QUANTIFICATION OF VISCERAL ADIPOSE TISSUE USING DUAL ENERGY X-RAY ABSORPTIOMETRY

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ABSTRACT

Accumulation of visceral adipose tissue is an independent marker for cardiovascular risk and insulin resistance in both children and adults (Gower et al, 1999; Neeland et al. 2012; Owens et al. 1998). As the accumulation of visceral adipose tissue increases the risk of cardiovascular also increases independently of total obesity (Nakamura et al. 1993). Given its location surrounding the visceral organs, accurate measurement of visceral adipose tissue presents a challenge. The first reliable methods for quantifying visceral adipose tissue were computed tomography and magnetic resonance imaging. These procedures, while valid and reliable, are associated with several limitations and generally rely on a single slice to estimate visceral adipose tissue. Recently, dual energy x-ray absorptiometry (DXA) has been validated against computed tomography as a valid and reliable estimate of visceral adipose tissue (Kaul et al. 2012) and a significant marker of cardiometabolic risk in adults (Katzmarzyk, et al. 2013; Rothney et al. 2013). This dissertation will investigate the use of this novel method in three distinct populations: children; adults; and professional football players. We hypothesize that DXA will provide a valid estimate of visceral adipose tissue in children and be associated with cardiometabolic risk factors. Secondly, we hypothesize that differences in regional body composition exist in professional football players. Finally, we hypothesize that a threshold exists in distribution of fat to the visceral region and that this threshold is associated with increased cardiometabolic risk. The results of this dissertation will provide further insight into the reliability and clinical utility of DXA for estimating visceral adipose tissue.

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CHAPTER 1. INTRODUCTION

Introduction

Visceral adipose tissue (VAT) is an independent marker of cardiometabolic risk in children and adults, independent of overall obesity (Krotkiewski et al. 1983; Brambilla et al. 1994; Caprio et al. 1995; Gower et al, 1999; Neeland et al. 2012; Owens et al. 1998). Given its location, surrounding the visceral organs, accurate measurement of visceral adipose tissue presents a challenge. Prior to sophisticated measurement techniques, Vague (1947) observed increased risk for cardiovascular disease and diabetes based on body shape and not obesity (Vague 1956). He was the first to use the terms android and gynoid obesity to refer to increased accumulation of fat in either the trunk (android) or hips (gynoid) (Vague 1947).

Computed tomography (CT) was the first reliable method validated for directly quantifying visceral adipose tissue (Borkan et al. 1983; Grauer et al. 1984; Heimsfield et al. 1981; Tokunaga et al. 1983). Later, magnetic resonance imaging (MRI) provided a method of determining visceral adipose tissue that did not use ionizing radiation (Fowler et al. 1991; Staten et al. 1989). Both CT and MRI, while valid and reliable, are associated with several limitations and generally rely on single slice estimations of visceral fat. While single slice VAT measurements, generally, are strongly associated with total volume VAT, several gender and ethic differences exist dependent on slice site (Araneta et al. 2005; Camhi et al. 2011; Carroll et al. 2008; Demerath et al. 2007; Katzmarzyk et al. 2010; Lear et al. 2007).

Disproportionate accumulation of fat in the visceral region is consistently associated with hypertriglyceridemia, dyslipidemia, glucose intolerance and hyperinsulinemia in both children and adults (Bacha et al. 2003; Brambilla et al. 1994; Caprio et al. 1995; Despres et al. 2001; Gower et al. 1999; Nakamura et al. 1993; Neeland et al. 2012; Owens et al. 1998; Rasmussen-Torvik et al. 2012; Syme et al. 2008; Taksali et al. 2008). In addition, the association of VAT and cardiometabolic risk factors is influenced by the slice site (Demerath et al. 2007; Greenfield et al. 2002; Irlbeck et al. 2010; Kuk et al. 2006; Shen et al 2004; Shen et al. 2007). Furthermore the use of a single-slice does not accurately identify changes in subcutaneous and visceral adipose tissue following weight loss (Shen et al. 2012).

Recent software advancements have made it possible to measure VAT using dual energy x-ray absorptiometry (DXA) (Kaul et al. 2012). This novel method estimates VAT within the entire android region. DXA was validated against CT (Kaul et al. 2012) and has been determined to be a significant and reliable marker of cardiometabolic risk in adults (Katzmarzyk et al. 2014, Rothney et al. 2013). Since this new method can reliably measure total volume VAT, it may be possible to identify redistribution of excess fat to the visceral region and what role this may play in the association with cardiometabolic risk factors. This dissertation will focus on the use of this novel method to quantify VAT in children as well as determine the patterning

of visceral fat accumulation with increasing adiposity. This dissertation will address the following gaps in the current knowledge:

a) Determine the validity of DXA derived VAT compared to single slice computed tomography in children.
    i) Hypothesis 1: DXA total volume VAT will be significantly correlated with CT single slice VAT area
    ii) Hypothesis 2: DXA total volume VAT will demonstrate similar associations with cardiometabolic risk factors compared to CT single slice VAT
b) Determine the level of VAT accumulation in professional football players.
    i) Hypothesis 1: VAT accumulation is significantly higher in offensive and defensive linemen.
c) Determine body fat distribution patterns and their relation to cardiometabolic risk factors in adults.
    i) Hypothesis 1: VAT accumulation is dependent on the level of adiposity and is not linear throughout the entire range of total fat mass.
    ii) Hypothesis 2: As adiposity increases, increases in VAT are more strongly associated with cardiometabolic risk factors than total body fat or subcutaneous abdominal fat.

The second chapter of this dissertation provides a review of the existing literature on visceral adipose tissue. The differences between visceral and subcutaneous adipose tissue will be introduced as well as the association between visceral adipose tissue and cardiometabolic risk factors. We will

detail the standard measurement methods of determining visceral adipose tissue in humans: computed tomography and magnetic resonance imaging. Finally we will discuss the use of DXA to measure total body and regional body composition and the development of the method to measure VAT.

The third chapter of this dissertation compares the DXA derived total volume VAT estimate with a single slice (L4-L5) estimate using computed tomography in children. The correlation and concordance between the methods will be examined as well as their association with cardiometabolic risk factors.

Differences between total and regional body composition, measured by DXA, in professional football positional groups is examined in chapter four. Specific focus is placed on fat differences in the abdominal region and how this may explain the position group differences observed in development of cardiovascular disease and insulin resistance.

The association between visceral accumulation and total adiposity will be addressed in chapter five. We will measure the slope of visceral accumulation at identified sex-specific thresholds. Unadjusted and adjusted associations between cardiometabolic risk factors and fat measurements will be compared above and below the identified VAT accumulation threshold to determine the relative importance of each fat depot.

The summary of the observation of each study are discussed in chapter six of this dissertation. Future research questions are identified as well as the

clinical application of DXA derived VAT estimates are also discussed in this chapter.

CHAPTER 2. LITERATURE REVIEW

Introduction

Obesity is a result of excess fat accumulation which accumulates in both subcutaneous and ectopic regions of the body. One common ectopic region is the visceral region; where visceral adipose tissue accumulates around the mesenteric and omental areas of the abdominal region (Shen et al. 2003). The visceral region is similar to subcutaneous adipose depots in that it functions as a storage depot for excess fat. However, the location of that accumulation appears to be influential in determining the association between excess adipose tissue and cardiometabolic dysfunction. There are distinct anatomical and functional differences that exist between subcutaneous and visceral fat (Ibrahim 2010). The location of each storage depot and the functional differences of each depot as an endocrine organ likely explain why visceral fat accumulation is associated with increased cardiometabolic risk.

Subcutaneous adipose depots can be found all over the body and are located superficial to the muscle layer and includes mammary adipose tissue (Shen et al. 2003). The subcutaneous depot comprises between 80-90% of total fat stores depending on the level of obesity. Storage in this depot is greater because subcutaneous adipocytes expand more than visceral adipose tissue, but more importantly subcutaneous adipocytes can expand the capillary network to match the increased adipocyte diameter (Drolet et al. 2008; Fried et al. 1987; Gealekman et al. 2011; Spalding et al. 2008). The ability to increase vascularity protects adipocytes from becoming hypoxic, which will eventually lead to dysfunction and cell death. Subcutaneous tissue has

endocrine functions that suggest a protective quality. Adiponectin and leptin, considered "good" adipokines have a positive association with subcutaneous fat (Ibrahim 2010; Tchernof & Després 2013). These adipokines increase insulin sensitivity and decreases inflammation (Hou & Luo 2011; Montague et al. 1997; Van Harmelen et al. 1998; Van Harmelen et al. 2002). Additionally, subcutaneous fat are sensitive to alpha2-adrenergic receptors that signal inhibition of lipolysis (Ibrahim 2010; Tchernof & Després 2013). These qualities, along with the increased storage area would allow for preferential storage subcutaneously. However, several of the protective qualities of subcutaneous adipose tissue are attenuated with increasing obesity.

Visceral adipose tissue on the other hand is located deep to the abdominal muscle wall and includes adipose surrounding the greater omentum, mesentery and retroperitoneal regions (Shen et al. 2003). The vascular supplies to the subcutaneous and visceral regions are different, visceral adipose tissue is drained by the hepatic portal vein. This is a key feature to the hepatic portal hypothesis, which proposes a link between visceral adipose tissue and the development of cardiometabolic diseases (Bergman et al. 2001; Bjorntorp 1990). Simply stated, anything secreted into the portal vein will go to the liver before returning to the heart and the rest of the systemic circulation. Thus, in relation to visceral fat accumulation, the liver becomes overloaded with excess free fatty acids and the inflammatory cytokines leading to dysfunction (Bergman et al. 2001; Bjorntorp 1990).Subcutaneous adipose tissue is drained by more superficial veins that run through several

other tissues before going to the liver. Another difference between these two regions is the size and organization of adipocytes. Subcutaneous adipocytes are efficiently organized and are similar in shape, whereas visceral adipocytes are irregularly shaped and less organized (Markman et al. 1987). A lack of organization would lead to an inefficient storage system within the visceral region because as fat accumulates adipocytes expansion is inhibited by other adipocytes in the same area, leading to adipocyte dysfunction through increased recruitment of macrophages.

Probably more important than the anatomical differences of these storage sites are the functional differences between subcutaneous and visceral adipose tissue. Adipose tissue was first identified as an endocrine organ in a mouse model (Zhang et al. 1994). Since then several studies have identified specific adipokines released from adipose tissue and their association with inflammation and cardiometabolic dysfunction (Awazawa et al. 2011; Cnop et al. 2003; Hoeg et al. 2013; Kershaw et al. 2004; Margaritis et al. 2006; Meyer et al 2013; Shanker et al. 2012; Whitehead et al. 2006). These studies identify adiponectin and leptin as beneficial adipokines that are positively associated with insulin sensitivity and decreases cardiovascular risk factors. However, differences exist between subcutaneous adipose tissue and visceral adipose tissue. Increased visceral adipose accumulation is negatively associated with both adiponectin and insulin secretion (Cnop et al. 2003; Meyer et al. 2013; Montague et al. 1997; Motoshima et al. 2002; Schinzari et al; 2013; Van Harmelen et al 1998; Whitehead et al. 2006).

Visceral adipose tissue secretes pro-inflammatory cytokines (exs. tumor necrosis factor-alpha, interleukin-6, C-reactive protein, and resistin) that disrupt several signaling pathways leading to insulin resistance, hyperlipidemia and atherosclerosis (Anty et al. 2006; Bastard et al. 2002; Berg et al. 2005; Hotamisligil et al. 1993; Ibrahim 2010; Rotter et al. 2003; Shoelson et al. 2003; Steppan et al. 2001; Swaroop et al. 2012; Yang et al. 2009). Many of these cytokines are released by macrophages that have been recruited to adipocytes during expansion by the endoplasmic reticulum in response to sensing a nutrient surplus. This leads to a feed-forward loop of further macrophage recruitment and cytokine release. A second way macrophages are recruited to visceral adipose tissue is during expansion. When adipocytes increase via hypertrophy (increased size), the vasculature cannot expand fast enough resulting in an inadequate blood flow. This activates the HIF-1 pro-inflammatory pathways increasing macrophage recruitment. Failure to reduce adipocyte size will result in cell death and the recruitment of more macrophages. This process inhibits the visceral adipose tissue from storing free-fatty acids and at the same time increasing lipolysis (Schenk et al. 2008).

In addition to its role as an endocrine organ, adipose tissue is a receptor for hormones, cytokines and catecholamines. Visceral adipose receptors function differently than subcutaneous adipose receptors. Visceral adipose receptors are not as sensitive to insulin as subcutaneous adipose receptors, but are highly receptive to beta-adrenergic hormones (i.e.,epinephrine,

norepinepherine) and cortisol. This leads to conflicting functionality by increasing storage free-fatty acids and lipolysis simultaneously (Bjorntop 1991; Halleux et al. 1998; Lundgren et al. 2004; Wajchenberg et al. 2002). These differences between visceral and subcutaneous adipose tissue explain the differing relationship between subcutaneous adipose tissue and visceral adipose tissue with cardiometabolic risk factors.

The following literature review will detail the history of central adiposity and visceral fat and its association with cardiometabolic risk factors. Furthermore the methodologies for directly measuring visceral adipose tissue and the limitations associated with each will be discussed. Finally, this review will identify the need for further research in children that examines the relationship between visceral accumulation and increased adiposity utilizing DXA to measure visceral adipose tissue.

Relationship with Cardiometabolic risk Factors

Various measures of obesity have long been used to estimate total body composition and abdominal obesity. These methods include body mass index (aka Quetelet index), waist circumference, waist-to-hip ratio, anthropometric skinfold measurement, hydrostatic weighing and air displacement plethysmography. Body mass index dates back to the nineteenth century when it was described by Adolphe Quetelet. Body mass index, waist circumference and waist to hip ratio are surrogate measurements of total body mass and abdominal obesity. They are still used today and remain significant markers

for cardiometabolic risk (Harrington et al. 2013), even though they are limited in their ability to determine contributions of fat and lean mass (BMI) or subcutaneous and visceral mass (WC, WHR). Skinfold, hydrostatic weighing and air displacement plethysmography are more direct measures of body composition. Skinfolds were the first method to provide regional estimates of fat accumulation, whereas the other two provide estimates of total body fat. These methods are not as commonly used today because they do not provide significantly more information than BMI, WC or WHR and dual energy x-ray absorptiometry provides a more valid measurement of total and regional tissue composition.

Jean Vague (1947) was the first person to suggest that that abdominal obesity had a significant role in the development of cardiometabolic diseases. He observed that obesity levels were similar in all patients, but those patients with more android obesity had a higher prevalence of cardiovascular disease and diabetes (Vague 1947). Vague utilized the index of masculine differentiation to describe differences between males and females (Vague 1956). The index was based on anthropometric measurements and adipose-muscle ratios. This method allowed differences to be tracked over the life span and classified obesity on a hyper-android to hyper-gynoid scale (Vague 1956). Even with substantial evidence, it took decades for his work to be recognized. Over twenty years later Feldman et al. (1969) reported that diabetic and non-diabetic patients' had differences in fat distribution patterns. Using skinfold measurements they observed that diabetic females had more

fat in the abdominal region compared to the gluteal region and diabetic males had excess abdominal obesity. However, they also observed that diabetic patients had more total fat compared to non-diabetic patients (Feldman et al. 1969). As such, total fat may play a role. In the early 1980's Ruderman et al. observed an important distinction that suggested an independent role of visceral adiposity, the existence of metabolically obese, normal weight individuals (Ruderman et al. 1981; Ruderman et al. 1982). This was one of the first studies to observe that metabolic complications could be independent of obesity.

Around the same time, two groups were publishing several studies in this field, one out of Gothenberg, Sweden and the other out of the University of Wisconsin-Milwaukee. These groups published several studies identifying regional distribution, using waist-hip ratio, and adipose morphology as important markers for cardiovascular disease and diabetes (Kissebah et al. 1982; Kissebah et al. 1985; Krotkiewski et al. 1983; Lapidus et al. 1984; Larsson et al. 1984; Ohlson et al. 1985). The waist-hip ratio measured the widest part of the waist divided by the widest part of the hip to create a ratio of abdominal obesity to lower extremity obesity. This became the standard way of quantifying centralized obesity in the early years and a portion of it (waist circumference) is still used as a surrogate of visceral adiposity. These studies provided some of the first prospective evidence of the association between regional adipose distribution, cardiovascular disease, and diabetes in men and women. The results became the catalyst for further investigation of

regional distribution leading to several studies published in the next few years (Bjorntorp 1991; Bouchard et al. 1990; Despres et al 1990; Gillum 1987; Haffner et al 1987; Kannel et al 1991; Kissebah et al 1989; Peiris et al. 1989; Pouliot et al. 1994; Stern et al. 1986). While these studies observed results consistent with previous work. They began to utilize more precise measurement techniques, computed tomography and magnetic resonance imaging, to segment the visceral and subcutaneous regions of the abdominal depot.

