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Determining the relationship between C-reactive protein, visceral fat, and the metabolic syndrome risk factors

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DETERMINING THE RELATIONSHIP BETWEEN C-REACTIVE PROTEIN, VISCERAL FAT, AND THE METABOLIC SYNDROME RISK FACTORS

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MASTER'S THESIS

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ABSTRACT

The metabolic syndrome (MetS) is a diagnosis that includes several clinical criteria that indicate a higher than normal risk of developing cardiovascular disease. Central adiposity is considered a risk factor for the MetS and is also associated with increased mortality and morbidity. The use of ultrasonography (US) has made it possible to measure the amount of visceral fat (VF) in a cost-effective and non-invasive manner as opposed to computed tomography or magnetic resonance imaging. C-reactive protein (CRP), an acute phase inflammatory marker, has been associated with increased risk for cardiovascular disease and has been shown to have a relationship with VF levels. The purpose of the study was to determine the relationship between VF, CRP, and the MetS. Visceral fat, CRP, MetS risk factors were evaluated in 34 participants who were over the age of 40. An ultrasound scan at the waist was conducted to determine VF levels by placing the wand 1 cm to the right of the umbilicus and performing a 10 cm scan towards the right hip. Waist circumference was measured at the superior portion of the iliac crest. CRP, blood glucose, HDL cholesterol, and triglycerides (TG) were measured using a blood analyzer. Pearson's Correlations were conducted to determine the relationships between VF, CRP, and MetS risk factors. All variables were tested with an alpha level of $p \leq .05$. There were significant positive correlations between CRP and VF ($r = .34$, $p = .05$) and CRP and TG ($r = .50$, $p < .002$). The results indicate the use of US as a cost-effective, non-invasive method of evaluating potential risk for increased inflammation as well as the development of the MetS and may be a viable alternative to traditional methods of measuring VF.

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Chapter 1

Introduction

The metabolic syndrome (MetS) is a series of clinical findings that when clustered together, increases the risk of developing type 2 diabetes and cardiovascular disease (Wilson, D'Agostino, Parise, Sullivan, & Meigs, 2005). Several organizations have provided clinical definitions for the diagnosis of the MetS, but the definitions between organizations differ (Grundy, Brewer, Cleeman, Smith, & Lenfant, 2004). The components of a MetS diagnosis are elevated triglycerides (TG), decreased high density lipoprotein cholesterol (HDL-C), hypertension, increased central obesity, and elevated fasting glucose levels. These risk factors are all independently related to an increased risk for cardiovascular disease, but the presence of the MetS increases the risk exponentially more than simply combining the individual risk factors (Ford, 2005; Isomaa et al., 2001; Malone et al., 2009). Individuals with the MetS also have an increased risk for developing type 2 diabetes (Lorenzo, Okoloise, Williams, Stern, & Haffner, 2003; Wilson et al., 2005). There is a smaller, but still significant increase in risk for cardiovascular disease and cardiovascular mortality for those with the MetS (Chambers et al., 2001; Isomaa et al., 2001; Ridker et al., 2003; Rutter, Meigs, Sullivan, D'Agostino, & Wilson, 2004; Wilson et al., 2005).

Recent data suggests that between 23% - 35% of all adult Americans have the MetS and approximately 40% of people over the age 60 have the diagnosis (Malone et al., 2009). In November of 2007, the American College of Sports Medicine (ACSM), in partnership with the American Heart Association, launched an initiative entitled *Exercise is Medicine*. The purpose of the initiative is for health care providers to include physical

activity as a vital sign during check-ups and to include physical activity as an avenue for chronic disease prevention and/or treatment. Lakka et al. (2003) concluded that a sedentary lifestyle and poor cardiorespiratory fitness led to an increase in risk for being diagnosed with the MetS. The researchers found that the least fit participants were nearly seven times more likely to have the MetS compared with the most fit participants (Lakka et al., 2003). On top of this finding, Lakka et al., (2003) concluded that low levels of moderate and vigorous physical activity were associated with the risk factors for the MetS as well as other factors related to the MetS such as inflammatory markers.

The *Exercise is Medicine* initiative can have a significant economic impact, but is dependent on allied health professionals and primary care givers committing to the core tenet that exercise can prevent and treat many chronic diseases. In a study done on the health care costs of the MetS, researchers compared the annual cost of health care for those with or without MetS and also compared those with and without type 2 diabetes (Boudreau et al., 2009). The total annual cost of health care is made up of four categories; inpatient, primary care, outpatient, and pharmacy. For patients with the MetS, the average cost for health care annually was $5,732 versus $3,581 for subjects without the MetS (Boudreau et al., 2009). Additionally, the inpatient and pharmacy cost for those with the MetS were double the cost of those without. Because there is a much greater risk for developing type 2 diabetes in the presence of the MetS, understanding the health care costs in patients with or without type 2 diabetes is also important. Those with type 2 diabetes had a much higher cost annually than those with the MetS at $6,038 (Boudreau et al., 2009). Overall, there is a clear link between the MetS and increased health care costs including even higher costs for those with the MetS and type 2 diabetes, thereby

increasing the need for the most practical and direct method of detecting, preventing, and treating the MetS.

There has been a relationship established between the MetS risk factors and C-reactive protein (CRP), but it is traditionally been assessed using the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) definition. The relationship between CRP and the MetS as defined by the World Health Organization (WHO) is also defined in the literature. In the larger scale, cross-sectional studies examining the relationship between CRP and the MetS, a strong correlation has been shown establishing a link between CRP and the MetS. Within these studies not all MetS risk factors were measured at the time of data collection and some factors were self-reported by participants (such as blood pressure). These studies also utilized waist circumference, waist-to-hip ratio, or BMI to measure central obesity, but none of those methods are able to differentiate between subcutaneous and visceral fat compartments. This is of significance as visceral fat has been shown to be more metabolically active than subcutaneous fat and has also been shown to be a stronger, more reliable predictor of future cardiovascular and metabolic disease (Ibrahim, 2009).

There is a well-documented relationship between obesity and an increased risk for metabolic and cardiovascular disease (Hamdy, Porramtikul, & Al-Ozairi, 2006; Ibrahim, 2009; Porter et al., 2009). Currently, approximately two out of three Americans are overweight or obese according to BMI classifications, which has steadily increased over the past 30 years (Low, Chew, & Deurenberg-Yap, 2009). Increased BMI creates an immediate and significant impact on our health care system costs as the cost per person, per year for someone who is overweight or obese ($498 and $1,630 respectively) is

significantly higher than a normal weight person's medical costs (Tsai, Williamson, & Glick, 2010). Obesity costs account for approximately 5% of health care spending in the United States (Tsai et al., 2010). Several studies suggest that central obesity is more strongly associated with increased risks for metabolic and cardiovascular disease when compared to total adiposity (Hamdy et al., 2006; Ibrahim, 2009; Liu et al., 2010; Smith et al., 2012). Additionally, visceral fat has a stronger correlation to the risk factors for MetS than that of subcutaneous fat even after the adjustment for weight (BMI; Liu et al., 2010).

