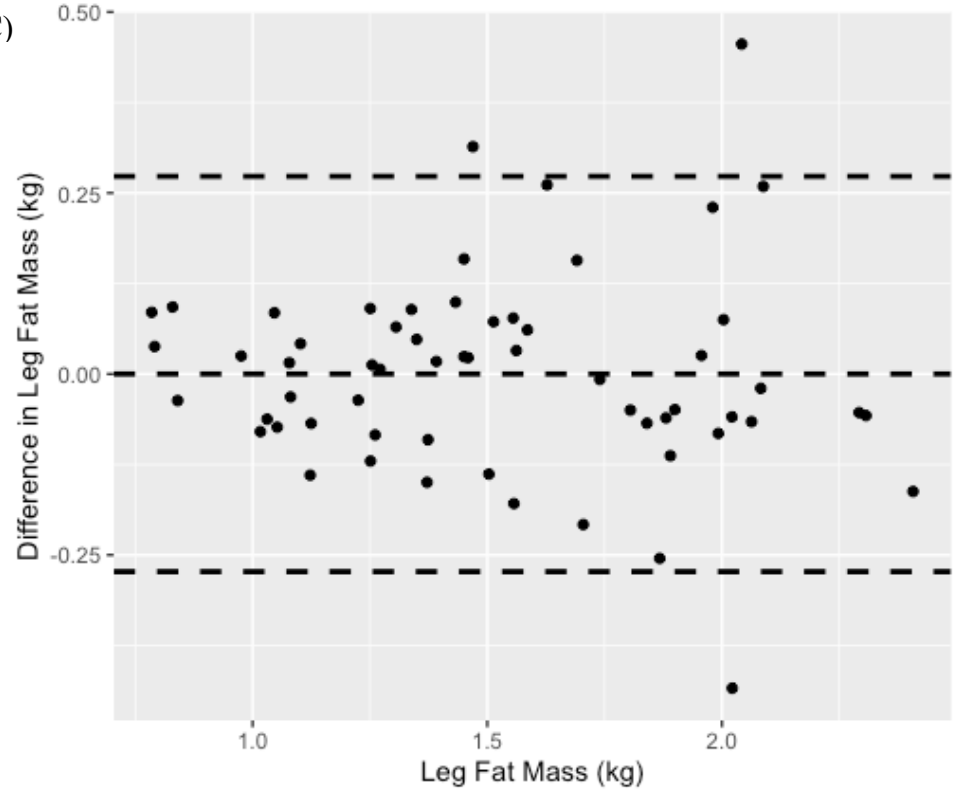


Figure 2. Differences between DXA derived 4-compartment model and traditional model for A) total body fat mass (mean difference = -10.86 kg), B) arm fat mass (mean difference = 0.78 kg), C) leg fat mass (mean difference = 0.16 kg).

CHAPTER 5: MUSCLE-TO-BONE AND SOFT TISSUE-TO-BONE RATIO IN CHILDREN AND ADOLESCENTS WITH OBESITY

Muscle-to-Bone and Soft Tissue-to-Bone Ratio in Children and Adolescents with Obesity

Madeline A. Czeck[1], William T. Juckett[1], Aaron S. Kelly[2,3], Donald R. Dengel[1,2,3]

[1]School of Kinesiology, University of Minnesota, Minneapolis, MN, 55455

[2] Center for Pediatric Obesity Medicine, University of Minnesota Medical School, Minneapolis, MN, 55414

[3]Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN, 55455

Short Title: MBR and SBR in Pediatrics with Obesity

Key Words: Dual X-ray absorptiometry; pediatric obesity; body composition ratios

Publication Reference: Czeck, MA, Juckett, WT, Kelly, AS, Dengel, DR. (2023) Muscle-to-bone and soft tissue-to-bone ratio in children and adolescents with obesity. *Journal of Clinical Densitometry, 26,* 101360.

Disclosures: ASK engages in unpaid consulting and educational activities for Boehringer Ingelheim, Eli Lilly, Novo Nordisk, and Vivus; receives donated drug/placebo for NIDDK-funded clinical trials from Novo Nordisk and Vivus.

Funding: Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number (R01HL110957 [to A.S.K.]).

Summary

Introduction: To explore the total and regional muscle-to-bone ratio in children and adolescents with obesity and compare the muscle-to-bone ratio (MBR) and soft tissue-to-bone ratio (SBR) to their peers with normal weight or overweight. **Methods:** A total of 219 male and female pediatrics (mean age=12.3±2.5 years) participated in this study. Body composition was assessed with a total body dual X-ray absorptiometry. The MBR was calculated by dividing lean mass by bone mineral content. The SBR was determined by dividing the soft tissue mass (i.e., lean mass+fat mass) and by bone mineral content. Differences in total and regional body composition measures between body mass index (BMI) percentile groups was assessed by ANOVA. **Results:** The obesity group had significantly higher MBR compared to the normal weight group for total (19.24±1.56 vs. 18.26±1.64), arm (17.11±1.67 vs. 15.88±1.81), and leg (18.41±1.68 vs. 16.62±1.55). Similarly, the obesity group had significantly higher MBR in the leg (18.41±1.68) compared to the overweight group (17.24±1.45). However, the overweight group was not significantly different from the normal weight or the obesity group for total and arm MBR. The total, arm, and leg SBR was significantly different between all BMI groups. Across the entire sample, MBR) and SBR were negatively associated with high-density lipoprotein. SBR was positively associated with insulin, HOMA-IR, low-density lipoprotein, very low-density lipoprotein, triglycerides, and systolic blood pressure. **Conclusions:** Children with obesity had a higher MBR and SBR compared to their normal weight peers. In addition, there were significant associations between SBR, higher levels of insulin, atherogenic lipoproteins, and increased systolic blood pressure. Thus, SBR may

be useful as a marker for increased cardiometabolic disease risk, though more research in this area is warranted.

Introduction

Obesity is characterized by excess fat mass and associated with decreased levels of physical activity (Maffeis et al., 1997; Stodden et al., 2008). Studies exploring muscular strength in children and adolescents with obesity have reported incongruent findings. Some studies have reported a positive relationship between obesity and grip strength, muscle thickness and cross-sectional area, voluntary contraction and activation, and elbow extensors (Deforche et al., 2003; Garcia-Vicencio et al., 2016; C. K. Lee et al., 2022; Thivel et al., 2016). However, other studies have reported a negative relationship between obesity and sit-ups, nine-minute walk test, knee extensor strength, plantar flexor strength, and 20 meter shuttle run (Bonney et al., 2018; Deforche et al., 2003; Musálek et al., 2020; Singh et al., 2021; Thivel et al., 2016; Tsiros et al., 2016). These differences in results may be due to differences in methodology for defining obesity. For example, some studies reported using guidelines from the World Health Organization and other studies used the International Obesity Task Force guidelines. However, despite these differences in muscular performance, previous studies have also shown higher levels of lean mass with children with obesity compared to their normal weight peers (Dimitri et al., 2010). Increased lean mass results in greater strain on bones during muscle contraction, ideally making them stronger. Therefore, increased lean mass as observed in children with obesity may have a positive effect on bone, actually making them stronger (L. Mosca et al., 2013; Rinonapoli et al., 2021) as well as increasing their bone mineral density (BMD) and bone mineral content (BMC) (Dimitri, 2019; Litwic et al., 2021; Rinonapoli et al., 2021; Rokoff et al., 2019). However, once normalized for body weight, children with obesity have lower BMD and BMC (Litwic et al., 2021; L. N. Mosca et al., 2014). Previous studies have also

reported significant positive correlations between bone measures (i.e., BMD, BMC, and BMD z-scores) and lean mass in males and females, and fat mass in females (Maïmoun et al., 2016; L. N. Mosca et al., 2014). Therefore, as lean mass and fat mass increase, so do measures of bone heath (i.e., BMC and BMD). However, once fat mass is normalized to lean mass, increased fat mass has been reported to be negatively associated with bone strength (Ducher et al., 2009).

Assessing BMC in relation to obesity is important, as previous studies have reported increased risk of fractures (Compston, 2013; Dimitri et al., 2010). A previous study normalizing BMC to lean mass reported lower values (i.e., less BMC per lean mass) were indicative of poor skeletal adaptation and individuals were at an increased risk of fractures (Golec & Chlebna-Sokół, 2014). Notably, obesity may be accompanied by cardiometabolic disease risks (i.e., insulin resistance, dyslipidemia, hypertension) that may negatively impact bone health. Previous studies have reported that BMC is negatively associated with insulin, homeostatic model assessment for insulin resistance (HOMA-IR) (do Prado et al., 2009; Hetherington-Rauth et al., 2019; Karimi et al., 2021; Kindler et al., 2019; Lawlor et al., 2012; K. Lee, 2013; Pollock et al., 2011; Shawar et al., 2022), fasting glucose and total cholesterol (Pollock et al., 2011). In addition, BMC is positively associated with high-density lipoprotein (HDL) (Pollock et al., 2011). Studies have also reported no significant associations between BMC and systolic or diastolic blood pressure, triglycerides, and low-density lipoprotein (LDL) (Hetherington-Rauth et al., 2019; Pollock et al., 2011). Similarly, individuals with prediabetes have lower BMC compared to individuals with normal-glucose levels (Pollock et al., 2010). Therefore, exploring the

relationship between cardiometabolic risk factors associated with obesity and bone measures may provide additional insight into bone health in children.

