THE ASSOCIATIONS OF REGIONAL BODY COMPONENTS WITH
CARDIOVASCULAR DISEASE METABOLIC RISK FACTORS IN THE
MORBIDLY OBESE

BY

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DEDICATION

This thesis is dedicated to my soon-to-be husband, Brennan MacMillan. This was the final big event between us and our marriage- bring on the wedding! Thanks for being supportive of my dream to move out of state and pursue a master's degree in a field I am so passionate about. Your genuine love for me allowed you to be so willing to let me go and I've never overlooked that. I've also not overlooked the fact that a part of you also went through this program over the last two years and I don't think you were anticipating that. Thanks for listening to my presentations, getting me through biomechanics, for being so in tune with everything I've been going through at school and being my biggest encourager. I'm excited to start our life together.
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ABSTRACT

It is well accepted that there is a growing obesity epidemic in the United States and around the world. Even more alarming are the rates at which morbid obesity is increasing. From 2000-2005 the prevalence of a BMI >40.0 kg/m² increased by 52% which is twice the rate moderate obesity increased and the prevalence of a BMI >50.0 kg/m² increased by 75%, which is 3 times this rate. Due to the rapidly expanding morbidly obese population and the further risk they are subjected to metabolically, physically and in terms of morbidity and mortality, there is an urgent need to better understand the relationships as well as causes between health risks and excessive adipose tissue in the body. The purpose of this study was to examine associations of percent body fat, thigh muscle volume and four different types of fat depots: visceral adipose tissue, abdominal and thigh intermuscular adipose tissue, abdominal and thigh subcutaneous adipose tissue and thigh intramuscular adipose tissue, to several cardiovascular disease metabolic risk factors: inflammation, blood lipids, glucose, insulin, HOMA and blood pressure. This observational, cross-sectional study provided pre-surgery body composition measures of 28 individuals scheduled to undergo bariatric surgery. Descriptive statistics, correlational analyses and multiple linear regression were performed on the data. There was a statistically significant positive correlation between percent fat and plasma triglycerides (r= .384, n= 27, p= .048). A positive correlation between the volume of thigh muscle and insulin (r= .67, n= 15, p= .007). There was a significant, positive correlation between the volume of visceral fat and the total number of comorbidities (r= .50, n= 18, p=}
.038), and a negative correlation with diastolic blood pressure (r= -.50, n= 18, p< .038). The multiple regression analyses revealed two statistically significant models. Thigh muscle volume and percent fat created a model explaining 52.6% of the variance in fasting plasma insulin. Visceral adipose tissue and percent fat created a significant model explaining 39% of the variance in diastolic blood pressure. Overall, this study found selected noteworthy correlations but was limited by a small, homogenous sample and was unable to make definite conclusions about the association of regional body components to cardiovascular disease metabolic risk factors in the severely obese population. These results and future studies in this area can shed light on potential improvements health care providers can expect when helping this population lose weight.
INTRODUCTION

OBESITY AND MORBID OBESITY

It is well accepted that there is a growing obesity epidemic in the United States and around the world. Even more alarming are the rates at which morbid obesity is increasing. In 2007, Sturm showed that over the past 20 years the heaviest BMI categories (BMI ≥45.0 kg/m² and >50.0 kg/m²) increased significantly faster than the class I (BMI 30.0-34.9 kg/m²) and II (BMI 35.0-39.9 kg/m²) categories. This is illustrated in Figure 1 with the lines increasing in slope with heavier BMI categories. From 2000-2005 the prevalence of a BMI >40.0 kg/m² increased by 52% which is twice the rate moderate obesity increased and the prevalence of a BMI >50.0 kg/m² increased by 75%, which is 3 times this rate. These rates are troublesome because the most threatening health conditions are associated with morbid obesity (BMI ≥40.0 kg/m²) \(^1\). As of 2010, the age-adjusted prevalence of obesity was 35.5% among adult men and 35.8% among adult women \(^2\).
The literature thoroughly documents obesity’s association with the development of type 2 diabetes mellitus, coronary heart disease and its risk factors (hyperlipidemia, hypertension, and impaired glucose tolerance), stroke, respiratory complications, osteoarthritis and an increased incidence of certain forms of cancers 3–6. Furthermore, obesity is a predictor of longevity, with risk of death increasing with each pound of excess weight 7,8. This evidence not only points out the danger of obesity, but highlights the increased risk that occurs with excessive weight gain seen in morbid obesity.

There is substantial research comparing obese to desired-weight persons and the risk that is involved in carrying excessive fat in amounts categorized as moderately obese. In a review by Hubert, he identifies several studies whose results consistently show a graded, linear relationship between some type of
obesity measure and coronary heart disease morbidity or mortality or show significant associations. For example, the Manitoba Study showed that men whose BMI was 22.6-25.0 kg/m² experienced almost twice the risk of coronary heart disease over 26 years compared to those who BMI was less than 22.6 kg/m². However, there lacks an equivalent comprehensive knowledge of the ramifications of morbid obesity and comparisons of health risks between obese and morbidly obese persons. An added emphasis needs to be placed specifically on those in the morbid obese category who have been shown to have an even greater mortality risk.

In a prospective cohort of U.S. adults, the risk for all-cause mortality increased throughout the range of moderate and severe overweight for both genders of all ages. In detail, Figure 2 and 3 show the all-cause mortality relative risk for each BMI category for both men and women and also compares risk within each gender between current or former smokers with disease history and without disease history and also nonsmokers with a disease history and without a disease history. The relationship between relative risk of death and BMI can most clearly be seen looking at the solid thick line on the graph of figure 2 which represents nonsmokers with no history of disease. In both men and women the slope of the line drastically starts to increase as BMIs start to exceed 30 kg/m². This same study also reported a general pattern of increased mortality risk across increasing BMI categories in both white and black men and women. In both white males and females the mortality risk more than doubled by the final BMI category (≥40 kg/m²), black women at this BMI category had a relative risk
of 1.21 and data was only available on black males until the 32.0 - 34.9 kg/m² category at which their relative risk was 1.35. However, both genders and races showed an increased mortality risk in all BMI categories beyond the referent category (BMI 23.5 - 24.9 kg/m²) ¹⁰.

**Figure 2. Multivariate Relative Risk Of Death From All Causes Among Men According To BMI, Smoking Status and Disease Status (Calle et al. 1999).**
Fontaine et al. illustrated the relationship between mortality and obesity through their study looking at the degree and age of onset of overweight or obesity and the impact this has on years of life lost. This study additionally emphasized the increased risk that occurs with greater degrees of obesity. Data was gathered from several large-scale epidemiological studies to estimate the expected number of years of life lost (YLL) due to overweight and obesity (as measured by BMI) over an adult’s lifespan. There were distinct race and sex differences in estimated YLL; however, across both races and genders for any given degree of overweight, the YLL were generally greater for younger adults than older adults. Seen especially in whites, was a J-shaped association between overweight and obesity and YLL. Thus, the more years one is overweight or obese, the greater YLL and the greater degree of overweight or
obesity, the greater YLL. The greatest YLL in white men and women was seen in men and women aged 20-30 years with a BMI >45, with men losing 13 years and women 8. This translates into a 17% and 10% reduction in total life expectancy respectively. In black men and women, the greatest YLL was seen in men aged 20-30 with 20 years lost and in women aged 20-40 with 5 years lost which translates into a 29% and 6.6% reduction in total life expectancy respectively.\textsuperscript{11}.

Individuals categorized by BMI as morbidly obese are also at an increased risk for many of the diseases and complications that plague the obese population. In the Nurse’s Cohort Study, BMI was the leading predictor of diabetes risk after age adjustment. Specifically, women with a BMI between 30-34.9 kg/m\(^2\), had a 28-fold increase in risk of diabetes while those with a BMI >35 kg/m\(^2\) had a 93-fold increased risk of diabetes compared to those with a BMI of less than 21 kg/m\(^2\).\textsuperscript{12} In men, compared to a BMI of less than 21 kg/m\(^2\), the risk of diabetes increased 6.7-fold with a BMI between 29 and 30 kg/m\(^2\) and 42-fold with a BMI of >35 kg/m\(^2\).\textsuperscript{13} Across genders, even approaching the morbid obese category indicates significantly greater risk for diabetes.

The Nurse’s Cohort Study also reported that the risk for coronary heart disease and stroke increases as BMI increases. Compared to women with a BMI <21 kg/m\(^2\), risk for coronary heart disease increased 3.6-fold in women with a BMI >29 kg/m\(^2\) while those with a BMI of 25 to 28.9 kg/m\(^2\) only had a twofold increase.\textsuperscript{14} Stroke risk increased 1.75-fold in those with a BMI of 27 to 28.9 kg/m\(^2\), 1.90-fold with BMI 29 to 31.9 kg/m\(^2\) and 2.37-fold for BMI ≥32 kg/m\(^2\), as
compared to those with a BMI <21 kg/m². In addition, a J-shaped relation between BMI and overall mortality was observed in women of the Nurse’s Health Study. Using a BMI of <19 kg/m² as the referent group, mortality risk increased with increasing BMI; specifically, risk increased from 1.2-fold with BMI 19 to 21.9 kg/m² to over 2-fold with BMI ≥32 kg/m².

Beyond mortality rates and metabolic dysfunction, severe obesity also impairs function. Recently, it was shown in middle-aged women that obesity impairs physical function, with the greatest impairments being in the most severe BMI category (≥40 kg/m²). These findings held true whether physical function was measured using performance based tests or questionnaire based in self-report.

Due to the rapidly expanding morbidly obese population and the further risk they are subjected to metabolically, physically and in terms of morbidity and mortality, there is an urgent need to better understand the relationships as well as causes between health risks and excessive adipose tissue in the body.
REVIEW OF THE LITERATURE

FAT DISTRIBUTION

Visceral Fat

The profound changes that occur physiologically with increased body fat can partially be determined by the distribution of the adipose tissue. It has been known since the 1950s that upper-body obesity is more closely associated with diabetes and atherosclerosis than lower body obesity. Today, fat in the body is further broken down into different categories. Visceral fat, also known as intra-abdominal or periomental fat is found within the body cavity and in and around the internal organs. These visceral fat depots represent about 20% of the total body fat in men and 6% in women. Excess visceral fat is commonly referred to as abdominal or central obesity and is hallmarked by the pot-belly and apple-shaped appearance.

There is accumulating evidence that central obesity is particularly predictive of metabolic syndrome and increased risk of cardiovascular disease. A number of other studies also point to visceral fat as the chief contributor to the development of hypertension, elevated plasma insulin concentrations and insulin resistance, diabetes mellitus and hyperlipidemia. Even when examined independently of total body obesity, visceral fat content was a strong indicator of insulin resistance. Waist circumference is highly correlated with visceral adipose tissue, and in a study by Janssen et al. they found that in...
both males and females across all BMI categories the prevalence of hypertension, type 2 diabetes, dyslipidemia and the metabolic syndrome tended to be higher in those with above normal waist circumference values (males >102 cm, females >88 cm). This study found that regardless of weight, the health risk is greater in those with high waist circumference values or greater amounts of visceral adipose tissue. The metabolic complications of obesity in general are attributed to increases in visceral adipose tissue and the central obesity pattern more than any other type of fat tissue and pattern. This is suggestive that there are unique properties of visceral adipose tissue that play a significant role in the development of these cardiovascular disease risk factors.

A number of studies have studied the unique properties of visceral adipose tissue and compared its effect on metabolic function with other fat deposits. Both Fain et al. and Kershaw and Flier compared the properties of abdominal visceral adipose tissue and subcutaneous adipose tissue. Their studies revealed similar findings; that visceral adipose tissue explants released more vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1). VEGF, IL-6 and PAI-1, when overexpressed, are all linked to the development of certain cancers. In addition, PAI-1 has been linked to the metabolic syndrome while IL-6 is also a pro-inflammatory cytokine. Kershaw’s study also showed that visceral adipose tissue was more inversely associated with adiponectin levels than subcutaneous adipose tissue and this was in agreement with another study by Kwon et al.
Other studies have shown visceral adipose tissue to have greater lipolytic activity and to be more resistant to the metabolic effects of insulin \(^{39–41}\).