The gold standards for measuring visceral fat are through computed tomography (CT) scans or magnetic resonance imaging (MRI), which are expensive and difficult to access for the general population. Recently, ultrasonography has been utilized and validated as a method to measure visceral fat levels (Alempijevic et al., 2011; Gong et al., 2007; Hirooka et al., 2005; Kim et al., 2004; Rolfe et al., 2010; Shojaei et al., 2010; Stolk, Meijer, Mali, Grobbee, & Van Der Graaf, 2003; Stolk et al., 2001). The relationship between visceral fat measured through ultrasonography have been compared to visceral fat using CT ($r = 0.82 - 0.86$) and MRI ($r = 0.77$) with strong correlations (Gong et al., 2007; Hirooka et al., 2005; Stolk et al., 2003). This comparison between the gold standards of measuring visceral fat and ultrasonography lend validity to ultrasonography as an alternative, accessible, and inexpensive measurement tool for central obesity that is more practical for allied health professionals. Therefore, the use of ultrasound to measure visceral fat as a replacement for waist circumference and other anthropometric measures of obesity may provide a valid, practical, and cost-effective measurement of central obesity in the definition of the MetS.

Purpose Statement

The primary purpose of this study was to determine the relationship between CRP, visceral fat, and the metabolic syndrome (MetS) risk factors.

Hypotheses

H_o: There will be no significant correlations between CRP and the MetS risk factors.

H_a: There will be significant, positive correlations between CRP and the following MetS risk factors: triglycerides, blood pressure, fasting glucose, and visceral fat.

H_a: There will be a significant, negative correlation between CRP and HDL-C.

Operational Definitions

The Metabolic Syndrome: The clustering of three or more of the following risk factors as defined by the National Cholesterol Education Program ATP III: elevated triglycerides, hypertension, low levels of high density lipoprotein cholesterol, visceral adiposity, and elevated fasting glucose. TG, HDL-C, and fasting glucose were measured with a Cholestech LDX Analyzer. Blood pressure was measured using an aneroid sphygmomanometer and waist circumference was measured with a Gulick tape measure. The specific thresholds for each of the risk factors are as follows (Grundy et al., 2004):

Elevated TG. Characterized by blood TG levels >150 mg/dl.

Hypertension. Characterized by systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg.

Reduced HDL-C. Characterized by < 40 mg/dl of HDL-C for males and <50 mg/dl for females.

Elevated waist circumference. Characterized by waist circumference >102 cm for males and >88 cm for females.

Elevated (impaired) fasting glucose. Characterized by fasting glucose levels >110 mg/dl.

Body Mass Index: Body mass index (BMI) is the ratio of height to weight. The formula used for BMI in this study was weight in kilograms divided by height in meters squared.

Assumptions

An important aspect of collecting the most accurate blood glucose and cholesterol information is for the participant to be fasting for 10-12 hours before testing. It is assumed that all participants followed this protocol to produce the most valid results possible.

Delimitations and Limitations

The sample population for this study was delimited to older adults. For the purpose of this study, those over the age of 40 were considered older adults. Also, the sample size was delimited to 50 participants due to issues surrounding cost of equipment and limited funding.

Significance

The importance of preventing the onset or the early detection and treatment of the MetS is increasing as our population ages, becomes more sedentary, and more obese. Currently, the estimates for Americans who have the MetS range from 23% - 35% with this number reaching over 40% in those who are over the age of 60 (Malone et al., 2009). By determining how CRP relates to the MetS risk factors in a relatively older population

(older than 40) as well as exploring the relationship between CRP and visceral fat, allied health professionals will gain important insight into potential methods of screening and monitoring for MetS risk factors. The use of ultrasonography in the current study is also significant because it may eliminate the need to use expensive, inaccessible methods of measurement such as MRI or CT scans.

Overall, the goal of the study was to determine the most practical, cost-effective method of identifying the MetS for the purpose of prevention, early detection, and early treatment. Allied health professionals, along with primary care givers such as physicians, are a crucial link to preventing the onset of chronic diseases such as the MetS, type 2 diabetes, and cardiovascular disease. In line with the *Exercise is Medicine* campaign, equipping these professionals with the methods to better help their clients will only have a favorable effect on the impact of chronic disease on their substantial health care costs.

Chapter 2

Review of Literature

Introduction

The purpose of this study was to determine the relationship between CRP, an inflammatory marker, and the metabolic syndrome (MetS) risk factors using the NCEP ATPIII definition while including visceral fat measurements through ultrasonography. This chapter provides a review of the metabolic syndrome, visceral fat, and inflammation as well as the interrelationships amongst them.

The Metabolic Syndrome

The metabolic syndrome (MetS) is characterized by a series of clinical findings including hypertension, dyslipidemia, impaired glucose sensitivity, and visceral adiposity and it is estimated that approximately one in four people in the United States are living with the MetS (Wilson et al., 2005). People diagnosed with the MetS are at a higher risk for developing type 2 diabetes as well as cardiovascular disease, which has a significant economic impact in terms of health care costs. The burden of type 2 diabetes and cardiovascular disease on United States health care costs is over $600 billion annually (Rice, Cifelli, Pikosky, & Miller, 2011). More specifically, the health care costs for individuals with and those without the MetS are substantially different. According to Boudreau et al. (2009), health care utilization as well as costs was significantly greater in patients with the MetS compared to patients without the MetS. Average annual total costs for patients who had the MetS were 1.6 times higher than patients without the MetS ($5,732 vs. $3,581; Boudreau et al., 2009). Therefore, it is both in the best interest of

individuals as well as the population as a whole to determine the most cost-effective, practical methods of screening, preventing, and treating the components of the MetS.

For the purpose of this study, the most recent definition for MetS provided by the NCEP ATPIII was used. There are other commonly used definitions, such as one defined by the WHO. The two definitions are described below for the purpose of comparison as they are the most commonly used in the MetS literature (Ford et al., 2005). The NCEP ATP III definition was chosen for the current study as the WHO standards are difficult to measure outside of a hospital or lab setting. The NCEP ATP III criteria are the most easily measured and identified and the thresholds for the criteria are less stringent than other definitions because having multiple risk factors that are marginal increases the risk for cardiovascular disease (Grundy et al., 2004).

NCEP ATP III MetS definition. The current definition for the MetS in the NCEP ATP III is the presence of any three (or more) of the following risk factors: elevated TG, hypertension, reduced HDL-C, elevated waist circumference, and elevated fasting glucose (Grundy et al., 2004).

WHO MetS definition. Defined as insulin resistance as classified by one of the following: Type 2 diabetes, impaired fasting glucose, impaired glucose tolerance, or those with glucose uptake below the lowest quartile for background population under investigation under hyperinsulinemic or euglycemic conditions. Along with insulin resistance, at least two of the following have to present as well: antihypertensive medication and/or high blood pressure, elevated plasma triglycerides, elevated BMI and/or waist to hip ratio, and elevated urinary albumin excretion rate or the albumin:creatinine ratio (Grundy et al., 2004).