Using these newer methodologies, researchers reported that the visceral region had a stronger association with dysfunctional glucose and lipid metabolism and that preferential subcutaneous accumulation of adipose tissue results in normal glucose and lipid metabolism (Després et al 1989; Després et al. 1990; Fujioka et al. 1987; Pouliot et al. 1992; Ross et al. 2002; Ross et al. 2002). These studies matched obese participants on either body mass index or total body fat and compared glucose and lipid levels in participants with low visceral accumulation and high visceral accumulation at the level of the umbilicus or L4-L5. Regardless of slice site, high visceral accumulation was consistently associated with higher glucose and lipids compared to participants with low visceral accumulation. Després et al. (1990) compared the results of an oral glucose tolerance test with visceral and subcutaneous fat and observed a much stronger association with visceral adipose tissue. While these studies were the first to provide strong evidence of the role of visceral accumulation with cardiometabolic disturbances, the sample sizes in each

study was relatively small. Since those initial studies, both CT and MRI methods have improved and allowed for larger sample sizes and prospective cohort studies which have resulted in strong evidence for the role of visceral accumulation with cardiometabolic risk in adults (Carey et al. 1996; Castro et al. 2014; Després et al. 2008; Fox et al. 2007; Goodpaster et al. 2003; Hanley et al 2009; Hayashi et al. 2003; Kuk et al. 2006; Liu et al. 2010; Matsuzawa et al. 2011; Neeland et al; 2012; Onat et al. 2004; Pou et al. 2007). The results of these studies consistently reported that elevated visceral adipose accumulation was associated with hypertriglyceridemia, hyperinsulinemia, low levels of high-density lipoproteins, elevated fasting glucose and elevated blood pressure all of which are important components of the metabolic syndrome. These studies covered a wide age range, both genders and a plethora of ethnicities. In each population increased visceral adipose tissue was associated with negative cardiometabolic consequences. However, no consistent slice site has been identified in the literature, as such, different groups use difference sites. This makes the applicability of the results difficult. MRI makes it easier to measure multiple slice sites, however, most studies still only report a single slice.

In adults visceral adipose tissue accumulation appears to be influenced by gender and ethnicity (Araneta et al. 2005; Camhi et al. 2011; Carroll et al. 2008; Demerath et al. 2007; Katzmarzyk et al. 2010; Lear et al. 2007). Furthermore, there is variability in the association between visceral fat and cardiometabolic risk depending on the slice site used (Demerath et al. 2007;

Greenfield et al. 2002; Irlbeck et al. 2010; Kuk et al. 2006; Shen et al 2004; Shen et al. 2007).These variations are not surprising; a single slice provides a relatively small estimate of total volume, it's possible that these differences do not exist if total volume is used.

Freedman et al. (1987) were the first to report a relationship between centralized obesity and glucose metabolism in children. They reported that insulin resistance was associated with an increase in central fat accumulation. This study utilized anthropometric skinfold measurements and oral glucose tolerance tests. Several anthropometric studies would follow (Baumgartner et al. 1988; Flodmark et al. 1994; Freedman et al. 1990; Gillum et al 1987; Kikuchi et al. 1992; Sangi et al. 1992; Wabitsch et al. 1994; Zonderland et al 1990; Zwiauer et al. 1992) demonstrating a significant association between regional fat distribution and cardiometabolic risk factors. Most of these studies used waist circumference or waist-to-hip ratio to measure regional fat distribution. While these methods are viable surrogates they do not differentiate between visceral and subcutaneous fat compartments.

CT and MRI were used to quantify visceral adipose tissue in children. (Bacha et al. 2003; Brambilla et al. 1994; Caprio et al. 1995; Cruz et al. 2002; Goran et al. 1995; Goran et al. 1997; Goran et al. 1999; Nagy et al. 1997; Gower et al. 1998; Gower et al. 1999; Owens et al. 1998) These studies reported that visceral fat was positively associated with triglycerides and fasting insulin levels independent of total body fat. However, when measuring insulin sensitivity using the hyperinsulinemic euglycemic clamp technique,

visceral fat was not associated with insulin resistance independent of total fat. These studies also identified ethnic differences in visceral adipose accumulation. Interestingly, they observed that African-American children had lower visceral adipose tissue but were more insulin resistant than Caucasian children. More recent work has detailed the relationship between visceral accumulation and cardiometabolic risk factors across genders and ethnicities (Ali et al. 2014; Asayama et al. 2002; Kelly et al. 2014; Kim et al. 2008; Syme et al. 2008; Rasmussen-Torvik et al. 2012; Taksali et al. 2008). While these studies observed a strong relationship between visceral adipose accumulation and cardiometabolic risk factors, in children, visceral adipose tissue is not always independent of total fat mass in relation to cardiometabolic risk factors. Kelly et al. (2014) observed that subcutaneous fat may be associated with similar cardiometabolic risk factors as visceral fat. This was consistent with previous research (Maffeis et al. 2008). Thus, subcutaneous adipose accumulation may play an important role in cardiometabolic dysfunction in children compared to adults. This may be a result of the decreased area for visceral accumulation in children However, the difference in visceral fat accumulation and cardiometabolic disease may also be a function of the location of the single slice being used to estimate visceral fat. Children do not accumulate a lot of visceral fat, except in cases of extreme obesity. It may be difficult to quantify the importance of visceral fat with the variability associated with a single slice. There is a need for a valid and

reliable method capable of accurately estimating total volume VAT in children.

Computed Tomography

Tokunaga et al. were the first to use computed tomography (CT) to measure body fat in specific compartments (Tokunaga et al. 1983). Their method segmented the body into eleven cylinders (head, chest, abdomen, and right and left forearms, upper arms, thighs and calves). Fat tissue was obtained at the mid-point of each segment. They further developed the standard method of visceral fat estimation using a single-slice at the level of the umbilicus and were able to distinguish between visceral fat and subcutaneous fat. Around the same time another study demonstrated the usefulness of CT for measurement of adipose tissue for the entire body (Sjostrom et al 1986). However, an important limitation of CT is that it emits ionizing radiation which limits scans to one or two slice sites. Currently estimates of abdominal visceral and subcutaneous adipose tissue are obtained by CT using a two separate 10 mm slices obtained at the L4-L5 interspace. The two images are than subdivided into five mm slices and the 1st and 3rd five mm slices were combined and analyzed for visceral adipose tissue. The upper limit of adipose tissue density was -30 Hounsfield units and the lower limit was -190 Hounsfield units. (Rasmussen-Torvik et al. 2012).

In addition to radiation concerns (Miglioretti et al. 2013), CT has several limitations in both children and adults. There is inconsistency in the reporting

of results; some studies adjust for visceral fat or match based on BMI and total body fat while others do not. These variables are all highly correlated with each other and require some sort of adjustment to account for this multi-collinearity. This makes the applicability of this research difficult. But more importantly, single slice is not a reliable measure of change in visceral fat during weight loss (Shen et al. 2012). A single slice is a small estimate and changes in visceral fat over time could come from anywhere within the visceral region. Thus, it is difficult to produce reliable longitudinal or intervention based research using a single slice. In addition, this review has detailed the variability in visceral fat from a single slice measurement (Ali et al. 2014; Asayama et al. 2002; Brown et al. 2014; Kelly et al. 2014; Kim et al. 2008; Lee et al. 2008; Lee et al. 2011; Rasmussen-Torvik et al. 2012; Satake et al. 2010; Staiano et al. 2013; Syme et al. 2008; Taksali et al. 2008) Furthermore, estimation of visceral fat requires manual analysis by a trained technician and is expensive to administer. This decreases the feasibility of this method in both research and a clinical setting. There is a clear need for a more feasible method to measure VAT. The use of computed tomography greatly advanced the knowledge base about the relationship of visceral fat and cardiometabolic risk. However, the limitations associated with CT have made it difficult to conclusively determine a causal pathway between visceral accumulation and increased cardiovascular risk and insulin resistance.

Magnetic Resonance Imaging

Another method to measure visceral fat was developed using magnetic resonance imaging (MRI) (Staten et al. 1989). Because there is no radiation risk, participants could be scanned at multiple areas and repeated measurements could be taken. This original method used a 0.5 Tesla with axial images obtained in a 256 x 256 matrix covering a 50 cm field of view. Each slice was 1.0 cm thick. This method demonstrated strong reproducibility and visceral fat measurement was correlated strongly with waist hip ratio. In addition, MRI allowed for multiple slice measurements as well as the measurement of visceral adipose total volume within the android region. Much of the research that has demonstrated the variability in single slice measurements utilized MRI to quantify visceral fat. Current methodology uses a 1.5 Tesla with T1- weighted, spin-echo sequence with a 210-ms repetition time, a 17-ms echo time, a 48-cm field of view, and a 256 x 256 matrix. Most studies acquire roughly 40 axial images of 10-mm thickness at 40-mm intervals using the L4-L5 intervertebral space as the point of origin. In each case, after image acquisition visceral fat is segmented and estimated manually using image analysis software. Similar to CT estimation, visceral fat quantified by MRI is dependent on slice site. Also, gender and ethnicity have a main effect on the amount of visceral fat estimated. Additionally, the association between visceral fat and cardiometabolic risk is affected by the slice site used. Interestingly, even though MRI has the ability to measure total volume, most studies still report one or two slice sites. This is likely to match the results reported in studies that used CT to measure visceral fat. Given the

variability in VAT when estimating on a single slice, a shift to total volume measurement is necessary to accurately assess the importance of visceral accumulation.

Even though MRI does not emit radiation, it shares several limitations with CT. First, most studies using MRI still report single slice visceral fat area, which has been observed to be affected by ethnicity and gender. Single slice measurement is not a reliable method for determining change in regional fat during weight loss. Additionally, while total volume assessment is possible with MRI, it is usually done using spiral analysis estimation and not measuring the entire region. As with CT, MRI requires manual analysis of images and is expensive to administer which limits the feasibility of visceral measurement in research and clinical settings.

Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) is an established method for quantifying total body composition and bone mineral density in adults and children (Brunton et al. 1993; Ellis et al. 1994; Going et al. 1993; Haarbo et al. 1991; Johnansson et al. 1993; Mazess et al. 1990; Pritchard et al. 1993; Svendsen et al. 1993). As DXA has been refined it has become able to reliably measure specific regions in addition to total body composition. These measurements included trunk and abdominal adiposity (Carey et al 1996; Daniels et al. 1999; Glickman et al. 2004; Goodpaster et al 1997; Lee et al. 2005; Park et al. 2002; Rissanen et al 1997; Teixeira et al 2001). These initial

measures used manual analysis of abdominal adiposity by creating a region of interest bordered by the T12/L1 intervertebral space and the L4/L5 intervertebral space. Fat mass was measured within this region to estimate abdominal adiposity. Software advancements allowed this process to become automated. Android and gynoid regions of interest are created based on reproducible landmarks and the participants' height (Henche et al. 2008; Lee et al. 2008; Novotny et al. 2007; Stults-Kolehmainen et al. 2013). Android fat was measured by a region-of-interest automatically defined with a caudal limit placed at the top of the iliac crest and its height set to 20% of the distance from the top of the iliac crest to the base of the skull. The gynoid region is located mid-pelvis to mid-thigh; the upper limit is set below the iliac crest a distance 1.5 times the height of the android region. The lower limit is set a distance of 2 times the height of the android region. These measurements were significantly associated with cardiometabolic risk (Aucouturier et al. 2009; Fu et al. 2013; Jahagirdar et al. 2012; Kang et al. 2011; Staiano et al. 2013; Vatanparast et al. 2009; Wiklund et al 2008). Recently, the quantification of total volume visceral fat by DXA was validated against total volume visceral fat by CT (Kaul et al. 2012). This method involves measuring the width of the subcutaneous fat layer on the lateral extent of the abdomen. Using geometric modeling parameters that were optimized for males and females respectively, the subcutaneous fat layer was modeled to estimate subcutaneous fat in the entire android region.

The visceral estimation was a result of subtracting subcutaneous fat in the android region from the total fat within the android region (Kaul et al. 2012). In developing this model the data set was split to initially develop the model estimate of visceral fat and then validate the estimate. This method is the first automated measurement of visceral fat within the entire android region. Results are available immediately after a full body DXA scan. Another study validated the use of DXA to measure a single slice estimate of visceral fat compared to CT (Micklesfield et al. 2012). They used a similar method to estimate visceral fat from DXA scan images but only used a five centimeter region of interest approximately at the L4/L5 intervertebral space (Micklesfield et al. 2012). This method was developed to provide a low cost, low radiation alternative to visceral fat measured by CT.

There are several benefits of using DXA to quantify visceral fat. First it is a low cost and low radiation alternative CT and MRI. It provides an automated measurement of VAT that is immediately available following a scan. Some DXA machines will provide a total volume visceral fat estimate that may provide a better measure of visceral fat than a single slice. However, limitations exist as well, while DXA is lower cost than CT and MRI it is still more expensive than anthropometric measures. The clinical utility of this new method must be examined to determine if it provides significantly more information than waist circumference or BMI. Also, because DXA uses subcutaneous fat to estimate visceral fat, differentiating the subcutaneous layer can be difficult in lean individuals owing to the relatively low volume of

subcutaneous fat, and as such a good estimate of visceral fat cannot be obtained. Because the use of DXA to quantify visceral fat is so new, there is limited research on its reliability as well as its association with cardiometabolic risk factors.

To date, this method has not been validated in children. Furthermore, while the association between visceral fat and cardiometabolic risk has been detailed at great lengths, with few exceptions, single slice estimates of visceral fat are used. The total volume measurement of visceral fat provides a unique measurement that may change the relationship with cardiometabolic risk. Further examination of this association is warranted as well as the effect of ethnicities and gender. Finally, the ability to measure the total volume of visceral fat may provide additional meaningful insight regarding how visceral fat accumulates with increasing total body fat accumulation.

While DXA has the ability to measure VAT, it is unable to measure other ectopic fat depots (i.e. liver, epicardial, intramuscular). These regions can only be measured using CT, MRI and echocardiography. These other depots have significant associations with cardiometabolic risk in children and adults (Alderete et al. 2013; Azza et al. 2012; Greif et al. 2009; Manco et al. 2013; Nguyen-Duy et al. 2003; Rosito et al. 2008; Schusterova et al. 2014; Taguchi et al. 2001; Westerbacka et al. 2012) and as such lead to debate over the importance of ectopic accumulation in various regions. To my knowledge, no study has definitively observed causality for any ectopic fat depot with cardiovascular disease or insulin resistance.

Summary

While the relationship between visceral fat and cardiometabolic risk has long been established, the new DXA volumetric method of visceral fat quantification may provide a better understanding of this relationship. The increased feasibility of DXA and total volume measurement could provide for more longitudinal and interventional research that may determine a causal role between visceral fat and cardiometabolic dysfunction. This dissertation will focus on examining the DXA method of visceral fat quantification in children and then reexamining the relationship between visceral fat accumulation with increasing total body adiposity in adults. Furthermore it will examine regional fat differences in professional football players, with specific focus on abdominal fat differences by positions.

CHAPTER 3. VISCERAL ADIPOSE TISSUE MEASURED BY DXA CORRELATES WITH MEASUREMENT BY CT AND IS ASSOCIATED WITH CARDIOMETABOLIC RISK FACTORS IN CHILDREN

Visceral Adipose Tissue Measured by DXA Correlates with Measurement by CT and is Associated with Cardiometabolic Risk Factors in Children.

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Data Analysis
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Drafting the article and subsequent revisions for critically important content
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Chapter 3 Summary

Background: Visceral adipose tissue (VAT) generally demonstrates a stronger relationship with cardiometabolic risk factors than total body fat or subcutaneous adipose tissue.

Objectives: The purpose of this study was to compare VAT estimated in children by total volume dual energy X-ray absorptiometry (DXA) with a gold standard measurement, single slice (L4-L5) computed tomography.

Methods: 329 (152 females, 177 males) children ages 6-18 years (mean age 12.3 ±3.6) and average BMI percentile of 54.9% (3-99%) had VAT estimated by both CT and DXA. Linear association between methods was measured using Pearson's correlation. Multiple linear regressions compared the associations between cardiometabolic risk factors and both CT-VAT and DXA-VAT, respectively.

Results: In children, DXA-VAT was correlated significantly with CT-VAT, with a stronger relationship in overweight and obese children. Multiple regression analysis showed that both estimates of VAT were significantly associated with lipids and insulin sensitivity, measured by euglycemic-hyperinsulinemic clamp. Additionally, DXA-VAT was associated with diastolic blood pressure, HOMA-IR and fasting insulin, but CT-VAT was not.