Muscle and bone form a functional unit for movement that ultimately strains and strengthens the bones (Anliker & Toigo, 2012; Schoenau et al., 2002). This unit, or relationship, can be explored by looking at muscle and bone as a ratio called the muscle-to-bone ratio (MBR). The MBR has been proposed as a way to look at the physiological adaptations of bone in response to the mechanical loading of muscle. Although MBR has been used extensively in animal science (Hopkins, 1996; Purchas et al., 2002), recently it has been used in athletes to examine possible adaptions to training and its relationship with performance outcomes (Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Holway & Garavaglia, 2009; Ireland et al., 2013; Withers et al., 1991). Studies in athletes have used the fractionation method to determine adipose, muscle, residual, skeletal, and skin tissue masses and ultimately the MBR (Holway & Garavaglia, 2009). Dual X-ray absorptiometry (DXA) allows for the direct measurement of lean and bone masses instead of estimation of these two masses through use of skinfolds, girths, and breadths. DXA has been validated, concordance coefficients of 0.58-0.91, as an estimate for total and regional lean and fat mass in children and adolescents (Bridge et al., 2009, 2011). A recent study used BMC and lean mass from DXA to look at the MBR between adolescent swimmers and controls (Gomez-Bruton et al., 2019). To the best of our knowledge only one study has used DXA to look at the effect of body fat percent on regional muscle to bone relationship in adolescents (Duran et al., 2019). However, they combined arms and legs and thus referred to it as appendicular muscle-to-bone unit. Assessing regional body composition allows for determination of asymmetries in body

composition that may result in increased risk of injury. Therefore, the purpose of this study was to use DXA to explore the total and regional muscle-to-bone relationship in children and adolescents with obesity. This study also further expands on the muscle-to-bone relationship by exploring the impact of lean mass and fat mass on bone through the soft tissue-to-bone ratio (SBR). To the best of our knowledge, this study is the first to explore the SBR in children and adolescents with obesity. We hypothesize that children with obesity will have higher MBR and SBR compared to their normal weight peers, indicative of lower bone mass to lean mass.

Methods

Participants

A total of 219 (101 male, 118 female) children and adolescents participated in this study (age: 12.3±2.5 years) between 2011 and 2016. Participants were recruited from various pediatric clinics within the Minneapolis and St. Paul metropolitan area. Exclusion criteria included untreated obstructive sleep apnea, obesity due to a genetic cause determined by physician diagnosis, previous medical history of weight loss surgery, current use of antihypertensive medications, type I and type II diabetes mellitus, medically documented history of hypercholesterolemia, chronic kidney disease, Kawasaki disease, autoimmune inflammatory diseases, and congenital heart disease. Study approval was given by the University of Minnesota institutional review board. Parents and participants provided informed consent and assent, respectively.

Body Composition

Age was self-reported. Sex and pubertal maturation stage were determined using classical Tanner staging by a trained nurse or physician (Tanner & Whitehouse, 1976).

Height and weight were measured using a wall-mounted stadiometer and a calibrated electronic scale. Body mass index (BMI) was calculated by dividing body mass in kilograms by height in squared meters. Obesity status was determined using BMI percentiles from the Center of Disease Control (CDC) guidelines and the categories were defined as: normal weight (i.e., ≥5th to <85th BMI percentile), overweight (i.e., ≥85th to <95th BMI percentiles), obesity (i.e., ≥95th BMI percentiles). Body composition was measured in the supine position using standard DXA (iDXA; General Electronic Medical Systems, Madison, Wisconsin) procedures. DXA has been shown to have high precision in individuals with and without obesity (Hind et al., 2011; Huizenga et al., 2007; Rothney et al., 2012). Data were analyzed by the same technician using enCore software (platform version 16.0; General Electric Medical Systems). DXA provided measures of lean mass, fat mass, BMC, and BMD of total body, arm, and leg. The MBR was calculated by lean mass divided by BMC. In addition, the SBR was calculated by adding lean mass and fat mass to create soft tissue and then dividing soft tissue by BMC.

Cardiometabolic Risk Factors

Fasting (>10 hours) blood samples were collected. Samples were analyzed in the Fairview Diagnostic Laboratories, Fairview-University Medical Center (Minneapolis, Minnesota). Blood samples were analyzed for glucose, insulin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides. HOMA-IR was calculated by taking fasting insulin (mU/L) times fasting glucose (mg/dL)/405 (Matthews et al., 1985). Seated blood pressure was obtained on the right arm after 5 minutes of quiet rest, using an automatic

sphygmomanometer and an appropriately fitted cuff. Three measurements were taken, and the average of the final two was used.

Statistical Analysis

Descriptive statistics were summarized by mean (SD) or n (%) for continuous and categorical covariates, respectively. Analysis of Variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) post hoc assessed differences between BMI percentile groups for measures of fat mass, lean mass, BMC, and BMD for total, arm, and leg. Similarly, ANOVA with Tukey's HSD assessed differences between BMI percentile group and MBR and SBR for total, arm, and leg. Differences between BMI percentile group and body composition measures, MBR, and SBR separated by sex were assessed by t-tests. The overweight group was excluded from these analyses due to the small n when separated by sex. Associations between MBR, SBR, and cardiometabolic risk factors were assessed with linear regressions, p-values were adjusted for multiple testing. All analyses were conducted using R, version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Descriptive statistics of the study population are presented in Table 1. Body composition variables of fat mass, lean mass, BMC, and BMD between BMI percentile groups for total body, arm, and leg are contained in Table 2. The obesity group had significantly higher MBR compared to the normal weight group for total body (19.24 ± 1.56 vs. 18.26 ± 1.64) and arm (17.11 ± 1.67 vs. 15.88 ± 1.81) (Figure 1). However, the overweight group was not significantly different from the normal weight and obesity group for total body and arm MBR. The obesity group had significantly higher MBR in the leg

(18.41 ± 1.68) compared to the normal weight (16.62 ± 1.55) and overweight group (17.24 ± 1.45) (Figure 1).

Figure 2 shows the SBR between BMI percentile groups for total body, arm, and leg. All groups were significantly different from each other for total body (35.04 ± 4.99 vs. 28.36 ± 3.14 vs. 24.48 ± 2.59), arm (32.83 ± 5.73 vs. 25.36 ± 3.67 vs. 22.32 ± 3.07), and leg (33.90 ± 5.36 vs. 27.55 ± 4.35 vs. 24.05 ± 3.19), with the obesity group having the highest SBR and the normal weight group with the lowest SBR (Figure 2).

Due to small number of adolescents in the overweight group in each sex, the overweight group was excluded from statistical analyses separated by sex. Table 3 is the body composition variables of fat mass, lean mass, BMC, and BMD between BMI percentile groups for total body, arm, and leg for males. The normal weight group had significantly lower total, arm, and leg fat mass, and BMD compared to the obesity group (Table 3). They also had significantly lower total lean mass, leg lean mass, and leg BMC. Total BMC, arm BMC, and arm lean mass were not significantly different between the two groups. Figure 3 is the MBR and SBR between the normal weight and obesity BMI group. Total (18.83 ± 1.50 vs. 19.46 ± 1.29, $p = 0.042$), arm (16.75 ± 1.44 vs. 17.50 ± 1.25, $p = 0.012$), and leg (17.00 ± 1.57 vs. 18.27 ± 1.79, $p<0.001$) MBR were significantly higher in the obesity group compared to the normal weight group for males (Figure 3a). Total (24.31 ± 2.77 vs. 34.20 ± 4.34, $p<0.001$), arm (22.53 ± 3.19 vs. 31.97 ± 4.99, $p<0.001$), and leg (23.53 ± 3.57 vs. 32.21 ± 4.66, $p<0.001$) SBR was also significantly higher in the obesity group compared to the normal weight group for males (Figure 3c).

Body composition variables for fat mass, lean mass, BMC, and BMD between BMI percentile groups for total body, arm, and leg for females are contained in Table 4. The

normal weight group had significantly lower total, arm, and leg fat mass, lean mass, and BMD compared to the obesity group (Table 4). Total, arm, and leg BMC was not significantly different between the two groups. Figure 3 also displays the MBR and SBR between the normal weight and obesity BMI group for females. Total (17.49 ± 1.52 vs. 19.09 ± 1.70), arm (14.72 ± 1.61 vs. 16.85 ± 1.86), and leg (16.11 ± 1.37 vs. 18.42 ± 1.67) MBR were significantly ($p<0.001$ for all) higher in the obesity group compared to the normal weight group for females (Figure 3b). Total (24.72 ± 2.35 vs. 35.51 ± 5.24), arm (22.04 ± 2.92 vs. 33.26 ± 6.03), and leg (24.74 ± 2.48 vs. 34.80 ± 5.48) SBR was also significantly higher ($p<0.001$ for all) in the obesity group compared to the normal weight group for females (Figure 3d).

The associations of cardiometabolic risk factors and MBR and SBR are in Table 5. Both MBR ($p = 0.007$) and SBR ($p<0.001$) were negatively associated with HDL (Table 5). In addition, SBR was positively associated ($p<0.001$ for all) with insulin, HOMA-IR, LDL, VLDL, triglycerides, and systolic blood pressure.

Discussion

The present study explored differences in body composition and body composition ratios between BMI percentile groups in children and adolescents. Notably, the present study observed significantly higher total and regional body composition measures in children with obesity compared to their normal weight peers. In addition, children with obesity had higher MBRs and SBRs compared to their normal weight peers. The present study also observed significant associations between SBR and cardiometabolic risk factors.

Muscle-to-Bone Ratio

The MBR is a way to explore bone health as a result of muscle mechanical loading. Studies in athletes have used the MBR (Bernal-Orozco et al., 2020; Brocherie et al., 2014; Carvajal et al., 2012; Holway & Garavaglia, 2009; Ireland et al., 2013; Withers et al., 1991), but have primarily used the 5-way fractionation method to determine bone measures. The 5-way fractionation method estimates adipose, muscle, residual, skeletal, and skin tissues through skinfolds, girths, and breadths (Holway & Garavaglia, 2009). However, a study in adolescent swimmers used DXA to determine BMC and lean mass for the muscle-bone unit (Gomez-Bruton et al., 2019). The authors reported swimmers had significantly lower total and regional ratios compared to controls, indicative of more lean mass to bone mass or less bone mass to lean mass.