The primary risk factors for the MetS have been strongly associated with risk for developing type 2 diabetes using both the NCEP ATP III and WHO definitions (Bernard et al., 2007; Ford, 2005; Hanson et al., 2002; Lorenzo et al., 2003; Rutter et al., 2004; Wilson et al., 2005). Research has shown that several risk factors for type 2 diabetes are strongly correlated with each other, which has led to the belief that they arise from common factors (Hanson, Imperatore, Bennett, & Knowler, 2002). Obesity, central adiposity, hypertension, insulin resistance, hypoalphalipoprotein, and hypertriglyceridemia have been described as primary risk factors for type 2 diabetes and because these risk factors are often clustered, it has been hypothesized that they stem from a limited number of or a single metabolic abnormality (Hanson et al., 2002). Of the aforementioned risk factors for type 2 diabetes, the combination of hypertension, insulin resistance, dyslipidemia, and obesity fall into the definition for the MetS. Previous research has shown that as the number of metabolic syndrome risk factors increases, the higher the risk there is for developing type 2 diabetes (Wilson et al., 2005).

In addition to the individual MetS risk factors being associated with type 2 diabetes, there is an increased relative risk for developing type 2 diabetes for those with the MetS (Ford, 2005; Hanson et al., 2002; Lorenzo et al., 2003; Wilson et al., 2005). Throughout the research, there is consensus that there is an increased risk for type 2 diabetes, but the relative risk values differ. The range of relative risk for type 2 diabetes in those who have the MetS is 1.5 to 17.9 (Ford, 2005; Hanson et al., 2002; Lorenzo et al., 2003; Wilson et al., 2005). These relative risk values include research studies that utilized both the NCEP ATP III definition and the WHO definition for the MetS, which is important to note. The range presented is significantly large, but Ford (2005) performed a

meta-analysis of studies that evaluated relative risk for type 2 diabetes in those with the MetS and concluded that the NCEP definition yielded a collective relative risk of 2.99. Much of the difference in relative risk values in the research can be attributed to the definition of the MetS being used. Hanson et al. (2002) showed that the relative risk for type 2 diabetes using the WHO definition was significantly higher than the relative risk using the NCEP ATP III definition within the same population (3.58 & 2.09). This difference in relative risk is credited to the fact that the WHO definition for the MetS requires impaired fasting glucose plus two additional risk factors whereas the NCEP ATP III definition simply requires any three risk factors (Hanson et al., 2002). The fact that the NCEP ATP III definition treats all five risk factors equally and does not require impaired fasting glucose for diagnosis plays a significant role in relative risk calculations for predicting the development of type 2 diabetes compared to the WHO definition.

Impaired fasting glucose, a primary risk factor for the MetS, is diagnosed when fasting blood glucose levels are greater than or equal to 110 mg/dL. The presence of impaired fasting glucose very often precedes the onset of type 2 diabetes (Lorenzo et al., 2003; Wilson et al., 2005). In a study done examining the MetS as a precursor of cardiovascular disease and type 2 diabetes, the researchers found that having the MetS is a good predictor of developing type 2 diabetes (Wilson et al., 2005). Within the same research, an 8-year follow-up study, the researchers found that participants who only had one component (impaired fasting glucose) of the MetS were at a much higher risk of developing type 2 diabetes when compared to participants who had any one of the other four MetS risk factors (Wilson et al., 2005). These results indicate that having impaired

fasting glucose is a good predictor of a future type 2 diabetes diagnosis and is an independent risk factor of incident type 2 diabetes.

The relative risk for type 2 diabetes for someone who only displays impaired fasting glucose is significantly higher (12.5) than the relative risks for type 2 diabetes for those who have elevated TG (2.9), hypertension (2.4), elevated waist circumference (4.1), and low HDL-C (2.7; Wilson et al., 2005). These specific results indicate that the appearance of the impaired fasting glucose risk factor for MetS has a larger impact on the relative risk and prevalence of future type 2 diabetes diagnoses, as the relative risk for non-impaired glucose fasting risk factors was three to five times lower (Wilson et al., 2005).

For those who had two MetS components with one being impaired fasting glucose, the relative risk for type 2 diabetes was two to three times higher than those who had two MetS components without having impaired fasting glucose (Wilson et al., 2005). Participants who had three MetS components, including impaired fasting glucose, also had two to three times higher relative risk for type 2 diabetes (Wilson et al., 2005). Interestingly, the relative risk for those with two compared to three MetS components (both including impaired fasting glucose) was not significantly different, which is important as it may indicate that any level of clustering of risk factors including impaired fasting glucose shows a central insulin-resistant pathophysiology. Although participants who showed the impaired fasting glucose component of MetS suffered from a higher relative risk than those who did not, all participants who had any form of risk factor clustering suffered from a higher relative risk.

Individuals who have been diagnosed with the MetS are also at an increased risk for developing cardiovascular disease (Chambers et al., 2001; Isomaa et al., 2001; Ridker et al., 2003; Rutter et al., 2004; Wilson et al., 2005). The risk factors for the MetS have all independently been linked to a greater risk for cardiovascular disease, but the presence of MetS increases that risk even further (Ford, 2005; Isomaa et al., 2001; Malone et al., 2009). The use of differing definitions of the MetS as well as different criteria within each definition has led to an inconsistency in the numbers related to the relative risk of cardiovascular disease for those with the MetS. In spite of these discrepancies, the overwhelming consensus is that being diagnosed with the MetS increases the risk for cardiovascular disease as well as cardiovascular mortality (Chambers et al., 2001; Ford, 2005; Isomaa et al., 2001; Malone et al., 2009; Ridker et al., 2003; Rutter et al., 2004; Wilson et al., 2005).

Isomaa et al. (2001) suggests that the presence of the MetS was related to an increase in cardiovascular disease, specifically coronary heart disease, acute myocardial infarction, and stroke. The relative risk for coronary heart disease using the WHO definition of the MetS was 2.96, whereas the risk for acute myocardial infarction was 2.63 and the risk for stroke was 2.27 (Isomaa et al., 2001). Using the NCEP ATPIII definition of the MetS, it was shown that the relative risk for developing cardiovascular disease ranged from 3.40 to 5.64 based on the number of MetS risk factors present (3-5 risk factors, respectively; Malone et al., 2009). Additionally, it was found that dyslipidemia was associated with a higher risk for cardiovascular disease in patients with type 2 diabetes and hypertension was associated with a higher risk in patients without type 2 diabetes (Isomaa et al., 2001). In addition to being at a higher risk for

cardiovascular disease, Isomaa et al. (2001) found that cardiovascular mortality was increased in patients with the MetS compared to those without the MetS (12% v. 2%). They also identified the combinations of obesity and hypertension and obesity and dyslipidemia as the most common risk factor combinations, which are similar to the findings of Malone et al., (2009).

Malone et al. (2009) proposes that the specific combination of risk factor clustering is the most important factor for an increased risk of cardiovascular disease. Malone et al. (2009) examined the specific combinations of risk factors and determined that low HDL-C levels were present in the combinations with the highest relative risks and that the combination of impaired fasting glucose, hypertension, and dyslipidemia inferred the highest risk for cardiovascular disease of any of the combinations. Also, their findings showed that hypertension was the greatest independent contributing risk factor for increase in risk for stroke whereas low HDL-C was the greatest contributing risk factor for the increase in risk for acute myocardial infarction (Malone et al., 2009). These results are significant as low levels of HDL-C was a stronger risk factor than type 2 diabetes for acute myocardial infarction and stroke, but the clusters of risk factors that contributed the most to increased risk of cardiovascular disease included both HDL-C and impaired fasting glucose (Malone et al., 2009).