Conclusion: In children, total volume DXA-VAT and single slice CT-VAT are significantly correlated and each demonstrates similar associations with cardiometabolic risk factors. This suggests that DXA is a useful and valid method for estimation of VAT in children.

Introduction

Obesity continues to be a major public health issue in the United States and many industrialized nations (Ogden et al. 2012) and has been linked to hypertension, insulin resistance, type 2 diabetes mellitus and coronary heart disease (Abbasi et al. 2002). It is well established in adults that visceral adipose tissue (VAT) has a stronger relationship with cardiometabolic risk factors and insulin resistance than other types of adipose tissue (Despres et al. 2001; Nakamura et al. 1993; Neeland et al. 2012). In children, conflicting results in the association of VAT with lipid markers, insulin and blood pressure have been reported (Bacha et al. 2003; Brambilla et al. 1994; Caprio et al. 1995; Gower et al. 1999; Owens et al. 1998; Rasmussen-Torvik et al. 2012; Syme et al. 2008; Taksali et al. 2008). Previous studies used either computed X-ray tomography (CT) or magnetic resonance imaging (MRI) to quantify VAT. Each has significant limitations for clinical research and practice due to cost, the need for manual analysis, extended scan times, and a limitation to the maximum size of participants that can be examined. Additionally, CT emits ionizing radiation making its use for VAT especially problematic in children (Miglioretti et al. 2013).

Because of these limitations most studies have used a single abdominal slice within the android region to estimate VAT, often at the umbilicus or L4-L5 (Bacha et al. 2003; Brambilla et al. 1994; Caprio et al. 1995; Despres et al. 2001; Gower et al. 1999; Nakamura et al. 1993; Neeland et al. 2012; Owens et al. 1998; Rasmussen-Torvik et al. 2012; Syme et al. 2008; Taksali et al. 2008). However, studies in adults demonstrated high intra-subject variability (Greensfield et al. 2002) depending on slice site, differences by sex and race (Demerath et al. 2007) and differences reported in the association

between VAT and metabolic syndrome (Irlbeck et al. 2010; Kuk et al. 2006; Shen et al. 2007) Similar variations in regional adipose tissue distribution by sex and race have been shown in pre-pubertal children (He et al. 2002).

Software advancements have made it possible for the DXA to estimate the amount of VAT and subcutaneous adipose tissue within the android region. Recently, dual energy X-ray absorptiometry (DXA) for quantification of VAT has been validated in adults (Kaul et al. 2012) and a study in adults observed DXA-VAT to be reproducible and useful as a clinical marker of cardiometabolic risk (Katzmarzyk et al. 2013). This new method is less expensive than CT or MRI, provides immediate quantification of regional body composition and scans participants weighing up to 450 lbs. [DXA (Lunar Prodigy, General Electric Medical Systems, Madison, WI, USA)]. In addition, the extremely low amount of radiation emitted by DXA is considered safe for use in children. To date, VAT estimation by DXA has not been examined in children. Furthermore, it has not been compared with current methods of single slice using CT or MRI in children. The purpose of this study was to compare total volume DXA-VAT with single slice CT-VAT in children. Given that DXA is currently the standard method used for measuring total body fat, confirmation of DXA as a useful estimate of visceral and subcutaneous adipose tissue would offer a valuable alternative to CT and MRI.

Methods

Study Design and Participants

The current study was a cross-sectional analysis of data obtained in 329 children ages 6-18 years. The range of adiposity ranged from lean to obese (body mass index (BMI) 13-45 kg/m^2 and BMI percentile from 3-99% mean 55%). Data were combined

from a community-based study evaluating cardiometabolic risk in children and a group of healthy siblings of childhood cancer survivors. Data were collected from 2007-2011(n = 555). Only participants with VAT measured by CT and a full body composition scan by DXA were included. The respective protocols were approved by the University of Minnesota Institutional Review Board and consent/assent was obtained from parents and participants, respectively.

Anthropometric and Blood Pressure Measurements

Testing was conducted at the University of Minnesota Clinical Translation Science Institute after participants had been fasting for a minimum of eight hours. Tanner stage of sexual maturation was determined by trained pediatricians. Height and weight were measured on a calibrated stadiometer and electronic scale, respectively, while participants were wearing light clothes and without shoes. BMI was calculated as kg/m^2. BMI percentile was calculated based on the Centers for Disease Control (CDC) growth charts using age and sex. Waist circumference was measured to the nearest 0.5 cm, taken in duplicate and the mean value reported. Blood pressure was measured in duplicate on the right arm after participants were sitting in a quiet room for at least five minutes using a digital blood pressure cuff and the average of the two values was reported.

Body Composition and Visceral Adipose Quantification

Total body composition was measured using DXA (Lunar Prodigy, General Electric Medical Systems, Madison, WI, USA) and analyzed using its enCore^{TM} software (platform version 13.6). Participants were scanned using standard imaging and positioning protocols while fasted. Estimates of abdominal visceral and subcutaneous adipose tissue were obtained using the method described previously for adults (Kaul et al.

2012). Android fat was measured by a region-of-interest automatically defined with a caudal limit placed at the top of the iliac crest and its height set to twenty percent of the distance from the top of the iliac crest to the base of the skull. Subcutaneous fat and visceral fat were estimated within the android region. All scans were reviewed for accurate placement of the android box by the same technician.

Estimates of abdominal visceral and subcutaneous adipose tissue were obtained by CT using a Siemens Sensation 16 (Siemens Medical Solutions, Malvern, PA, USA) with two separate 10 mm slices obtained at the L4-L5 interspace. The two images were subdivided into five mm slices and the 1st and 3rd five mm slices were combined and analyzed for visceral adipose tissue. The upper limit of adipose tissue density was -30 Hounsfield units and the lower limit was -190 Hounsfield units. Image slices were individually analyzed by one trained technician using a computer program (Fat Scan version 3.0; N2 System, Osaka, Japan). A lack of inter-rater and intra-rater reliability may be a limitation; however the technician is extremely experienced in CT imaging analyses. DXA and CT measurements were taken within one week of each other.

Measurement of Blood Markers

Insulin sensitivity was measured by the euglycemic hyperinsulinemic clamp as previously described (Sinaiko et al. 2001). Insulin was infused at a constant rate of 1mU/kg/min for 3 hours, and glucose was infused at a variable rate to maintain euglycemia. Insulin sensitivity (M) was expressed as the glucose infusion rate (mg/kg/min of glucose) during the last 40 minutes of the clamp, with adjustment for lean body mass ($M_{/LBM}$). Low $M_{/LBM}$ represents insulin resistance. Fasting blood samples were collected for lipids, glucose and insulin and assays were conducted with standard

procedures at the Fairview Diagnostic Laboratories, Fairview-University Medical Center (Minneapolis, MN), a Centers for Disease Control and Prevention-certified laboratory. Homeostasis model of insulin resistance (HOMA-IR) was calculated as described previously (Matthews et al. 1985).

Statistical Analysis

Unpaired t-tests were used to compare males and females for demographic and cardiometabolic characteristics. Pearson correlation was used to assess the linear relationship between the two measures of VAT. Both CT and DXA VAT were right skewed so data were log transformed before correlation was measured. Since these two measures differed in measurement units, Kendall tau correlation coefficient was used to measure the concordance of the ranks between the two measures to assess their independence. Kendall's tau correlation coefficient measures the probability of concordance of the rank values for each method. Multiple linear regression analysis for the whole sample, adjusted for age, sex, race, BMI percentile, Tanner stage and total fat mass was used to evaluate associations of VAT with cardiometabolic risk factors. Sex, race and Tanner stage were factors with the reference levels set as male, Caucasian and Tanner stage 1, respectively. Interaction terms were included in the model and removed if they failed to show significance. Covariates were chosen based upon correlation analysis. Variables with a significant association ($p< 0.05$) with cardiometabolic risk factors were included within the model. Additionally, age and Tanner stage were used to control for pubertal status. Models were reduced if covariate variables were not significantly associated with the dependent variable. Variables were first removed at a modest level of significance ($p>0.5$). This cut point decreased ($p>0.3$ and 0.1) after variables were

removed. Akaike information criterion (AIC) was calculated for each model to determine if the reduced, final model had the best fit. Separate analysis was completed for CT-VAT and DXA-VAT to compare the associations of each method with cardiometabolic risk factors. A variance inflation factor (VIF) was calculated for each model to monitor collinearity between covariates. Given the smaller variance explained by our models, models were reexamined if variables of interest had a VIF greater than 2.5 or if covariates had a VIF greater than 4. The results for each regression are presented in Table 2 and 3 the estimate, standard error, adjusted R^2 for the final model and p-value for the estimate are reported with the individual R^2 (proportion of explain variance for that variable) for DXA-VAT, CT-VAT and DXA-TFM. The covariates that remained in the final models are listed at the bottom of tables 2 and 3. The complete results of each model including covariates are presented in Supplementary Tables 1 and 2. All analyses were done using R (R Foundation for Statistical Computing, www.R-project.org).

Results

Data from 329 children ages 6-18 years old (152 females, 177 males) were included. Demographic data and clinical measures for females, males and the total sample are presented in Table 1 as mean$\pm$SE. Based on CDC classifications the number of participants classified in each category are: 5(<5%-underweight); 207(5-84.9%-normal weight); 64(85-94.9%-overweight); 53(>94.9%-obese). Log transformation was performed for CT-VAT, DXA-VAT, DXA-TFM, triglycerides, insulin, BMI percentile, and HOMA-IR because of the lack of normal distribution as determined by Anderson-Darling test. These results are presented as the geometric mean and 95% confidence interval. Systolic blood pressure, glucose, DXA-VAT and total fat mass were

significantly different between males and females. CT-VAT was not significantly different between sexes.

Figure 1 presents the scatterplot of DXA-VAT volume and CT-VAT area. The association does not appear to be directly linear. The Pearson correlation for CT and DXA (log transformed) within the total sample was 0.626 (95% CI = 0.554, 0.689). Due to the wide age range, we measured the correlations for younger (age 6-11 years) and older (age 12-18 years) children. The Pearson correlations for these groups were 0.55 (95% CI = 0.43, 0.65) and 0.63 (95% CI = 0.53, 0.71), respectively. This demonstrates similar significant moderate linear relationships between CT-VAT and DXA-VAT across each age subgroup. Additionally, we calculated the correlations based on BMI percentile, above or below the 85^{th} percentile. The Pearson correlation for these groups were 0.859 (95% CI = 0.802, 0.900) and 0.226 (95% CI = 0.092, 0.353) respectively. This demonstrates a strong positive relationship between each method in the heavier participants. The correlations between DXA-VAT and total fat mass, waist circumference and BMI are 0.564, 0.652, and 0.656 respectively. The correlations between CT-VAT and the same measures are 0.863, 0.795 and 0.786 respectively. The Kendall tau correlation coefficient for the total sample was 0.451 ($p<0.001$). This suggests that VAT values for each participant would be ranked similarly by each method (the highest VAT by DXA is the highest VAT value by CT).

Table 2 presents the regression analysis for lipid and blood pressure variables for both CT-VAT and DXA-VAT. Regression analysis for glucose metabolism variables for both CT-VAT and DXA-VAT is presented in Table 3. The R^2 value represents the final

model which may have included some of the covariate variables. These final models always produced the lowest AIC values.

Regression analysis for CT-VAT

CT-VAT was associated with adverse lipid levels and insulin resistance. There was a positive association with triglycerides and low density lipoproteins and an inverse association with high density lipoproteins and $M/_{LBM}$ (all below $p<0.05$). No significant association was observed between CT VAT and systolic blood pressure, diastolic blood pressure, fasting glucose, fasting insulin or HOMA-IR.

Regression analysis for DXA-VAT

DXA-VAT was also associated with adverse lipid levels, insulin resistance, and additionally diastolic blood pressure. A positive association was observed with triglycerides, diastolic blood pressure, HOMA-IR and fasting insulin; and an inverse association is observed with high density lipoprotein-cholesterol and $M_{/LBM}$ (all below $p<0.05$). No associations were observed between DXA-VAT, systolic blood pressure and fasting glucose.

DXA-total fat mass and Tanner stage were consistently included within the final models for DXA-VAT. Tanner stage, sex, race and age, remained in several final models for CT-VAT. Tanner stage was significantly associated with all glucose metabolism variables (fasting glucose, fasting insulin, HOMA-IR and $M/_{LBM}$) in models for both CT-VAT and DXA-VAT. The full results for each final model are presented in supporting information Tables S1 and S2.

Discussion

To our knowledge this is the first study to use DXA to estimate VAT in children. Because of this, we used the current gold standard, single slice CT-VAT estimation, to compare the total volume DXA method. The purpose of this study was not to demonstrate that these methods are interchangeable, but rather to determine if DXA-VAT provides an accurate estimate of VAT that demonstrates an association to cardiometabolic risk factors in children.

This study compared the linear relationship and concordance of each method. The log transformed sample correlation provided evidence of a significant linear relationship between CT-VAT and DXA-VAT estimates. This relationship was consistent across all ages. While the correlation between the two measures was lower than expected; the strength of this relationship increases in the largest children, which is important given increased risk in heavier children, suggesting that the relationship is dependent on total adiposity. The correlation observed in overweight/obese children is consistent with correlations observed in adults between L4-L5 and total volume (Irlbeck et al. 2010; Shen et al. 2007) To measure the concordance between each method we ranked both VAT estimates for each participant. We observed that the rankings were similar to one another based on the Kendall's tau correlation which compares the rankings for concordance. This suggests that these two methods for estimating VAT classify participants similarly despite the differences in measurement units.

While the results of the Kendall's tau suggest that the measurements are not independent from each other, the plot of the ranks suggests that this relationship may be influenced by the largest participants. This is expected given the increased association demonstrated in heavier participants in this study. A leaner individual may accumulate

VAT in different areas, which could lead to discordant relationship between a single slice and total volume measurement, suggesting that a single slice at L4-L5 may not be representative of total VAT volume in lean children. This is consistent with research in adults that observed a stronger association between single slice and total volume 5-10cm above L4-L5 (Shen et al. 2004). This may explain the differences observed between the relationships of each method with certain cardiometabolic risk factors.

To our knowledge, this is the first study in children utilizing concurrent measurement of VAT by both CT and DXA. This allowed the associations of each method with several cardiometabolic risk factors to be indirectly compared. While the two measurement units are different, significant associations were observed between cardiometabolic risk factors for both DXA-VAT and CT-VAT. These results suggest that VAT quantified by DXA provides similar information about the relationship between VAT and measures of cardiometabolic risk. Each method resulted in a similar proportion of explained variance with metabolic risk factors (individual R^2). Furthermore DXA-VAT demonstrates a significant association with additional variables (diastolic blood pressure, fasting insulin, HOMA-IR) for which, CT shows no significant association. This may be due to the slice site used in this study; L4-L5 is not the site with the strongest association with cardiometabolic risk factors, in adults (Irlbeck et al. 2010; Shen et al. 2007). While we believe this is the first study to use DXA derived VAT to measure the association with risk factors in children, a previous study observed that total fat measured at L1-L4 and regional fat at other depots, using DXA, provided the best predictive measure of insulin resistance and other cardiovascular risk factors independent of total body mass in children (Daniels et al. 1999; Teixeira et al. 2001)

The gender differences in VAT observed in this study were apparent by DXA-VAT but not with CT-VAT. This is similar to a recent study which observed ethnic and sex differences by total volume MRI in children (Staiano et al. 2013). Adolescence is a time where adipose tissue accumulation differences develop between the sexes, in part due to introduction of sex specific hormones; puberty results in shifts in accumulation of adipose tissue to specific depot (Guo et al. 1998). Females, generally, store more adipose tissue in the gynoid region and males, generally, store more adipose tissue in the android region. In this study, and many others, CT-VAT estimates visceral adipose tissue in a single slice at L4-L5, near the umbilicus. This location is near the inferior border of the android region. It is possible that accumulation of adipose tissue in the visceral region starts higher than L4-L5 and thus sex specific differences cannot be identified by a single slice at this region. The fact that with obesity more storage of adipose tissue is likely to accumulate through the whole android region, including the lower regions, would explain the stronger linear relationship in the heavier participants. Thus, for most children who have relatively small amounts of VAT, total volume of VAT by DXA, may provide important information that is missed in the single slice approach.