Body Composition Ratios between BMI Percentile Groups

Notably, the current study observed significant differences in the MBR between the BMI percentile groups, specifically with the obesity group having higher ratios. These relationships were maintained when separating males and females. A higher ratio suggests that children with obesity have higher proportions of lean mass to bone mass or lower proportions of bone mass to lean mass. To the best of our knowledge only one study has looked at the effect of body fat percent on total and regional MBR (Duran et al., 2019). However, they combined arms and legs and thus referred to it as appendicular muscle to bone unit. This study observed a negative correlation between body fat percentage and appendicular BMC for lean body mass (Duran et al., 2019). Therefore, as body fat percent increased, the appendicular BMC for lean body mass decreased, indicating more lean mass for bone mass. These results are similar to the present study where higher amounts of adiposity are indicative of a higher proportion of lean mass to bone mass.

This relationship between muscle and bone is important as bone strengthening occurs because of mechanical loading from muscle. The MBR provides a possible way to examine the mechanical adaptations of bone from muscular strain. However, a higher amount of muscular strain on the bones, indicative of a higher ratio, may overload the bone. Therefore, the excess mass observed in children with obesity may negatively affect bone strength. A lower MBR would suggest that the proportion of muscle to bone are more equal and that the bone can handle the torque from muscle. The negative impact of obesity on bone health has been reported in previous studies. Specifically, after normalizing BMD and BMC to weight, researchers have reported lower values in children with obesity (Litwic et al., 2021; L. N. Mosca et al., 2014). This is important as lower amounts of BMD may put children with obesity at a greater risk of obtaining fractures.

This study further expands on the relationship of muscle-to-bone by including the SBR. The present study observed higher SBRs in children with obesity compared to the overweight and normal weight BMI percentile group. A previous study explored the relationship between fat mass, lean mass, and bone strength. They observed increased amounts of fat mass, normalized for lean mass, was negatively associated with bone strength (Ducher et al., 2009). Similarly, a previous study normalized BMC in regard to lean mass and they reported that children with decreased bone mass had lower total body BMC (Golec & Chlebna-Sokół, 2014). Thus, increased loading of the bone as a result from lean mass and fat mass may negatively impact bone mass. These results are indicative of bone poorly adapting to increased loading and children with obesity may have an increased risk of fractures.

Muscle-to-Bone and Soft Tissue-to-Bone Ratio Associations with Cardiometabolic Risk Factors

This study adds to the current literature by expanding on the association of obesity and cardiometabolic risk factors with bone health. A previous study also reported a positive association between HDL and BMC (Pollock et al., 2011). Thus, as HDL increases, so does BMC. The present study observed a similar association between MBR and SBR with HDL, as MBR and SBR decreased, HDL increased. Also previous studies have observed negative associations between insulin, HOMA-IR (do Prado et al., 2009; Hetherington-Rauth et al., 2019; Karimi et al., 2021; Kindler et al., 2019; Lawlor et al., 2012; K. Lee, 2013; Pollock et al., 2011; Shawar et al., 2022), fasting glucose, total cholesterol and BMC (Pollock et al., 2011). Indicating the higher the insulin, HOMA-IR, glucose and total cholesterol levels, the lower the BMC values. The current study reported similar results with SBR having a significant positive association with insulin, HOMA-IR, LDL, VLDL, and triglycerides. Indicating that as SBR increases (i.e., more lean and fat mass relative to BMC), so do insulin, HOMA-IR, LDL, VLDL, and triglyceride levels. Higher levels of insulin, insulin resistance as assessed by HOMA-IR, and atherogenic lipoproteins are potential cardiometabolic disease risk factors associated with obesity. Notably, the associations of cardiometabolic risk factors are with SBR and not MBR. These observations suggest that the addition of fat mass in the ratio is driving the significant associations. Thus, as the SBR increases we observe a relative decrease in functional tissue (i.e., increasing fat mass relative to lean mass and BMC). This decrease in functional tissue as assessed by the SBR may be clinically significant as it may be used as an indicator for cardiometabolic disease risk in children and adolescents.

Body Composition between BMI Percentile Groups

The results of body composition differences between BMI percentile groups in the current study are similar to those in previous studies. Children with obesity have been reported to have increased fat mass, lean mass, BMC, and BMD compared to their normal weight peers (Dimitri, 2019; Dimitri et al., 2010; Litwic et al., 2021; L. Mosca et al., 2013; Rinonapoli et al., 2021; Rokoff et al., 2019). This relationship of increased fat mass, lean mass, BMC, and BMD in children with obesity was also observed in females, but males with obesity had increased fat mass, lean mass, and BMD compared to their normal weight peers. However, when looking at bone measures, some studies normalized BMD and BMC for body weight (Litwic et al., 2021; L. N. Mosca et al., 2014). They reported that once normalized for body weight, children with obesity had lower BMC and BMD compared to their normal weight peers (Litwic et al., 2021; L. N. Mosca et al., 2014). Thus, normalizing bone measures to other body composition variables, such as fat mass and lean mass, may provide additional insight into how obesity impacts bone health. One way to do this is by looking at the impact of muscle on bone health through the MBR.

Limitations of the study include the cross-sectional nature of the study; therefore, causality of the relationships of MBR and SBR with cardiometabolic risk factors cannot be determined. The purpose of the current study was exploring MBR and SBR in a population of children with obesity, however, due to the composition of the sample, the interpretation of the results may be limited in their generalizability. Also, normative data for MBR and SBR exist, thus limiting clinical interpretation.

In conclusion, the present study demonstrates higher total and regional MBR and SBR in children with obesity compared to their normal weight peers. This study also

observed significant associations of SBR with cardiometabolic risk factors. These results suggest a relationship between increased adiposity and bone health in children and adolescents with obesity.

Table Legends

Table 1. Descriptive characteristics of the study population.

Table 2. Body Composition Variables between BMI Percentile Groups.

Table 3. Body Composition Variables (mean ± standard deviation) between BMI Percentile Groups for Males.

Table 4. Body Composition Variables (mean ± standard deviation) between BMI Percentile Groups for Females.

Table 5. Associations of cardiometabolic risk factors with MBR and SBR.

Tables

Table 1. Descriptive characteristics of the study population.

	Overall (n=219)	**Females (n=118)**	**Males (n=101)**
Age (yrs.)	12.3 (2.5)	12.3 (2.7)	12.4 (2.2)
Tanner Stage			
Stage I	59 (26.9)	24 (20.3)	35 (34.6)
Stage II/III/IV	127 (58.0)	78 (66.1)	49 (48.5)
Stage V	25 (11.4)	12 (10.2)	13 (12.9)
Missing Tanner	8 (3.7)	4 (3.4)	4 (4.0)
Race/Ethnicity			
African American or Black	17 (7.8)	12 (10.2)	5 (5.0)
Asian	1 (0.5)	0 (0)	1 (1.0)
White	182 (83.1)	96 (81.3)	86 (85.1)
Other	6 (2.7)	4 (3.4)	2 (2.0)
Latino/Hispanic	13 (5.9)	6 (5.1)	7 (6.9)
BMI percentile category			
Normal weight	103 (47)	44 (37.3)	59 (58.4)
Overweight	18 (8.2)	11 (9.3)	7 (6.9)
Obesity	98 (44.7)	63 (53.4)	35 (34.7)

Descriptive statistics were summarized by mean (SD) or n (%) for continuous and categorical covariates, respectively. BMI, Body Mass Index

Table 2. Body Composition Variables between BMI Percentile Groups

	Normal (n=103)	**Overweight (n=18)**	**Obesity (n=98)**
Total			
Fat mass (kg)	11.18 ± 3.83^{a}	18.71 ± 4.57^{b}	32.29 ± 9.36^{c}
Lean mass (kg)	34.18 ± 10.41^{a}	41.40 ± 12.72^{b}	39.92 ± 9.89^{b}
BMC (kg)	1.89 ± 0.60^{a}	2.20 ± 0.67^{ab}	2.09 ± 0.54^{b}
BMD (g/cm^2)	0.99 ± 0.16^{a}	1.08 ± 0.17^{b}	1.07 ± 0.16^{b}
Arm			
Fat mass (kg)	1.39 ± 0.43^{a}	2.23 ± 0.54^{b}	3.81 ± 0.99^{c}
Lean mass (kg)	3.81 ± 1.51^{a}	4.62 ± 1.75^{ab}	4.34 ± 1.26^{b}
BMC (kg)	0.24 ± 0.10^{a}	0.28 ± 0.10^{a}	0.26 ± 0.08^{a}
BMD (g/cm^2)	0.68 ± 0.15^{a}	0.77 ± 0.14^{ab}	0.78 ± 0.14^{b}
Leg			
Fat mass (kg)	4.87 ± 1.64^{a}	7.57 ± 1.70^{b}	11.86 ± 3.53^{c}
Lean mass (kg)	11.51 ± 3.69^{a}	14.52 ± 4.57^{b}	14.45 ± 3.95^{b}
BMC (kg)	0.70 ± 0.25^{a}	0.85 ± 0.30^{ab}	0.79 ± 0.23^{b}
BMD (g/cm^2)	1.03 ± 0.19^{a}	1.14 ± 0.20^{ab}	1.13 ± 0.18^{b}

Abbreviations: BMI, Body Mass Index; BMC, Bone Mineral Content; BMD, Bone Mineral Density. Note: Significant differences denoted by a letter correspond to with-in group analyses. BMI percentile groups that share a letter within the same row denote no significant difference.

Table 3. Body Composition Variables (mean ± standard deviation) between BMI Percentile Groups for Males.