Although there are discrepancies in relative risk values for the MetS throughout the literature and future risk for cardiovascular disease and mortality due to differing definitions, there is a consensus that a relationship exists between the MetS and an increased risk for cardiovascular disease and mortality (Chambers et al., 2001; Ford, 2005; Isomaa et al., 2001; Malone et al., 2009; Ridker et al., 2003; Rutter et al., 2004;

Wilson et al., 2005). It also appears that the clustering of MetS risk factors contributes more to an increased risk for cardiovascular disease and mortality than the individual components of the MetS alone (Malone et al., 2009). Also, the specific clusters of risk factors seems to play a significant role in the development of cardiovascular disease, with the specific combination of impaired fasting glucose, dyslipidemia, and hypertension inferring the highest risk (Malone et al., 2009).

Inflammation

There is a relationship between inflammatory markers, such as CRP, and cardiovascular disease in both men and women (McLaughlin et al., 2002; Yudkin, Stehouwer, Emeis, & Coppack, 1999). There is inconclusive evidence identifying the cause of the inflammation and the exact role that it plays, but there are several hypotheses for the etiology of the relationship between inflammation and cardiovascular disease.

Several epidemiological studies suggest that a strong relationship exists between CRP and the MetS, however, it is not well understood (Frohlich et al., 2000; Kahn et al., 2006; Ridker et al., 2003; Rutter et al., 2004; Wilson et al., 2005). A prominent theme is that adipose tissue and the associated cytokines produced play a strong role. The primary inflammatory markers that have been explored in relationship to cardiovascular disease and the MetS are CRP, interleukin-6 (Il-6), and tumor necrosis factor alpha (TNFa; Frolich et al., 2000). Il-6 and TNFa are both pro-inflammatory cytokines which are directly related to levels of CRP, an acute phase inflammatory protein (Hak et al., 1999).

Adipocytes are the primary producer of TNFa within the human body and have paracrine properties (Hak et al., 1999). The production of TNFa by adipose tissue leads to the production of Il-6, which has endocrine properties and is a prime regulator of CRP

(Hak et al., 1999). According to Yudkin et al. (1999), blood concentrations as well as production of Il-6 increases with increased adiposity and approximately 30% of circulating Il-6 stems from adipose tissue. In the same study, the researchers identified a statistically significant relationship between CRP and both Il-6 and TNFa (Yudkin et al., 1999). TNFa also inhibits insulin-stimulated glucose uptake in cells and also plays a role in decreasing lipoprotein lipase activity, which are important processes in the development of several of the MetS risk factors (Frolich et al., 2000). The relationship between the pro-inflammatory cytokines that are initiated by adipose tissue and CRP has important implications for the development of the MetS.

Several studies investigating the relationship between the MetS and CRP have suggested that the components of the MetS and the MetS itself are associated with an inflammatory response (Frohlich et al., 2000; Kahn et al., 2006; Ridker et al., 2003; Rutter et al., 2004). The stratification of CRP levels and the number of MetS risk factors showed that there was a positive, statistically significant relationship between the two (Frolich et al., 2000; Rutter et al., 2004). Also, researcher has shown that CRP levels were higher among women than men in those with and without the MetS (Rutter et al., 2004).

Researchers have been unable to identify the cause and effect relationship between CRP and the MetS, but a clear relationship between the two has been identified (Frohlich et al., 2000; Kahn et al., 2006; Ridker et al., 2003; Rutter et al., 2004; Wilson et al., 2005). Additionally, recent research exploring the relationship between CRP and the MetS has shown that CRP levels increase as the number of MetS risk factors increases (Frolich et al., 2000; Rutter et al., 2004). Also of importance is the fact that the current

research on the relationship between CRP and the MetS has been done using laboratory testing and analysis for CRP (requiring a blood draw). Researchers in the current study were only able to analyze CRP levels through a finger stick and a small amount of blood by using the Cholestech LDX Analyzer.

Visceral Fat

Adiposity is classified as two different components based on primary storage site, visceral fat and subcutaneous fat. Visceral fat is a specific type of adipose tissue that is located around internal organs and is also known as intraabdominal fat. Visceral fat accounts for 10-20% of total fat in males and 5-8% in females, making it far less common than subcutaneous fat (Ibrahim, 2009). It has been shown that visceral fat has a stronger correlation to the risk factors for MetS than that of subcutaneous fat and remains so even after the adjustment for weight (BMI; Liu et al., 2010). Also, the use of these common anthropometric measurements ignores the variability that is present between the distribution of fat within ethnic groups as well as males and females. For example, it has been shown that African-Americans have higher rates of obesity as defined by BMI than European Americans, but have lower amounts of visceral fat (Liu et al., 2010). Similarly, Nazare et al. (2012) showed that East Asians had lower BMI values but higher visceral fat content than other ethnic groups.

There are anatomical and structural differences between visceral and subcutaneous fat that are crucial to understanding the role they play in the development of the MetS. One of the primary anatomical differences between visceral and subcutaneous fat is the location of venous blood drainage. Subcutaneous fat has venous drainage through systemic veins, which is in contrast to visceral fat venous drainage

which occurs via the hepatic portal vein (Ibrahim, 2009). This is significant because the portal drainage that occurs with visceral fat provides the liver with immediate access to free fatty acids as well as adipokines. Adipokines are responsible for hepatic immune mechanisms which produce important inflammatory markers such as CRP (Ibrahim, 2009).

The structural differences in visceral fat and subcutaneous fat have a significant role in the development of the MetS. In general, adipose tissue is composed of a large number of adipocytes as well as a variety of other non-fat cells such as macrophages and immune cells (Ibrahim, 2009). A distinguishing difference in structure between visceral fat and subcutaneous fat is the size of the adipocytes. The role of adipocytes within adipose tissue is to store energy, which is in the form of triglyceride droplets. Research has indicated that visceral fat contains a greater number of large adipocytes when compared to subcutaneous fat, which contains a greater number of smaller adipocytes (Ibrahim, 2009). This is important because when adipocytes get too large, they become dysfunctional and are more resistant to insulin itself as well as to the anti-lipolytic effect of insulin, which directly affects the development of the MetS (Ibrahim, 2009).

There have been a number of studies conducted to assess the predictive ability of visceral and subcutaneous fat for cardiometabolic diseases (Fox et al., 2007; Liu et al., 2010; Smith et al., 2012). The research indicates that correlations between visceral and subcutaneous fat and the risk factors for MetS were statistically significant (Fox et al., 2007; Liu et al., 2010; Smith et al., 2012). However, the correlations for visceral fat were consistently stronger than the correlations for subcutaneous fat. Additionally, only visceral fat correlations remained strong after adjusting for waist circumference and body

mass index, which suggests that quantifying visceral fat provides more information than anthropometric measurements provide (Fox et al., 2007; Liu et al., 2010). Also, research has shown that visceral fat is correlated to future risk for cardiovascular disease (Smith et al., 2012). Results from these studies indicate that visceral fat is better correlate to the MetS than subcutaneous fat and gives more information regarding MetS risk factors than common anthropometric measurements such as body mass index and waist circumference. Furthermore, these studies suggest that the relationship between visceral fat is stronger and more persistent than measures of subcutaneous fat when assessing central obesity.