Subcutaneous Fat

Subcutaneous fat is most commonly measured in the thigh and abdomen and consists of adipose tissue located directly below the skin. Past studies have not been as consistent showing an association between subcutaneous fat and insulin resistance as they have with visceral fat and insulin resistance. McLaughlin et al. studied overweight and moderately obese men and women and after controlling for BMI and visceral adipose tissue, it was found that increased levels of subcutaneous adipose tissue at the abdomen and the thigh actually decreased an individual’s risk for insulin resistance \(^{30}\). In a separate study, subcutaneous adipose tissue of the thigh was shown to have no association with insulin resistance \(^{42}\). In contrast, three other studies using both lean and obese subjects found a positive association between abdominal subcutaneous adipose tissue and insulin resistance \(^{43–45}\), while Kelley and colleagues additionally showed that abdominal visceral and subcutaneous adipose tissue have similar associations with insulin resistance. However, in Kelley’s study, the measures of abdominal subcutaneous adipose tissue were broken down into deep and superficial subcutaneous adipose tissue, and it was this deep subcutaneous adipose tissue that showed similar associations as visceral adipose tissue. Superficial subcutaneous adipose tissue did not show any significant associations \(^{46}\). In a very similar study to Kelly et al., it was also found that
abdominal visceral and subcutaneous adipose tissue were both good predictors of metabolic risk. Most recently, the Framingham Heart Study which examined nearly 3,000 individuals showed that both visceral and subcutaneous adipose tissue were correlates of insulin resistance measures, however visceral adipose tissue had a greater strength of association than subcutaneous adipose tissue.

Decreased levels of adiponectin, an antiinflammatory hormone released from adipose tissue is characteristic of obesity and the metabolic syndrome; several studies have shown that serum adiponectin is inversely associated with insulin resistance. Studies found that subcutaneous adipose tissue was inversely associated with serum adiponectin while visceral adipose tissue was not. It should be noted however; that there is still contradicting research whether or not subcutaneous adipose tissue does indeed have a negative association with serum adiponectin.

Intermuscular Fat

Intermuscular fat, which is adipose tissue located between muscle cells, is commonly associated with physical functioning levels, especially in the elderly, as age-related changes in regional body composition occurs. In the last several years however, intermuscular fat has been shown to affect metabolic function. Most often, intermuscular fat is assessed and refers to fat within the thigh or leg muscles. In 2000, Goodpaster et al. found that in middle-aged men and women with type 2 diabetes, increased adipose tissue existed between the muscles. The intermuscular fat in these individuals was significantly
associated with insulin resistance while subcutaneous adipose tissue was not\textsuperscript{59}. Later, Goodpaster and colleagues also found that even in subjects with similar levels of subcutaneous fat, intermuscular fat was higher in those with type 2 diabetes and impaired glucose tolerance\textsuperscript{29}. Recently, a study showed that in terms of metabolic variables, intermuscular thigh fat had similar effects as visceral fat\textsuperscript{58}.

**Intramuscular Fat**

Intramuscular fat is the final classification of fat commonly measured and is also referred to as attenuation fat or fatty infiltration. This type of fat is found within the muscle fibers, rather than between the muscle fibers. It used to be this type of fat could only be assessed through a muscle biopsy; however several years ago it was found that CT scans could assess this type of fat through attenuation values\textsuperscript{42}. Fat has a strongly negative attenuation value on a CT imaging where muscle has a higher attenuation value; a lower attenuation value found in muscle is indicative of fat deposition within the muscle. However, some researchers argue this is still a very rough estimate and muscle biopsies are required to make more accurate conclusions\textsuperscript{60}.

Several studies show that there is an association between the amount of fat infiltration into the mid-thigh muscle and mobility performance and muscular strength. Greater fat attenuation resulted in poorer function and fat infiltration levels of the mid-thigh muscle and could additionally predict mobility limitations\textsuperscript{55,56,61}. Most of the research involving intramuscular fat describes its effect on
function rather than metabolic variables because of its close association with sarcopenia. Generally with age, the muscles lose mass and the lipid content within the muscle increases\(^2\)\(^3\).

However, there is a growing body of evidence that shows an association between intramuscular fat and obesity-related insulin resistance and type 2 diabetes\(^2\)\(^9\),\(^4\)\(^5\),\(^6\)\(^4\),\(^5\)\(^9\),\(^6\)\(^4\). In addition to finding a relationship between intramuscular fat and insulin resistance, one study also reported a relationship between intramuscular fat and the central obesity weight pattern\(^6\)\(^5\). Further, Goodpaster and colleagues showed intramuscular fat to have a positive association with BMI\(^6\)\(^0\).

**METABOLIC DYSFUNCTION**

**Inflammation**

Obesity’s association with insulin resistance, dyslipidemia, hypertension and an overall reduced life expectancy can be explained by the chronic low-grade inflammatory state the body is under when there is excessive adipose tissue\(^6\)\(^6\),\(^6\)\(^7\). Besides being involved in energy storage, adipose tissue also functions as an endocrine organ. As an endocrine organ, adipose tissue secretes bioactive substances, generally referred to as adipokines or adipocytokines, that maintain normal metabolism through regulation of food intake, insulin action, lipid and glucose metabolism, regulation of blood pressure and coagulation\(^6\)\(^8\). However, these substances can promote inflammatory responses and metabolic
dysfunction when dysregulated. Thus, several of these adipokines are involved in the pathophysiology of various obesity-linked metabolic diseases and complications. Pro-inflammatory adipokines have been found to be upregulated in those with obesity and diabetes\textsuperscript{69}. Dysregulated expression of adipokines can occur from excessive adipose tissue in the body and adipocyte cell dysfunction\textsuperscript{70,71}.

Several studies in this area of research have identified various adipokines as either pro or anti-inflammatory agents. Pro-inflammatory adipokines include leptin, tumor necrosis factor-alpha (TNF-\(\alpha\)), IL-6, resistin, retinol-binding protein 4 (RBP4), lipocalin 2, IL-18, angiopoietin-like protein 2 (ANGPTL2), CC-chemokine ligand 2 (CCL2), CXC-chemokine ligand 5 (CXCL5) and nicotinamide phosphoribosyltransferase (NAMPT)\textsuperscript{69}. On the other hand, adiponectin has been identified as having a protective effect against numerous metabolic and cardiovascular disorders, and levels are decreased in obesity\textsuperscript{72–74}.

The inflammatory process begins with excessive adipose tissue gain accompanied by adipocyte hypertrophy. Adipocytes are the main cellular component of adipose tissue. With the onset of obesity, adipocytes start to hypertrophy as they attempt to store more triglycerides\textsuperscript{69}. Despite the pliable nature of adipocytes, there is a breaking point to how large the adipocyte can expand before becoming dysfunctional. In an attempt to restore the now damaged cell, the remodeling process begins with macrophages infiltrating the adipose tissue; these macrophages alone account for a majority of the pro-
inflammatory cytokines that are released \(^{75}\). At this point stress signals are released and it is the variety of released stress signals that activate the inflammatory pathways which begin upregulating the pro-inflammatory agents \(^{76–78}\). The adipokine profile is now completely changed.

Adipokines such as leptin and resistin cause inflammation specifically through promoting the production and expression of other pro-inflammatory adipokines, with resistin additionally countering the effects of adiponectin \(^{79–82}\). IL-6 levels, along with positively correlating with adipose tissue, are also increased in those with type 2 diabetes \(^{83}\). Additionally, IL-6 elicits increments in C-reactive protein levels. C-reactive protein (CRP) is a pro-inflammatory marker found in the blood. Excess adipose tissue has been found to be associated with elevated levels of CRP. CRP levels are also predictive of the development of type 2 diabetes \(^{84}\). Other adipokines operate under similar pathways, but the exact mechanisms through which each of these pro-inflammatory adipokines cause inflammation is beyond the scope of this study. These findings demonstrate that adipokines play an important role in proper metabolic function. Metabolic dysfunction results when there is an imbalance in the expression of pro- and anti-inflammatory adipokines, as occurs with excessive adipose tissue.

There are several depots in the body where adipose tissue is found and adipocytokine expression can vary depending on location \(^{85,86}\). In conjunction with this, the exact consequence that results from excessive adipose tissue in terms of adipocytokine expression also vary with the site where it accumulates,
highlighting the importance of studying the relationship between fat distribution and metabolic variables.

Insulin Resistance

Insulin resistance can also be defined as altered insulin sensitivity, type 2 diabetes mellitus, and impaired glucose tolerance. Insulin is released from beta cells in the pancreas when elevated levels of glucose are detected in the blood, signaling muscle and adipose tissues to absorb glucose. This process lowers blood glucose levels until 5mmol/L of glucose is maintained in the blood. Insulin resistance is when normal levels of insulin fail to have the desired effect on the muscle and adipose cells causing glucose levels to remain high. The pancreas then continues to release more insulin which can have damaging effects. This leads to hyperinsulinemia. Prolonged insulin resistance can progress to type 2 diabetes and leads to dysregulation of a number of metabolic processes in the cell.

One mechanism for the development of insulin resistance begins with excessive adipose tissue gain and the inflammation process. The bioactive substances released by adipose tissue, among other functions, are fundamental to proper regulation of insulin action and lipid and glucose metabolism. Signaling by membrane receptors is what regulates several of the cell functions in the body. Through a highly integrated network, insulin signaling controls several processes. In the presence of insulin, the insulin receptor (IR) phosphorylates insulin receptor substrate proteins (IRS proteins) which are a part of the
activation of two main signaling pathways: the phosphatidylinositol 3-kinase (PI3K)- AKT/protein kinase B (PKB) pathway, responsible for almost all of the metabolic actions of insulin, and the Ras-mitogen-activated protein kinase (MAPK) pathway, which regulates expression of some genes and in conjunction with another pathway controls cell growth and differentiation. Tanaguchi and colleagues identified three main critical nodes involved in the insulin-signaling pathway: the IR/IRS, the PI3K heterodimer and AKT/PKB. Thus, when just one substance becomes dysregulated, many pathways are affected which ultimately disturbs communication with the central and peripheral tissues.
During metabolic dysregulation (created by obesity and a high-fat diet) the IKK-beta/NF-kappa-B and JNK pathways are activated in adipocytes, hepatocytes and associated macrophages (see Figure 4). There are a variety of stimuli responsible for activating these pathways which include: tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1beta), toll-like receptors (TLRs), receptor for advanced glycation endproducts (RAGE), advanced glycation endproducts (AGE), tumor necrosis factor receptor (TNFR), interleukin-1 receptor
(IL-1R), reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, ceramides and protein kinase C enzymes (PKCs); all of which are described below.

The first possible stimuli include ligands for proinflammatory cytokines: TNF-alpha and IL-1beta, both released by adipocyte cells. These cytokines activate IKK-beta/NF-kappa-B and JNK pathways through classical receptor-mediated mechanisms. The IKK-beta/NF-kappa-B and JNK pathways can also be activated by pattern recognition receptors which are surface proteins that recognize foreign substances; these include the TLRs which include microbial products and lipopeptides and the RAGE. RAGE can also bind to a variety of ligands including endogenous AGEs, TNFR and IL-1R. AGEs in excess production can activate the NF-kappa-B pathway which occurs during conditions of prolonged hyperglycemia.

Cellular stresses, including the ROS and ER stress, can additionally activate JNK and NF-kappa-B pathways. ROS activates these pathways through the following mechanism: As a result of lipid accumulation in the adipocyte, systemic markers of oxidative stress increase which triggers an increase in ROS production. This mechanism increases the production of TNF-alpha, IL-6, and monocyte chemoattractant protein-1 (MCP-1) and decreases the production of adiponectin.

Increased levels of nonesterified fatty acids (NEFAs) initiate the ER stress due to the processing demand. Lipid accumulation also increases ER stress by
activating the unfolded protein response. The ER is well described by Ozcan et al. as a membranous network that synthesizes and processes secretory and membrane proteins and transports materials through the cell. When the ER is under stress, signals are sent that disrupt insulin metabolism leading to inhibition of insulin action. This stress specifically activates the JNK pathway leading to serine phosphorylation of insulin receptor substrate-1 (IRS-1), while also activating the NF-kappa-B pathway. As a result, the liver increases its production of glucose, a pathway normally suppressed by insulin. Additionally, the inflammatory cytokines, TNF-alpha, and IL-6, secreted by macrophages disturb proper insulin signaling and its pathways.

Saturated fats and cell stress both contribute to the formation of ceramides which is another stimulus for activating the JNK and NF-kappa-B pathways. Ceramides promote cell signaling and regulation of apoptosis and accumulate in tissues such as muscle. They have been found to possibly correlate with the degree of insulin resistance. Excess lipids also increase the activities of various protein kinase C (PKC) isoforms which activate IKK-beta and NF-kappa-B.

The main pathways that are activated by the stimuli described above are the IKK-beta/NF-kappa-B and JNK pathways and can be clearly seen in Figure 4. The IKK-beta activation leads to NF-kappa-B translocation and the increased expression of several markers and potential mediators of inflammation that cause insulin resistance. The JNK activation promotes the phosphorylation of insulin.
receptor substrate-1 (IRS-1) at serine sites that disrupt normal signaling through the insulin receptor/IRS-1 axis. Examples include serine-302 (pS302) and serine-307 (pS307). (There is no evidence for obesity-induced effects regulated by JNK on transcription factors such as AP-1, hence the question mark on this pathway in Figure 4). Further, IKK-beta and NF-kappa-B are inhibited or repressed by the actions of salicylates, thiazolidinediones and statins which are sometimes used to improve insulin resistance.