DXA offers several advantages over CT in the pediatric population. DXA is less expensive and associated with significantly lower radiation exposure than CT. In addition to VAT, DXA provides additional useful information including bone mineral density, total and regional body composition. A third method, MRI does not involve exposure to radiation but the cost and feasibility concerns are similar to CT in this population. Also, neither CT nor MRI provides a measure of total body composition. The results of this study suggest that DXA-VAT is significantly associated with CT-VAT, especially in

overweight and obese children. Furthermore, DXA-VAT was associated with several measures of cardiometabolic risk. This demonstrates that DXA-VAT, estimated in children, maintains the independent relationship with cardiometabolic risk factors observed previously using single slice VAT estimation (Bacha et al. 2003; Brambilla et al. 1994; Caprio et al. 1995; Gower et al. 1999; Owens et al. 1998; Rasmussen-Torvik et al. 2012; Syme et al. 2008; Taksali et al. 2008). These results provide evidence that DXA is an acceptable method for quantification of VAT in children.

Limitations

A limitation of this study is that it was an analysis of previously collected data. This did not allow for comparison of equal sized VAT regions, or multiple slice sites. Additionally, there is limited evidence as to the best slice site in children; however the evidence in adults suggests a slice higher than L4-L5 may provide a stronger association to total volume and metabolic risk factors.

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JS, AS and AM developed and carried out the experiments. TB analyzed the data and wrote the manuscript. All authors were involved in editing the manuscript and had final approval of the submitted and published versions. We would also like to acknowledge Derran Bedward and Joanna Liu for their support in conversion of data.

Table Legend

Table 1: Demographics and Clinical measures table for females, males and the total sample

Table 2: Regression Analysis for Lipid and Blood Pressure Variables

Table 3: Regression Analysis for Glucose Metabolism Variables

Table 1: Demographic and Clinical Measurements by Sex and Total (mean(±SE)) or geometric mean (x,x 95% confidence interval)				
	Females (n=152)	Males (n=177)	p-value	Total (n=329)
Age (years)	12.4(0.3)	12.2(0.3)	0.55	12.3(0.2)
Height (cm)	150.2(1.0)	153.4(1.1)	0.14	151.9(1.1)
Weight (kg)	50.2(1.2)	52.5(1.4)	0.37	51.4(1.3)
BMI Percentile (%)	55.0(50,61)	54.9(49,62)	0.44	54.9(51,60)
Race (%)				
Non-Hispanic white	108(71)	127(72)	-	235(72)
Non-Hispanic black	29(19)	28(16)	-	57(17)
Other	15(10)	22(12)	-	37(11)
Tanner Stage (%)				
1	56(37)	78(44)	-	134(41)
2	6(4)	23(13)	-	29(9)
3	12(8)	13(7)	-	25(7)
4	29(19)	19(11)	-	48(15)
5	49(32)	44(25)	-	93(28)
SBP (mmHg)	102*(0.8)	106(0.8)	0.002	104(0.6)
DBP (mmHg)	59(0.6)	60(0.7)	0.39	60(0.5)
HDL-C (mmol/L)	1.29(0.3)	1.25(0.3)	0.33	1.27(0.3)
LDL-C (mmol/L)	2.24(0.6)	2.25(0.6)	0.90	2.24(0.6)
Triglycerides (mmol/L)	0.77(0.7,0.8)	0.76(0.7,0.8)	0.90	0.77(0.7,0.8)
Insulin (pmol/L)	43.8(38,51)	41.8(37,47)	0.55	42.8 (39,47)
Glucose (mmol/L)	4.4*(0.7)	4.6(0.8)	0.01	4.5(0.8)
$M/_{LBM}$ (mg/kg/min)	12.8(0.3)	13.2(0.4)	0.48	13.0(0.3)
HOMA-IR	1.2(1.0,1.4)	1.2(1.0,1.4)	0.73	1.2(1.1,1.3)
CT-VAT (cm^2)	15.9(15,17)	15.5(14,17)	0.80	15.7(15,17)
DXA-VAT (cm^3)	80.5*(66, 98)	120.9(103,142)	0.002	113(100,129)
DXA-TFM (kg)	11.7*(10,13)	8.9(8,10)	0.003	10.1(9,11)

BMI = Body Mass Index, SBP = systolic blood pressure, DB= diastolic blood pressure

HDL-C = High density lipoprotein cholesterol, LDL-C = Low Density lipoprotein cholesterol
M_{lbm} = glucose utilization per minute per kg of lean body mass

CT VAT = Computed Tomography derived visceral adipose tissue

DXA VAT = Dual X-ray absorptiometry derived visceral adipose tissue, TFM = total fat mass

*Significantly different from males at p=0.05

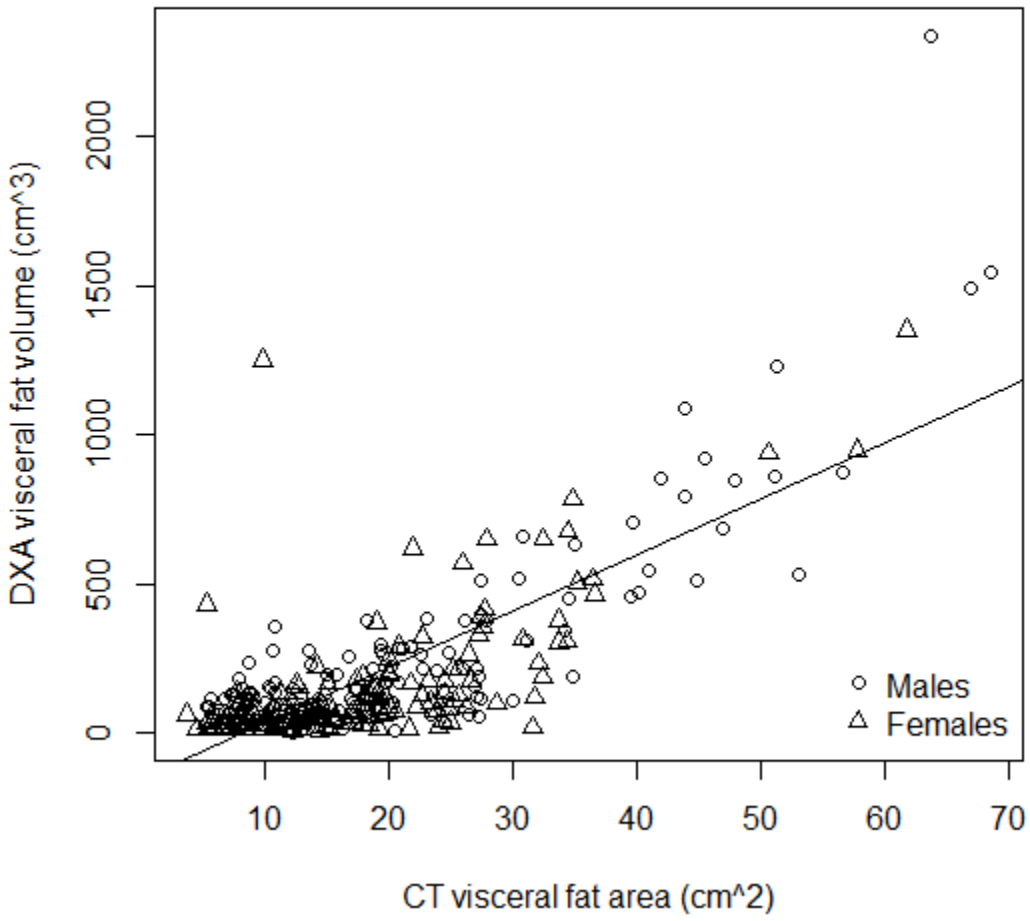

Figure 1: Scatter plot of CT versus DXA visceral fat estimation in the sample population

Table 2: Regression analysis for blood lipid variables and blood pressure					
DXA			CT		
Variables	Estimate (±SE)	p-value	Variables	Estimate (±SE)	p-value
log TG (model adjusted R^2 = 0.187)			**log TG (Model adjusted R^2 = 0.1984)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.107)	0.11(.02)	<0.001	log CT VAT (R^2 = 0.198)	0.35(0.04)	<0.001
log DXA TFM (R^2 = 0.086)	0.13(.04)	<0.001			
HDL-C (model adjustedR^2 = 0.1416)			**HDL-C (model adjusted R^2 = 0.1181)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.082)	-2.39(0.6)	<0.001	log CT VAT (R^2 = 0.118)	-7.0(1.1)	<0.001
log DXA TFM (R^2 = 0.063)	-2.66(0.9)	<0.001			
LDL-C (model* adjusted R^2 =0.1103)			**LDL-C (model* adjusted R^2 = 0.1185)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.025)	3.0(1.4)	0.027	log CT VAT (R^2 = 0.038)	13.4(2.7)	<0.001
log DXA TFM (R^2 = 0.020)	7.0(2.4)	0.003			
SBP (model* adjusted R^2 = 0.3671)			**SBP (model* adjusted R^2 = 0.3633)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.031)	0.84(.5)	0.107	log CT VAT (R^2 = 0.082)	3.1(1.7)	0.077
log DXA TFM (R^2 = 0.139)	3.4(.9)	<0.001			
DBP (model* adjusted R^2 = 0.073)			**DBP (model* adjusted R^2 = 0.074)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.026)	1.0(0.4)	0.022	log CT VAT (R^2 = 0.009)	-0.09(1.8)	0.96
TG=triglycerides, HDL-C= high density lipoproteins cholesterol, LDL-C= low density lipoproteins cholesterol					
SBP=systolic blood pressure, DBP= diastolic blood pressure, VAT=visceral adipose tissue					
TFM = Total fat mass, CT VAT = computed tomography visceral adipose tissue					
The parentheses and r^2 next to each variable indicates the individual variance explained by that variable					
* other covariates included within this final model are not presented					
DXA models: LDL-C = Tanner stage; SBP = Tanner stage, Sex, Race; DBP = Tanner stage, Age					
CT models: LDL-C = Race, Age; SBP = Tanner stage, Age, Sex, Race; DBP = Tanner stage, Age					

Table 3: Regression analysis for glucose metabolism variables.					
DXA			CT		
Variables	Estimate (±SE)	p-value	Variables	Estimate (±SE)	p-value
Glucose (model* adjusted R^2 = 0.4361)			**Glucose (model* adjusted R^2 = 0.4418)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.014)	0.5(0.7)	0.467	log CT VAT (R^2 = 0.035)	0.8(2.1)	0.71
log DXA TFM (R^2 = 0.244)	4.3(1.1)	<0.001	log DXA TFM (R^2 = 0.244)	4.4(1.7)	0.008
log HOMA-IR (model* adjusted R^2 = 0.5329)			**log HOMA-IR (model* adjusted R^2 = 0.5234)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.068)	0.1(0.04)	0.023	log CT VAT (R^2 = 0.077)	0.14(0.1)	0.334
log DXA TFM (R^2 = 0.267)	0.55(0.1)	<0.001	log DXA TFM (R^2 = 0.257)	0.56(0.1)	<0.001
log Insulin (model* adjusted R^2 = 0.5137)			**log Insulin (model* adjusted R^2 = 0.5014)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2= 0.067)	0.11(0.04)	0.002	log CT VAT (R^2= 0.062)	0.1(0.1)	0.43
log DXA TFM (R^2 = 0.286)	0.47(0.1)	<0.001	log DXA TFM (R^2 = 0.287)	0.52(0.1)	<0.001
M_{lbm} (model* adjusted R^2 =0.1801)			**M_{lbm} (model* adjusted R^2 = 0.1543)**		
Independent Variables			Independent Variables		
log DXA VAT (R^2 = 0.068)	-0.87(0.3)	<0.001	log CT VAT (R^2 = 0.077)	-1.4(0.6)	0.03
HOMA-IR = homeostasis model for insulin resistance, M_{lbm}= insulin sensitivity m-value per lean body mass					
DXA VAT = dual x-ray absorptimetry visceral adipose tissue, TFM = total fat mass					
CT VAT = computed tomography visceral adipose tissue					
* other covariates included within this final model are not presented					
DXA models: Gluc = Tanner stage, Age, Sex, Race; HOMA = Tanner stage; INS = Tanner stage ; Mlbm = Tanner stage					
CT models: Gluc = Tanner stage, Age, Sex, Race; HOMA = Tanner stage; INS = Tanner stage; Mlbm = Tanner stage, Race					

CHAPTER 4. ABDOMINAL BODY COMPOSITION DIFFERENCES IN NFL FOOTBALL PLAYERS

Abdominal Body Composition Differences in NFL Football Players

Short Running Title: Regional Distribution in football players

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Chapter 4 Summary

Background: A few studies have reported total body composition characteristics in NFL football players. Similarities have been reported in both total fat and total lean mass between positions that mirror each other, while differences exist between non-mirrored positions. To date, no study has examined fat and lean mass in the abdominal region, especially the visceral region. The purpose of this study was to examine abdominal body composition, including visceral mass in NFL position groups.

Methods: Three hundred and seventy NFL football players were measured prior to the start of the season using dual energy x-ray absorptiometry. Players were categorized into three groups: Linemen; linebackers/tight ends/running backs and wide receivers/defensive backs. An analysis of variance was used to measure the differences in visceral adipose tissue (VAT) and other regional fat and lean mass variables.

Results: Weight and percent total body fat has a strong positive association (0.90, $p<0.001$). The only non-significant difference ($p>0.05$) was observed for visceral fat mass between the LB/TE/RB group and the WR/DB group. A clear cut-point was observed between VAT and percent body fat, with a linear increase in visceral accumulation after twenty-two percent body fat.

Conclusions: The results suggest that as players get larger there is more fat than lean mass accumulation. Additionally, VAT appears to accumulate distinctly from other regional fat depots. Fat mass is disproportionately allocated to the visceral depot after twenty-two percent body fat. This suggests that as NFL players get larger more fat will be stored in the visceral region.

Introduction

Previously, we had reported on positional differences in overall body composition in National Football League (NFL) professional players. (Dengel et al. 2014) While the majority of NFL players would be considered overweight or obese based on body mass index (BMI), their percent body fat for their weight is much closer to a normal or lean range. We and others observed the similarity in body composition of positions that mirror each other (i.e., offensive lineman vs. defensive linemen; wide receivers vs. defensive backs) (Dengel et al. 2014; Gleim 1984; Kraemer et al 2005; Snow et al 1998). To date, studies in this population have focused on total body composition measurements. To our knowledge, this is the first study to measure the abdominal and other regional body composition, including visceral adipose tissue (VAT), in NFL players. Previous work has observed a higher prevalence of cardiovascular disease and metabolic syndrome in retired NFL linemen (Baron et al. 1994; Harp et al. 2005; Miller et al. 2008; Tucker et al. 2009). Additionally, several studies, in other adult populations, have observed a link between VAT and cardiometabolic risk (Després et al. 2008; Fox et al. 2007; Katzmarzyk et al. 2013; Preis et al. 2010; Tulloch-Reid et al. 2004). Thus, it is of value to assess VAT values within this population to determine if differences exist between positions.

Several studies have used aerobic training as an intervention to measure change in body composition and VAT (Lesser et al. 2012; Murphy et al. 2012; Vissers et al. 2013). These studies observed lower overall body fat and VAT after training. These results suggest that football players, while having increased total body weight, may be protected from accumulating VAT because of their high levels of physical activity. The purpose of

this study was to provide an observational profile of abdominal fat using measures of VAT and trunk body composition in NFL football players prior to the season.

METHODS

Subjects

We assessed NFL players from the Green Bay Packers professional football team from 2006 to 2011. Players were either active on the roster, free-agents or prospective draft choices. One thousand three hundred and twenty-eight scans were performed during this time period. Three hundred and seventy-one NFL players had one measurement between April and August. If players had more than one scan, the scan used for analysis was randomly chosen using a pre-designated randomization scheme.

Experimental Procedures

Height and weight were measured by a standard wall stadiometer and medical beam scale, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Total body imaging was acquired using a GE Healthcare Lunar iDXA (GE Healthcare Lunar, Madison, Wisconsin, USA) dual energy X-ray absorptiometry and analyzed using enCore software version 13.6, rev. 2. No hardware or software changes were made during the duration of the study. Participants were scanned using standard imaging and positioning protocols. Two regions of interest were determined after the scan to measure fat and lean composition within the android and gynoid areas.

The base of the android region is placed at the iliac crests with the height of the region of interest being determined by calculating 20% of the distance between the iliac crests and the participants' chin (Stults-Kolehmainen et al. 2013). The gynoid region is located mid-pelvis to mid-thigh; the upper limit is set below the iliac crest a distance 1.5 times the height of the android region. The lower limit is set a distance of 2 times the height of the android region (Stults-Kolehmainen et al. 2013). VAT was determined in the android region by subtracting subcutaneous fat from total fat (Kaul et al. 2012). Subcutaneous fat was determined using an algorithm and measurements of total abdominal thickness and the width of the subcutaneous fat layer along the lateral extent of the abdomen along with empirically derived geometric constants to estimate the subcutaneous fat in the android region (Kaul et al. 2012).