As well as being strongly correlated to the MetS risk factors, visceral fat has also been shown to be strongly related to inflammatory markers such as CRP (Forouhi, Sattar, & McKeigue, 2001; Khera et al., 2009; Lemieux et al., 2001; Park et al., 2010; Saijo et al., 2004). These correlations were found in a variety of populations, including Canadians, Europeans, South and East Asians as well as in overweight and obese populations (Forouhi et al., 2001; Khera et al., 2009; Lemieux et al., 2001; Park et al., 2010; Saijo et al., 2004). The relationship between CRP and visceral fat suggests that visceral fat is an important contributor to chronic inflammation, especially in those who are overweight or obese (Forouhi et al., 2001; Khera et al., 2009; Lemieux et al., 2001; Park et al., 2010; Saijo et al., 2004). It is important to note that these correlations between the MetS and visceral fat were obtained primarily through the use of CT. There is a lack of scientific data that shows the relationship between visceral fat measured through ultrasonography and CRP levels.

Visceral Fat Measurement

Body composition methodology and risk identification has traditionally been in the form of hydrostatic weighing and more recently air displacement plethysmography. However, these forms of measuring body composition fail to account for different fat compartments and just report an overall amount or percentage of fat. Octopolar bioelectrical impedance devices have the ability to measure fat amounts in different regions of the body including the trunk, but fail to differentiate between subcutaneous and visceral fat. Similarly, external anthropometric methods of assessing abdominal obesity such as waist circumference and waist-to-hip ratio are unable to differentiate the fat compartments.

Both the NCEP ATPIII and the WHO definitions for the MetS utilize the following anthropometric measurements to evaluate obesity: BMI, waist-to-hip ratio, and waist circumference (Grundy et al., 2004). These anthropometric measurements are commonly utilized because of their cost-effectiveness and ease of use, but fail to differentiate fat compartments (visceral vs. subcutaneous) or in the case of BMI, fail to account for any sort of distribution of fat. Although these measurements are easy to administer and are correlated with other measures of central obesity, they fail to specifically account for the more harmful and metabolically active visceral fat tissue (Taylor et al., 2010). It has been shown that central obesity is more strongly associated with increased risks for metabolic and cardiovascular disease when compared to total adiposity (Hamdy et al., 2006; Ibrahim, 2009; Liu et al., 2010; Smith et al., 2012). As such, estimates of obesity within populations being studied as it relates to the MetS are inconsistent and are heavily dependent on the definitions being used. Isomma et al.

(2001) showed that obesity defined by waist-to-hip ratio was more common than obesity defined by BMI classifications. The differences were not subtle as only 10% of males and 14% of females were classified as obese using BMI classifications whereas 76% of males and 36% of females were classified as obese using waist-to-hip ratio classifications within the same population (Isomma et al., 2001).

Currently, CT is considered one of the gold standards of measuring visceral and subcutaneous fat compartments along with MRI techniques. Although these measurement methodologies are the most direct way of differentiating the fat compartments, they are also extremely expensive, non-portable, and can expose people to harmful radiation. The use of ultrasonography to measure visceral and subcutaneous fat compartments may be a more practical, cost-effective, and portable method. In addition to these benefits, the use of ultrasonography has been shown to be a valid and reproducible method of measuring visceral fat (Alempijevic et al., 2011; Gong et al., 2007; Hirooka et al., 2005; Kim et al., 2004; Rolfe et al., 2010; Shojaei et al., 2010; Stolk et al., 2003; Stolk et al., 2001). The use of ultrasonography has also been used in older populations (Rolfe et al., 2010) as well as those with chronic disease (Shojaei et al., 2010) to measure visceral fat. Additionally, it has been used to estimate risks for cardiovascular and metabolic diseases (Kim et al., 2004). Stolk et al., (2003) utilized ultrasonography measurements of visceral fat and showed that it was a better predictor of the MetS than the commonly used waist circumference measurement, which is an important finding that was used for the foundation of the current study. Although ultrasonography has been shown to be a valid method of measuring visceral fat, it has been validated in settings with clinical grade equipment and technicians which can be cost-prohibitive. Recently, an ultrasound

machine has been produced for use by allied health professionals. This provides a more cost-effective alternative to clinical ultrasound testing and needs to be validated against the MetS risk factors.

Visceral fat is a more metabolically active fat depot (Ibrahim, 2009) and is more strongly related to the MetS than subcutaneous fat (Fox et al., 2012; Fox et al., 2007; Liu et al., 2010). Therefore, it is essential to differentiate the two fat compartments when determining the relationship to the MetS rather than using common anthropometric measurements. There is also a relationship between visceral fat and inflammatory markers such as CRP, but they have only been established through the use of CT. The use of CT and MRI are the gold standards for measuring different fat compartments, but both have a high cost and can contribute to radiation exposure. The use of ultrasonography has been proposed as a valid, reproducible technique to measure visceral fat in a variety of populations and has strong correlations to measurements of visceral fat measured through both CT and MRI techniques (Alempijevic et al., 2011; Gong et al., 2007; Hirooka et al., 2005; Kim et al., 2004; Rolfe et al., 2010; Shojaei et al., 2010; Stolk, Meijer, Mali, Grobbee, & Van Der Graaf, 2003; Stolk et al., 2001).

Summary

Reviewing the current literature it is apparent that the MetS is a strong risk factor associated with elevated health care costs as well as two major lifestyle related diseases type 2 diabetes and cardiovascular disease. The syndrome is strongly associated with the level of visceral fat and inflammation and ultrasound appears to be an effective method to

assess visceral fat. If that is the case it is possible that correlating a non-invasive measure of visceral fat with inflammation may be used as method for diagnosis of MetS.

Chapter 3

Methods

The primary purpose of the current study was to determine the relationship between CRP, visceral fat, and the MetS risk factors using a modified NCEP ATPIII definition. This chapter provides a description of the methodology that was used to test the hypotheses for the current study. More specifically, this chapter provides a background of the participants, information on the instrumentation used, an overview of the procedures, and a description of the statistical analyses performed.

Participants

This study was delimited to participants over the age of 40 and testing was limited to a maximum of 50 participants. The participants were recruited through a variety of methods including informational flyers, word of mouth, and referrals from in and around the community.

Instrumentation

To conduct this study, the following variables were measured in each participant: height, weight, age, sex, triglyceride levels, HDL-C, fasting blood glucose levels, blood pressure, hip and waist circumference, visceral and subcutaneous fat, and CRP. Demographic information was collected through an interview with each participant.

Blood pressure was measured with an aneroid sphygmomanometer (ADC Diagnostix, Hauppage, NY) after being seated for at least five minutes. Height and weight were measured by a physician's scale (Cardinal Scale Manufacturing Co., Webb City, MO) and stadiometer. Hip and waist circumference were measured with the use of a

Gulick tape measure. TG, HDL-C, fasting blood glucose, and CRP was measured through the use of a Cholestech LDX blood analyzer (Cholestech Corporation, Hayward, CA). Visceral and subcutaneous fat levels were measured with a BodyMetrix (IntelaMetrix, Inc., Livermore, CA) ultrasonography machine.