Insulin resistance is accepted as a key contributor to cardiovascular disease risk factors and the underlying cause for metabolic syndrome. Metabolic syndrome is a clustering of several cardiovascular disease risk factors, primarily central obesity, type 2 diabetes, dyslipidemia and hypertension. It is crucial to study this condition and the diseases that influence it because of the serious threat it poses to health. In a recent meta-analysis compiling data from over 950,000 patients, it was found that metabolic syndrome was associated with an increased risk for cardiovascular disease (RR: 2.35; 95% CI: 2.02 to 2.73), cardiovascular disease mortality (RR: 2.40; 95% CI: 1.87 to 3.08) and all-cause mortality (RR: 1.58; 95% CI: 1.39 to 1.78).

Another recent study studying overweight and moderately obese individuals found that after adjustment for BMI, greater absolute or relative visceral adipose tissue increases the risk for insulin resistance, whereas increased absolute or relative subcutaneous adipose tissue actually decreased the risk for insulin resistance. The results of the logistic regression analysis
showed that each subcutaneous adipose tissue increment was associated with a 42% decreased risk of being insulin resistant, while each visceral adipose tissue increment was associated with an 80% increased risk of insulin resistance.30

Research supports a strong link between visceral adipose tissue and insulin resistance. The main mechanism supporting this link being visceral adipose tissue’s capability to produce proinflammatory cytokines such as TNF-alpha and IL-1 and-6 which are players in the processes that result in whole body insulin resistance. These cytokines can activate the dangerous IKK-beta/NF-kappa-B and JNK pathways which promote transcription of cytokine genes and lead to insulin resistance.67 Other articles agree, and were touched on previously, that visceral fat is a far more important risk factor to control than subcutaneous fat, especially for metabolic syndrome.6,21,25,28,29 A few articles even point out that visceral compared to subcutaneous adipose tissue secretes far greater amounts of the proinflammatory cytokines such as CRP, IL-6 and TNF-alpha.36,94 Recent research has shown visceral fat’s involvement in the pathogenesis and prognosis of nonalcoholic fatty liver disease (NAFLD).95,96 One of the main mechanisms being that the livers of obese subjects show a dramatic upregulation of inflammatory cytokines which can be explained by the livers close proximity and downstream position to the visceral fat pads.70

There are several methods used to diagnose insulin resistance including the glucose or hyperinsulinemic-euglycemic clamp technique, the insulin-suppression test, measuring plasma insulin concentrations during either a fasted
or post-glucose state, the insulin-tolerance test, the oral glucose tolerance test, the constant glucose infusion with model assessment (CIGMA) and the homeostatic model assessment (HOMA) which was used to assess insulin resistance in the present study.

The glucose clamp is considered the gold standard for evaluating insulin sensitivity. This technique requires a steady IV infusion of insulin into one arm of the subject to create a hyperinsulinaemic plateau and in the other arm the plasma glucose concentration is clamped at the normal fasting level by performing exogenous infusion of glucose. Numerous blood samples are then collected to monitor comparable conditions of stimulus (insulin) and substrate (glucose). When a steady state is reached and the exogenous glucose infusion rate equals the amount of glucose disposal by all the tissues, the overall insulin sensitivity can be quantified. The insulin-suppression test is a reverse clamp where during infusion of insulin, glucose is infused exogenously at a constant rate while the plasma glucose concentration varies. When steady state is reached, the insulin levels will be similar, but the plasma glucose levels will vary and these values will provide estimates of insulin resistance.

Measuring the circulating level of insulin in plasma can also be performed using a fasting or post-glucose condition. Insulin resistance is determined based on standard values. The insulin-tolerance test is based on the measurement of the rate of decay of plasma glucose after an IV bolus of regular insulin. Several insulin and glucose levels are sampled over a period of time following the
injection and are plotted; the slope of the plotted line can be calculated and used to rank insulin sensitivity. The oral glucose tolerance test involves ingesting a glucose-rich load, usually in the form of a beverage, with blood drawn at various intervals after consumption to measure glucose levels. This test is measuring the body’s ability to metabolize glucose.

CIGMA and HOMA both determine insulin sensitivity from steady glucose and insulin concentrations; CIGMA measures concentrations after a standardized one-hour intravenous glucose infusion, while HOMA measures concentrations under basal conditions. Calculations are then performed for relative insulin resistance (R) using functions from a model of glucose homeostasis. This model accounts for glucose distribution, production and utilization. In HOMA, R equals the product of the fasting values of glucose (expressed as mmol/L) and insulin (expressed as µU/mL) divided by a constant of 22.5. HOMA values will be increased in insulin resistant subjects and have been shown to correlate well with the gold standard clamp technique.97

The role that each of the main fat depots play in the development of insulin resistance needs to be further examined as much of the research has focused primarily on visceral and subcutaneous adipose tissue. Of the research that has been done, there is still conflicting results across studies as to which fat depot plays the biggest role. Partly contributing to this lack of consensus is the wide range of study participant BMI and age. Since those with morbid obesity are
most affected by morbidity and mortality it is imperative to understand the underlying mechanisms to these diseases particularly in this population.

Dyslipidemia

Obesity, especially the central obesity weight pattern, is a risk factor for dyslipidemia. Dyslipidemia refers to an abnormal amount of lipids in the blood. Lipids are organic compounds used for energy storage, as structural components of the cell membrane and also for cell signaling. The discussion of dyslipidemia related to obesity will focus on hyperlipidemia or an abnormally high amount of lipids in the blood and decreased high density lipoproteins (HDLs). Hyperlipidemia can further be classified into subgroups: high cholesterol, hypercholesterolemia or high triglycerides, hypertriglyceridemia.
Insulin resistance is a main player in the pathophysiology of dyslipidemia as related to obesity. During insulin resistance, carbohydrate and lipid metabolism are impaired. Insulin normally facilitates lipid synthesis while inhibiting lipolysis and regulating glucose production by the liver. When insulin is inhibited, the liver increases its production of glucose and increased lipolysis releases non-esterified fatty acids (NEFAs). TNF-alpha, a cytokine that increases its circulation in obesity, also increases the concentration of NEFAs through stimulating adipocytes. These NEFAs soon infiltrate the liver, contributing to fat
accumulation and triglyceride synthesis in the liver. Very low density lipoprotein (VLDL) concentrations increase accordingly as they must transport the abundance of triglycerides which increases the blood triglyceride concentration.

Another key factor resulting in increased VLDLs is the rate of apolipoprotein B-100 (apoB-100) degradation. If this protein is not degraded it contributes to VLDL production. Insulin actually is very important in the degradation of this protein, and with its action inhibited the body is unable to suppress apoB-100 production. On top of greater VLDL production, VLDL clearance is compromised. VLDL clearance is accomplished through lipoprotein lipase (LPL) or by the LDL receptor (LDLR). Insulin stimulates lipoprotein lipase (LPL) activity by increasing its rate of synthesis. LPL activity in insulin resistant individuals is lower due to defective insulin. The LDLR capabilities also become hindered as the pathways in which it operates are insulin dependent. Lipoprotein metabolism then becomes disrupted, resulting in decreased clearance of VLDL.

Both the increase in VLDL production and decrease in clearance contribute to consistently increased levels of other triglyceride-rich lipoproteins (TRLs) including chylomicrons. The abundance of TRLs changes the metabolism of VLDL to LDL causing an increased exchange between cholesterol esters in LDL and triglycerides in VLDL. This results in LDL particles that are enriched in triglycerides and stripped of their cholesterol and phospholipid content, making them smaller and denser. The modifications to the LDL particles cause the
particle to more easily move through openings in the endothelial where they can contribute to plaque formation. The particles are also more difficult to clear from the body which contributes to elevated levels in plasma in obese individuals.99

Further contributing to dyslipidemia is a decrease in HDL. Decreased HDL levels are caused by the impaired exchange between cholesterol esters in LDL and triglycerides in VLDL. This happens through decreasing the transfer of apolipoproteins and phospholipids from VLDL to the HDL compartment. Additionally, HDL size is decreased and HDL clearance increased to due greater concentrations of TRLs and increased activity of hepatic lipids. Per increased triglycerides, abnormal LDL composition and decreased HDL levels the lipid profile is completely altered and an increased risk of cardiovascular disease is present.99

Hypertension

Hypertension, or high blood pressure, is another risk factor for cardiovascular disease and the final main metabolic complication discussed here. Similar to the previous metabolic abnormalities, the development of hypertension cannot be attributed to a single mechanism; however obesity and the inflammation process play a major role. The principle mechanism responsible for obesity-related hypertension is thought to be an increased renal tubular sodium and water reabsorption. Factors contributing to this malfunction include the activation of the sympathetic nervous system (SNS), the renin-angiotensin system (RAS) and the hormone aldosterone.102
Hemodynamic changes initially take place with increases in regional blood flow, cardiac output and heart rate. Part of the reason these changes take place are to accommodate the additional blood flow needed by extra adipose tissue. In order to meet the increased metabolic demands of carrying excessive adipose tissue, there is also an increase in blood flow to organs such as the heart and kidneys and also to the gastrointestinal tract and skeletal muscle\(^{103}\).

One of the major players in obesity-related hypertension is an increase in sympathetic nervous system (SNS) activity. The SNS is mainly responsible for
initiating the stress (fight-or-flight) response and counteracting the parasympathetic nervous system which maintains the body at a resting state. As a result of sympathetic stimulation, the heart increases the rate and force of contraction, blood vessels are constricted and the kidneys increase renin secretion, amongst several other changes to organ activity\textsuperscript{104}. However, some studies have observed increased sympathetic stimulation only at the kidneys\textsuperscript{105,106}. This kind of chronic stimulation, especially at the resting state is what can cause an increase in blood pressure as the body attempts to overcome the changes.

There are several proposed mechanisms for SNS activation. High caloric intake can activate the SNS through increasing norepinephrine turnover in peripheral tissues. This raises resting norephinephrine concentrations and intensifies the release of this hormone in response to even the smallest stimuli such as standing up\textsuperscript{104}. There is also evidence of high fat and carbohydrate diets stimulating peripheral a1- and b-adrenergic receptors which increases sympathetic activity\textsuperscript{107}. However, central stimulation by leptin and hyperinsulinemia are proposed as the central mechanisms for increased SNS activity\textsuperscript{102}.

Leptin is a protein hormone derived from adipose tissue which acts on the hypothalamus to regulate energy intake and expenditure\textsuperscript{108}. This is accomplished primarily through leptin’s ability to decrease food consumption and upregulate thermogenesis and energy expenditure by stimulating SNS activity.
after eating. Leptin is secreted from adipose tissue in direct proportion to the amount of adiposity in an individual; therefore obese individuals have high levels of circulating leptin. Despite these high levels, the normal metabolic actions of leptin are absent during obesity. Leptin no longer signals to reduce food intake and increase energy expenditure, yet it still stimulates the SNS; this is known as selective resistance, and while a few explanations have been proposed, it is not fully understood why this happens 104.

Insulin resistance and hyperinsulinemia are common features of obesity and represent another mechanism through which hypertension develops. Insulin directly acts on the renal tubes and normally exhibits a sodium retaining effect. During hyperinsulinemia there is enhanced sodium retention placing a greater processing demand on the kidneys. Another pathway through which insulin can cause hypertension is by activating the SNS. Insulin release leads to hypoglycemia which is an activator of the SNS. Additionally, hyperinsulinemia increases muscular glucose uptake and oxygen demands which create a vasodilator response and activation of the baroreceptor reflex and enhanced muscle SNS activity. In some individuals this vasodilator response is impaired, creating vascular dysfunction 104.

Abnormal activation of the renin-angiotensin system (RAS) can also raise blood pressure mainly through the effects of angiotensin II and aldosterone. The RAS is a system of hormones that regulates blood pressure and fluid balance. When the system senses low blood volume renin is secreted from the kidneys
which initiates the process of creating angiotensin II. Angiotensin II enhances vascular tone by causing blood vessels to constrict, increasing blood pressure. Angiotensin II also promotes aldosterone secretion from the adrenal cortex. Aldosterone is a hormone that triggers the kidneys to increase the reabsorption of sodium and water into the blood. This increases the volume of fluid in the body and blood pressure \(^{102}\).

Additionally, fat accumulation around the kidneys can cause physical compression and can even penetrate into the renal medulla causing increased intrarenal pressure. This affects the flow rate of fluids through this system; less volume detected will lead to activation of the RAS to secrete renin \(^{109}\). Another mechanism through which the RAS can be activated is chronic activation of the SNS which causes renal vasoconstriction limiting blood volume flow \(^{104}\). It is also important to consider that aldosterone alone can have potent effects without working through the RAS. Adipose tissue secretes mineralocorticoid-releasing factors into circulation which upon reaching the adrenal glands cause the secretion of aldosterone. Fatty acids released by adipose tissue and oxidized via the liver will also increase aldosterone secretion \(^{102}\).