Statistical Analysis

Participants were categorized by position into one of seven categories: defensive backs (DB); defensive lineman (DL); linebackers (LB); offensive lineman (OL); running backs (RB); tight ends (TE); and wide receivers (WR). They were then placed into groups of positions that mirror each other: Linemen; LB/TE/RB; and WR/DB. This was done to increase the power for testing comparisons between groups. These groups were determined based on our previous work that observed similar body composition between positions that mirror each other. Descriptive statistics were calculated using means$\pm$standard deviation by position group. An analysis of variance (ANOVA) was used to test if positional group means were equal to each other. The TukeyHSD (honest significant difference) method was used to compare each positional group mean against the next to correct for type I error from performing multiple comparisons. Boxplots were

used to present the median (black line), variation and range of VAT variables and each ratio. The boxplot displays the middle fifty percent of the data (box), range of the data (dashed lines) and possible outliers (open circles). All analysis was completed by TB. All analysis was completed using R (R Foundation for Statistical Computing Vienna, Austria).

Results

Table 1 presents the characteristics for each position group for the cross-sectional sample. If position groups share a letter (A,B,C) within the same row then they are not significantly different ($p>0.05$) from each other. Each position group had spent similar time in the NFL at the time of the scan. According to standard body mass index (BMI) classifications, the Linemen position group would be classified as severely obese (BMI > 35 kg/m^2) , the LB/TE/RB position group would be classified as moderately obese (BMI 30-34.9 kg/m^2), and the WR/DB position group would be classified as overweight (BMI 25-29.9 kg/m^2). Not one group had a mean BMI that was considered to be normal.

Table 1 also presents total and trunk body composition characteristics for each position group. Unlike the BMI classifications, only the Linemen are classified as obese (>24%) using standard percent body fat classifications (Jeukendrup & Gleeson 2010). The other two position groups would be classified as acceptable (15-20%) or healthy (11-14%) (Jeukendrup & Gleeson 2010). The Linemen position group had significantly more fat and lean mass ($p<0.05$) across all trunk composition variables compared to the other position groups. Compared to the WR/DB group, the LB/TE/RB group had significantly more fat and lean mass for all variables except VAT. Figure 1 presents the relationship of

android fat mass and weight. As weight increases there is an exponential increase in android fat mass. Figure 2 presents a boxplot of VAT by position groups. Linemen had a much greater range of VAT values compared to other position groups.

Figure 3 presents the relationship between VAT mass and percent total body fat. There is not a complete linear relationship between percent fat and VAT mass, but rather, it becomes linear after twenty-two percent body fat. An analysis of variance determined a significantly different slope in VAT accumulation before and after the twenty-two percent threshold. Figure 4 presents the relationship between subcutaneous android fat and percent total fat mass. A distinct change to a positive linear relationship occurs after 14% body fat, prior to this there is no relationship between data points. An analysis of variance determined a significant difference between the slopes of subcutaneous accumulation before and after this threshold.

Discussion

NFL players are a unique population because their body composition is so different than the average population. Their BMI classifications are all extremely high, yet their percent fat classifications are relatively normal or lean in a majority of players. This suggests that the amount of lean mass all players had is much higher than the average population. This is evident in the lean mass differences between position groups. While significantly different, the magnitude of difference between position groups for lean mass variables is, on average, between 10-15%. Conversely, the average fat mass difference between position groups is close to 200%, or a two-fold difference. This suggests there is a limit to lean mass that can be accumulated and most of the weight

differences between position groups were the result of fat mass accumulation.

Abdominal fat accumulation increased as weight increased. The increase was more exponential than linear. This would suggest that as weight increases, more fat is being stored in the abdominal region, likely because other areas, legs or arms cannot hold any more. When fat accumulates in the abdominal region it can be stored in the visceral region or the subcutaneous region. Because of the metabolic consequences (Després et al. 2008; Fox et al. 2007; Katzmarzyk et al. 2013; Preis et al. 2010; Tulloch-Reid et al. 2004), visceral accumulation is not ideal. Each position group was significantly different from the other groups for both weight and fat mass in the abdominal (android) region. Interestingly, no difference was observed in VAT mass between the LB/TE/RB and WR/DB groups. This means an increase in weight and abdominal fat accumulation does not mean an increase in VAT accumulation.

Although there is no difference in VAT mass between LB/TE/RB and WR/DB groups, the Linemen group had significantly higher VAT mass than the other two groups. These differences are a result of the differences in percent body fat between groups. The Linemen had a higher average percent body fat than the other two groups. This average value was above twenty-two percent where VAT accumulation increases linearly with percent body fat. Linemen would be classified as obese based on percent body fat. The body will shift fat accumulation to various subcutaneous depots in the arms, legs and abdomen. These data would suggest that these adipose stores have a limit and additional fat beyond this limit will be sent to the visceral region for storage. This non-linear relationship was observed in both subcutaneous and visceral depots within the android region. Examining the data it appears that the subcutaneous depot in the android region

fills first, starting to accelerate around 14%. On the other hand, significant, VAT accumulation appears to begin around twenty-two percent. In addition, there does not appear to be a direct relationship between subcutaneous android fat mass and VAT mass until 22%. This suggests that the body will prevent a shift to VAT accumulation until all other depots have been filled. This is intuitive given the metabolic consequences with VAT accumulation. VAT has been observed to be an independent risk factor for cardiometabolic dysfunction (Després et al. 2008; Fox et al. 2007; Katzmarzyk et al. 2013; Preis et al. 2010; Tulloch-Reid et al. 2004). Thus the higher VAT mass observed in the Linemen group may explain the higher prevalence of metabolic syndrome and cardiovascular disease observed in retired NFL linemen (Baron et al. 1994; Harp & Hecht 2005; Miller et al. 2008; Tucker et al. 2009).

Interestingly, compared to the WR/DB group, the LB/TE/RB group has two times as much android fat mass, yet their VAT mass is similar. These observations can be explained by the relationship of each regional depot with total percent mass. Linear accumulation in these depots occurs at different levels of percent fat. These levels correspond very closely with standard classifications (Jeukendrup & Gleeson 2010). Each increasing classification level is an increase in fat mass. This excess fat must be allocated to a storage depot; it appears allocation to the subcutaneous android region begins around fourteen percent body fat. Allocation to the visceral depot occurs at a higher percent fat, around 22%. The LB/TE/RB and WR/DB groups were below this threshold, which likely resulted in minimal VAT accumulation. Additionally, the significantly lower percent body fat for the WR/DB group explains the difference in android fat mass with the LB/TE/RB group. This observation suggests an independent relationship between VAT

and subcutaneous android fat. To our knowledge, this is the first study to observe distinct cut-points of linear accumulation for regional fat depots. These cut-points may help identify players at risk for metabolic complications. It may also serve to educate players, post retirement, about the importance of maintaining low body fat or loosing excess mass.

Our previous study observed similarities in positions that mirror each other for measures of total body composition and bone mass and differences compared to positions that do not mirror. This current study observed a similar pattern in regional trunk composition. However, a unique pattern was observed for VAT. There were no differences in VAT observed between the LB/TE/RB and WR/DB groups. Furthermore, VAT appears to dramatically accumulate after twenty-two percent body fat. Prior to that, there is little relationship between percent fat and VAT. These observations indirectly suggest a minimal VAT accumulation independent of other fat depots and further VAT accumulation is influenced by excess accumulation in other areas.

Future research should examine activity differences between position groups to determine the role of physical activity on limiting VAT accumulation. The physical demands of these positions may also explain the similarities in VAT mass. Both groups are highly active and require several short explosive bursts with minimal rest. Low percent body fat and thus low VAT accumulation, combined with high activity levels may explain why these position groups do not show the same increased prevalence of cardiovascular risk factors. Additionally more research should be completed in players post-retirement. Of interest, would be to follow players after retirement and to track changes in body composition and how that relates to cardiometabolic risk.

In conclusion, NFL position groups exhibit differences between fat and lean body composition variables. However, the Linemen have significantly more VAT than both the LB/TE/RB group and the WR/DB group. The key may be the excess body fat exhibited by the Linemen group, whom were classified as obese using both BMI and percent body fat. These excess body fat needs to be allocated to various storage depots and these data suggest it was distributed to the visceral region in addition to various subcutaneous depots. Increases in VAT accumulation are associated with increased cardiometabolic risk.

FIGURE LEGEND

Figure 1: Android fat mass by weight in kilograms

Figure 2: Visceral adipose tissue by position group

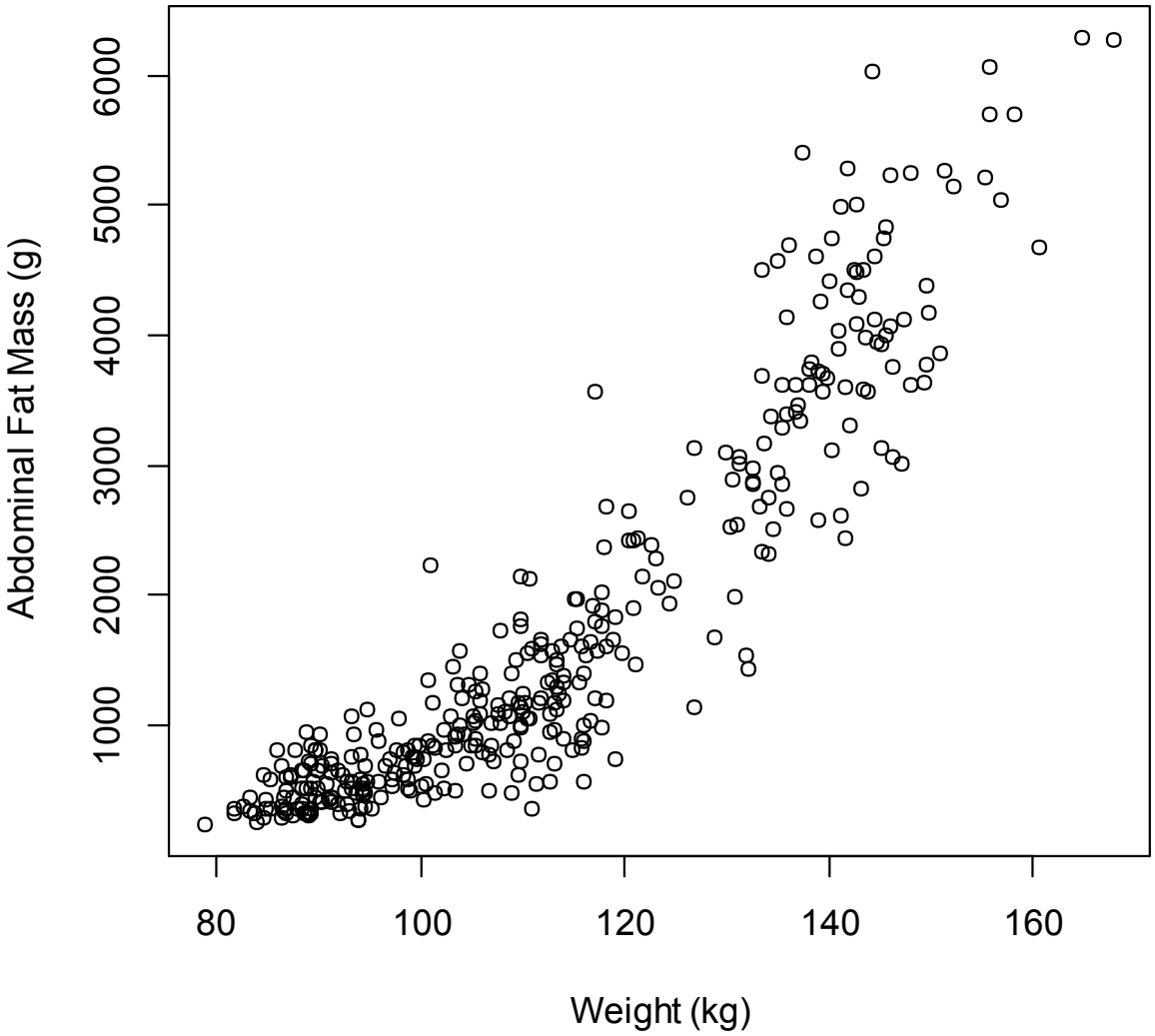
Figure 3: Relationship of visceral adipose tissue by percent body fat

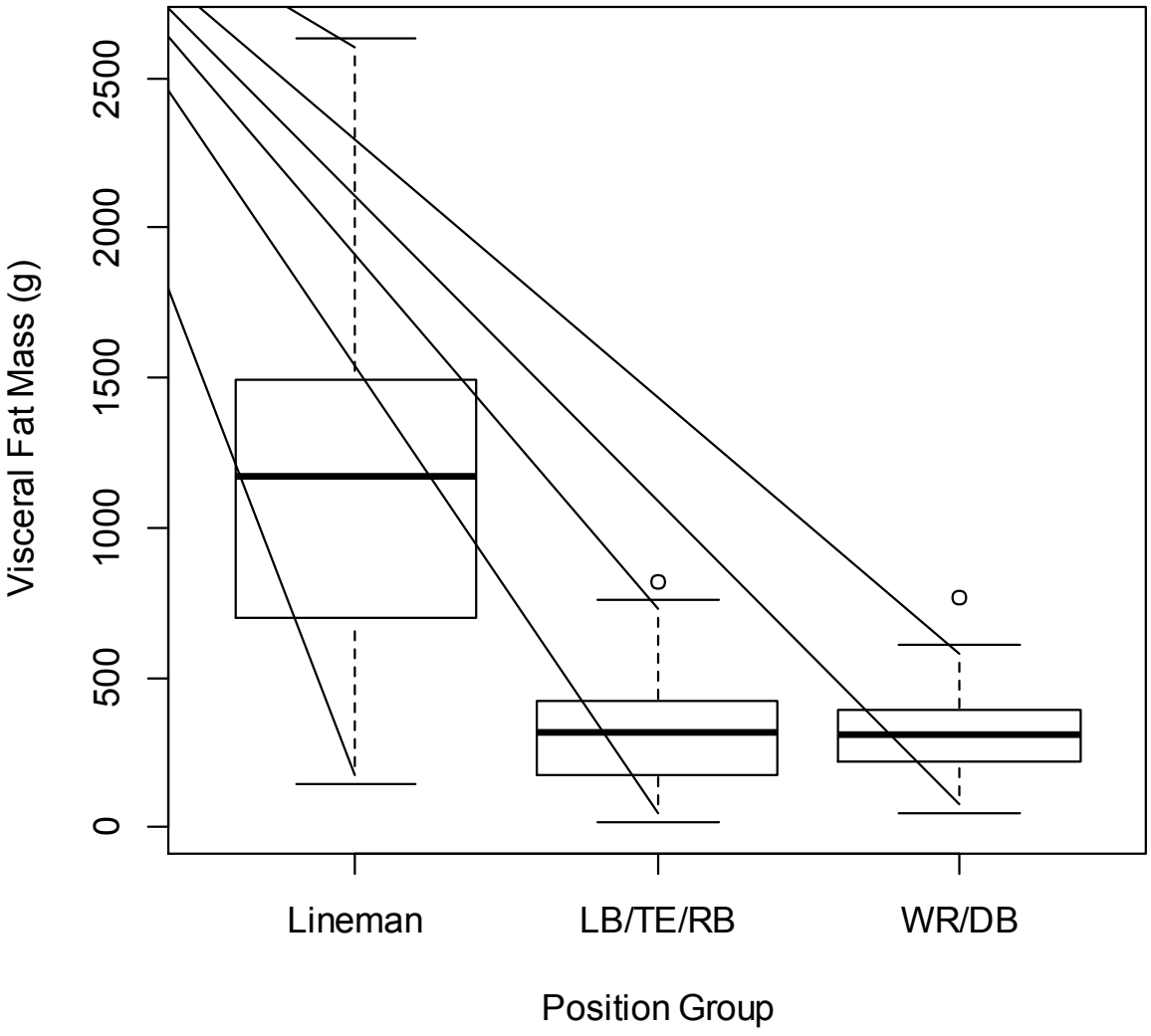
Figure 4: Relationship of subcutaneous android fat and percent body fat