Procedure

This study was approved through the Institutional Review Board (IRB) at Eastern Washington University. Once the study was approved through the IRB, participants were recruited to participate in the study. Prior to participation, an informational meeting was held for all potential participants to go over informed consent. Participants were given 24-48 hours to decide whether they wanted to participate after full disclosure of the study had been given.

All testing was completed in the Human Performance Lab at Eastern Washington University. Blood pressure was taken after the participant was seated for at least five minutes. Participants' height and weight were measured using a physician's scale and stadiometer. A finger stick blood sample using a sterile lancet was performed to analyze TG, HDL-C, blood glucose, and CRP. TG, HDL-C, and blood glucose were measured in one cassette (35 ml blood) and CRP was measured in a separate cassette (40 ml blood). The two cassettes were then placed in the Cholestech LDX Analyzer for analysis. Participants had their waist circumference measured through the use of a Gulick tape measure, which was placed at the superior portion of the iliac crest for measurement. The last procedure that participants went through was an ultrasound scan of their abdomen to assess visceral and subcutaneous fat levels. The abdomen scan was performed by placing the gelled wand 1 cm to the right of the umbilicus and slowly moving it three to four

inches towards the hip while maintaining a perpendicular position to the surface of the skin. The measurement depths for visceral and subcutaneous fat were not calculated by the machine, so they were determined by the researchers utilizing the BodyMetrix software. The different fat layers are not consistent throughout the body, so the depth varied across the scan. Because of this, two researchers agreed on the greatest depth for each participant.

Data was collected on a separate data collection sheet, void of any identifiers that was later entered into a Microsoft Excel file before being imported into SPSS for analysis. Participants had the option of receiving their results from all the tests that were performed. Educational materials were also available for participants who wanted more information about their results.

Analysis

Statistical analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL). Data was screened for outliers as well as checked for normality. Descriptive statistics were provided on all of the MetS risk factors and frequencies were run to determine the number of participants with and without the MetS. To examine the relationship between the MetS risk factors, visceral fat, and CRP, Pearson's correlations were utilized. T-tests were also used to identify any differences between groups. Alpha levels were set at $p \leq .05$ for all statistical tests.

Summary

Using the outlined procedures and methodology, the researchers were able to address the primary purpose of the current study, which was to determine the relationship between CRP, an inflammatory marker, and MetS risk factors. Conclusions drawn from

the statistical analyses were used to further the understanding of the interrelationships between CRP and MetS risk factors (including visceral fat).

Chapter 4

Results

Introduction

The purpose of this study was to determine the relationship between CRP, visceral fat, and the MetS risk factors. This chapter provides a summary of the results of the statistical analysis described in the previous chapter.

Descriptive Statistics

Forty-five participants were initially recruited for data collection. Five participants were excluded from analysis due to the CRP samples not reacting. Of the remaining forty participants, another five were excluded from the analysis due to missing TG values. Additionally, one participant was excluded due to having an abnormally high CRP value. The overall means and standard deviations for the remaining 34 participants included in the analysis are summarized in Table 1 as well as the means and standard deviations for those with and without the MetS. In this study, the NCEP ATP III definition of the MetS was used as previously described. Participants who were taking hypertension medication were considered positive for the BP risk factor, regardless of their measured values. Similarly, participants who were taking medication for diabetes were considered positive for the impaired fasting glucose risk factor, regardless of their measured values.

MetS Risk Factor Distribution

Twenty-one percent (n = 7) of our sample population had the MetS. The most common risk factor was increased BP (62%) while the least common was increased WC (6%). A similar number of participants had impaired fasting glucose (38%) compared to

those with elevated TG (32%). Low HDL occurred in only 18% of the participants in the current study.

Table 1. Descriptive statistics of sample population (Mean ± SD)

Variable	Total (n = 34)	With MetS (n = 7)	Without MetS (n = 27)
Age	57.47 ± 9.96	63.29 ± 8.36	55.96 ± 9.91
Weight (kg)	74.77 ± 13.58	75.40 ± 12.20	76.32 ± 14.13
Height (m)	1.70 ± 0.09	1.63 ± 0.08	1.71 ± 0.09*
Waist Circumference (cm)	88.37 ± 11.92	96.14 ± 9.58	86.35 ± 11.78
Visceral Fat Depth (mm)	17.44 ± 7.16	18.81 ± 10.32	17.08 ± 6.31
Systolic Blood Pressure (mmHg)	116.65 ± 9.06	123.71 ± 10.55	114.82 ± 7.85*
Diastolic Blood Pressure (mmHg)	75.88 ± 8.90	79.43 ± 12.53	74.96 ± 7.75
High Density Lipoproteins (mg/dL)	61.77 ± 19.44	51.14 ± 16.80	64.52 ± 3.43
Triglycerides (mg/dL)	102.88 ± 64.88	182.43 ± 51.21	82.26 ± 50.91**
Blood Glucose (mg/dL)	98.82 ± 14.23	116.86 ± 16.28	94.15 ± 9.26**
C-reactive protein (mg/L)	1.46 ± 1.11	2.71 ± 1.37	1.14 ± 0.78**

**p < .05, **p < .01

Pearson's Correlations

Correlations between the MetS risk factors, visceral fat, and CRP are reported in Table 2. Significant moderate correlations were found between CRP and TG (r = .50, p = .05). No other MetS risk factors were found to be significantly correlated. There was a significant, moderate correlation between CRP and visceral fat depth as measured by ultrasound (r = .34, p = .05). There was a weak to moderate non-significant correlation between CRP and FG (r = .31, p = .08), CRP and WC (r = .29, p = .10), and CRP and systolic BP (r =

.28, p = .11). Also, there were weak to moderate, non-significant correlations between visceral fat and WC and visceral fat and systolic BP (r = .31, p = .07; r = .31, p = .08).

Table 2. Correlations between MetS risk factors, visceral fat, and CRP

	WC	SPB	DPB	CRP	HDL	TG	VF	FG	LDL
WC	-								
SBP	.42*	-							
DBP	.07	.64**	-						
CRP	.29	.28	.00	-					
HDL	-.12	-.05	-.34	.09	-				
TG	.40*	.42*	.29	.50**	-.09	-			
VF	.31	.31	.18	.34*	.06	.12	-		
FG	.38*	.11	-.03	.31	.01	.64**	-.07	-	
LDL	.14	.15	.16	.21	-.38*	.32	-.10	.37	-

*p < .05, **p < .01

Summary

A significant correlation was found between CRP and visceral fat as well as between CRP and TG. Non-significant weak to moderate correlations were found between CRP and three MetS risk factors (BP, FG, and WC). There were also non-significant, weak to moderate correlations between visceral fat and WC and visceral fat and systolic BP. Overall, 21% of the studied population had the MetS and BP was the most common MetS risk factor while WC was the least common risk factor.

Chapter 5

Discussion

Introduction

The primary purpose of this study was to determine the relationship between CRP, visceral fat, and the MetS risk factors. The following chapter discusses the results of the current study as they relate to the current literature.

Summary of results

A total of 34 participants over the age of 40 were used in the analysis. In the current study, 21% (n = 7) of the population had at least three risk factors, classifying them as having the MetS. The frequency of the MetS in this population is slightly lower than previously reported in the literature (23% - 35%; Malone et al., 2009).