The increased renal tubular reabsorption of sodium and water that results from activation of both the SNS and RAS then disturbs renal-pressure natiuresis. Also altering pressure natiuresis are the physical changes that occur in the renal structure due to fat accumulation. This altered state is both a result and cause of hypertension. Pressure natiuresis is a mechanism through which the kidneys
regulate blood pressure by increasing the amount of sodium along with water that is excreted in urine when there is increased arterial pressure. When pressure natiuresis is altered, increases in sodium intake do not lead to the normal response of sodium excretion which leads to chronic increases in extracellular fluid volume and blood volume, causing hypertension 110.

According to the Framingham Heart Study, approximately 78% of cases of hypertension in men and 65% of cases in women can be directly attributed to obesity 111. Other epidemiological evidence suggests that hypertension is the most common comorbidity associated with obesity, indicating that more than 60% of obese persons are hypertensive 112. Despite the research gains that have been made in the pathophysiology of hypertension, research is still incomplete in this area and the exact mechanisms leading to the obesity-associated changes need to be identified. Particularly, more needs to be learned of hypertension in the morbidly obese since they are affected to a greater degree by the effects of excessive adipose tissue.

LIMITATIONS OF PREVIOUS RESEARCH

The impact of body fat stores in obesity, namely morbid obesity, on health risks is clearly documented. Although several studies have examined the relationships between various fat depots, there still lacks a clear consensus as to the influence of each type of fat on the cardiovascular metabolic profile. Each of the main regional sites for fat distribution have been studied and related to at least one metabolic variable in separate studies; however no single study has
looked at visceral, subcutaneous, intermuscular and intramuscular adipose tissue sites, as well as other body components such as thigh muscle volume and percent body fat and examined their relationship to a host of cardiovascular disease risk metabolic variables in this morbidly obese cohort.

As documented above, several studies have used CT scanning or MRI imaging to compare abdominal visceral and subcutaneous adipose tissue’s associations with cardiovascular disease metabolic risk factors, particular insulin resistance. Most of these studies show visceral adipose tissue has a stronger association than thigh and abdominal subcutaneous adipose tissue with insulin resistance 28,31,32,36; however there is still conflicting studies showing that subcutaneous adipose tissue is more closely associated with the pathways that cause metabolic dysfunction 25,44,53. Several studies have examined thigh subcutaneous adipose tissue along with abdominal visceral and subcutaneous adipose tissue, but again the focus is on associations with insulin and glucose measures. Most of these studies show visceral adipose tissue to have a stronger association to metabolic function than thigh subcutaneous adipose tissue 27,30,33 while there are still conflicting results shown in other studies 45,46. There were only two studies found that examined three or more types of fat, with no studies examining intermuscular fat of the abdomen 29,58. Yim et al. examined total body visceral, subcutaneous and intermuscular adipose tissue while Goodpaster examined abdominal visceral and subcutaneous adipose tissue, thigh subcutaneous intermuscular adipose tissue as well as intramuscular fat of the thigh. Results in both of these studies focused on associations with insulin and
glucose and all three fat compartments. Further, almost all of these studies were in a moderate obese population. Thus, there is clearly a lack of work in this area in the morbidly obese population. With the growing prevalence of this level of obesity in our country, this area of research is important; identifying the main fat deposits that are strongly associated with metabolic dysfunction would facilitate in developing strategies to reduce the fat compartments that pose the biggest threat. This area of research would especially benefit the morbid obese population who are often restricted from conventional weight loss strategies due to severe comorbidities and complications.

Therefore the specific purpose of this study was to examine associations of percent body fat, thigh muscle volume and four different types of fat depots: visceral adipose tissue, abdominal and thigh intermuscular adipose tissue, abdominal and thigh subcutaneous adipose tissue and thigh intramuscular adipose tissue, to several cardiovascular disease metabolic risk factors: inflammation, blood lipids, glucose, insulin, HOMA and blood pressure.

Based on the previous research described and the subsequent aim, the main hypothesis is that total fat and all fat depots (subcutaneous fat and intermuscular fat of the thigh and abdomen, intramuscular fat of the thigh and visceral fat), assessed by CT scanning, will show associations with the inflammatory marker CRP, blood lipids, glucose, insulin and HOMA such that higher fat will have a worsening of these risk factors. An additional hypothesis is
that visceral adipose tissue will have the strongest correlations to the cardiovascular disease metabolic risk factors as compared to all other fat depots.
METHODOLOGY

STUDY POPULATION

Participants for this analysis were combined from two separate studies. The cohort of women from the first study were part of an observational pilot designed to investigate the effect of laparoscopic Roux-en-Y gastric bypass (RYGB) on performance and self-report based on physical function in those with extreme obesity. Participants consisted of 18 female patients recruited from the general surgery clinic at Wake Forest University Baptist Medical Center (WFUBMC) scheduled for laparoscopic RYGB. Eligibility criteria included a BMI ≥40 kg/m² or ≥35 kg/m² with an obesity-related comorbidity, such as diabetes, hypertension or dyslipidemia. In addition, they had to have reported a sedentary lifestyle and to have self-reported difficulty in performing at least one of the following activities attributed to back, hip, knee, or ankle pain: lifting and carrying groceries, walking one-quarter mile, getting in and out of a chair, or going up and down stairs.

Data from the second study came from a randomized controlled trial pilot investigating the effect of exercise training on body composition, inflammation and physical function following bariatric surgery. Subjects included 10 women (ages between 30-50 years) with a BMI ≥ 35.0 kg/m² who underwent adjustable gastric banding or RYGB for weight loss through the WFUBMC general surgery department. Individuals were excluded if they had an unstable medical condition or condition where exercise was contraindicated (ex. unstable angina, frailty,
unstable diabetes) as well as currently undergoing treatment for cancer. In addition, those that were unwilling to modify exercise patterns, those living farther than 50 miles from the exercise training center, those with severe depression or known excessive alcohol consumption were excluded from the study. Individuals had to agree to report to the testing facilities three times a week for six months in order to effectively comply with the study. The primary recruiting strategy was through their surgeon at WFUBMC, co-investigator of this study, Dr. Fernandez and his staff. Initial screening for major eligibility criteria was made at a pre-surgery appointment with the physician and medical staff. If interested, eligible contacts were invited to a clinic visit for further eligibility screening at which time all participants were given written informed consent to participate in the study according to the guidelines of the Wake Forest University Institutional Review Board.

STUDY DESIGN

This observational, cross-sectional study provided baseline (pre-surgery) body composition measures of 28 individuals that were scheduled to undergo bariatric surgery. The independent variables were body composition measures which included: percent body fat, regional muscle volume of the thigh, intermuscular fat of the thigh and abdomen, intramuscular fat of the thigh, subcutaneous adipose tissue of the thigh and abdomen and visceral adipose tissue. The outcome variables for this analysis were cardiovascular disease metabolic risk factors which included: the inflammatory biomarker CRP, blood
lipids (total cholesterol, LDL cholesterol, HDL cholesterol, total triglycerides), plasma glucose, plasma insulin, HOMA, number of comorbidities, resting blood pressure and mean arterial pressure.

PROCEDURES

Upon meeting eligibility criteria and consenting to the study, participants were scheduled for testing visits. Participants reported to the Geriatric Research Center (GRC) and the General Clinical Research Center (GCRC) for testing. The following assessments were obtained at these visits: demographics, a 12-hour fasting blood sample to measure serum concentrations of CRP, blood lipids, glucose, insulin and resting systolic and diastolic blood pressure. At a second visit a bioelectrical impedance analysis and computed tomography (CT) scan yielded body composition information.

Demographics and Blood Pressure

Initially, medical history was collected using chart records. Participants were asked to bring in their current medications and these were recorded by research staff. Body mass and height were measured using standard techniques. Briefly, weight and height were determined with shoes and jackets or outer garments removed. Instruments were calibrated on a weekly basis. BMI was calculated from these measures (weight in kg, divided by height in squared meters). Resting systolic and diastolic blood pressure were determined using standard techniques. From the resting systolic (SBP) and diastolic blood
pressure (DBP) mean arterial pressure (MAP) was calculated using the following formula: MAP = DBP + 1/3 (SBP - DBP).

C-Reactive Protein

CRP was assessed from aliquots of serum obtained at baseline. Enzyme-linked immunosorbent assays (ELISA) were used to determine the serum concentrations of CRP. A reference interval of 0.00-3.00 was used. All samples were measured in duplicate and the average of the two values was used for data analyses. Laboratory Corporation of America (LabCorp) performed all analyses. (Laboratory Corporation of America Holdings 531 S. Spring Street, Burlington, NC 27215).

Lipids, Glucose and Insulin Measures

Concentrations of lipids, glucose, and insulin were measured from 12 hour fasted blood. Blood was drawn in the morning after an overnight fast, during which time the individual consumed only water. These assessments were used to determine the cardiovascular metabolic health risk. Triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol were obtained for the lipoprotein profile. Specifically for analyzing plasma glucose, a minimum volume of 0.5 mL of plasma was collected in a gray-top (sodium fluoride) tube. In the plasma insulin analysis, 0.8 mL of plasma was collected in a red-top or gel-barrier tube. A reference interval of 2.6-24.9 μU/mL was used. Laboratory Corporation of America (LabCorp) performed all analyses. HOMA was calculated using the following formula: fasting insulin (μU/mL) x fasting glucose (mmol/L) / 22.5$^{113}$. 
Comorbidities and the Metabolic Syndrome

The presence of the following physician diagnosed comorbidities was also found from each subject's medical records by the GCRC staff: diabetes, gallbladder disease, hypertension, dyslipidemia, cardiovascular disease, degenerative joint disease, sleep apnea, liver disease, gastroesophageal reflux disease and depression. Additionally, metabolic syndrome was considered a comorbidity if the subject met the criteria established by the American Heart Association. The American Heart Association's definition of metabolic syndrome is a cluster of metabolic risk factors. They diagnose metabolic syndrome when a person has three or more of the following measurements: abdominal obesity (waist circumference >40 in for males; >35 in for females), triglyceride level of 150 mg/dL of blood or greater, HDL cholesterol less than 50 mg/dL (for women), systolic blood pressure of 130 mm Hg or greater, diastolic blood pressure of 85 mm Hg or greater, fasting glucose of 100 mg/dL or greater, insulin resistance or glucose intolerance.

Body Composition

Total body fat mass and lean body mass (relative and absolute levels) were determined using bioelectrical impedance assessment (BIA) (RJL Quantum II Desktop, Clinton Township, Michigan). Body composition estimates for this instrument have been validated based on NHANES III dataset. Individuals were in a supine position for this test. Regional assessments of the thigh and abdomen areas were made by computed tomography to assess thigh muscle volume, as well as regional volume of fat in visceral adipose tissue of the abdomen, subcutaneous adipose tissue of the thigh and abdomen.
Intermuscular adipose tissue volume of the thigh and abdomen and intramuscular adipose tissue attenuation of the thigh were also measured by computed tomography. Relative and absolute levels of total body fat mass and lean body mass

For computed tomography (CT) scans, (LightSpeed Plus™, General Electric Medical Systems, Milwaukee) subjects were positioned supine with the arms above the head and legs positioned flat. The scan parameters for the thigh were set at 120 kV and 350 mA. Approximately 50 scans were obtained covering the entire femur from the hip through the knee joint. Abdominal scans were obtained for visceral adipose tissue with the participant in the same position and scan parameters were set at: helical mode, 120 kVp, 250 mA, 4 x 2.5 mm collimation, standard reconstruction kernel, and a display field-of-view of 500 mm. CT slices within 15 mm centered at the L4-5 level were used to calculate volume.