	Normal Weight (n=59)	**Obesity (n=35)**	**p-value**	**95% CI**
Total				
Fat Mass (kg)	9.63 ± 2.66	31.04 ± 8.67	<0.001	-23.81, -19.00
Lean Mass (kg)	35.84 ± 11.41	42.05 ± 10.97	0.011	-10.98, -1.44
BMC (kg)	1.91 ± 0.62	2.16 ± 0.54	0.054	-0.50, 0.004
BMD (g/cm^2)	0.99 ± 0.15	1.07 ± 0.14	0.013	-0.14, -0.02
Arm				
Fat Mass (kg)	1.22 ± 0.33	3.65 ± 0.94	<0.001	-2.70, -2.17
Lean Mass (kg)	4.14 ± 1.73	4.68 ± 1.44	0.126	-1.23, 0.15
BMC (kg)	0.25 ± 0.11	0.27 ± 0.08	0.362	-0.06, 0.02
BMD (g/cm^2)	0.69 ± 0.16	0.77 ± 0.13	0.008	-0.15, -0.02
Leg				
Fat Mass (kg)	4.19 ± 1.18	11.17 ± 2.84	<0.001	-7.80, -6.14
Lean Mass (kg)	12.04 ± 4.03	15.29 ± 4.41	<0.001	-5.03, -1.49
BMC (kg)	0.72 ± 0.27	0.84 ± 0.24	0.030	-0.23, -0.01
BMD (g/cm^2)	1.03 ± 0.19	1.13 ± 0.18	0.008	-0.19, -0.03

Abbreviations: BMI, Body Mass Index; BMC, Bone Mineral Content; BMD, Bone Mineral Density.

Table 4. Body Composition Variables (mean ± standard deviation) between BMI Percentile Groups for Females.

	Normal Weight (n=44)	**Obesity (n=63)**	**p-value**	**95% CI**
Total				
Fat Mass (kg)	13.25 ± 4.19	32.98 ± 9.72	<0.001	-22.82, -16.62
Lean Mass (kg)	31.96 ± 8.53	38.75 ± 9.11	<0.001	-10.24, -3.32
BMC (kg)	1.86 ± 0.58	2.05 ± 0.54	0.080	-0.41, 0.02
BMD (g/cm^2)	1.00 ± 0.17	1.08 ± 0.17	0.014	-0.15, -0.02
Arm				
Fat Mass (kg)	1.62 ± 0.45	3.90 ± 1.02	<0.001	-2.60, -1.95
Lean Mass (kg)	3.37 ± 1.02	4.16 ± 1.11	<0.001	-1.21, -0.37
BMC (kg)	0.23 ± 0.08	0.25 ± 0.08	0.294	-0.05, 0.01
BMD (g/cm^2)	0.68 ± 0.15	0.78 ± 0.15	<0.001	-0.16, -0.05
Leg				
Fat Mass (kg)	5.77 ± 1.74	12.24 ± 3.83	<0.001	-7.70, -5.24
Lean Mass (kg)	10.8 ± 3.08	13.98 ± 3.62	<0.001	-4.51, -1.85
BMC (kg)	0.68 ± 0.23	0.77 ± 0.22	0.057	-0.17, -0.003
BMD (g/cm^2)	1.03 ± 0.20	1.12 ± 0.18	0.017	-0.16, -0.02

Abbreviations: BMI, Body Mass Index; BMC, Bone Mineral Content; BMD, Bone Mineral Density.

Table 5. Associations of cardiometabolic risk factors with MBR and SBR.

	Estimate	**p-value**	**Adjusted p-value**	**R^2-value**
MBR				
Glucose (mg/dL)	0.010	0.452	>0.999	-0.002
Insulin (mU/L)	0.051	0.002	0.050	0.038
HOMA-IR	0.231	0.003	0.058	0.036
Total Cholesterol (mg/dL)	0.002	0.639	>0.999	-0.004
HDL (mg/dL)	-0.028	<0.001	0.007	0.054
LDL (mg/dL)	0.008	0.099	>0.999	0.008
VLDL (mg/dL)	0.031	0.022	0.443	0.027
Triglycerides (mg/dL)	0.006	0.010	0.204	0.026
Systolic Blood Pressure (mmHg)	0.015	0.131	>0.999	0.006
Diastolic Blood Pressure (mmHg)	-0.019	0.177	>0.999	0.004
SBR				
Glucose (mg/dL)	0.117	0.015	0.293	0.023
Insulin (mU/L)	0.519	<0.001	<0.001	0.308
HOMA-IR	2.352	<0.001	<0.001	0.293
Total Cholesterol (mg/dL)	0.036	0.018	0.364	0.021
HDL (mg/dL)	-0.231	<0.001	<0.001	0.269
LDL (mg/dL)	0.076	<0.001	<0.001	0.084
VLDL (mg/dL)	0.343	<0.001	<0.001	0.247
Triglycerides (mg/dL)	0.072	<0.001	<0.001	0.262

Systolic Blood Pressure (mmHg)	0.216	<0.001	<0.001	0.147
Diastolic Blood Pressure (mmHg)	0.127	0.015	0.290	0.023

Abbreviations: MBR, muscle-to-bone ratio; SBR, soft tissue-to-bone ratio; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein

Figures

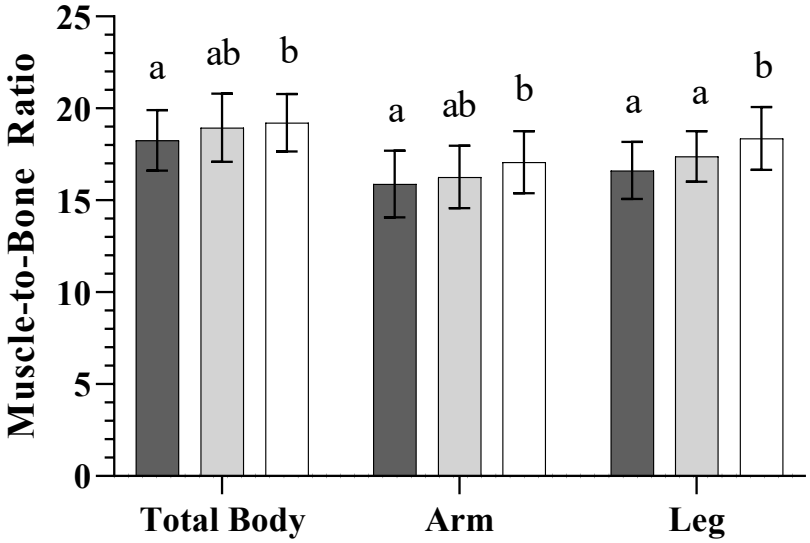


Figure 1. Muscle-to-bone ratio between BMI percentile groups for total, arm, and legs. BMI percentiles that share a letter within group analyses are not significantly different. BMI percentiles that do not share a letter are significantly different, within group (i.e., total, arm, and leg) analyses. Normal weight is represented by a solid dark grey bar, overweight by a solid light gray bar, and obesity by an open bar.

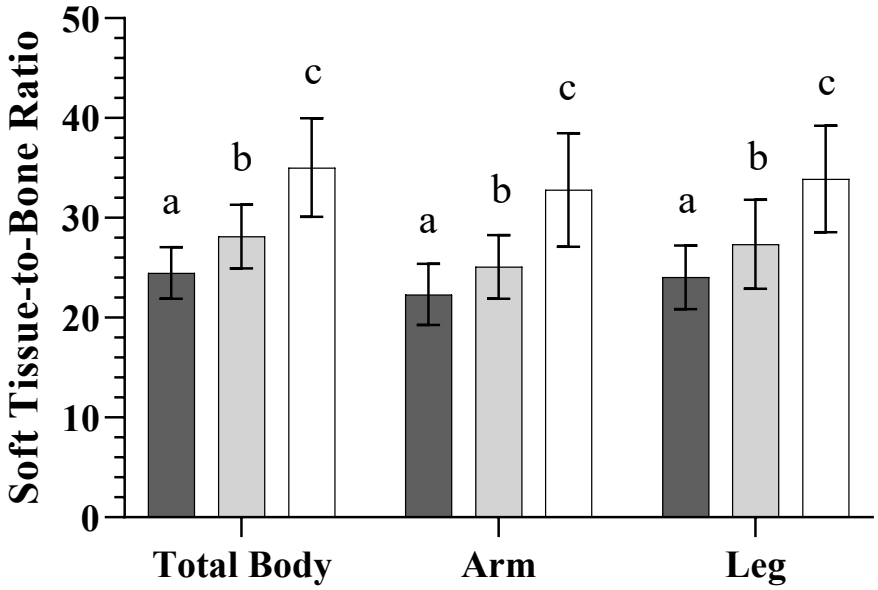


Figure 2. Soft tissue-to-bone ratio between BMI percentile groups for total, arm, and leg. BMI percentiles that do not share a letter are significantly different, within group (i.e., total, arm, and leg) analyses. Normal weight is represented by a solid dark grey bar, overweight by a solid light gray bar, and obesity by an open bar.