CRP and visceral fat

A significant positive correlation was found between CRP and visceral fat as measured through ultrasonography. These results are in accordance with previous studies that have identified a relationship between CRP and visceral fat (Forouhi et al., 2001; Lemieux et al., 2001; Park et al., 2010; Saijo et al., 2004; Stolk et al., 2003). Previous studies have assessed the validity and reliability of using ultrasonography to measure different fat compartments, but none of them included CRP measures in their comparisons (Gong et al., 2007; Hirooka et al., 2005; Stolk et al., 2003).

Although CRP and visceral fat have been shown to have a positive relationship the mechanism for this relationship is unclear. One suggested mechanism is related to the cytokines produced by adipose tissue. Both IL-6 and TNFa are secreted by adipose tissue as proinflammatory cytokines, which can stimulate the liver to produce larger quantities

of CRP (Park et al., 2010). Visceral fat has been shown to be the more metabolically active adipose tissue in terms of production of both IL-6 and TNFa when compared to subcutaneous fat (Park et al., 2010).

Similar to previous findings in the literature, women in the current study exhibited significantly higher amounts of CRP compared to men (1.90 vs. 0.77 mg/L; $p = 0.002$; Park et al., 2010). Lear et al., (2003) also showed that women, independent of age, waist to hip ratio, smoking, and alcohol consumption, had consistently higher CRP values when compared to men. One possible explanation is women who have reduced amounts of estrogen tend to have higher amounts of visceral fat (Spangenburg, Wohlers, & Valencia, 2012). In the current study, the average age of women was 59.38 years old and the majority of women tested were post-menopausal. A potential outcome of age and menopausal status of women tested is reduced estrogen and increased visceral fat or increased fat cell size, which may contribute to the statistically higher amounts of CRP in women.

In the current study, a relationship between visceral fat and the MetS risk factors was identified through the use of an ultrasonography tool, which found similar results of Stolk et al., (2003). Of further note, Stolk et al., (2003) used a clinical grade ultrasonography machine within a clinical setting, whereas the current study used an inexpensive, consumer-grade, the BodyMetrix handheld ultrasonography wand that is portable and compatible with most computers. Because the BodyMetrix ultrasound machine produced similar results as a clinical-grade ultrasound machine as well as CT and MRI scans, it has the potential to be used as a screening tool for those who are at risk of the MetS. It may also be used by individuals or professionals to assess changes in the

different types of fat (visceral and subcutaneous), which are known to have an influence on CRP levels.

CRP and MetS risk factors

Associations between CRP and the components of a lipoprotein-lipid profile have been identified in previous literature, yet TG was the only component with a significant relationship in the current study (Lemieux et al., 2001). The significant relationship between CRP and TG values may suggest that there is a link between inflammation and the body's ability to clear fat from the blood. A potential explanation of the relationship between inflammation and fat clearance is the effect of the IL-6 cytokine. Approximately 30% of circulating IL-6 stems from adipose tissue in the body (Yudkin et al., 1999). In addition to stimulating CRP production in the liver, IL-6 and TNFa also affect the activity of lipoprotein lipase, which is the protein that is responsible for the breakdown of lipoproteins (Lemieux et al., 2001).

There were also weak non-significant correlations between CRP and WC, CRP and systolic BP, and CRP and blood glucose. These same relationships have been identified as significant correlations in previous studies (Ridker et al., 2002). The lack of significance in the current study may be attributed to the relatively small sample size compared to the larger, epidemiological studies in which significance was found and also the fact that only seven out of 34 participants had the MetS in the current study.

Future Research Directions

Further research should include a larger sample size to ensure that the correlations persist. Also, technicians who utilize the BodyMetrix software should become accustomed to the process ahead of time and screen each image before moving on to the

next participant, as the software does not allow the users to go back and track the interfaces later. During data reduction, the interfaces on the ultrasound images from several participants were difficult to identify, which may have led to inaccurate visceral fat values. It may not be possible to produce images with easily identifiable interfaces, but the technique can be improved upon for future research.

The validity of the BodyMetrix as compared to clinical grade ultrasound machines using strict protocols should be explored. It would also be beneficial to validate the BodyMetrix against the gold standards of CT and/or MRI scans of fat compartments. These validations against the gold standards of measuring the different fat compartments would strengthen the value of the BodyMetrix machine for use with clinical populations as well as in research settings.

In order to explore a possible explanation for the relationship between CRP and visceral fat through ultrasonography, it would be beneficial to include TNFa and IL-6 measurements as well. It has been proposed that these cytokines play a role in the development of the MetS risk factors as well as elevated levels of CRP.

Finally, it is of future interest to determine if changes in the amounts of visceral fat lead to a reduction in CRP levels over time. To assess this, an exercise protocol could be established to target visceral fat loss and the BodyMetrix machine could be used to identify changes in fat levels over the course of the experiment.

Conclusion

The results of the current study suggest that there is a relationship between visceral fat and CRP. This finding suggests that elevated levels of visceral fat plays a role in chronic inflammation. This relationship did not exist between WC, a crude

measurement of visceral fat, and CRP, suggesting that more direct measurements of visceral fat such as ultrasonography are more predictive of chronic inflammation. It has been shown that chronic inflammation increases the risk for future cardiovascular disease, which also gives weight to the potential use of the BodyMetrix ultrasonography tool to detect changes in visceral fat levels over time.

To add to and strengthen the results of the current study, it is necessary to conduct a study with a larger number of participants and to include the measurement of proinflammatory cytokines such as IL-6 and TNFa. Also, exploring the reliability and sensitivity of the BodyMetrix machine to detect changes in visceral fat over time may be useful in showing that the machine can be used as a preventive tool for the MetS. Implementing an exercise protocol targeted at visceral fat loss can help determine if a change in fat levels will also lead to a reduction in CRP values over time. Finally, using venous blood draws to assess lipid profiles, glucose, and CRP may be preferred as they produce more sensitive results.

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Appendix 1: IRB Approval

To: Evan Hilberg, Department of Physical Education, Health and Recreation, 200 PEB
From: Sarah A.C. Keller, Chair, Institutional Review Board

Date: February 21, 2013

Subject: Expedited Review of *Determining the Relationship Between C-Reactive Protein and the Metabolic Syndrome Risk Factors* (HS-4172)

The Institutional Review Board for Human Subjects' Expedited Review Committee has reviewed your proposal to evaluate the use of handheld ultrasonography as a method in the measurement of visceral fat as part of an assessment of cardiovascular risk and compare it to the most common methods of estimating body composition (bioelectrical impedance analysis and three site skin folds).

The Expedited Review Committee has approved your application subject to the conditions noted below; a signed, approved copy of your application is enclosed.

Before you begin:

1. We will need a copy of the recruiting flyer that you will be distributing.
2. Is Dr. Peterson going to be available at EWU for training of technicians and lifestyle counseling?
3. You may provide the results of your tests to the subjects if they want them but as a student you may not provide them your analysis of their results. If they are to receive any analysis or lifestyle counseling it must be provided by a professional such as Drs. Peterson or Repovich or their own physician.
4. Is the health questionnaire you are using the Par-Q? If not will you please provide us a copy of the instrument for our files.