Quantitative measures of muscle and adipose tissue, as well as CT attenuation values for intramuscular fat, were determined with the GE Healthcare, Advantage Windows 4.2 Volume Viewer (Waukesha, WI). The total volume of non-adipose, non-bone tissue within the deep fascial plane was used as a measure of muscle volume (cm³). The average value across both legs was used in data analyses. For visceral adipose tissue, the inner and outer aspects of the abdominal wall were traced, and visceral fat was defined as fat enclosed by the inner aspect of the abdominal wall. Subcutaneous fat was defined as the fat outside the outer aspects of the abdominal wall.
STATISTICAL ANALYSIS

All data were checked for normality using the Kolmogorov-Smirnov test of normality. Descriptive statistics (including means, standard deviations, frequency and minimum and maximum values) were determined. Pearson correlations were used to determine the relationships between body composition variables (muscle thigh volume, intermuscular fat of the thigh, intramuscular fat of the thigh, abdominal visceral adipose tissue and abdominal intermuscular fat) and cardiovascular metabolic risk factors (inflammation, blood lipids, glucose, insulin, HOMA, blood pressure and CRP). All participants were included for determining correlations. Differences were deemed significant at P< 0.05. In addition, multiple linear regression was used to examine associations between body composition variables that were significantly associated with the cardiovascular metabolic risk factors and to determine the amount of variance in the cardiovascular disease metabolic risk factors explained by the fat depots in our sample of severely obese women. All analyses were performed using the Statistical Package for Social Sciences, version 19.0 (SPSS, Chicago, IL).
RESULTS

DEMOGRAPHICS

Baseline characteristics for the 28 individuals in the study are categorized as demographic information, body composition and metabolic variables and are displayed in Tables I, II and III. Participants were all female with the race being predominantly Caucasian (86%), and the remainder African American. The mean age of the group was 45.0 years with a range from 27.1 to 59.1 years. The number of obesity related co-morbidities ranged from 0 to 9 with each individual having on average about 4 conditions. Some of the most common comorbidities included metabolic syndrome, degenerative joint disease, gastroesophageal reflux disease, hypertension, depression, sleep apnea, dyslipidemia and diabetes. All participants were scheduled for bariatric surgery prior to informing them about the study and consenting them into the study.
### Table I. Demographic Characteristics (n=28)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
<th>Minimum - Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Years)</strong></td>
<td>45.0 (8.3)</td>
<td>27.1- 59.1</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>86% Caucasian</td>
<td>Caucasian= 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>African American= 4</td>
</tr>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td>139.6 (25.6)</td>
<td>92.3- 193.9</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td>4.2 (2.4)</td>
<td>0- 9</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>78.6%</td>
<td></td>
</tr>
<tr>
<td>Degenerative Joint Disease</td>
<td>67.9%</td>
<td></td>
</tr>
<tr>
<td>Gastroesophageal Reflux Disease</td>
<td>67.9%</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>60.7%</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Sleep Apnea</td>
<td>42.9%</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>39.3%</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>32.1%</td>
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</tr>
</tbody>
</table>

n=27 for Body Weight
BODY COMPOSITION

Table II presents the BMI and body composition data for each participant. The mean BMI was 51.4 kg/m² with a mean percent body fat of 57.5%. All but 3 subjects fell into the Class III or morbid obesity category (BMI ≥40.0 kg/m²) and these 3 were all in the Class II obesity category (BMI ≥35.0- 39.9 kg/m²) with significant comorbidities. In terms of thigh composition the average amount of intermuscular fat of the thigh was 65.2 cm³, the average muscle volume in the thigh was 768.6 cm³. Attenuation of the thigh muscle, as a measure of intramuscular fat, was 60.8 HU. Abdominal visceral fat volume was 1466.5 cm³ and intermuscular abdominal fat was 108.0 cm³. For both thigh and abdominal areas, subcutaneous fat was not obtained due to part of the adipose tissue lying outside the scanner for the majority of participants.
Table II. Body Composition Measures

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
<th>Minimum - Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>51.4 (8.2)</td>
<td>36.1 - 65.9</td>
</tr>
<tr>
<td><strong>Total- BIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat (kg)</td>
<td>79.0 (24.2)</td>
<td>40.8 - 127.9</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>55.4 (7.8 )</td>
<td>41.0 - 66.1</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>60.8 (6.3 )</td>
<td>49.4 - 70.8</td>
</tr>
<tr>
<td>Lean Mass (%)</td>
<td>44.7 (7.8 )</td>
<td>33.9 - 59.0</td>
</tr>
<tr>
<td><strong>Thigh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle Volume (cm³)</td>
<td>768.6 (159.6)</td>
<td>539.1 - 1167.7</td>
</tr>
<tr>
<td>Intermuscular Fat (cm³)</td>
<td>65.2 (32.3)</td>
<td>27.3 - 152.8</td>
</tr>
<tr>
<td>Intramuscular Fat (HU)</td>
<td>60.8 (9.1)</td>
<td>40.3 - 80.7</td>
</tr>
<tr>
<td><strong>Abdominal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral Fat (cm³)</td>
<td>1466.5 (563.3)</td>
<td>573.7 - 2580.5</td>
</tr>
<tr>
<td>Intermuscular Fat (cm³)</td>
<td>108.0 (54.7)</td>
<td>44.9 - 224.8</td>
</tr>
</tbody>
</table>

n= 27 for BMI, Body Fat (kg), Body Fat (%), Lean Mass (kg) and Lean Mass (%)

n=17 for Thigh Muscle Volume and Thigh Intermuscular Fat

n=18 for Intramuscular Fat, Visceral Fat and Abdominal Intermuscular Fat.
CARDIOVASCULAR DISEASE METABOLIC RISK FACTORS

Specific values for the metabolic variables can be viewed in Table III. Whereas the mean systolic and diastolic blood pressure were 132.6 and 75.5 mmHg, respectively, according to patients’ self-report of physician diagnosed hypertension almost 61% were hypertensive. According to the ACSM classification of blood pressure for adults 55.6% of the sample was prehypertensive (SBP 120-139 mm Hg or DBP 80-89), 18.5% of the sample was classified as having Stage 1 hypertension (SBP 140-159 mm Hg or DBP 90-99 mm Hg), and 7.4% of the sample was classified as having Stage 2 hypertension (SBP ≥160 mm Hg or DBP ≥100 mm Hg). The average CRP value was 6.2 mg/L as compared to the 3.0 mg/L cut point which indicates elevated concentration and high risk for a future cardiovascular event according to the American Heart Association. There was a wide range of CRP values (0.47-31.73 mg/L) in our sample, however over half (53.6%) were in the high risk for a future cardiovascular event category based on the American Heart Association guidelines.

Wide ranges of values were also present in the variables constituting the blood lipid profile, plasma glucose and plasma insulin. Based on a physician diagnosis, 39.3% of subjects had dyslipidemia. According to the guidelines set forth by the American Heart Association, (endorsed by the National Cholesterol Education Program (NCEP) guidelines for detection of high cholesterol) for cardiovascular disease risk solely based on triglyceride levels, about 60% of the
sample fell within normal ranges interpreted as low risk \[^{114}\]. Using ACSM guidelines for defining dyslipidemia, 42.9% of the sample had high total cholesterol \((\geq 200 \text{mg/dL})\), 7.1% of the sample had low HDL cholesterol \((<40 \text{mg/dL})\) and 28.6% of the sample had high LDL cholesterol \((\geq 130 \text{mg/dL})\) \[^{116}\].

Average plasma glucose was 108.8 mg/dL which is indicative of prediabetes according to the American College of Sports Medicine criteria \((\text{fasting plasma glucose} \geq 100 \text{mg/dL})\) \[^{116}\]. Looking at individual scores, 45.8% of the sample was prediabetic using the same criteria from above. About 17% of the sample was classified as having diabetes according to the American Diabetes Association guidelines \((\text{fasting plasma glucose} > 126 \text{mg/dL})\) \[^{117}\]. According to self-reported physician diagnosis, 32% of our sample was diabetic. The average plasma insulin level was 26.8 µU/mL. There are not firmly established norms for fasting plasma insulin levels, however 12 µU/mL is commonly used by authors as the cutoff between subjects with and without insulin resistance \[^{118,119}\]. Our average levels are above this cutoff as well as 78.6% of the sample. The average HOMA value was 7.1 for our sample. There is a lack of consensus on the cut-off points for classification of insulin resistance using HOMA. Recently, data from the third National Surveillance of Risk Factors of Non-Communicable Disease concluded that 1.775 was the optimal cut-off for the diagnosis of metabolic syndrome in non-diabetic individuals. In those with diabetes, this cut-off was determined to be 3.875 \[^{120}\]. According to these criteria, our average HOMA value was higher than this cut-off as well as about 83% of the sample. Combining some of these metabolic risk factors we were able to estimate that almost 80% of
our sample had metabolic syndrome based on the criteria set forth by the American Heart Association\textsuperscript{114}. 
### Table III. Metabolic Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
<th>Minimum - Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood Pressure (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>132.6 (14.6)</td>
<td>108- 166</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.5 (10.0)</td>
<td>52- 93</td>
</tr>
<tr>
<td>Mean Arterial Pressure</td>
<td>94.5 (9.9)</td>
<td>74- 112</td>
</tr>
<tr>
<td><strong>C-Reactive Protein (mg/L)</strong></td>
<td>6.2 (7.6)</td>
<td>0.47- 31.73</td>
</tr>
<tr>
<td><strong>Blood Lipids (mg/dL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>196.9 (40.5)</td>
<td>144- 304</td>
</tr>
<tr>
<td>Low Density Lipoprotein</td>
<td>118.3 (34.9)</td>
<td>56- 220</td>
</tr>
<tr>
<td>High Density Lipoprotein</td>
<td>50.1 (10.6)</td>
<td>38- 82</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>142.1 (60.7)</td>
<td>57-279</td>
</tr>
<tr>
<td><strong>Plasma Glucose (mg/dL)</strong></td>
<td>109.8 (34.4)</td>
<td>81- 243</td>
</tr>
<tr>
<td><strong>Plasma Insulin (µU/mL)</strong></td>
<td>26.8 (15.1)</td>
<td>3.5- 54.7</td>
</tr>
<tr>
<td><strong>HOMA (units)</strong></td>
<td>7.1 (6.5)</td>
<td>0.84- 26.82</td>
</tr>
<tr>
<td>(Homeostatic Model Assessment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic Syndrome</strong></td>
<td>78.6%</td>
<td></td>
</tr>
</tbody>
</table>

n= 27 for Systolic and Diastolic Blood Pressure and Mean Arterial Pressure
n= 24 for Plasma Glucose
n= 26 for Plasma Insulin
n= 23 for HOMA

CORRELATION ANALYSIS

The relationships between body composition (as measured by thigh muscle volume, thigh intermuscular fat volume, thigh intramuscular fat, abdominal visceral fat volume and abdominal intermuscular fat volume) and cardiovascular disease metabolic risk factors (as measured by number of total comorbidities, presence of metabolic syndrome, systolic blood pressure, diastolic blood pressure, mean arterial pressure, insulin, total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and HOMA) was investigated using Pearson product-moment correlation coefficient and can be viewed in Table IV. Preliminary analysis indicated two variables were not normally distributed, glucose and CRP, and for these variables Spearman rank order correlations were performed and can be viewed in Table V.

There was a statistically significant positive correlation between percent fat as determined by DEXA and plasma triglycerides, \( r = .384, n = 27, p = .048 \). A positive correlation is also seen between the volume of thigh muscle and insulin, \( r = .67, n = 15, p = .007 \), with higher muscle volume in the thigh associated with higher insulin levels. There was a significant, positive correlation between the volume of visceral adipose tissue and the total number of comorbidities, \( r = .50, n = 18, p = .038 \), and a negative correlation with diastolic blood pressure, \( r = -.50, n = 18, p < .038 \), with the higher volume of visceral adipose tissue associated with a greater number of comorbidities and a lower diastolic blood pressure.
Additionally, because of the small sample size and the preliminary analysis these correlations serve, trends towards significance \((p<.15)\) were also noted. There were several additional associations using this criteria, including between percent fat and total number of comorbidities \((r= .318, p= .106)\), metabolic syndrome \((r= .318, p= .106)\), diastolic blood pressure \((r= -.307, p= .119)\) and fasting plasma insulin \((r= .380, p= .061)\). Between thigh muscle volume and HOMA \((r= .514, p= .088)\), between thigh intermuscular fat volume and metabolic syndrome \((r= .385, p=.127)\), and between thigh intramuscular fat and systolic blood pressure \((r= -.389, p=.111)\), fasting plasma insulin \((r= .489, p=.055)\) and triglycerides \((r= -.354 p=.150)\). Of the two abdominal fat depots only intermuscular fat volume had trends toward significant correlations and those were between total comorbidities \((r= .375, p= .125)\), total cholesterol \((r=.397, p= .103)\) and triglycerides \((r=.452, p= .060)\).
Table IV. Pearson Product Moment Correlations Of Regional Body Components With Cardiovascular Disease Metabolic Risk Factors [r value, (p value)]