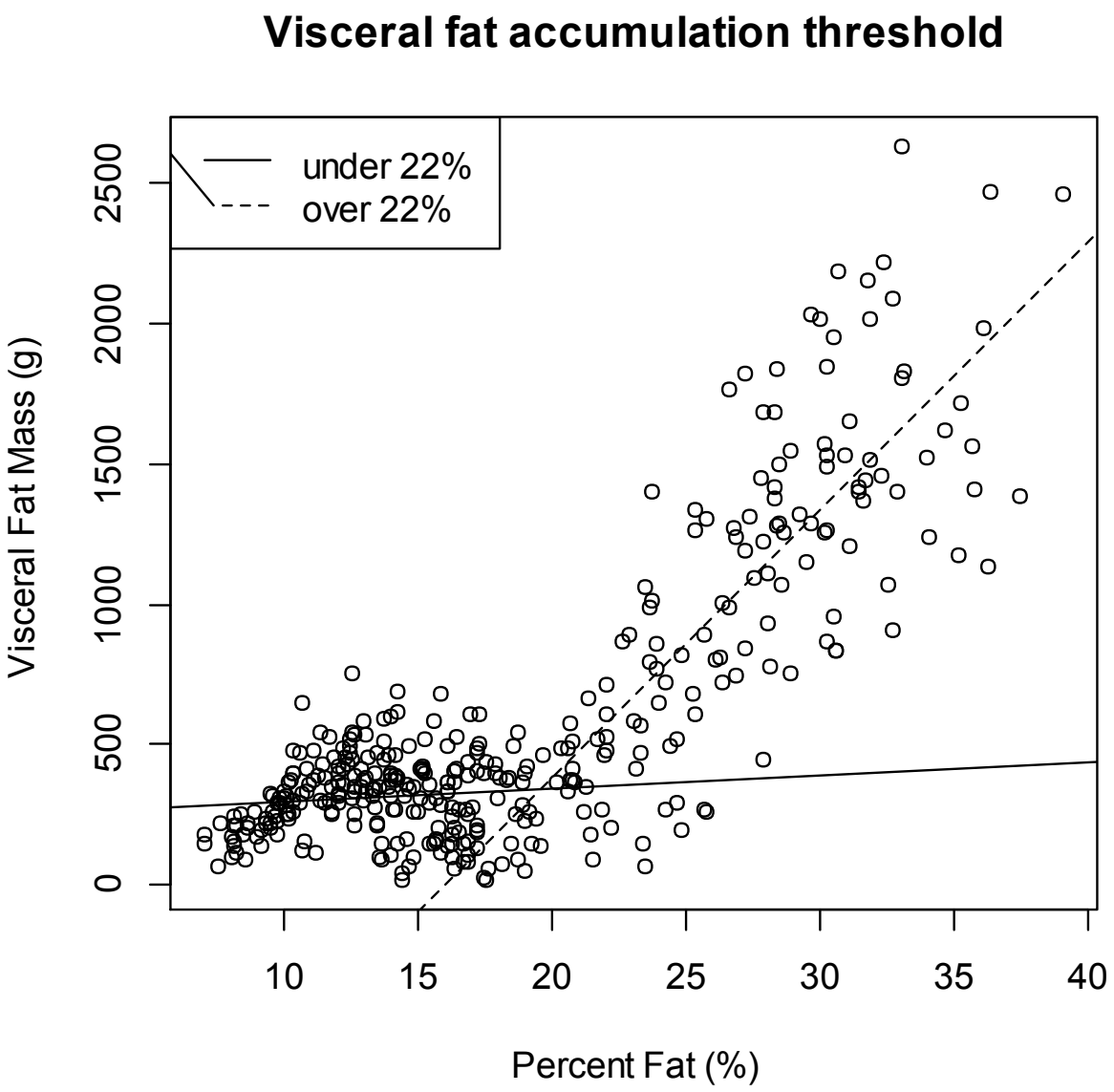
Table 1: Descriptive and body composition measurements means (±standard deviation) for the sample

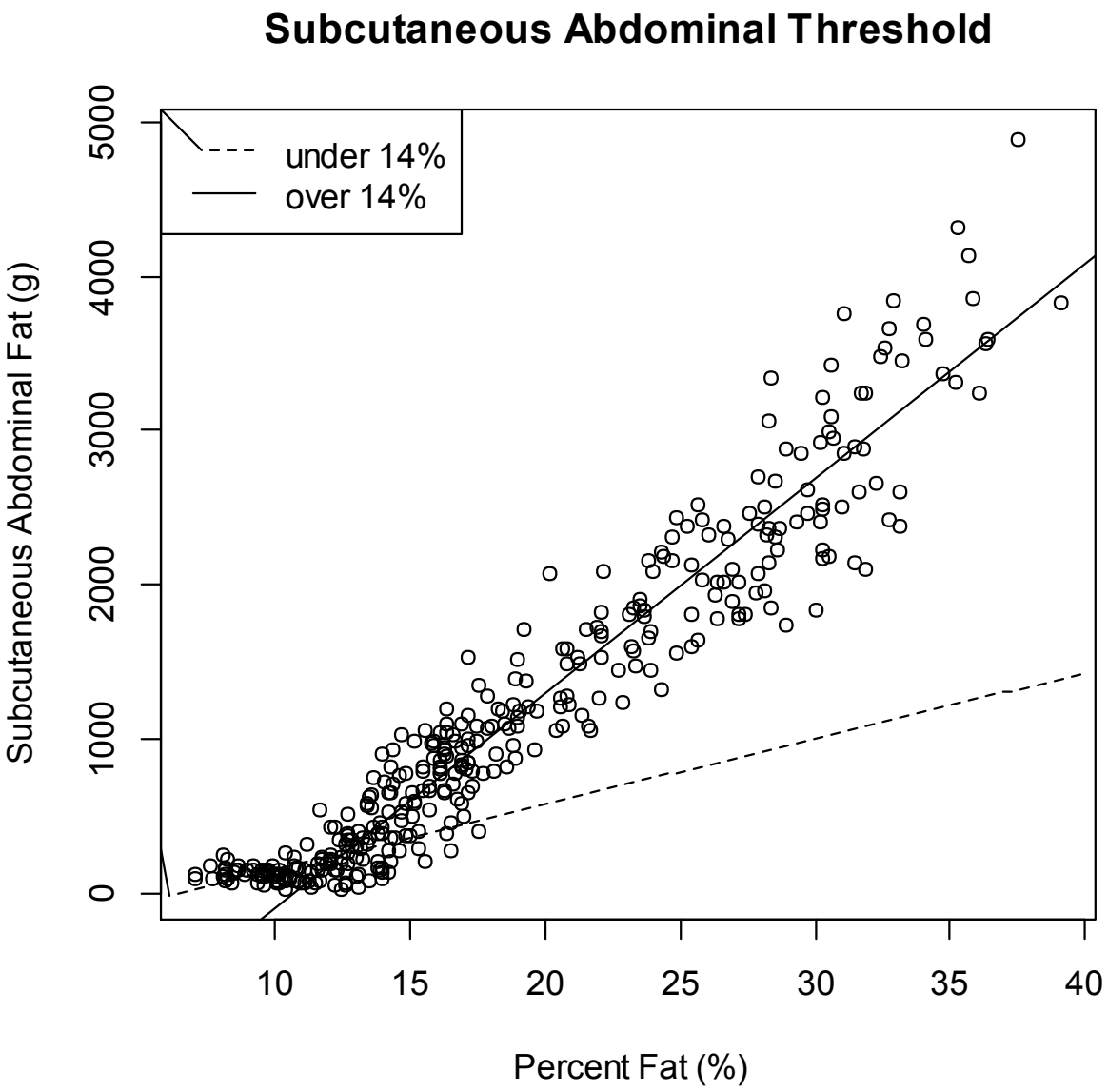
	Linemen (n=123)	LB/TE/RB (n=122)	WR/DB (n=125)
Age (yrs)	24.0^{A}(2.4)	23.8^{A}(2.2)	23.6^{A}(2.0)
Height (cm)	191.9^{A}(3.7)	186.7^{B}(5.7)	183.8^{C}(3.9)
Weight (kg)	137.1^{A}(11.7)	109.6^{B}(6.6)	92.3^{C}(6.2)
BMI (kg/m^2)	37.3^{A}(3.5)	31.5^{B}(1.9)	27.3^{C}(1.8)
Years Played (yrs)	2.4^{A}(2.3)	2.2^{A}(1.9)	2.1^{A}(2.0)
Percent Fat (%)	27^{A}(6)	17^{B}(4)	12^{C}(3)
Total Fat (kg)	36.4^{A}(10.0)	17.6^{B}(4.5)	10.9^{C}(3.4)
Total Lean (kg)	95.9^{A}(5.0)	87.3^{B}(4.7)	77.1^{C}(4.4)
Trunk Fat (kg)	19.9^{A}(6.3)	8.3^{B}(2.6)	4.8^{C}(2.0)
Trunk Lean (kg)	42.7^{A}(3.2)	39.0^{B}(2.7)	34.5^{C}(2.0)
Android Fat (kg)	3.4^{A}(1.3)	1.2^{B}(0.5)	0.6^{C}(0.3)
Android Lean (kg)	6.3^{A}(0.5)	5.6^{B}(0.5)	4.8^{C}(0.4)
Gynoid Fat (kg)	6.0^{A}(1.7)	3.0^{B}(0.9)	1.7^{C}(0.7)
Gynoid Lean (kg)	16.0^{A}(1.2)	14.4^{B}(1.0)	12.5^{C}(1.0)
Visceral Fat (kg)	1.2^{A}(0.6)	0.3^{B}(0.2)	0.3^{B}(0.1)

BMI = Body mass index
If the variables share a letter within each row they are not significantly different than one another at $\alpha=0.05$.




Visceral Fat Mass (g)
0
500
1000
1500
2000
2500
Lineman
LB/TE/RB
WR/DB
Position Group


Visceral fat accumulation threshold
under 22%
over 22%
Visceral Fat Mass (g)
0
500
1000
1500
2000
2500
10
15
20
25
30
35
40
Percent Fat (%)


Subcutaneous Abdominal Threshold
under 14%
over 14%
Subcutaneous Abdominal Fat (g)
0
1000
2000
3000
4000
5000
10
15
20
25
30
35
40
Percent Fat (%)

CHAPTER 5: Sex specific thresholds for accumulation of visceral adipose tissue are associated with increased cardiometabolic risk factors in adults.

Identification of sex-specific thresholds for accumulation of visceral adipose tissue in adults

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Short Running Title: Threshold accumulation of VAT in adults

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Abbreviations

VAT – visceral adipose tissue, DXA – dual energy X-ray absorptiometry, BMI – body mass index, M-glucose utilization, $M/_{LBM}$- glucose utilization per kg of lean body mass, HOMA-IR – homeostasis model of insulin resistance

Author Contributions:

Conception and design of the experiments
- Tyler A. Bosch

Collection, analysis and interpretation of data
- Julia Steinberger, Alan R. Sinaiko, Antoinette Moran, Donald R Dengel, Tyler A. Bosch

Drafting the article or revising it critically for important intellectual content
- Tyler A Bosch, Julia Steinberger, Alan R. Sinaiko, Antoinette Moran, Aaron S. Kelly Donald R. Dengel,

Chapter 5 Summary

Introduction: The body preferentially stores fat subcutaneously over the visceral region; however at some point the body distributes fat to the visceral region. To date, an adiposity threshold has never been demonstrated for increased visceral accumulation. The purpose of this study was to measure the linearity of visceral adipose tissue (VAT) accumulation with measures of total body adiposity and the association with cardiometabolic risk factors in adults.

Methods: Data were obtained from 735 (330 females) adults' ages 19-47 years. Body mass index ranged from 15-52 kg/m^2. An analysis of variance compared the slopes of VAT mass with percent body fat before and after the threshold was identified using scatter plots. T-tests compared differences of cardiometabolic risk factors and body composition above each sex specific threshold. Partial correlations measure the association of VAT mass, total fat mass and subcutaneous android fat with cardiometabolic risk factors.

Results: Adiposity thresholds were identified at 25% body fat in males and 40% body fat in females beyond which VAT accumulation increased significantly with increased adiposity. Above these adiposity thresholds males and females had significantly higher lipids ($p<0.001$), increased insulin resistance ($p<0.001$), and higher fat mass across all depots. However, of the various fat depots VAT mass provided the strongest association with and cardiometabolic risk factors in both males and females.

Conclusion: Accumulation of VAT mass is not linear with increasing adiposity; distinct sex specific thresholds exist at which visceral accumulation increases. Adjusting for the increase in VAT diminishes the association between total fat mass and cardiometabolic risk.

Introduction

Over a third of adults are obese in the United States (Flegal et al. 2010). While the prevalence of obesity has stabilized recently (Flegal et al. 2010), the overall number of obese individuals is alarming considering both the financial and the health consequences of obesity (Kopelman 2000). The accumulation of total body fat that occurs with obesity results in excess fat in several subcutaneous and ectopic depots located around the body. The body preferentially stores excess fat subcutaneously (Bjorntorp 1991; Velilleux et al. 2001). However, at some point fat is stored ectopically. One ectopic depot is the visceral region. Visceral adipose tissue (VAT) surrounds the internal organs in the android region and has a significant association with cardiometabolic risk factors (Carey et al. 1996; Castro et al. 2014; Despres et al. 2001; Lima et al 2013; Matsuzawa et al 2011; Nakamura et al 1993; Neeland et al. 2012; Rothney et al. 2013), independent of total fat mass and subcutaneous fat. Thus, measurement of VAT has become an important marker for identifying cardiometabolic risk. Recently dual energy x-ray absorptiometry (DXA) was validated, against CT, as an accurate and reliable method for estimating VAT within the entire android region and as a reliable marker of cardiometabolic risk (Kaul et al. 2012; Katzmarzyk et al. 2013; Rothney et al. 2013). In addition to VAT, DXA provides measurement of regional fat, lean body mass, and bone mass. We recently observed a non-linear relationship between percent body fat and VAT in professional football players (Bosch et al. Submitted 2014). VAT accumulation increased at a steeper rate in athletes with 22 or more percent body fat. Prior to that threshold, there was minimal VAT accumulation associated with increases in total body fat. Professional athletes have diet and exercise patterns that are very different from those of the general population. The primary aim of this study was to determine if a similar threshold for VAT accumulation

existed in a community population of adults. The secondary aim of this study was to measure the association between fat depots with cardiometabolic risk factors above the identified threshold.

Methods

Data were obtained from two population based studies tracking cardiovascular and metabolic changes over the lifespan (n = 950). Data were from follow-up visits occurring between 2005-2011 when participants were between the ages of 19-47 years. A total of 735 (330 females and 405 males) participants were included in the current study who completed a full body DXA scan, blood draw, and had insulin sensitivity measured by hyperinsulinemic, euglycemic. Participants were excluded if they were missing data or DXA was unable to determine VAT mass. The respective protocols were approved by the University of Minnesota Institutional Review Board and consent was obtained from each participant.

Anthropometric and Blood Pressure Measurements

Testing was conducted at the University of Minnesota Clinical Translation Science Institute after participants had been fasting for a minimum of eight hours. Height and weight were measured on a calibrated stadiometer and electronic scale, respectively, while participants were wearing light clothes and without shoes. Body mass index (BMI) was calculated as kg/m^2. Waist circumference was measured to the nearest 0.5 cm, taken in duplicate and the mean value reported. Blood pressure was measured in duplicate on the right arm after participants were sitting in a quiet room for at least five minutes using a digital blood pressure cuff and the average of the two values was reported.

Body Composition and Visceral Adipose Quantification

Total body composition was measured using DXA (Lunar Prodigy, General Electric Medical Systems, Madison, WI, USA) and analyzed using its enCore^{TM} software (platform version 13.6). Participants were scanned using standard imaging and positioning protocols while fasted and hydrated. Subcutaneous fat and visceral fat were estimated within the android region. Estimates of abdominal visceral and subcutaneous adipose tissue were obtained using the method described previously for adults (Kaul et al. 2012). The android region was a region of interest automatically defined with a caudal limit placed at the top of the iliac crest and its height set to 20% of the distance from the top of the iliac crest to the base of the skull (Stults-Kolehmainen et al. 2013). The gynoid region is located mid-pelvis to mid-thigh; the upper limit was set below the iliac crest a distance 1.5 times the height of the android region. The lower limit was set a distance of 2 times the height of the android region (Stults-Kolehmainen et al. 2013). All scans were reviewed for accurate placement of the android box by the same technician.

Measurement of Blood Markers

Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp as previously described (Sinaiko et al. 2001). Insulin was infused at a constant rate of 1 mU/kg/min for 3 hours, and glucose was infused at a variable rate to maintain euglycemia. Insulin sensitivity (M) was expressed as the glucose infusion rate (mg/kg/min of glucose) during the last 40 minutes of the clamp, with adjustment for lean body mass ($M_{/LBM}$). Fasting blood samples were collected for lipid levels (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density

lipoprotein cholesterol), glucose and insulin. All assays were conducted with standard procedures at the Fairview Diagnostic Laboratories, Fairview-University Medical Center (Minneapolis, MN), a Centers for Disease Control and Prevention-certified laboratory.

Identification of the threshold

Identification of percent fat threshold for change in slope was accomplished by using a standard receiver operating characteristic (ROC) analysis with an area under the curve (AUC) measurement to identify the percent fat value that best predicts visceral fat greater than the mean for males and females. This method identified a threshold of 25.4% in males (AUC = 0.904 CI(0.873-0.934), sensitivity = 0.965, specificity = 0.755) and a threshold of 40.3% in females (AUC = 0.838 CI(0.795-0.881), sensitivity = 0.881, specificity = 0.689). For the purpose of this study we will use 25% and 40%.