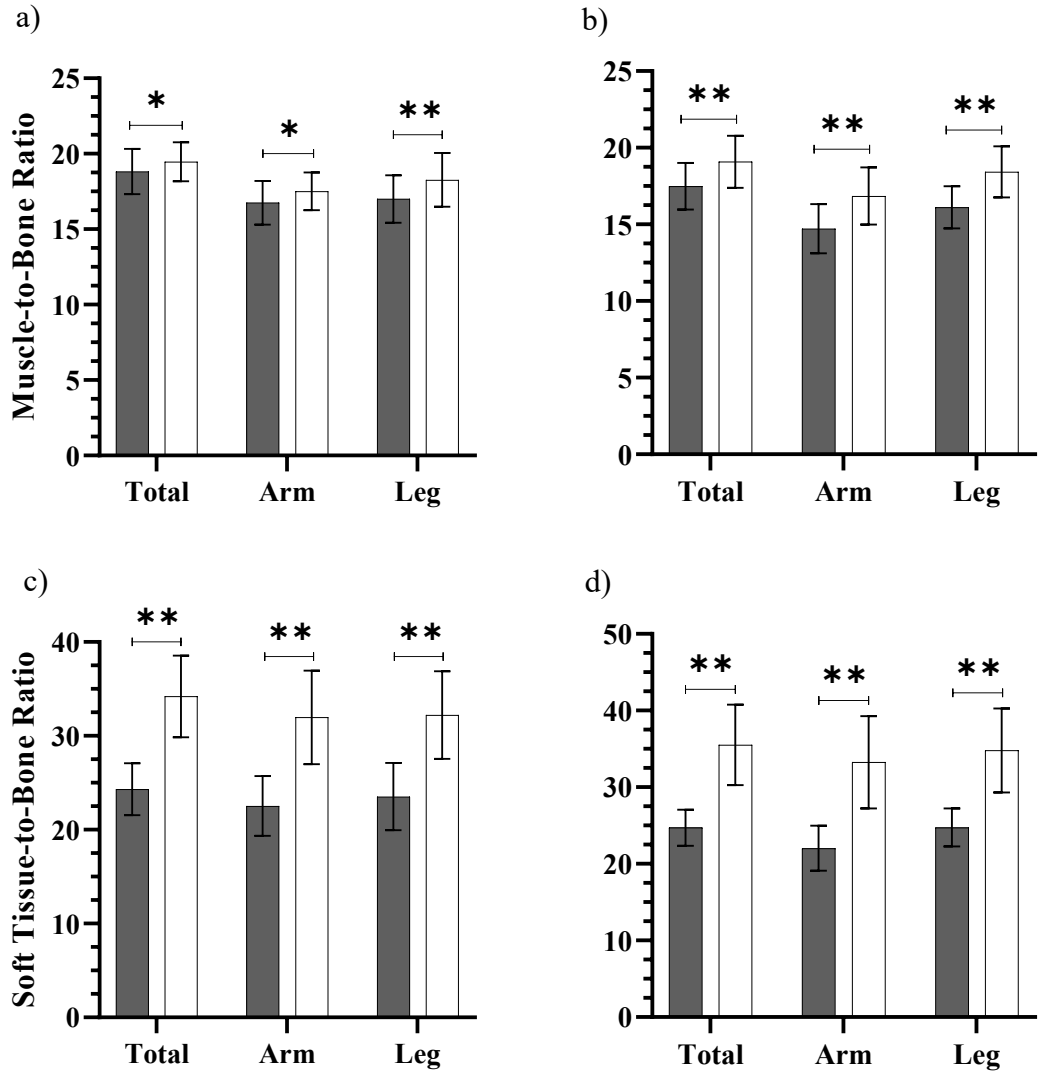


Figure 3. Muscle-to-bone ratio for (Panel a) males and (Panel b) females by BMI percentile group. Soft tissue-to-bone ratio for (Panel c) males and (Panel d) females by BMI percentile group. In all figures normal weight is represented by solid gray bar and obesity represented by an open bar. Significance denoted by * for $p<0.05$ and ** for $p<0.001$.

CHAPTER 6: CONCLUSION

Research Results and Implications

DXA has been widely used to measure total and regional body composition measures of lean mass, fat mass, and bone mass. While previous studies have explored the use of DXA to measure total body volume, few studies have explored the use of DXA in regional volume. Similarly, no studies have explored the use of DXA derived regional volumes in a regional 4-compartment model. While studies have explored the relationship of lean mass and bone health in children with obesity, few studies have explored the ratio of muscle-to-bone and no studies have explored the relationship of soft tissue and bone. Thus, this dissertation offered greater understanding of the use of DXA for regional volumes and 4-compartment model and the use of DXA to explore bone health in children with obesity.

First, DXA was used to assess total and regional volumes. Previous studies have explored the use of DXA for total volume derived from a DXA scan. This dissertation's first study expanded on this topic by examining the use of regional DXA derived volumes. In this study, total DXA derived volume was compared to total volume measured from UWW. In addition, this study compared regional DXA derived volumes to the traditional method of water displacement for regional volumes. The observations from the first study suggest that DXA can be used to assess total and regional volumes as it was not significantly different from the total (i.e., UWW) and regional (i.e., water displacement) volumes measured. Thus, DXA derived regional volumes may be used when monitoring body composition that may be impacted by changes in volume, such as, treatment or injury. The observations from the first study allowed for the use of DXA derived total and regional volumes for this dissertation's second study.

Second, this dissertation used the total and regional DXA derived volumes from the first study to explore total and regional 4-compartment models. To the best of our knowledge, this study was the first to explore a regional 4-compartment model. These DXA derived regional 4-compartment models were compared to a traditional method where regional volumes were measured from water displacement. Observations indicated that the regional 4-compartment model with DXA derived regional volumes were not significantly different from a traditional regional 4-compartment model. These observations indicate that DXA can be used to develop a "convenient" 4-compartment model, which requires only two devices (DXA and BIS) and thus is a quick assessment of body composition. Also, due to the increased number of tissues measured, the 4-compartment model makes fewer assumptions than a 2 and 3-compartment model. Ultimately, using DXA in a total and regional 4-compartment model increases the utility of DXA technology.

Finally, the MBR and SBR were examined in a group of children and adolescents with and without obesity. These ratios were also examined for their associations with cardiometabolic disease risk factors. Observations revealed significantly higher MBRs and SBRs in children with obesity compared to their normal weight peers. These observations indicated that children with obesity have more muscle mass/soft tissue mass to bone mass, suggesting greater strain on the skeletal system. This investigation also observed significant associations between SBR and insulin, HOMA-IR, the lipid profile, and systolic blood pressure. Observations indicated the impact that excess mass (i.e., lean and fat mass) has on cardiometabolic risk factors such as insulin resistance, dyslipidemia, and hypertension.

This dissertation explored innovative ways in which DXA may be used to assess body composition, ultimately expanding DXA's capabilities. First, this dissertation established that DXA may be used to as a method to estimate regional volumes. Also, that these DXA derived regional volumes may be implemented into a regional convenient 4-compartment model to determine body composition. In addition, this dissertation established the utility of body composition ratios (i.e., MBR and SBR) for the assessment of bone health in pediatrics with and without obesity.

Future Research

Although this dissertation provided insight into the use of DXA derived regional volumes in a "convenient" 4-compartment model, future studies should examine these regional DXA derived volume equations in a variety of populations (i.e., age, BMI groups, diseases). Further, while body composition ratios differ between BMI percentile groups in children, future research should explore these ratios in adults. Also, how these ratios may change in response to diseases, injury, or treatment. Lastly, future research should examine the longitudinal change the associations between these body composition ratios and cardiometabolic disease risk factors into adulthood.

CHAPTER 7: REFERENCES

Ackland, T. R., Lohman, T. G., Sundgot-Borgen, J., Maughan, R. J., Meyer, N. L., Stewart, A. D., & Müller, W. (2012). Current status of body composition assessment in sport. *Sports Medicine*, *42*(3), 227–249. https://doi.org/10.2165/11597140-000000000-00000

Anliker, E., & Toigo, M. (2012). Functional assessment of the muscle-bone unit in the lower leg. *Journal of Musculoskeletal and Neuronal Interactions*, *12*(2), 46–55.

Bantle, A. E., Bosch, T. A., Dengel, D. R., Wang, Q., Mashek, D. G., & Chow, L. S. (2019). DXA-determined regional adiposity relates to insulin resistance in a young adult population with overweight and obesity. *Journal of Clinical Densitometry*, *22*(2), 287–292. https://doi.org/10.1016/j.jocd.2018.06.001

Bernal-Orozco, M. F., Posada-Falomir, M., Quiñónez-Gastélum, C. M., Plascencia-Aguilera, L. P., Arana-Nuño, J. R., Badillo-Camacho, N., Márquez-Sandoval, F., Holway, F. E., & Vizmanos-Lamotte, B. (2020). Anthropometric and body composition profile of young professional soccer players. *Journal of Strength and Conditioning Research*, *34*(7), 1911–1923. https://doi.org/10.1519/JSC.0000000000003416

Bilsborough, J. C., Greenway, K., Opar, D., Livingstone, S., Cordy, J., & Coutts, A. J. (2014). The accuracy and precision of DXA for assessing body composition in team sport athletes. *Journal of Sports Sciences*, *32*(19), 1821–1828. https://doi.org/10.1080/02640414.2014.926380

Blake, G. M., & Fogelman, I. (1997). Technical principles of dual energy X-ray absorptiometry. *Seminars in Nuclear Medicine*, *27*(3), 210–228. https://doi.org/10.1016/S0001-2998(97)80025-6

Blue, M. N. M., Hirsch, K. R., Trexler, E. T., & Smith-Ryan, A. E. (2018). Validity of the 4-compartment model using dual energy X-ray absorptiometry-derived body volume in overweight individuals. *Applied Physiology, Nutrition, and Metabolism*, *43*(7), 742–746. https://doi.org/10.1139/apnm-2017-0804

Bonney, E., Ferguson, G., & Smits-Engelsman, B. (2018). Relationship between body mass index, cardiorespiratory and musculoskeletal fitness among South African adolescent girls. *International Journal of Environmental Research and Public Health*, *15*(6), 1087. https://doi.org/10.3390/ijerph15061087

Bosch, T. A., Chow, L., Dengel, D. R., Melhorn, S. J., Webb, M., Yancey, D., Callahan, H., De Leon, M. R. B., Tyagi, V., & Schur, E. A. (2015). In adult twins, visceral fat accumulation depends more on exceeding sex-specific adiposity thresholds than on genetics. *Metabolism*, *64*(9), 991–998. https://doi.org/10.1016/j.metabol.2015.06.002

Bosch, T. A., Dengel, D. R., Ryder, J. R., Kelly, A. S., & Chow, L. (2015). Fitness level is associated with sex-specific regional fat differences in normal weight young adults. *Journal of Endocrinology and Diabetes*, *2*(3), 01–05. https://doi.org/10.15226/2374-6890/2/3/00122

Bosch, T. A., Steinberger, J., Sinaiko, A. R., Moran, A., Jacobs, D. R., Kelly, A. S., & Dengel, D. R. (2015). Identification of sex-specific thresholds for accumulation of

visceral adipose tissue in adults. *Obesity*, *23*(2), 375–382. https://doi.org/10.1002/oby.20961