<table>
<thead>
<tr>
<th></th>
<th>Percent Fat (DEXA)</th>
<th>Thigh Muscle Volume</th>
<th>Intermuscular Thigh Fat Volume</th>
<th>Intramuscular Thigh Fat (Attenuation)</th>
<th>Visceral Fat Volume</th>
<th>Intermuscular Abdominal Fat Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r value</td>
<td>.318 (*)</td>
<td>-.143</td>
<td>.233</td>
<td>-.275</td>
<td>.492*</td>
<td>.375</td>
</tr>
<tr>
<td>P value</td>
<td>(.106)</td>
<td>(.583)</td>
<td>(.368)</td>
<td>(.270)</td>
<td>(.038)</td>
<td>(.125)</td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td>.318</td>
<td>.169</td>
<td>.385</td>
<td>-.082</td>
<td>.265</td>
<td>.296</td>
</tr>
<tr>
<td></td>
<td>(.106)</td>
<td>(.516)</td>
<td>(.127)</td>
<td>(.746)</td>
<td>(.288)</td>
<td>(.233)</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>.071</td>
<td>-.076</td>
<td>-.327</td>
<td>-.389</td>
<td>.107</td>
<td>-.064</td>
</tr>
<tr>
<td></td>
<td>(.726)</td>
<td>(.771)</td>
<td>(.200)</td>
<td>(.111)</td>
<td>(.673)</td>
<td>(.801)</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>-.307</td>
<td>.164</td>
<td>-.313</td>
<td>-.022</td>
<td>-.493*</td>
<td>-.353</td>
</tr>
<tr>
<td></td>
<td>(.119)</td>
<td>(.529)</td>
<td>(.221)</td>
<td>(.931)</td>
<td>(.038)</td>
<td>(.151)</td>
</tr>
<tr>
<td>Mean Arterial Pressure</td>
<td>-.165</td>
<td>.087</td>
<td>-.357</td>
<td>-.179</td>
<td>-.305</td>
<td>-.290</td>
</tr>
<tr>
<td></td>
<td>(.411)</td>
<td>(.740)</td>
<td>(.159)</td>
<td>(.478)</td>
<td>(.218)</td>
<td>(.243)</td>
</tr>
<tr>
<td>Fasting Plasma Insulin</td>
<td>.380</td>
<td>.667*</td>
<td>.277</td>
<td>.489</td>
<td>-.028</td>
<td>-.329</td>
</tr>
<tr>
<td></td>
<td>(.061)</td>
<td>(.007)</td>
<td>(.318)</td>
<td>(.055)</td>
<td>(.918)</td>
<td>(.214)</td>
</tr>
<tr>
<td>Plasma Total Cholesterol</td>
<td>.133</td>
<td>-.095</td>
<td>-.212</td>
<td>-.256</td>
<td>-.040</td>
<td>.397</td>
</tr>
<tr>
<td></td>
<td>(.509)</td>
<td>(.717)</td>
<td>(.414)</td>
<td>(.306)</td>
<td>(.876)</td>
<td>(.103)</td>
</tr>
<tr>
<td>Plasma Triglycerides</td>
<td>.384*</td>
<td>-.315</td>
<td>.292</td>
<td>-.354</td>
<td>.374</td>
<td>.452</td>
</tr>
<tr>
<td></td>
<td>(.048)</td>
<td>(.218)</td>
<td>(.255)</td>
<td>(.150)</td>
<td>(.218)</td>
<td>(.060)</td>
</tr>
<tr>
<td>Plasma High Density Lipoprotein</td>
<td>.004</td>
<td>-.295</td>
<td>-.151</td>
<td>-.560</td>
<td>.105</td>
<td>.132</td>
</tr>
<tr>
<td></td>
<td>(.985)</td>
<td>(.250)</td>
<td>(.563)</td>
<td>(.825)</td>
<td>(.678)</td>
<td>(.601)</td>
</tr>
<tr>
<td>Plasma Low Density Lipoprotein</td>
<td>.019</td>
<td>.092</td>
<td>-.317</td>
<td>-.169</td>
<td>-.179</td>
<td>.281</td>
</tr>
<tr>
<td></td>
<td>(.924)</td>
<td>(.724)</td>
<td>(.215)</td>
<td>(.503)</td>
<td>(.478)</td>
<td>(.259)</td>
</tr>
<tr>
<td>Homeostatic Model Assessment (HOMA)</td>
<td>.292</td>
<td>.514</td>
<td>.143</td>
<td>.360</td>
<td>-.062</td>
<td>-.403</td>
</tr>
<tr>
<td></td>
<td>(.187)</td>
<td>(.088)</td>
<td>(.657)</td>
<td>(.226)</td>
<td>(.841)</td>
<td>(.172)</td>
</tr>
</tbody>
</table>

*. Correlation is significant at the 0.05 level
Table V. Spearman Rank Correlations Of Regional Body Components With Cardiovascular Disease Metabolic Risk Factors, Glucose And C-Reactive Protein [rho value, (p value)]

<table>
<thead>
<tr>
<th></th>
<th>Percent Fat (DEXA)</th>
<th>Thigh Muscle Volume</th>
<th>Intermuscular Thigh Fat Volume</th>
<th>Intramuscular Thigh Fat (Attenuation)</th>
<th>Visceral Fat Volume</th>
<th>Intramuscular Abdominal Fat Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R value</td>
<td>.250</td>
<td>.190</td>
<td>.176</td>
<td>-.051</td>
<td>.249</td>
<td>-.018</td>
</tr>
<tr>
<td>P value</td>
<td>(.249)</td>
<td>(.534)</td>
<td>(.564)</td>
<td>(.863)</td>
<td>(.391)</td>
<td>(.952)</td>
</tr>
<tr>
<td>C-Reactive Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R value</td>
<td>-.063</td>
<td>-.120</td>
<td>.093</td>
<td>-.278</td>
<td>.265</td>
<td>-.100</td>
</tr>
<tr>
<td>P value</td>
<td>(.755)</td>
<td>(.646)</td>
<td>(.722)</td>
<td>(.265)</td>
<td>(.287)</td>
<td>(.693)</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level

MULTIPLE REGRESSION ANALYSIS

Multiple regression was used for further analysis of the relationship between body composition variables and common cardiovascular disease metabolic risk factors. Because of the nature of this study in that it only examined baseline data, this analysis was not used to predict cardiovascular disease metabolic risk factors from body composition variables, but to demonstrate relationships between variables. Preliminary analyses were conducted to ensure no violation of the assumptions of multiple regression. From the Pearson product-moment correlation coefficients, only body composition variables significantly associated with cardiovascular metabolic risk factors were entered; the variables were: percent body fat and triglycerides, thigh muscle volume and insulin; visceral adipose tissue and total number of comorbidities and visceral adipose tissue and diastolic blood pressure. Due to only one independent
variable being significant for each dependent variable, percent body fat was forced into all the equations in order to get a true multiple regression analysis. No multiple regression test was performed for percent body fat and triglycerides due to the lack of another independent variable. Three separate multiple regression tests were run with insulin, total number of comorbidities and diastolic blood pressure being the dependent variables for each individual test.

After entry of thigh muscle volume and percent body fat into the equation for insulin, the total variance explained by the model was 52.6% (p= .011). Thigh muscle volume did not have an independent association, with a p value of .179. Percent body fat remained significantly associated with plasma insulin (p= .007), with a standardized beta value of .648. The next model, using visceral adipose tissue and percent body fat to show associations with total number of comorbidities, was not significant (p= .072). Neither visceral fat (p= .147) nor percent body fat (p= .301) had significant independent associations. A final multiple regression analysis was run entering visceral adipose tissue and percent body fat into the equation for diastolic blood pressure. This model was significant, p= .025, and explained 39% of the variance in diastolic blood pressure. Neither visceral adipose tissue (p= .215) or percent body fat (p= .076) had independent associations.
Table VI. Multiple Regression Analysis: Association Between Insulin And Thigh Muscle Volume, Between Total Comorbidities And Visceral Fat And Between Diastolic Blood Pressure And Visceral Fat

<table>
<thead>
<tr>
<th>Variable</th>
<th>R</th>
<th>R²</th>
<th>Adjusted R²</th>
<th>Standardized Beta</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Thigh Muscle Volume</td>
<td>.725</td>
<td>.526</td>
<td>.447</td>
<td>.285</td>
<td>.011</td>
</tr>
<tr>
<td>-Percent Body Fat</td>
<td></td>
<td></td>
<td></td>
<td>.648</td>
<td>.179</td>
</tr>
<tr>
<td>Comorbidites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Visceral Fat</td>
<td>.544</td>
<td>.296</td>
<td>.203</td>
<td>.372</td>
<td>.072</td>
</tr>
<tr>
<td>-Percent Body Fat</td>
<td></td>
<td></td>
<td></td>
<td>.261</td>
<td>.147</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Visceral Fat</td>
<td>.624</td>
<td>.390</td>
<td>.309</td>
<td>-.294</td>
<td>.025</td>
</tr>
<tr>
<td>-Percent Body Fat</td>
<td></td>
<td></td>
<td></td>
<td>-.432</td>
<td>.215</td>
</tr>
</tbody>
</table>

Table represents three separate linear regression models using the dependent variables: Fasting plasma insulin, total comorbidities and diastolic blood pressure.
DISCUSSION

The primary purpose of this study was to examine the associations between percent body fat, thigh muscle volume and regional fat depots and several common cardiovascular disease metabolic risk factors in the severely obese population. Overall, this study found selected noteworthy correlations. The relevance of these results is discussed below.

Obesity is a complex physiological state, likely due to the endocrine properties of adipose tissue. In excessive levels, this endocrine organ has the ability to disrupt cell metabolism, initiate inflammatory responses and promote whole body metabolic dysfunction. In the last several years attention has been given to understanding the significance of the location of adipose tissue and if these various depots are equal in consequence. However, data are still inconclusive as to the relative influence of each type of fat on the cardiovascular metabolic profile; furthermore, the severely obese population specifically has not been included in a majority of the research in this area. This cohort deserves further attention and research based on the greatly elevated health risks and increased prevalence of this condition. These findings will provide further insight into understanding the impact of regional fat on health, specifically in severe obesity, which have a greatly increased risk for comorbid conditions.

Numerous previous studies have shown that visceral fat is predictive of metabolic syndrome and increased risk of cardiovascular disease. Substantial research has also shown that visceral fat is the chief contributor to the development of hypertension, elevated plasma insulin concentrations and
insulin resistance, diabetes mellitus and hyperlipidemia in obesity \cite{22,27-33}.

Research supporting these findings has shown visceral adipose tissue to behave differently metabolically, releasing more adipokines and being more inversely associated with adiponectin levels compared to subcutaneous adipose tissue \cite{36-38}.

Intermuscular fat of the thigh has not been as thoroughly studied and while some research has shown this type of fat to affect metabolic function, conflicting studies prevent this concept from wide acceptance \cite{29,42,58}. Intermuscular fat of the abdomen has not been previously studied.

Intramuscular fat of the thigh also has little research to support conclusions about its association with metabolic risk factors. It has been previously shown to be associated with insulin resistance and type 2 diabetes \cite{29,45,59,64}. Different metabolic profiles within the morbidly obese population raise the question whether different fat depots have similar effects. A cursory look at the body composition and metabolic profiles of a morbidly obese sample and the relationships between these variables are discussed below.

Body Composition

Mean BMI for our sample was 51.4 kg/m\(^2\) which is significantly greater than any of the studies reviewed (most studies involving obese subjects had mean BMIs less than 40 kg/m\(^2\)). As mentioned below, there are special logistical challenges working with this cohort, specifically with regards to not fitting in the scanner once they are above a certain dimension. A challenge that arose in the
The present study was the accurate measurement of subcutaneous adipose tissue. The initial intention of measuring subcutaneous adipose tissue of the thigh and abdomen was not possible due to a large amount of this adipose tissue lying outside the scanning parameters. In the previous literature, most researchers utilized a single scan in the targeted area to determine regional body composition. Thus, the resultant unit of fat or muscle is expressed as area. In this current work, the imaging protocol used multiple scans in each region; the unit of tissue measured is in volumes. Mean volumes were used because prior work has demonstrated that volumetric measurements, especially of subcutaneous adipose tissue and visceral adipose tissue, are superior to single-slice measurements \(^{121}\). Because of these differences in units and no accurate measure of subcutaneous adipose tissue, direct comparisons with other studies are limited.

Preis et al. determined mean volumes of fat and in their sample of men and women with a mean BMI of 27.6 kg/m\(^2\), they had a mean abdominal visceral fat volume of 1,326 cm\(^3\). Not surprisingly based on BMI values, the visceral fat in the current study, mean volume 1,466.5 cm\(^3\), was much higher than reported by Preis et al. However comparisons of mean muscle volume and intermuscular fat values of the thigh as well as the mean intermuscular fat values of the abdomen could not be compared with Preis et al. and other studies due to a lack of similar measuring techniques used across the different studies. Intramuscular fat was measured using attenuation values. Lower attenuation values as measured with CT indicate greater fat content within muscle because lipid is characterized by
negative attenuation values on CT. This is due to the attenuation value being a representation of tissue density and muscle is more dense (registers a higher attenuation value) than adipose tissue. The mean intramuscular fat or attenuation value of the thigh found in this study, 60.8 HU, was substantially greater than seen in previous literature. This was unexpected since our sample was composed of individuals with much higher BMIs and in turn, expected higher amounts of adipose tissue than compared studies.

Brochu et al. in a sample of 44 women with an average BMI of 35.4 kg/m² found mean thigh attenuation values of 43.5 HU. In a study by Goodpaster and colleagues, their sub sample of 40 obese (21 women, 19 men) individuals had mean thigh muscle attenuation values of 35.9 HU. And Ross et al. used a sample of 40 females with an average BMI of 32.3 kg/m² and found a mean attenuation value of 43.2 HU. Due to the lack of studies performing this measure on severely obese individuals, there is currently not a sufficient explanation as to why other studies had much lower attenuation values. It can only be speculated that perhaps this type of measure is not appropriate in the severely obese population and excessive adipose tissue skews the results of this particular imaging technique.