Statistical Analysis

Unpaired t-tests compared males and females for demographic, body composition, and cardiometabolic characteristics. Data are presented as means$\pm$standard deviation. Normality of data that were right skewed was tested using the Anderson-Darling test. If significantly skewed, data were log transformed for comparisons and data were presented as geometric mean and 95% confidence intervals. Body composition data were log transformed to measure if non-normality affected the comparison and all comparisons resulted in the same sex differences whether data was log transformed or not. Pearson's correlation was used to assess the linear relationship between total fat mass and regional fat mass. A scatter plot matrix was used to observe the linearity of DXA fat measurements (total fat mass, VAT mass, gynoid fat mass, subcutaneous

android fat, percent body fat), against each other. An adiposity threshold was determined using ROC analysis with AUC threshold. An analysis of covariance was used to determine if the slopes were significantly different above and below threshold. Unpaired t-tests compared males and females above and below the threshold for anthropometric, cardiometabolic and body composition measurements. Partial Pearson's correlation measured the association between VAT mass, total fat mass and subcutaneous android fat mass with cardiometabolic risk factors. All variables were adjusted for age. VAT mass and subcutaneous android fat mass were adjusted for total fat mass and total fat mass was adjusted for VAT mass. All analyses were completed using R (R Foundation for Statistical Computing, www.R-project.org).

Results

Table 1 presents the demographic and body composition comparison of males and females from the sample population. As expected Males were taller and heavier than females, had greater lean body mass and had a lower percent fat, BMI, but larger waist circumferences than females. Females had higher level of android fat than males, but males had higher VAT mass. Females had higher total fat mass, subcutaneous fat mass, gynoid fat mass and subcutaneous-visceral ratio compared to males. While females had higher levels of android fat than males their VAT mass was less.

Males had worse cardiovascular profiles than females with higher low-density lipoprotein cholesterol, triglycerides and blood pressure, lower high density lipoprotein cholesterol, higher fasting glucose, and lower insulin sensitivity. (Table 2).

All correlations were significantly positive between DXA fat measures. However, VAT mass displayed a non-linear relationship with percent body fat (Figure 1a-b) for both males and females. This non-linear relationship suggests a threshold whereby visceral accumulation significantly increases non-linearly before and after the threshold. The male threshold appears at 25% body fat and the female threshold appears at 40% body fat. The slope of the relationship between VAT mass and percent body fat was significantly steeper ($p<0.001$) after the threshold in males and females. The slopes above and below threshold were not significantly different between males and females ($p=0.112$, $p=0.385$). A threshold was also observed between VAT Mass and BMI in males (≈ 24 kg/m^2) but not in females. There was not an observed threshold for waist circumference in males or females.

Within males significant differences in body composition and cardiometabolic risk factors were observed after the adiposity threshold was reached. Males above threshold were significantly ($p<0.001$) older. There was no difference in height of males above and below threshold ($p=0.457$), but males above threshold were heavier, had a higher BMI, waist circumference and percent fat mass ($p<0.001$). Additionally, males above threshold had significantly ($p<0.001$) more fat mass in all regions, but the same amount of lean mass ($p=0.06$) compared to males below threshold. Males above the adiposity threshold had significantly higher low-density lipoprotein cholesterol and triglyceride levels, but lower high-density lipoprotein cholesterol, higher systolic and diastolic blood pressure, higher fasting glucose and insulin, and lower insulin sensitivity [M/lbm] ($p<0.001$).

Similarly, females above threshold demonstrated significant differences from females below threshold. Age (p=0.314) and height (0.630) were similar above and below threshold. Similar to males, females above threshold were heavier, had a higher BMI, waist circumference and percent fat mass (p<0.001) than females below threshold. All measurements of fat mass were significantly higher in females above threshold (p<0.001), but there was no difference in lean mass (p=0.641). Females above threshold had higher triglycerides, low-density lipoprotein cholesterol and lower high-density cholesterol (p<0.001), but total cholesterol were similar (p=0.09). Females above threshold had higher systolic blood pressure (p<0.001) but similar diastolic blood pressure (p=0.146) to females below threshold. Similar to males, females above threshold had higher fasting glucose, insulin and lower insulin sensitivity [M/lbm], (p<0.001).

A comparison of males and females above their respective thresholds indicated that males had higher VAT mass (p<0.001), but females had higher fat mass in all other regions (p<0.001). Cardiovascular risk was worse in males compared to females above the adiposity threshold. Males had higher triglycerides, total cholesterol, low-density lipoprotein cholesterol, lower high-density lipoprotein cholesterol and higher systolic and diastolic blood pressure (p<0.001). Males had significantly lower insulin sensitivity (M/lbm) and higher fasting glucose (p<0.001) than females above threshold.

Table 3 presents the adjusted and unadjusted correlations of fat measurements with cardiometabolic risk factors above threshold. Prior to adjustment, VAT mass, total fat mass and subcutaneous android fat mass were similarly associated with cardiometabolic risk factors in men and women. Negative associations were consistently observed for high-density lipoprotein cholesterol and insulin sensitivity (M/lbm) and

positive associations were consistently observed for the other risk factors. With a few exceptions, adjusted correlations for VAT mass remained significantly associated with cardiometabolic risk factors in men and women. Conversely, with only a few exceptions, adjusted correlations between cardiometabolic risk factors with either total fat mass and subcutaneous android fat mass were non-significant (Table 3).

Table 4 presents the adjusted and unadjusted correlations of fat measurements with cardiometabolic risk factors below threshold. Prior to adjustment visceral fat was associated with more metabolic risk factors than both total fat mass and subcutaneous abdominal fat. After adjustment VAT remained significantly associated with several cardiometabolic risk factors in both males and females. However, adjustment of total fat mass and subcutaneous abdominal fat reduced many of the associations with cardiometabolic risk factors (Table 4).

Discussion

To our knowledge, this is the first study to identify sex-specific adiposity thresholds for VAT accumulation in adults. In both males and females, reaching threshold results in an increased accumulation of excess fat in the visceral region. Increased visceral adiposity had a stronger association with cardiometabolic risk factors independent of total fat mass. Conversely, after accounting for VAT mass, total fat mass was not significantly associated with cardiometabolic risk or insulin resistance. These results suggest increased distribution to the visceral region after 25% fat mass in males and 40% fat mass in females, which coincides with increased insulin resistance and increased cardiometabolic risk.

Females have a higher adiposity threshold than males. This may be a result of sex differences in subcutaneous depots, hormonal differences or differences in adipocyte characteristics. Estrogen promotes distribution to peripheral subcutaneous adipose tissue, whereas testosterone shifts more fat to abdominal and visceral regions (Tchernof et al. 2013). Additionally, females have greater expandability of adipocytes since increased volume can occur through adipocyte hyperplasia (Drolet et al. 2008). Adipocytes do not need to expand as much to accommodate excess fat accumulation. Conversely in males, increased fat volume generally occurs via adipocyte hypertrophy (Drolet et al. 2008) which limits the expandability of the region because as adipocyte increases, angiogenesis cannot keep up and the adipocyte becomes hypoxic which leads to lipolysis (Hosogai et al. 2007). It is likely that all of these factors influence the higher percent fat adiposity threshold observed in females. However, after threshold is reached the slope of VAT accumulation is not different between males and females, suggesting that the rate of increase in VAT is not different.

Above threshold, VAT mass is a stronger marker of cardiometabolic risk than total fat mass or subcutaneous android fat mass. This suggests that it is not excess fat, per se, but rather where fat is distributed that influences cardiometabolic risk and insulin resistance. This is consistent with previous research (Carey et al. 1996; Castro et al. 2014; Despres et al. 2001; Lima et al 2013; Matsuzawa et al 2011; Nakamura et al 1993; Neeland et al. 2012; Rothney et al. 2013). The novel observation in this current study is that stronger relationships between cardiometabolic risk factors and VAT mass were present among participants who had reached or exceeded their percent fat adiposity threshold. Differences in subcutaneous fat mass between males and females remained

consistent regardless of whether the adiposity threshold had been reached. Compared to males, females had better cardiometabolic profiles despite higher subcutaneous fat mass, suggesting a protective mechanism of their higher percent fat adiposity threshold. Adipose tissue is an endocrine organ, but differences exist between subcutaneous and visceral adipocytes. VAT releases several pro-inflammatory cytokines (Kershaw & Flier 2004; Mohamed-Ali et al. 1998) and increases lipolytic activity even in high insulin states (McLaughlin et al. 2011) whereas subcutaneous adipocytes decrease lipolytic activity and release adipokines considered to be protective (Kershaw & Flier 2004; Mohamed-Ali et al. 1998). For a give fat mass, males had a much smaller subcutaneous-visceral ratio which might result in more cytokines being released. This greater cytokine release may play a role in cardiometabolic health; however future research is needed to describe whether concentrations of cytokines are higher above these identified adiposity thresholds.

To our knowledge, this is the first study to demonstrate the presence of an adiposity threshold at which visceral fat accumulation steeply increases with increasing adiposity in males and females. This threshold appears to be unique to VAT since all subcutaneous depots display a linear relationship with total fat mass. Above this threshold VAT mass is a stronger marker for cardiovascular risk and insulin resistance compared to total fat mass or subcutaneous android mass. These results suggest that males above 25% fat and females above 40% fat should be carefully monitored for cardiometabolic risk factors and perhaps targeted for more intensive weight management approaches. Clinically it may be more relevant to use the threshold observed with BMI in

males ($\geq$24 kg/m^2), however it should be noted that a given BMI could be associated with a wide range of percent body fat values.

The population of this study was predominantly Caucasian and African American. These adiposity thresholds may not be applicable across other races. However, different threshold may explain why some populations have more visceral fat at a given level of adiposity.

Table 1: Demographic, body composition and clinical measurements mean(sd)			
	Female	Male	p-value
Age (yrs)	34(7)	33(8)	0.04
Race (%)	Caucasian (70) African Am. (21) Other (9)	Caucasian (71) African Am (21) Other (8)	NA
Height (cm)	164.3(9.0)	177.6(7.8)	<0.001
Weight (kg)	79.1(19.8)	87.2(19.5)	<0.001
Percent Fat (%)	41.4(8.9)	26.8(10.0)	<0.001
BMI (kg/m^2)	29.5(8.7)	27.6(5.7)	<0.001
Waist Circumference (cm)	94.8(17.9)	96.8(15.9)	0.001
Total Fat (kg)	31.5(13.9)	23.9(12.7)	<0.001
Total Lean (kg)	46.2(14.5)	48.4(15.3)	0.05
Subcutaneous Fat (kg)	2.0(1.1)	1.3(0.9)	<0.001
Visceral Fat (kg)	0.6(0.6)	0.9(0.9)	<0.001
Gynoid Fat (kg)	5.7(2.3)	3.8(2.0)	<0.001
Subcutaneous-Visceral ratio	4.8* (4.3, 5.3)	2.4* (2.1, 2.7)	<0.001
Triglycerides* (mg/dL)	88(82,94)	113(105,122)	<0.001
HDL-C (mg/dL)	55(14)	44(11)	<0.001
LDL-C (mg/dL	104(27)	108(29)	0.03
SBP (mmHg)	118(17)	122(16)	0.002
DBP (mmHg)	68(11)	71(10)	<0.001
Glucose* (mg/dL)	97(93,101)	102(98,106)	<0.001
Insulin* (mg/dL)	6(5,7)	6(5,7)	0.8992
Insulin Sensitivity [Mlbm] ($mg/kg_{lbm}/min$)	11.9(5.1)	9.5(4.1)	<0.001

*Indicates log transformed data presented as the geometric mean and 95% confidence interval

BMI = body mass index, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol SBP = systolic blood pressure, DBP = diastolic blood pressure

Table 2: Body composition and cardiometabolic variables above and below thresholds				
	Males <25% (n=158)	Females <40% (n=133)	Males >25% (n=242)	Females>40% (n=191)
Age (yrs)	30^{A}(9)	34^{B}(8)	35^{B}(7)	35^{B}(7)
Height (cm)	177.2^{A}(8.2)	164^{B}(8.4)	177.8^{A}(7.6)	164.7^{B}(6.2)
Weight (kg)	73.3^{A}(12.2)	64.9^{B}(11.1)	96.3^{C}(17.8)	89.3^{D}(18.2)
BMI (kg/m^2)	23.3^{A}(3.1)	24^{A}(4.0)	30.4^{B}(5.2)	33^{C}(6.5)
Waist (cm)	82.9^{A}(7.0)	81.9^{A}(12.5)	105.9^{B}(13.1)	104.0^{B}(15.3)
Body Fat (%)	16.4^{A}(5.4)	32.8^{B}(5.6)	33.7^{B}(5.3)	47.5^{C}(4.7)
Total Fat mass (kg)	13.4^{A}(4.8)	20.5^{B}(5.8)	30.9^{C}(11.2)	39.4^{D}(12.6)
Total Lean mass (kg)	50.1^{A}(14.2)	45.7^{B}(14.6)	47.3^{B}(16.0)	46.4^{B}(14.4)
Andoid Fat mass (kg)	0.8^{A}(0.5)	1.4^{B}(0.7)	3.1^{C}(1.5)	3.5^{D}(1.4)
Gynoid Fat mass (kg)	2.2^{A}(0.9)	4.0^{B}(1.1)	4.8^{C}(1.7)	6.9^{D}(2.2)
Subq Fat mass(kg)	0.6^{A}(0.4)	1.1^{B}(0.5)	1.8^{C}(0.9)	2.6^{D}(1.0)
Visceral Fat mass (kg)	0.3^{A}(0.2)	0.3^{A}(0.3)	1.4^{B}(0.8)	0.9^{C}(0.6)
SV ratio	3.6^{A}(3.0,4.4)	7.2^{B}(6.0,8.6)	1.8^{C}(1.6,2.1)	3.6^{A}(3.1,4.1)
Triglycerides (mmol/L)	0.9^{A}(0.8,1.0)	0.9^{A}(0.8,1.0)	1.6^{B}(1.4,1.8)	1.1^{C}(1.0,1.2)
Total Chol (mmol/L)	4.3^{A}(0.8)	4.5^{A}(0.8)	4.9^{B}(0.9)	14.6^{A}(0.8)
HDL-C (mmol/L)	1.3^{A}(0.3)	1.5^{B}(0.4)	1.1^{C}(0.3)	1.3^{A}(0.3)
LDL-C (mmol/L)	2.5^{A}(0.7)	2.6^{A}(0.7)	3.0^{B}(0.7)	2.8^{C}(0.7)
SBP (mmHg)	116^{A}(13)	114^{A}(13)	126^{B}(17)	121^{C}(18)
DBP (mmHg)	68^{A}(10)	67^{A}(11)	74^{B}(10)	69^{A}(11)
Glucose (mmol/L)	5.4^{A}(5.1,5.8)	5.2^{B}(4.9,5.6)	5.8^{C}(5.5,6.1)	5.5^{A}(5.2,5.8)
Insulin (pmol/L)	22.2^{A}(19.2, 25.2)	25.2^{A}(22.2,29.4)	49.8^{B}(44.4,55.8)	46.8^{B}(41.4,52.8)
Mlbm (mg/kglbm/min)	12.5^{A}(4.5)	13.8^{B}(4.7)	10.0^{C}(4.1)	12.1^{D}(4.9)