Bridge, P., Pocock, N. A., Nguyen, T., Munns, C., Cowell, C. T., Forwood, N., & Thompson, M. W. (2011). Validation of longitudinal DXA changes in body composition from pre- to mid-adolescence using MRI as reference. *Journal of Clinical Densitometry*, *14*(3), 340–347. https://doi.org/10.1016/j.jocd.2011.04.005

Bridge, P., Pocock, N. A., Nguyen, T., Munns, C., Cowell, C. T., & Thompson, M. W. (2009). Prediction of appendicular skeletal and fat mass in children: Excellent concordance of dual-energy X-ray absorptiometry and magnetic resonance imaging. *Journal of Pediatric Endocrinology and Metabolism*, *22*(9), 795–804. https://doi.org/10.1515/JPEM.2009.22.9.795

Brocherie, F., Girard, O., Forchino, F., Al Haddad, H., Dos Santos, G. A., & Millet, G. P. (2014). Relationships between anthropometric measures and athletic performance, with special reference to repeated-sprint ability, in the Qatar national soccer team. *Journal of Sports Sciences*, *32*(13), 1243–1254. https://doi.org/10.1080/02640414.2013.862840

Brorson, H., Ohlin, K., Olsson, G., & Karlsson, M. K. (2009). Breast cancer-related chronic arm lymphedema is associated with excess adipose and muscle tissue. *Lymphatic Research and Biology*, *7*(1), 3–10. https://doi.org/10.1089/lrb.2008.1022

Cannon, T., & Choi, J. (2019). Development of a segmental bioelectrical impedance spectroscopy device for body composition measurement. *Sensors*, *19*(22), 4825. https://doi.org/10.3390/s19224825

Carvajal, W., Betancourt, H., León, S., Deturnel, Y., Martínez, M., Echevarría, I., Castillo, M. E., & Serviat, N. (2012). Kinanthropometric profile of Cuban women Olympic volleyball champions. *MEDICC Review*, *14*(2), 7.

Compston, J. (2013). Obesity and bone. *Current Osteoporosis Reports*, *11*(1), 30–35. https://doi.org/10.1007/s11914-012-0127-y

Czeck, M. A., Roelofs, E. J., Juckett, W. T., & Dengel, D. R. (2022). Dual X-ray absorptiometry-derived total and regional body volume. *Clinical Nutrition ESPEN*, *52*, 100–104. https://doi.org/10.1016/j.clnesp.2022.10.011

Deforche, B., Lefevre, J., De Bourdeaudhuij, I., Hills, A. P., Duquet, W., & Bouckaert, J. (2003). Physical fitness and physical activity in obese and nonobese Flemish youth. *Obesity Research*, *11*(3), 434–441. https://doi.org/10.1038/oby.2003.59

Dimitri, P. (2019). The impact of childhood obesity on skeletal health and development. *Journal of Obesity & Metabolic Syndrome*, *28*(1), 4–17. https://doi.org/10.7570/jomes.2019.28.1.4

Dimitri, P., Wales, J. K., & Bishop, N. (2010). Fat and bone in children: Differential effects of obesity on bone size and mass according to fracture history. *Journal of Bone and Mineral Research*, *25*(3), 527–536. https://doi.org/10.1359/jbmr.090823

do Prado, W. L., de Piano, A., Lazaretti-Castro, M., de Mello, M. T., Stella, S. G., Tufik, S., do Nascimento, C. M. O., Oyama, L. M., Lofrano, M. C., Tock, L., Caranti, D. A., & Dâmaso, A. R. (2009). Relationship between bone mineral density, leptin and insulin concentration in Brazilian obese adolescents. *Journal of Bone and Mineral Metabolism*, *27*(5), 613–619. https://doi.org/10.1007/s00774-009-0082-6

Ducher, G., Bass, S. L., Naughton, G. A., Eser, P., Telford, R. D., & Daly, R. M. (2009). Overweight children have a greater proportion of fat mass relative to muscle mass in the upper limbs than in the lower limbs: Implications for bone strength at the distal forearm. *The American Journal of Clinical Nutrition*, *90*(4), 1104–1111. https://doi.org/10.3945/ajcn.2009.28025

Duran, I., Martakis, K., Bossier, C., Stark, C., Rehberg, M., Semler, O., & Schoenau, E. (2019). Interaction of body fat percentage and height with appendicular functional muscle-bone unit. *Archives of Osteoporosis*, *14*(1), 65. https://doi.org/10.1007/s11657-019-0610-5

Fuller, N. J., Jebb, S. A., Laskey, M. A., Coward, W. A., & Elia, M. (1992). Four-component model for the assessment of body composition in humans: Comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clinical Science*, *82*(6), 687–693. https://doi.org/10.1042/cs0820687

Fuller, N. J., Laskey, M. A., & Elia, M. (1992). Assessment of the composition of major body regions by dual-energy X-ray absorptiometry (DEXA), with special reference to limb muscle mass. *Clinical Physiology*, *12*(3), 253–266. https://doi.org/10.1111/j.1475-097X.1992.tb00831.x

Garcia-Vicencio, S., Coudeyre, E., Kluka, V., Cardenoux, C., Jegu, A.-G., Fourot, A.-V., Ratel, S., & Martin, V. (2016). The bigger, the stronger? Insights from muscle architecture and nervous characteristics in obese adolescent girls. *International Journal of Obesity*, *40*(2), 245–251. https://doi.org/10.1038/ijo.2015.158

Gjorup, C. A., Zerahn, B., & Hendel, H. W. (2010). Assessment of volume measurement of breast cancer-related lymphedema by three methods: Circumference measurement, water displacement, and dual energy X-ray absorptiometry. *Lymphatic Research and Biology*, *8*(2), 111–119. https://doi.org/10.1089/lrb.2009.0016

Gjorup, C. A., Zerahn, B., Juul, S., Hendel, H. W., Christensen, K. B., & Hölmich, L. R. (2017). Repeatability of volume and regional body composition measurements of the lower limb using dual-energy X-ray absorptiometry. *Journal of Clinical Densitometry*, *20*(1), 82–96. https://doi.org/10.1016/j.jocd.2016.08.009

Glickman, S. G., Marn, C. S., Supiano, M. A., & Dengel, D. R. (2004). Validity and reliability of dual-energy X-ray absorptiometry for the assessment of abdominal adiposity. *Journal of Applied Physiology*, *97*(2), 509–514. https://doi.org/10.1152/japplphysiol.01234.2003

Golec, J., & Chlebna-Sokół, D. (2014). Assessment of the influence of body composition on bone mass in children and adolescents based on a functional analysis of the muscle-bone relationship. *Journal of Orthopaedics, Traumatology, and Rehabiliations*, *16*, 153–163.

Gomez-Bruton, A., Gonzalez-Aguero, A., Matute-Llorente, A., Lozano-Berges, G., Gomez-Cabello, A., Moreno, L. A., Casajus, J. A., & Vicente-Rodríguez, G. (2019). The muscle-bone unit in adolescent swimmers. *Osteoporosis International*, *30*(5), 1079–1088. https://doi.org/10.1007/s00198-019-04857-3

Haarbo, J., Gotfredsen, A., Hassager, C., & Christiansen, C. (1991). Validation of body composition by dual energy X-ray absorptiometry (DEXA). *Clinical Physiology*, *11*(4), 331–341. https://doi.org/10.1111/j.1475-097X.1991.tb00662.x

Hetherington-Rauth, M., Bea, J. W., Blew, R. M., Funk, J. L., Lee, V. R., Roe, D. J., Sardinha, L. B., & Going, S. B. (2019). Relationship of cardiometabolic risk biomarkers with DXA and pQCT bone health outcomes in young girls. *Bone*, *120*, 452–458. https://doi.org/10.1016/j.bone.2018.12.013

Heymsfield, S. B., Adamek, M., Gonzalez, M. C., Jia, G., & Thomas, D. M. (2014). Assessing skeletal muscle mass: Historical overview and state of the art. *Journal of Cachexia, Sarcopenia and Muscle*, *5*(1), 9–18. https://doi.org/10.1007/s13539-014-0130-5

Heymsfield, S. B., Ebbeling, C. B., Zheng, J., Pietrobelli, A., Strauss, B. J., Silva, A. M., & Ludwig, D. S. (2015). Multi-component molecular-level body composition reference methods: Evolving concepts and future directions. *Obesity Reviews*, *16*(4), 282–294. https://doi.org/10.1111/obr.12261

Hind, K., Oldroyd, B., & Truscott, J. G. (2011). In vivo precision of the GE Lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *European Journal of Clinical Nutrition*, *65*(1), 140–142. https://doi.org/10.1038/ejcn.2010.190

Holway, F. E., & Garavaglia, R. (2009). Kinanthropometry of Group I rugby players in Buenos Aires, Argentina. *Journal of Sports Sciences*, *27*(11), 1211–1220. https://doi.org/10.1080/02640410903207408

Hopkins, D. L. (1996). The relationship between muscularity, muscle:bone ratio and cut dimensions in male and female lamb carcasses and the measurement of muscularity using image analysis. *Meat Science*, *44*(4), 307–317. https://doi.org/10.1016/S0309-1740(96)00032-0

Huizenga, R., Schoeller, M., Krueger, D., & Vallarta-Ast, N. (2007). Precision of Lunar iDXA total body BMC and composition measurements on obese subjects. *Journal of Clincial Densitometry*, *10*(2), S208.