Cardiovascular Disease Metabolic Risk Factors

The present analysis contributed to the literature by providing the most extensive analysis of body composition components with cardiovascular disease metabolic risk factors. While no study included the same panel of cardiovascular
disease metabolic risk factors, several analyses included at least two common risk factors that could compare with our values. Overall, this study’s values were comparable to other studies with similar samples. The mean fasting plasma glucose for this sample was 109.8 mg/dL, studies reviewed had means ranging from 86 mg/dL in Yim et al.’s sample subset of African American women with a mean BMI of 28.9 kg/m² to 107 mg/dL in Janssen et al.’s sample of men and women with a mean BMI of 32 kg/m² and high waist circumference. The mean fasting plasma insulin in the present study was 26.8 µU/mL, this value was higher than all studies reviewed which ranged from 1.04 µU/mL in Goodpaster’s study involving older adults with a mean age of 73.6 years and a mean BMI of 26.5 kg/m² to 22 µU/mL found in Brochu et al whose sample was quite similar to ours; women with a mean age of 56.5 years and a mean BMI of 35.4 kg/m². The higher fasting plasma glucose and plasma insulin values and ~40% prevalence rate of self-reported diabetes found in this study involving severely obese individuals can be expected based on previous research which identifies inflammation caused by excessive adipose tissue as one of the main mechanisms for insulin resistance. Increased levels of bioactive substances by excessive adipose tissue disturb the proper regulation of insulin action and lipid and glucose metabolism and activate the IKK-beta/NF-kappa-B and JNK pathways in adipocytes, hepatocytes and associated macrophages.

Inflammation and excessive adipose tissue also are mechanisms through which hypertension manifests itself. The principle mechanism responsible for
obesity related hypertension is thought to be increased renal tubular sodium and water reabsorption; each of the factors contributing to this malfunction are initiated by increased fat mass \(^{102}\). This process was evident in the current sample with over 60% of the sample diagnosed as hypertensive. Mean systolic and diastolic blood pressure were \(~133\) and \(76\) mmHg, respectively.

The lipid profile means of the present study were similar to those of the reviewed literature with several studies having total cholesterol near \(197\) mg/dL \(^{20,52,58}\), several studies having LDL and HDL cholesterol matching the present study’s data of \(118\) mg/dL and \(50\) mg/dL, respectively \(^{20,46,52}\), and similar values in triglyceride levels of \(142\) mg/dL \(^{20,25,46}\). Almost 40% of the current sample was diagnosed with dyslipidemia. Dyslipidemia is also affected by obesity; however, insulin resistance is a critical component in its pathophysiology. During insulin resistance, carbohydrate and lipid metabolism are impaired. Insulin normally facilitates lipid synthesis while inhibiting lipolysis and regulating glucose production by the liver. With insulin not functioning properly, fat accumulates in the liver and very low density lipoproteins increase accordingly which ultimately begins the disruption in a healthy lipid balance \(^{98}\).

CRP values were not found in the studies reviewed, however the present study’s mean CRP value of \(6.2\) mg/L is above the \(3.0\) mg/L cut point for elevated levels established by the American Heart Association. In establishing these guidelines, the American Heart Association used data from the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection,
Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report, which are the most current and widely used data. Based on these criteria, over half of the current sample is at high risk for a future cardiovascular event. CRP is a pro-inflammatory marker and research has shown that higher BMIs across adults are associated with higher CRP concentrations. While the data from the current study does not show this association, data from the Third National Health and Nutrition Examination Survey (NHANES III) which studied over 16,000 men and women aged 17 years or older, found with increasing BMI, the prevalence of elevated CRP level increased across both genders, suggesting a state of low-grade systemic inflammation present in overweight and obese persons. This study supports the earlier work showing that individuals with large amount of excessive adipose tissue (severely obese) are expected to have high CRP values.

Relationships Between Body Composition Variables and Cardiovascular Disease Metabolic Risk Factors

Volume of thigh muscle was the only body composition variable significantly correlated with fasting plasma insulin (r = .667). While it may be reasonable that since muscle is the largest organ for disposing of glucose via insulin dependent pathways, there would be an association between these variables, it is surprising to see a positive association such that those with a higher muscle volume had higher plasma insulin concentrations. The direction of this relationship is difficult to explain. Although speculative, this could be due to
an underlying effect of intermuscular and intramuscular adipose tissue in and around the muscle which have been previously shown to be associated with insulin resistance. This cannot be firmly concluded however, since the present results did not find these correlations. Furthermore, it is likely that the muscle volume is a surrogate marker for another variable not assessed and/or a result of the small homogenous sample. In addition to these explanations, Dohm and colleagues have recently been investigating a novel explanation for diabetes which proposes that diabetes could be due to an issue of hyperinsulinism rather than the traditional thought of an issue of hyperglycemia. Preliminary research by this team has suggested that the muscle of obese persons does not transport glucose as fast as healthy individuals due to fat accumulation impairing insulin signaling. As a result, these persons have increased insulin levels (East Carolina University Summit on Childhood Obesity Prevention and Treatment, August 18, 2003). This is a potential explanation as to why we saw increased fasting plasma insulin concentrations with increased thigh muscle volume in our severely obese population. Percent body fat did show a trend towards significance in positively correlating with plasma insulin \((r= .384, p= .061)\) which is in the direction predicted.

Visceral fat of the abdomen was the only body composition variable associated with number of comorbidities \((r= .492)\) and diastolic blood pressure \((r= -.493)\), however trends toward significance were observed between both of these variables and percent body fat. Whereas others suggest that blood pressure is tied with visceral fat due to production of adipokines and increased
blood volume, the direction of the association found in this study contradicts this traditional view with visceral fat. The negative trend observed between percent fat and diastolic blood pressure and also the negative trend observed between intramuscular fat of the thigh and systolic blood pressure are also in a notable direction with higher levels of fat being associated with lower systolic and diastolic blood pressure. One possible explanation of this lies in the over 60% prevalence of self-reported physician diagnosed hypertension. Having hypertension, a majority of the sample was most likely on medication to control blood pressure which potentially skewed the results. This can only be speculation as this information was not provided and thus, was not controlled in the analysis. To further examine these associations scatterplots were used. Figures 7-12 show the individual data points for those body composition variables that had significant associations or trends toward significant associations with either diastolic or systolic blood pressure and are split according to those subjects with and without a self-report physician diagnosis of hypertension. Due to the small number of subjects in each category after splitting the figures by hypertension diagnosis, the scatterplots do not help in explaining the perplexing direction of the associations. Intermuscular fat and intramuscular fat of the thigh and intermuscular fat of the abdomen had no significant correlations to any of the cardiovascular disease metabolic risk factors.
Figure 7. Relationship Of Visceral Fat With Diastolic Blood Pressure In Those Subjects Without A Physician Diagnosis Of Hypertension

![Graph showing relationship between visceral fat and diastolic blood pressure in subjects without hypertension.]

Figure 8. Relationship Of Visceral Fat With Diastolic Blood Pressure In Those Subjects With A Physician Diagnosis Of Hypertension

![Graph showing relationship between visceral fat and diastolic blood pressure in subjects with hypertension.]

Figure 9. Relationship Of Intramuscular Fat With Systolic Blood Pressure In Those Subjects Without a Physician Diagnosis of Hypertension

![Graph showing relationship between intramuscular fat and systolic blood pressure in subjects without a physician diagnosis of hypertension.]

Figure 10. Relationship Of Intramuscular Fat With Systolic Blood Pressure In Those Subjects With a Physician Diagnosis of Hypertension

![Graph showing relationship between intramuscular fat and systolic blood pressure in subjects with a physician diagnosis of hypertension.]

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Figure 11. Relationship Of Percent Body Fat With Diastolic Blood Pressure
In Those Subjects Without a Physician Diagnosis of Hypertension

Figure 12. Relationship Of Percent Body Fat With Diastolic Blood Pressure
In Those Subjects With a Physician Diagnosis of Hypertension
With the multiple regression analysis, further understanding of the degree to which each regional body component was associated with the cardiovascular disease metabolic risk factors when 2 or more significantly associated components, as determined by Pearson correlations, were examined in the equation was not possible due to the limited significant associations observed. To overcome this, percent body fat was added to the models along with the body composition component that was significantly correlated with the cardiovascular disease risk factor forcing both into the equation.

The multiple regression analysis yielded 2 significant models, the model for plasma insulin (p= .011) with thigh muscle volume and percent body fat explaining about 53% if the variance in this risk factor and the model for diastolic blood pressure (p= .025) with visceral fat and percent body fat explaining 39% of the variance in this risk factor. Of these 2 significant models only the model for plasma insulin had significant independent predictors when the variables were examined separately. In this model, thigh muscle volume was not independently associated with plasma insulin (p= .179), however percent body fat was (p= .007).

Muscle attenuation or intramuscular fat is altered in obesity\textsuperscript{122,123} and has been linked to insulin resistance\textsuperscript{64,65,124,125}. Studies have shown that a reduced attenuation value of skeletal muscle in obesity is a strong marker of insulin resistance\textsuperscript{45,64}. In Simoneau’s study, a stepwise multiple regression model using components of mid-thigh composition and visceral fat content, with leg
glucose storage as the dependent variable revealed muscle with low attenuation (high intramuscular fat) as the strongest predictor for insulin resistance. Together with visceral fat these components explained 57% of the variance in leg glucose storage.

Intermuscular fat of the thigh has also been shown to correlate with insulin sensitivity and has been shown to be a marker of insulin resistance in those with obesity and type 2 diabetes\textsuperscript{59}. Our findings were contrary to these results, we found no association between mean intramuscular fat of the thigh and fasting plasma insulin or glucose levels. Brochu et al. examined this relationship in 44 older, obese females (mean age 56.5 years) and also found no relationship between mean muscle attenuation and rates of glucose disposal\textsuperscript{28}. This could be due to the smaller sample sizes and relative homogenous sample that the current study used. Goodpaster et al. also found no association between thigh muscle area, intermuscular fat or intramuscular fat of the thigh and fasting insulin in obese women (BMI \(>29.9\) kg/m\(^2\)). Interestingly though, they found that in normal weight men and women, intermuscular thigh fat was associated with fasting insulin. Moreover, Goodpaster and colleagues found that the associations between intermuscular thigh fat and abdominal visceral fat and fasting insulin were weaker in the highest BMI groups\textsuperscript{29}. This is in agreement with our study where we did not find significant associations between either of these fat depots and fasting insulin in our sample of severely obese women.
When looking at other cardiovascular disease metabolic risk factors, Yim et al. evaluated the most similar panel of cardiovascular disease metabolic risk factors as the present study. The population of their study was quite different from the present sample in that it retrospectively evaluated healthy African American and Caucasian men and women with mean BMIs ranging from 24.8 kg/m² to 28.9 kg/m² across racial and gender subgroups. The age of their sample was similar to this study with means at approximately 45 years across each of the racial and gender subgroups. Similar to the current study, they found no independent associations between intermuscular thigh fat and insulin, HOMA, triglycerides and HDL cholesterol. In contrast with the current findings, significant independent associations between intermuscular thigh fat and total cholesterol were found. It was proposed by Yim et al. that possible regional difference in the secretion of adipokines between abdominal and thigh areas could contribute to the different associations of these fat depots with lipid levels. Aside from this study, there are no previous reports on the relationship between intermuscular fat and plasma lipids. More work is needed in studying both intermuscular fat of the thigh and abdomen and intramuscular fat.

There is substantial research showing abdominal visceral fat's association with measures of glucose, measures of insulin and type 2 diabetes risk, and with cardiovascular disease metabolic risk factors. Banerji et al. used a multiple regression analysis examining visceral and subcutaneous abdominal adipose tissue to predict insulin-mediated glucose disposal and found visceral adipose tissue per meter squared of body surface.
area explained 33% of the variance with subcutaneous adipose tissue adding 1%. Multiple regression was used by Preis et al. with abdominal subcutaneous and visceral fat entered into the equation for insulin resistance. Visceral fat had a greater effect size (beta= .20 vs. .16) and explained 25% of the variance in insulin whereas subcutaneous adipose tissue only explained 17%. Visceral adipose tissue was also a stronger predictor of HOMA than subcutaneous adipose tissue, with similar beta and R squared values. In Smith et al.’s multiple regression analysis using cross-sectional areas of visceral adipose tissue and deep and superficial abdominal subcutaneous adipose tissue adjusted for total percent body fat, they found visceral adipose alone explained 35% of the variance in triglycerides which was higher than any other fat compartment. Visceral adipose tissue coupled with superficial subcutaneous adipose tissue explained the most variance, 31%, in fasting insulin. The current study was unable to support these past results from other studies; however it did contribute the association of abdominal visceral adipose tissue to number of comorbidities which has not been shown in previous literature.