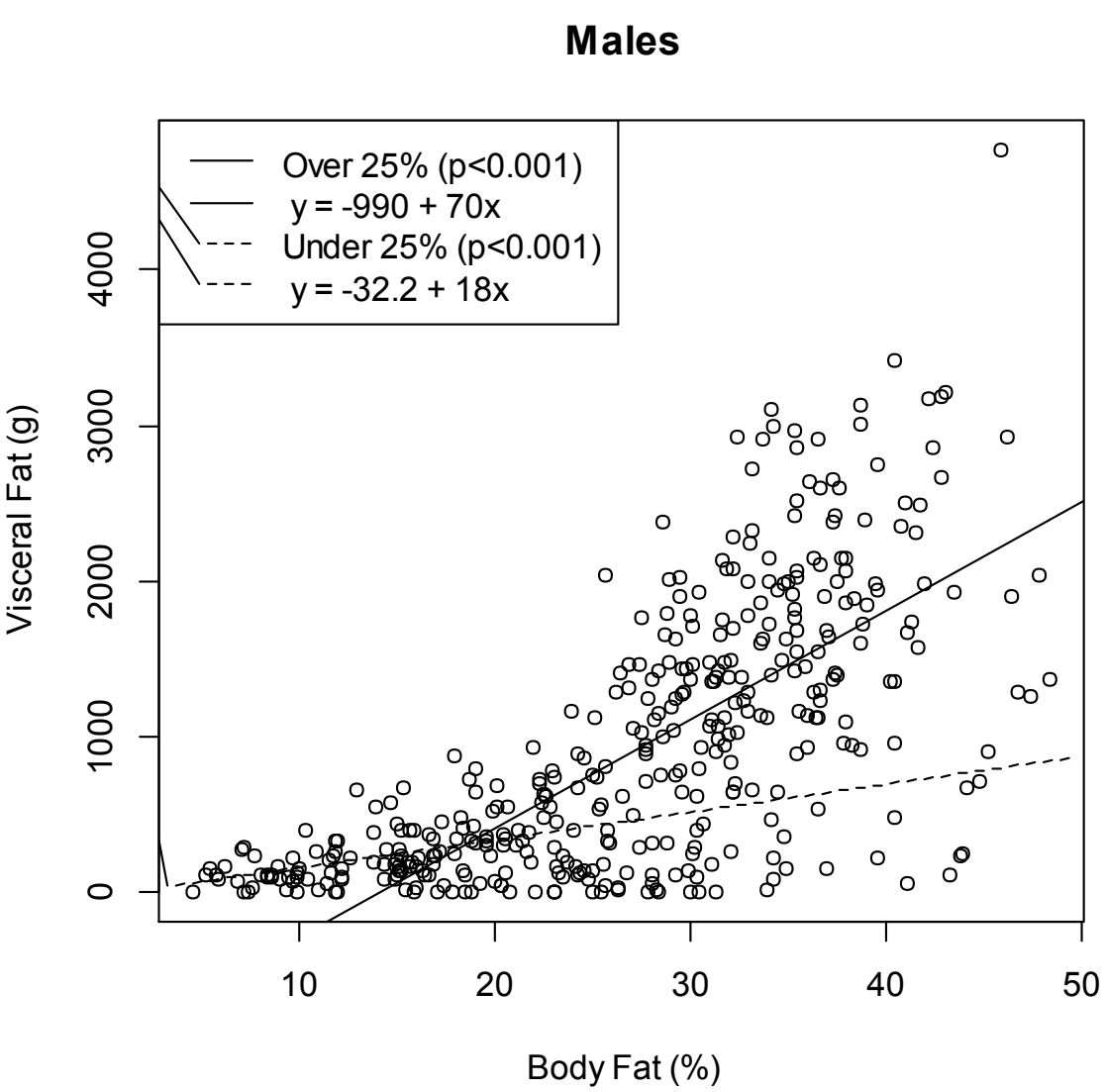
If groups do not share a letter in the same row they are significantly different $p<0.025$

BMI = body mass index, HDL-C = high density lipoprotein cholesterol,

LDL-C = low density lipoprotein cholesterol, SBP = systolic blood pressure, DBP = diastolic blood pressure

Figure Legend

Figure 1: (A) Percent body fat threshold for visceral fat accumulation in males. (B) Percent body fat threshold for visceral fat accumulation in females


Males
Over 25% (p<0.001)
y = -990 + 70x
Under 25% (p<0.001)
y = -32.2 + 18x
Visceral Fat (g)
0
1000
2000
3000
4000
10
20
30
40
50
Body Fat (%)

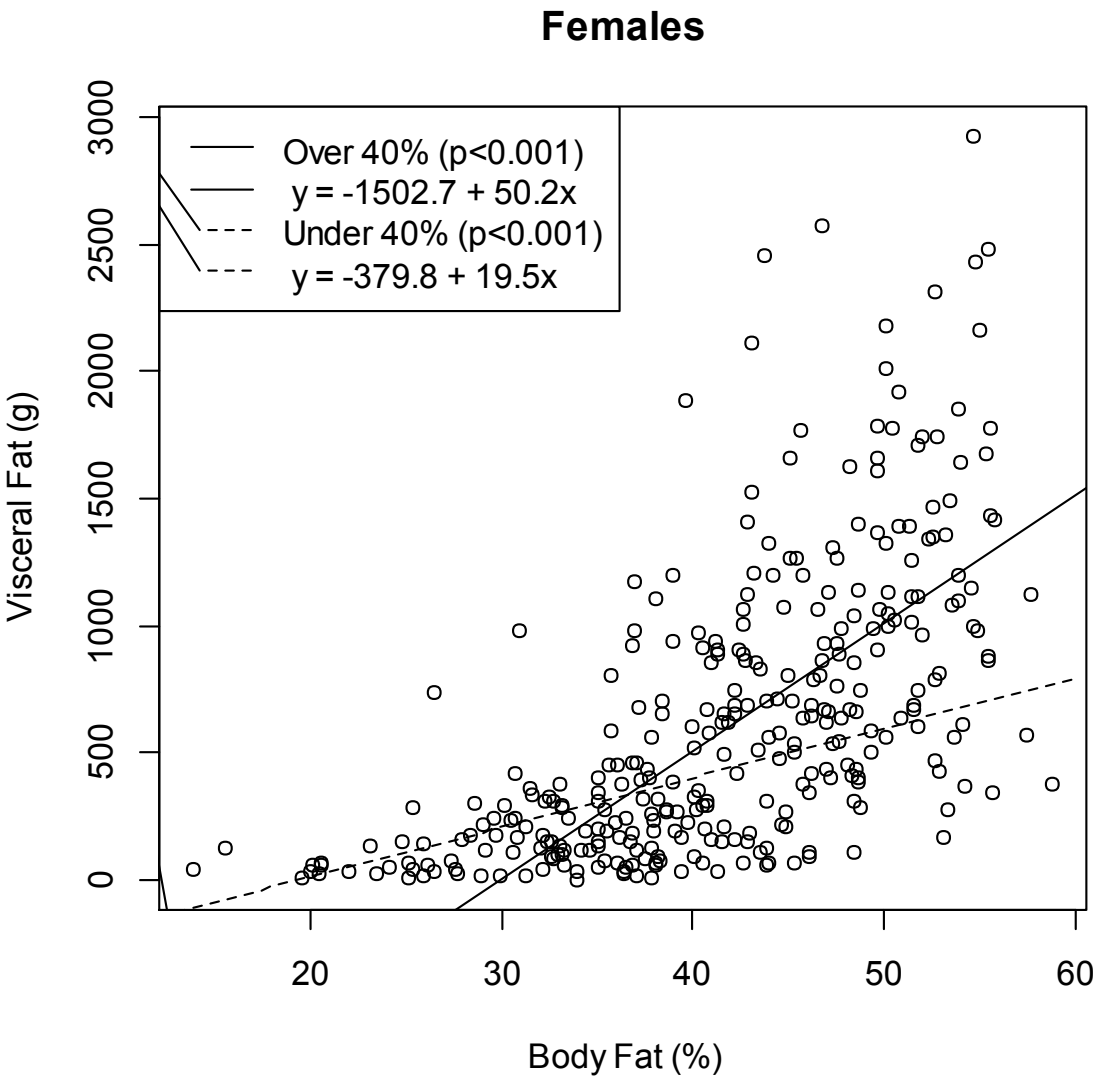

Females
Over 40% (p<0.001)
y = -1502.7 + 50.2x
Under 40% (p<0.001)
y = -379.8 + 19.5x
Visceral Fat (g)
0
500
1000
1500
2000
2500
3000
20
30
40
50
60
Body Fat (%)

Table 3: Correlation and partial correlation of DXA fat measurements in male above and below threshold

	TG	Chol	HDL-C	LDL-C	SBP	DBP	M/lbm	Glucose	Insulin
Males > 25% Body Fat									
VAT	**0.41**	**0.253**	**-0.254**	**0.193**	**0.442**	**0.370**	**-0.363**	**0.306**	**0.361**
VAT*	**0.342**	0.107	**-0.284**	0.051	**0.169**	**0.156**	**-0.318**	0.112	**0.288**
TFM	**0.193**	0.116	**-0.213**	**0.143**	**0.199**	**0.205**	**-0.232**	**0.224**	**0.375**
TFM†	**-0.150**	-0.029	0.052	0.045	-0.036	-0.011	0.078	0.050	0.079
Subq	**0.132**	0.066	**-0.201**	0.107	0.144	**0.166**	**-0.205**	**0.208**	**0.338**
Subq*	-0.089	-0.066	0.010	-0.039	-0.015	-0.001	0.013	-0.042	-0.043
Males <25% Body Fat									
VAT	**0.334**	**0.352**	0.066	**0.307**	**0.316**	**0.262**	-0.028	**0.180**	-0.121
VAT*	**0.391**	**0.351**	-0.027	**0.228**	**0.280**	**0.314**	-0.133	**0.225**	**-0.188**
TFM	-0.018	0.096	0.087	**0.157**	0.152	-0.036	0.159	-0.036	0.091
TFM†	**-0.217**	-0.038	0.064	0.007	0.006	-0.184	0.12	-0.14	**0.17**
Subq	0.013	0.120	-0.056	**0.182**	**0.183**	**-**0.047	0.160	-0.032	0.064
Subq*	0.010	0.082	0.064	0.091	0.114	**-0.37**	0.032	0.002	-0.051

* Indicates a partial correlation after adjustment for total fat mass and age

† Indicates a partial correlation after adjustment for VAT mass and age

Values that are bold indicate significance at $p<0.05$

VAT = visceral adipose tissue, TFM = total fat mass, subq = subcutaneous android fat

Table 4: Correlation and partial correlation of DXA fat measurements in females above and below threshold

	TG	Chol	HDL-C	LDL-C	SBP	DBP	M/lbm	Glucose	Insulin
Females > 40 % Body Fat									
VAT	**0.342**	0.125	**-0.263**	**0.148**	**0.264**	**0.202**	**-0.396**	0.131	**0.430**
VAT*	**0.357**	0.075	**-0.267**	0.073	-0.045	-0.056	**-0.397**	0.036	**0.276**
TFM	0.127	0.065	**-0.149**	0.111	**0.328**	**0.275**	**-0.202**	0.128	**0.384**
TFM†	**-0.186**	-0.022	0.082	0.016	**0.269**	**0.227**	0.117	0.056	0.101
Subq	**0.185**	0.066	**0.163**	0.093	**0.368**	**0.279**	**-0.222**	**0.168**	**0.389**
Subq*	**0.149**	-0.028	-0.103	-0.046	0.094	0.002	-0.124	0.104	0.141
Females < 40 % Body Fat									
VAT	**0.392**	0.083	**-0.256**	0.128	**0.195**	0.148	**-0.210**	**0.394**	**0.353**
VAT*	**0.362**	0.141	-0.104	0.108	**0.253**	**0.177**	**-0.244**	**0.369**	0.129
TFM	**0.182**	-0.047	**-0.288**	0.068	-0.006	0.015	-0.029	**0.174**	**0.426**
TFM†	-0.094	-0.119	**-0.172**	-0.005	**-0.165**	-0.101	0.081	-0.092	**0.281**
Subq	**0.187**	0.011	**-0.190**	0.076	0.030	0.054	0.015	**0.176**	**0.422**
Subq*	0.051	0.119	0.152	0.041	0.078	0.088	0.110	0.046	0.101

* Indicates a partial correlation after adjustment for total fat mass and age

† Indicates a partial correlation after adjustment for VAT mass and age

Values that are bold indicate significance at $p<0.05$

VAT = visceral adipose tissue, TFM = total fat mass, subq = subcutaneous android fat

CHAPTER 6: CONCLUSION

Research Implications

The relationship between visceral fat and cardiometabolic risk factors has been described in great detail. However the development of visceral fat derived from DXA introduces a new methodology that may provide more information about how visceral

accumulation effects cardiometabolic risk. DXA provides a reliable, total volume estimation of visceral fat that is low cost and automated. This greatly increases the feasibility of measuring visceral fat during longitudinal or intervention research. DXA should reliably measure changes in VAT since it is measuring the entire android region.

Three main findings are taken from Chapters 3, 4 and 5 that have implications for future research. First, quantification of visceral fat using DXA is a valid measurement in children and this measurement has a significant association with cardiometabolic risk factors even during childhood. A single slice estimate may not be the best method for measuring visceral fat in children. Children are small and have relatively low visceral accumulation, except in morbidly obese children. Using a single slice likely underestimates the variability in visceral fat accumulation between participants. Being able to use a total volume measurement of both visceral and subcutaneous fat may allow for a better estimation of the relative contribution of each fat compartment with cardiometabolic risk. Second, highly active fit populations still accumulate visceral fat, however, there is a percent fat adiposity threshold at which visceral mass begins to accumulate at a much steeper rate. This is an important because it may help identify visceral fat cut-points associated with increased metabolic risk. Finally, males have a lower adiposity threshold than females at which visceral fat levels begin to non-linearly increase. This difference may explain why females, generally, have lower cardiometabolic risk factors. It may also help clarify the notion of metabolically healthy obesity. This group may still close to the adiposity threshold but have a BMI greater than 30 kg/m^2.

Summary of Chapter 3

Chapter 3 described the relationship between total volume visceral fat measured by DXA (DXA-VAT) and single slice (L4-L5) visceral fat measured by CT in children. It also demonstrated the association between DXA-VAT and cardiometabolic risk factors. It demonstrated that DXA-VAT is a valid measurement in children and that it is associated with cardiometabolic risk factors. The results provide a low cost, automated method to estimated visceral fat in children that can be used by researchers.

A limitation of this study is that it compared different sized regions. There was a weak correlation between DXA-VAT and single slice CT in normal weight (BMI percentile < 85) children. This is likely caused by minimal visceral fat accumulation in these participants. A much stronger correlation was observed in overweight/obese children. This is important considering they would be most at risk for future complications. Future research could examine the role of visceral fat and its association with cardiometabolic risk factors in children. This method could also be used to measure visceral fat reduction during interventions.

Summary of Chapter 4

Chapter 4 described differences in regional body composition between professional football players. There was a specific focus on abdominal fat differences between position groups. The only fat measurement that was not significantly different between position groups was visceral fat. Further examination demonstrated that an adiposity threshold exists at which there is increases distribution of fat to the visceral region. In this population, this threshold was at 22% body fat. The adiposity threshold could be examined to see if it exists in other populations. It may help explain the

relationship between visceral fat and cardiometabolic risk factors. It may also explain why different ethnicities have different amounts of visceral adipose tissue when matched on BMI, waist circumference or total body fat. A limitation of this study is that professional football players are a unique population. It is possible that this threshold only exists in this population of highly active young males.

Summary of Chapter 5

Chapter 5 describes the existence of sex specific adiposity thresholds in a population of adults. Males have a lower threshold than females. Visceral fat accumulation above the adiposity threshold is significantly associated with cardiometabolic risk factors independent of total fat mass in males and females. Adjustment of total fat mass for visceral fat reduces the association between total fat mass and cardiometabolic risk factors. This threshold may explain the metabolically healthy obese phenotype. Those people may have a BMI over 30, but could still be near the adiposity threshold which would mean they have not started accumulating additional visceral fat. Future research could examine how these thresholds influence changes in cardiometabolic risk factors.

A limitation of this study is that the population was mainly Caucasian and African American. The adiposity thresholds observed in this population may not be applicable in other ethnicities. I hypothesize that the adiposity threshold may be different in other ethnicities, Asian populations have demonstrated higher VAT at a given level of adiposity, and this suggests they may have a lower threshold compared to the threshold observed in our population.

Clinical Significance of the Research

This dissertation establishes DXA-VAT as a valid method for estimation of visceral fat in children. This provides clinicians a more feasible method to monitor visceral fat accumulation in patients. DXA-VAT could be used to monitor at risk patients with high BMIs to determine if they have started accumulating excess visceral fat. The demonstration of adiposity thresholds associated with increased visceral fat accumulation could be used to identify at risk patients and determine if they have accumulated visceral fat associated with increased cardiometabolic risk. Additionally, it could be used to monitor visceral fat changes in patients attempting weight loss. This study did identify that males had a similar adiposity threshold at a BMI of 24 kg/m^2. This is more clinically significant. However there was not a clear threshold observed in women.

Suggestions for Future Research

Future research directions need to identify the clinical utility of visceral fat measured by DXA. Previous research has demonstrated that while visceral fat is the best measurement for predicting the presence of metabolic syndrome, it is not any better than waist circumference. This needs to be determined for DXA. If DXA does not provide any better prediction than waist circumference than the usefulness of this new method is diminished in the clinical setting. An examination of the change in inflammatory cytokines should also be examined. It would be interesting to measure the differences in serum concentration of cytokines in populations above and below the adiposity threshold. This may provide a mechanism by which visceral fat accumulation causes an increase in cardiometabolic risk. Finally, the adiposity threshold should be compared to

accumulation in other ectopic regions to determine if there is accumulation in the liver and muscle at the same time, or if there is a delayed threshold for accumulation in these areas.

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