Ireland, A., Maden-Wilkinson, T., Mcphee, J., Cooke, K., Narici, M., Degens, H., & Rittweger, J. (2013). Upper limb muscle–bone asymmetries and bone adaptation in elite youth tennis players. *Medicine & Science in Sports & Exercise*, *45*(9), 1749–1758. https://doi.org/10.1249/MSS.0b013e31828f882f

Jaffrin, M. Y., & Morel, H. (2008). Body fluid volumes measurements by impedance: A review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. *Medical Engineering & Physics*, *30*(10), 1257–1269. https://doi.org/10.1016/j.medengphy.2008.06.009

Karimi, F., Ranjbar Omrani, G., & Dabbaghmanesh, M. H. (2021). Insulin resistance and bone health in adolescents. *Archives of Osteoporosis*, *16*(1), 66. https://doi.org/10.1007/s11657-021-00917-6

Kaul, S., Rothney, M. P., Peters, D. M., Wacker, W. K., Davis, C. E., Shapiro, M. D., & Ergun, D. L. (2012). Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity*, *20*(6), 1313–1318. https://doi.org/10.1038/oby.2011.393

Kelly, A. S., Kaizer, A. M., Bosch, T. A., Rudser, K. D., Ryder, J. R., Gross, A. C., Chow, L. S., Fox, C. K., & Dengel, D. R. (2020). Reaching the tipping point:

Identification of thresholds at which visceral adipose tissue may steeply increase in youth. *Obesity*, *28*(1), 139–145. https://doi.org/10.1002/oby.22679

Kindler, J. M., Lobene, A. J., Vogel, K. A., Martin, B. R., McCabe, L. D., Peacock, M., Warden, S. J., McCabe, G. P., & Weaver, C. M. (2019). Adiposity, insulin resistance, and bone mass in children and adolescents. *The Journal of Clinical Endocrinology & Metabolism*, *104*(3), 892–899. https://doi.org/10.1210/jc.2018-00353

Lawlor, D. A., Sattar, N., Sayers, A., & Tobias, J. H. (2012). The association of fasting insulin, glucose, and lipids with bone mass in adolescents: Findings from a cross-sectional study. *The Journal of Clinical Endocrinology & Metabolism*, *97*(6), 2068–2076. https://doi.org/10.1210/jc.2011-2721

Lee, C. K., Sim, Y. K., Lee, J.-H., Yook, J. S., Ha, S.-M., Seo, E. C., So, W.-Y., Kim, H. R., Jeong, W.-M., Goo, B. O., Chung, J.-W., & Ha, M.-S. (2022). The relationship between body composition and physical fitness and the effect of exercise according to the level of childhood obesity using the MGPA model. *International Journal of Environmental Research and Public Health*, *19*(1), 487. https://doi.org/10.3390/ijerph19010487

Lee, K. (2013). Sex-specific relationships between insulin resistance and bone mineral content in Korean adolescents. *Journal of Bone and Mineral Metabolism*, *31*(2), 177–182. https://doi.org/10.1007/s00774-012-0396-7

Lee, S. Y., & Gallagher, D. (2008). Assessment methods in human body composition. *Current Opinion in Clinical Nutrition & Metabolic Care*, *11*(5), 566–572. https://doi.org/10.1097/MCO.0b013e32830b5f23

Litwic, A. E., Westbury, L. D., Ward, K., Cooper, C., & Dennison, E. M. (2021). Adiposity and bone microarchitecture in the GLOW study. *Osteoporosis International*, *32*(4), 689–698. https://doi.org/10.1007/s00198-020-05603-w

Lohman, T. G., & Going, S. B. (1993). Multicomponent models in body composition research: Opportunities and pitfalls. In K. J. Ellis & J. D. Eastman (Eds.), *Human Body Composition* (pp. 53–58). Springer US. https://doi.org/10.1007/978-1-4899-1268-8_10

Lohman, T. G., Houtkooper, L., & Going, S. B. (1997). Body fat measurement goes high-tech. *Health & Fitness*, *1*(1), 30–35.

Maffeis, C., Zaffanello, M., & Schutz, Y. (1997). Relationship between physical inactivity and adiposity in prepubertal boys. *The Journal of Pediatrics*, *13*(2), 288–292.

Maïmoun, L., Mura, T., Leprieur, E., Avignon, A., Mariano-Goulart, D., & Sultan, A. (2016). Impact of obesity on bone mass throughout adult life: Influence of gender and severity of obesity. *Bone*, *90*, 23–30. https://doi.org/10.1016/j.bone.2015.11.020

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and beta cell funciton from fasting plasma glucose and insulin concentrations in man. *Diabetologia*.

Mazess, R. B., Barden, H. S., Bisek, J. P., & Hanson, J. (1990). Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue

composition. *The American Journal of Clinical Nutrition*, *51*(6), 1106–1112. https://doi.org/10.1093/ajcn/51.6.1106

McLester, C. N., Nickerson, B. S., Kliszczewicz, B. M., Hicks, C. S., Williamson, C. M., Bechke, E. E., & McLester, J. R. (2018). Validity of DXA body volume equations in a four-compartment model for adults with varying body mass index and waist circumference classifications. *PLOS ONE*, *13*(11), e0206866. https://doi.org/10.1371/journal.pone.0206866

Moon, J. R., Smith, A. E., Tobkin, S. E., Lockwood, C. M., Kendall, K. L., Graef, J. L., Roberts, M. D., Dalbo, V. J., Kerksick, C. M., Cramer, J. T., Beck, T. W., & Stout, J. R. (2009). Total body water changes after an exercise intervention tracked using bioimpedance spectroscopy: A deuterium oxide comparison. *Clinical Nutrition*, *28*(5), 516–525. https://doi.org/10.1016/j.clnu.2009.04.025

Moon, J. R., Tobkin, S. E., Roberts, M. D., Dalbo, V. J., Kerksick, C. M., Bemben, M. G., Cramer, J. T., & Stout, J. R. (2008). Total body water estimations in healthy men and women using bioimpedance spectroscopy: A deuterium oxide comparison. *Nutrition & Metabolism*, *5*(1), 7. https://doi.org/10.1186/1743-7075-5-7

Mosca, L., da Silva, V., & Goldberg, T. (2013). Does excess weight interfere with bone mass accumulation during adolescence? *Nutrients*, *5*(6), 2047–2061. https://doi.org/10.3390/nu5062047

Mosca, L. N., Goldberg, T. B. L., da Silva, V. N., da Silva, C. C., Kurokawa, C. S., Bisi Rizzo, A. C., & Corrente, J. E. (2014). Excess body fat negatively affects bone

mass in adolescents. *Nutrition*, *30*(7–8), 847–852. https://doi.org/10.1016/j.nut.2013.12.003

Musálek, M., Clark, C. C. T., Kokštejn, J., Vokounova, Š., Hnízdil, J., & Mess, F. (2020). Impaired cardiorespiratory fitness and muscle strength in children with normal-weight obesity. *International Journal of Environmental Research and Public Health*, *17*(24), 9198. https://doi.org/10.3390/ijerph17249198

Ng, B. K., Liu, Y. E., Wang, W., Kelly, T. L., Wilson, K. E., Schoeller, D. A., Heymsfield, S. B., & Shepherd, J. A. (2018). Validation of rapid 4-component body composition assessment with the use of dual-energy X-ray absorptiometry and bioelectrical impedance analysis. *The American Journal of Clinical Nutrition*, *108*(4), 708–715. https://doi.org/10.1093/ajcn/nqy158

Nickerson, B. S., Esco, M. R., Bishop, P. A., Kliszczewicz, B. M., Park, K.-S., & Williford, H. N. (2017). Validity of four-compartment model body fat in physically active men and women when using DXA for body volume. *International Journal of Sport Nutrition and Exercise Metabolism*, *27*(6), 520–527. https://doi.org/10.1123/ijsnem.2017-0076

Peiffer, J. J., Galvão, D. A., Gibbs, Z., Smith, K., Turner, D., Foster, J., Martins, R., & Newton, R. U. (2010). Strength and functional characteristics of men and women 65 years and older. *Rejuvenation Research*, *13*(1), 75–82. https://doi.org/10.1089/rej.2009.0916

Pietrobelli, A., Formica, C., Wang, Z., & Heymsfield, S. B. (1996). Dual-energy X-ray absorptiometry body composition model: Review of physical concepts. *American*

Journal of Physiology-Endocrinology and Metabolism, *271*(6), E941–E951. https://doi.org/10.1152/ajpendo.1996.271.6.E941

Pollock, N. K., Bernard, P. J., Gutin, B., Davis, C. L., Zhu, H., & Dong, Y. (2011). Adolescent obesity, bone mass, and cardiometabolic risk factors. *The Journal of Pediatrics*, *158*(5), 727–734. https://doi.org/10.1016/j.jpeds.2010.11.052

Pollock, N. K., Bernard, P. J., Wenger, K., Misra, S., Gower, B. A., Allison, J. D., Zhu, H., & Davis, C. L. (2010). Lower bone mass in prepubertal overweight children with prediabetes. *Journal of Bone and Mineral Research*, *25*(12), 2760–2769. https://doi.org/10.1002/jbmr.184

Purchas, R. W., Fisher, A. V., Price, M. A., & Berg, R. T. (2002). Relationships between beef carcass shape and muscle to bone ratio. *Meat Science*, *61*(3), 329–337. https://doi.org/10.1016/S0309-1740(01)00201-7

Raymond-Pope, C. J., Bosch, T. A., & Dengel, D. R. (2020). Assessing agreement of lateral leg muscle and bone composition using dual X-ray absorptiometry. *Journal of Clinical Densitometry*, *23*(3), 451–458. https://doi.org/10.1016/j.jocd.2019.04.007

Rinonapoli, G., Pace, V., Ruggiero, C., Ceccarini, P., Bisaccia, M., Meccariello, L., & Caraffa, A. (2021). Obesity and bone: A complex relationship. *International Journal of Molecular Sciences*, *22*(24), 13662. https://doi.org/10.3390/ijms222413662

Rokoff, L. B., Rifas-Shiman, S. L., Switkowski, K. M., Young, J. G., Rosen, C. J., Oken, E., & Fleisch, A. F. (2019). Body composition and bone mineral density in childhood. *Bone*, *121*, 9–15. https://doi.org/10.1016/j.bone.2018.12.009

Rothney, M. P., Martin, F.-P., Xia, Y., Beaumont, M., Davis, C., Ergun, D., Fay, L., Ginty, F., Kochhar, S., Wacker, W., & Rezzi, S. (2012). Precision of GE Lunar iDXA for the measurement of total and regional body composition in nonobese adults. *Journal of Clinical Densitometry*, *15*(4), 399–404. https://doi.org/10.1016/j.jocd.2012.02.009