No other study compared fat depots to the extensive number of major cardiovascular disease metabolic risk factors, however Smith et al. did find associations between abdominal visceral adipose tissue and many of the same risk factors we used including: triglycerides, fasting insulin, systolic and diastolic blood pressure. Similar to our study, they found no association between abdominal visceral adipose tissue and HDL cholesterol. Again, our small sample size and homogenous sample was probably our biggest limitation in
finding significant associations. Another reason why we may not have found significant associations between the various fat depots and insulin resistance is due to the younger age of our sample in which most women were premenopausal. It has been suggested that estrogen may protect women from the negative effects of excessive body fat on insulin sensitivity \(^{127}\).

Further limitations to our study included a predominantly white sample, thus results may not be generalizable to other racial or ethnic groups. The sample was also all female making these results specific to women. Although the present study only investigated those undergoing bariatric weight loss surgery, the use of only morbidly obese individuals may limit the application of these findings. Furthermore, we used HOMA instead of the gold standard insulin clamp measure to assess insulin sensitivity. We were also not able to assess abdominal or thigh subcutaneous adipose tissue due to limitations in our CT scanning device in accommodating the size of our participants. In addition to this, our intramuscular fat values assessed through attenuation characteristics were not agreement with previous literature, with only speculations as to why. Further research using CT imaging needs to be done in the severely obese population in order to gain a better understanding of the accuracy of CT scanning in evaluation regional fat deposits.

While this study was exploratory and did not have substantial power to make definite conclusions about the association of regional body components to cardiovascular disease metabolic risk factors in the severely obese population, it
did contribute to the literature. This study incorporated several of the body composition variables and cardiovascular disease metabolic risk factors commonly used across studies into one study to evaluate how these relationships would hold true in the severely obese population. The severely obese population has not been studied extensively especially a sample comprised entirely of subjects who fall into this category. Some obvious associations were not seen with the current study when compared with others, possibly due in large part to a small sample size and a relatively homogenous sample with all but three having a BMI greater than 40 kg/m² and similar values in all body composition measures.

This study helps to better understand associations between regional fat deposits in the severely obese population and major cardiovascular disease metabolic risk factors. Practically speaking, this information and future studies in this area can shed light on potential improvements health care providers can expect when helping this population lose weight. Future work in pharmaceuticals could potentially target specific fat depots associated with the most cardiovascular disease risk. With a comprehensive knowledge of what variables in the cardiovascular disease risk factor profile can be expected to change with gains or losses in adipose tissue in specific regions of the body, the severely obese population can be more effectively assisted in improving their health and ultimately their mortality.
This comprehensive knowledge will not come from this one study, rather the present study has functioned to stimulate research in this population and highlight areas that require further investigation. For example, thigh intramuscular fat evaluation had never been done in a sample comprised mostly of severely obese individuals before this study. As a result, a potential obstacle to evaluating this fat depot using CT scanning has been identified. In addition, an obstacle to evaluating subcutaneous adipose tissue in this population has been identified and future studies could focus on improving imaging techniques to better understand regional body components in the severely obese.
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SCHOLASTIC VITA

Renee D. Privette

Education

Graduate, 2010-2012
- Master of Science, Wake Forest University Anticipated: May 2012
  Department of Health and Exercise Science Winston Salem, NC

Undergraduate, 2006-2010
- Bachelor of Science, Saginaw Valley State University University Center, MI
  Major: Exercise Science
  Minor: Entrepreneurship and Health Science
  GPA: 3.9, Summa Cum Laude

Teaching Experience

- Wake Forest University
  - Graduate Assistant Instructor, Lecture/Lab Course HES 101 Exercise for Health (Fall 2010-Present)
    - Delivered lectures and instructed during laboratory assignments
    - Assigned and graded student’s coursework; official record holder of grades

- Saginaw Valley State University
  - Teaching Assistant, Lecture Course KINE 270 Physical Activity and Fitness Assessment (Winter 2010)
    - Created and delivered lectures
    - Assigned and graded student’s coursework

  - Laboratory Assistant, Human Performance Lab (Winter 2010)
    - Prepared for and aided in student laboratory assignments
    - Graded student’s coursework

  - Fitness Instructor, Healthy-U Program (2009-2010)
    - Aerobics class (biweekly)
- Cardiovascular training class (biweekly)

- Saginaw Valley State University, Coaching
  - Summer Soccer Camp Coach (2006-2009)
  - Summer Soccer Camp Counselor (2006-2008)

- Community, Coaching
  - Tactical Instructor, U-15 Travel Soccer Team: Bay City Spirit (2007)
  - Training Instructor, Bay Area Soccer, Bay City, MI (2006-2007)
  - Summer Conditioning Coach/Coordinator, Port Huron High Varsity Girls Soccer (2006-2007)
  - Assistant Coach, Port Huron High Varsity Girls Soccer (2007)

**Job Experience**

- Clinical Exercise Specialist, Wake Forest University Healthy Exercise and Lifestyle Programs (HELPs), Winston Salem, NC (2010- Present)
  - Monitored patient exercise through EKG operation, blood pressure and vital signs
  - Aided in patient stress testing
  - Lead group exercise classes

- Soccer Trainer, Winston Salem Soccer Plex, Winston Salem, NC (2011- Present)
  - Co-led group clinics and provided tactical instruction
  - Provided personal one-on-one training and tactical instruction

- Personal Soccer Coach, Saginaw, MI (2008-2010)
  - Provided personal one-on-one training and tactical instruction

- Summer Soccer Camp Director/Coach, Port Huron and Bay City, MI (2007-2008)
  - Designed, directed and coached a soccer team camp

- Entrepreneurship Intern, College of Business and Management, Saginaw Valley State University (2007-2008)
  - Promoted and created opportunities for entrepreneurship with the Workforce Innovation in Regional Economic Development (WIRED) team; funded by the Department of Labor
Research Experience

Wake Forest University Research

- Research Assistant under Dr. Gary Miller
  - Pilot: Exercise Training Following Weight Loss Surgery
    - Lead exercise interventionist
    - Other Responsibilities: Project coordination, schedule patient visits, data collection, management and analyses.
  - Brenner’s Children’s Hospital Pediatric Obesity Clinic
    - Responsibilities: Data management and analyses

- Research Assistant under Dr. Stephen Messier
  - The IDEA Study (Intensive Diet and Exercise for Osteoarthritis
    - Responsibilities: exercise interventionist and data management

Saginaw Valley State University Research

- Student Researcher in the Human Performance Laboratory, Saginaw Valley State University (2007- Present)
  - Fitness Testing of Saginaw Firefighters (2010)
    - Station Coordinator
  - Wellness Testing on Faculty and Staff (2008- 2010)
    - Site leader for submaximal testing
    - Site leader for body composition
  - Professional Hockey Team Fitness Testing, Flint Generals (2008- 2009)
    - Station Coordinator: hydrostatic weighing (2009)
    - Station Coordinator: agility measures (2008)
    - Station Coordinator: blood lactate measures (2008)
  - Wellness Testing and Data Collection of College Students (2008)
  - Athlete Testing of Saginaw Valley Men’s Basketball Team (2008)
    - Co-coordinator of VO2 maximal testing on 10 players

- Other Research
  - Symposium Presentation to Saginaw Valley Faculty and Students: The Role of a Structured Physical Activity Program in Treating Autistic Individuals (2009)
  - Wellness Testing of Elementary School Children (2009)
    - Francis Reh Academy
Academic Presentations

  - National ACSM, San Francisco, CA, May 2012

  - Southeast ACSM, Jacksonville, FL, February 2012

  - National American Association for Teaching and Curriculum (AATC), St. Louis, MS October 2010

- **Privette R**, Bowlby K, Knous J, Ode J. The Comparison of Field Tests to the Wingate Test in Professional Hockey Players.
  - National ACSM, Baltimore MA, May 2010

- **Privette R**, Bowlby K, Knous J, Ode J. The Comparison of Field Tests to the Wingate Test in Professional Hockey Players.
  - Michigan ACSM, Gaylord, MI, February 2010

Achievements

- Most Outstanding Graduate in the Department of Kinesiology, Saginaw Valley State University (2010)
- Women’s Soccer All-GLIAC Second Team Honors (Great Lakes Intercollegiate Athletic Conference) (2009)
- National College Athlete Honor Society (2008- 2009)
- All-Academic GLIAC Soccer Team (2007- 2009)
- Saginaw Valley State University, Bob Becker Endowed Scholarship (2009)
- Saginaw Valley State University Presidential Scholarship (2006- 2010)
- Saginaw Valley State University Athletic Scholarship (2006- 2010)

Service

- Community Service
  - Salem Chapel Church K-5 Instructor
    - Weekly instructor for K-5 children’s church
  - Kinship Program: Eleanor Frank Senior Center (2010)
    - Instructor for “Exercise Can Be Fun” event, taught exercise activities to 15 kids ages 5-13
  - Hidden Harvest Canned Food Drive (2009)
    - Team leader during the distribution of fliers and collection of canned goods
- Salvation Army Adopt a Family (2008-2009)
  - Member of a team that collected money, shopped for and delivered gifts to an adopted Saginaw family


  - Coordinate monthly fundraising campaigns at SVSU sporting events

- Camp Counselor with Christian Youth Camp (2009)

- Relay for Life Participant (2006-2009)

- Guest Fitness Educator, Landmark Academy (2007-2008)
  - Led girls’ gym classes in aerobics
  - Educated children on important aspects of fitness

- Alternative Break with Orphanage Outreach, Monte Cristi, Dominican Republic, 7 days (2008)
  - Created lesson plans and taught English in the public schools of Monte Cristi
  - Daily lessons in four classrooms
  - Care of orphanage children

- Mission Trip, Montego Bay, Jamaica, 15 days (2005)
  - Activity coordinator and Bible School teacher
  - Part of a construction project that repaired a flood damaged church building

- Mission Trip, Matagalpa, Nicaragua, 10 days (2003)
  - Coordinated activities for children
  - Part of a construction project that made repairs to a church building

- Member of Hillside Wesleyan Church (1996-Present)

  - Saginaw Valley State University Service
    - Member of SVSU Women’s Varsity Soccer Team (2006-2010)

    - Member of SVSU Business and Entrepreneurship Skills Training Program (BEST) (2006-present)

    - Member of SVSU Student Athlete Advisory Committee (SAAC) (2007-2010)
      - Team representative of Women’s Soccer
- Extend volunteer opportunities to teammates, organize volunteer efforts
  - Participate in over a dozen volunteer campaigns each semester

  o Member of SVSU Student Exercise Science Association (SESA) (2008-2010)
    - Participate in wellness fairs and wellness testing on faculty, students and athletes
    - Attend ACSM meetings

  o Community Youth Days, Saginaw Valley (2007-2010)
    - Coach free soccer clinic given to area children grades K-8

  o Kid’s Night, Sponsored by Saginaw Valley Women’s Soccer (2007-2010)
    - Supervisor of Friday night activities for area children

  o Personal Trainer, Healthy-U Program (2009)
    - Performed wellness measures on clients
    - Designed weekly workouts
    - Created weekly health-tip newsletter

  o Cardinal Cabaret, fundraising dinner auction for SVSU Athletics (2008-2010)
    - Team representative and station coordinator

  o Extreme Entrepreneurship Tour (EET), Hosted by Saginaw Valley (2008)
    - Experience with a national organization that promotes entrepreneurship
    - In charge of logistics and coordinating events with EET crew

  o Attended Family Business Program Events (2006-2008)
    - Lectures by local business executives and entrepreneurs

- Conferences Attended
  o ACSM National Conference, San Francisco, CA (2012)
  o ACSM Regional Southeast Conference, Jacksonville, FL (2012)
  o ACSM National Conference, Baltimore, MA (2010)
  o ACSM Conference, Michigan Chapter, Gaylord, MI (2010)
  o ACSM National Conference, Seattle, WA (2009)
  o ACSM Conference, Michigan Chapter, Gaylord, MI (2009)
  o NSCA Clinic, University of Detroit Mercy (2009)
  o NSCA Clinic, Delta College, MI (2007)
  o Chicago Business Experience through SVSU’s BEST Program (2007)
    - Toured facilities with renewable energy features
    - Attended lectures highlighting alternative energy options and resources
    - Networked with business leaders
Professional Affiliations/Certifications

- ACSM Clinical Exercise Specialist (2011- Present)
- Collaborative Institutional Training Initiative (CITI) Training in Human Subjects Research (2010- Present)
- Member of ACSM (American College of Sports Medicine) (2009- Present)
- Certified CPR/AED, American Red Cross (2007-Present)

Academic and Professional References

Letters available upon request

- Dr. Gary Miller, Associate Professor of Health and Exercise Science
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