Roubenoff, R., & Kehayias, J. J. (1991). The meaning and measurement of lean body mass. *Nutrition Reviews*, *49*(6), 163–175. https://doi.org/10.1111/j.1753-4887.1991.tb03013.x

Schoenau, E., Neu, C. M., Beck, B., Manz, F., & Rauch, F. (2002). Bone mineral content per muscle cross-sectional area as an index of the functional muscle-bone unit. *Journal of Bone and Mineral Research*, *17*(6), 1095–1101. https://doi.org/10.1359/jbmr.2002.17.6.1095

Shawar, R. S., Puyau, M., Shypailo, R., Musaad, S., Butte, N. F., & Bacha, F. (2022). Adiposity, insulin resistance, cardiorespiratory fitness, and bone health in Hispanic children. *The Journal of Clinical Endocrinology & Metabolism*, *107*(9), e3797–e3804. https://doi.org/10.1210/clinem/dgac344

Singh, B., Takeda, M. M., Niino, M. F., Goulart, J. D., Hammons, A. J., Roos, J. M., & Yack, H. J. (2021). The effects of adiposity, muscular strength, cardiorespiratory fitness, and fatigue on gait biomechanics in overweight and obese children. *Clinical Biomechanics*, *84*, 105332. https://doi.org/10.1016/j.clinbiomech.2021.105332

Smith-Ryan, A. E., Mock, M. G., Ryan, E. D., Gerstner, G. R., Trexler, E. T., & Hirsch, K. R. (2017). Validity and reliability of a 4-compartment body composition model

using dual energy X-ray absorptiometry-derived body volume. *Clinical Nutrition*, *36*(3), 825–830. https://doi.org/10.1016/j.clnu.2016.05.006

Stodden, D. F., Langendorfer, S. J., Goodway, J. D., Roberton, M. A., Rudisill, M. E., Garcia, C., & Garcia, L. E. (2008). A developmental perspective on the role of motor skill competence in physical activity: An emergent relationship. *Quest*, *60*(2), 290–306. https://doi.org/10.1080/00336297.2008.10483582

Sullivan, K., Hornikel, B., Holmes, C. J., Esco, M. R., & Fedewa, M. V. (2022). Validity of a 3-compartment body composition model using body volume derived from a novel 2-dimensional image analysis program. *European Journal of Clinical Nutrition*, *76*(1), 111–118. https://doi.org/10.1038/s41430-021-00899-1

Tanner, J. M., & Whitehouse, R. H. (1976). Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Archives of Disease in Childhood*, *51*, 170–179.

Thivel, D., Ring-Dimitriou, S., Weghuber, D., Frelut, M.-L., & O'Malley, G. (2016). Muscle strength and fitness in pediatric obesity: A systematic review from the European Childhood Obesity Group. *Obesity Facts*, *9*(1), 52–63. https://doi.org/10.1159/000443687

Tinsley, G. M. (2018). Reliability and agreement between DXA-derived body volumes and their usage in 4-compartment body composition models produced from DXA and BIA values. *Journal of Sports Sciences*, *36*(11), 1235–1240. https://doi.org/10.1080/02640414.2017.1369556

Tsiros, M. D., Buckley, J. D., Olds, T., Howe, P. R. C., Hills, A. P., Walkley, J., Wood, R., Kagawa, M., Shield, A., Taylor, L., Shultz, S. P., Grimshaw, P. N., Grigg, K.,

& Coates, A. M. (2016). Impaired physical function associated with childhood obesity: How should we intervene? *Childhood Obesity*, *12*(2), 126–134. https://doi.org/10.1089/chi.2015.0123

Wang, Z., Pi-Sunyer, F. X., Kotler, D. P., Wielopolski, L., Withers, R. T., Pierson, R. N., & Heymsfield, S. B. (2002). Multicomponent methods: Evaluation of new and traditional soft tissue mineral models by in vivo neutron activation analysis. *The American Journal of Clinical Nutrition*, *76*(5), 968–974. https://doi.org/10.1093/ajcn/76.5.968

Wang, Z., Shen, W., Withers, R. T., & Heymsfield, S. B. (2005). Multicomponent molecular-level models of body composition analysis. *Human Body Composition*, 163–176.

Wilson, J. P., Fan, B., & Shepherd, J. A. (2013). Total and regional body volumes derived from dual-energy X-ray absorptiometry output. *Journal of Clinical Densitometry*, *16*(3), 368–373. https://doi.org/10.1016/j.jocd.2012.11.001

Wilson, J. P., Mulligan, K., Fan, B., Sherman, J. L., Murphy, E. J., Tai, V. W., Powers, C. L., Marquez, L., Ruiz-Barros, V., & Shepherd, J. A. (2012). Dual-energy X-ray absorptiometry–based body volume measurement for 4-compartment body composition. *The American Journal of Clinical Nutrition*, *95*(1), 25–31. https://doi.org/10.3945/ajcn.111.019273

Withers, R. T., Craig, N. P., Ball, C. T., Norton, K. I., & Whittingham, N. O. (1991). The Drinkwater-Ross anthropometric fractionation of body mass: Comparison with measured body mass and densitometrically estimated fat and fat-free masses.

Journal of Sports Sciences, *9*(3), 299–311.

https://doi.org/10.1080/02640419108729891

APPENDICES

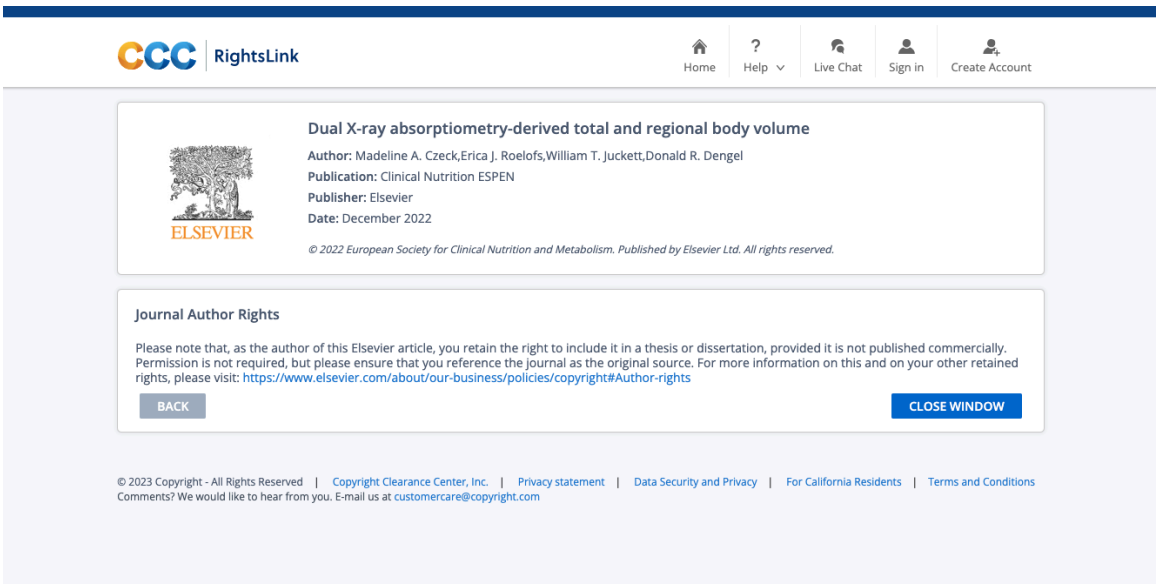


Figure 1. Copy right for chapter 3 study.

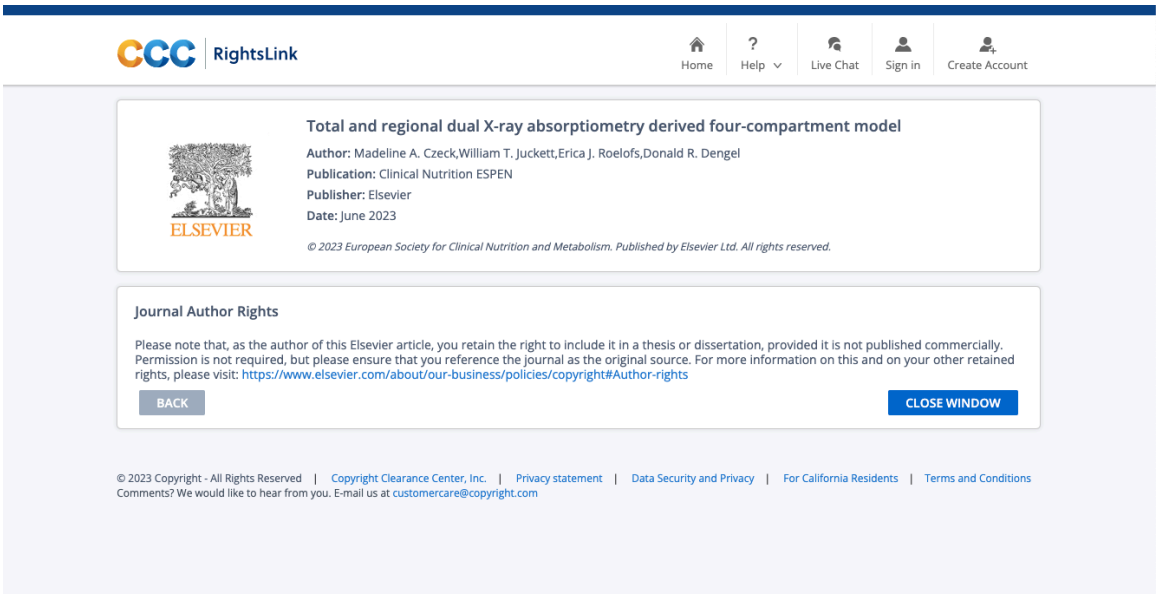


Figure 2. Copy right for chapter 4 study.

Figure 3. Copy right for chapter 5 study.