Human subjects research approval granted by the IRB is good for one year from the date of approval, to February 21, 2014. If research is to continue, with no substantial changes, beyond that date, a renewal of IRB approval must be obtained prior to continuation of the project (contact OGRD for procedure). If, subsequent to initial approval, a research protocol requires minor changes, the OGRD should be notified of those changes. Any major departures from the original proposal must be approved by the appropriate review process before the protocol may be altered. A Change of Protocol application must be submitted to the IRB for any substantial change in the protocol. The Director, Grant and Research Development, or the Chair of the IRB will determine whether or not the research must then be resubmitted for approval.

If you have additional questions please contact me at 509-359-7039; fax 509-359-2474: email: skeller@ewu.edu. It would be helpful if you would refer to HS-4172 if there were further correspondence as we file everything under this number. Thank you.

cc: R.Galm
J.Kawaguchi
W.Repovich
Graduate Office

Appendix 2: Informed Consent

Informed Consent Form

"Determining the relationship between C-reactive protein and the metabolic syndrome risk factors"

In partial fulfillment of Master's Thesis for Evan Hilberg

Principal Investigator
Evan Hilberg
1604 3rd Street Cheney, WA 99004
541-760-5072
evanhilberg@gmail.com

Responsible Project Investigator
Wendy Repovich, Ph.D., FACSM
Physical Education, Health and Recreation Dept.
200 Physical Education Bldg.
Cheney, WA 99004-2476

Purpose and Benefits

Risk for metabolic and cardiovascular disease is assessed through several biometric measures such as body composition, glucose, cholesterol, blood markers of inflammation (C-Reactive Protein), blood pressure, physical activity and visceral fat. The purpose of this study is to determine the relationship between c-reactive protein and the metabolic syndrome.

Procedures

Participation in this study will require the following procedures:

1) A questionnaire regarding current physical activity levels will be completed.
2) Height and weight will be measured on a standard scale.
2) Hip and waist circumference will be measured using a measuring tape.
3) Blood pressure will be measured on my right arm.
4) A finger prick will be conducted to collect 6 drops of blood for Cholesterol, C-Reactive protein and blood glucose tests.
5) Body fat percentage will be measured using skin folds, ultrasonography and bioelectrical impedance analysis as described below:
   - **Skin Fold:** a small fold of skin will be measured by a trained investigator at three sites: men- chest, abdomen and thigh and women- triceps, suprilium (hip) and thigh.
   - **Ultrasonography**: A dime-sized amount of ultrasound gel will be placed on each site and the scan will be performed by moving the ultrasound wand over the site – back and forth three or four times. The sites are the same for skin folds: men- chest, abdomen and thigh and women- triceps, suprilium (hip) and thigh.
   - **Bioelectrical Impedance Analysis:** A machine (similar to a scale) will be used to estimate body fat percentage by sending a small electrical current through my body.

7) Visceral Fat will be estimated by Ultrasonography. A dime-sized amount of ultrasound gel will be placed on my abdomen and the scan will be performed by moving the device back and forth three to four inches above the right hip.

Risk, Stress or Discomfort

There is a minimal risk of an allergic reaction to the ultrasound gel. Bruising can occur from the skin folds, ultrasonography, blood pressure readings, and finger stick. There is risk of an infection at the finger stick site. Body composition results may cause some stress for certain individuals. Lifestyle counseling will be available for my benefit at the time of the measurements if needed.

Inquiries

Any questions about the procedures used in this study are encouraged. If you have any concerns, questions, or would like more information please contact Wendy Repovich or Evan Hilberg prior to signing the informed consent form. We can be reached at (509)-359-7960; wrepovich@ewu.edu and (541) 760-5072 evanhilberg@gmail.com respectively.

Other Information

You are requested to not engage in an alternate training program or to alter your diet while you are taking part in the study. If you have any concerns about your rights as a participant in this research or any complaints you wish to make, you may contact Ruth Galm, Human Protection Administrator, (509) 359-6567 or rgalm@ewu.edu.

_____ _____

Signature of Principal Investigator Date

Subject Statement

My participation in this study is completely voluntary. I am free to refuse participation and to stop at any point in this study. I understand the study procedures that I will perform, and the possible risks that go along with the testing and training. Knowing all of the risks and discomforts, and being allowed to ask questions that have been answered to my satisfaction, I consent to take part in this study. I am not waiving my legal rights by signing this form. I understand I will receive a signed copy of this consent form.

_____ _____

Signature of Participant Date

Appendix 3: Data Collection Sheet

Date _____ Subject ID# _____

Demographics

Age	Sex	Ht (in)	Wt (lbs)	WaistCir	HipCir	BP	Smoke

Physical Activity	Mod(min)	Vig (min)

Medications	HyperTen	Cholest	Diabetes	HRT	Estrogen
Yes					
No					

Clinical Values

Time Fasted _____

CRP	TC	HDL-C	LDL-C	TG	FG

Body Composition

	Skin Folds		**Ultrasound**
Male/Female	**Trial 1**	**Trial 2**	**Trial 1**
Triceps/Chest			
SupIliac/Abd			
Thigh/Thigh			

Bioeletrical Impedance

BF %	
Trunk %	

Ultrasound Measurements

BF%	
VAT	
SCAT	
Total	

Appendix 4: Health History Questions

Family History/Medication Questions:

- Do you have a family history of myocardial infarction, coronary revascularization, or sudden death?
    - before 55 years of age in father or other male first degree relative (i.e., brother or son)
    - before 65 years of age in mother or other female first degree relative (i.e., sister or daughter)
- Are you currently on any of the following medications?
    - Hypertension
    - Diabetes
    - Cholesterol
    - Hormone replacement therapy
    - Estrogen

CURRICULUM VITAE

Evan Hilberg

Education:

M.S., Eastern Washington University, Physical Education-Exercise Science, 2013
B.S., Linfield College, Exercise Science, 2011

Employment History:

09/2012- Present: Graduate Assistant, PEHR, Eastern Washington University
09/2011-08/2012: *Let's Move, Cheney* Program Specialist, AmeriCorps
05/2010-05/2011: Student Center Director, Associated Students of Linfield College
08/2008-05/2010: Resident Advisor, Linfield College

Teaching Experience:

01/2013-03/2013- Eastern Washington University

PHED 125- Basketball (3), Tennis (2), Floor Hockey- created and distributed syllabi, taught skills and rules of sport. Facilitated classes to be safe and competitive.

EXSC 490- Senior Capstone in Exercise Science- Supervised a student research project and assisted with laboratory (YMCA Bike Test and Skinfolds). Assisted with grading.

EXSC 460- Physiology of Exercise- Graded assignments and exams, moderated in-class discussions, provided lab testing demonstration (VO2 max and Wingate).

EXSC 349-Anatomical Kinesiology- Assisted with lectures and tutored students.

09/2012-12/2012- Eastern Washington University

PHED 125- Basketball (3), Racquetball, Lacrosse, Flag Football- created and distributed syllabi, taught skills and rules of sport. Facilitated classes to be safe and competitive.

EXSC 460- Physiology of Exercise- Graded assignments and exams, moderated in-class discussions, provided lab testing demonstration (VO2 max and Wingate).

EXSC 455- Research and Analysis- Graded assignments and exams.

02/2011-05/2011- Linfield College

BIO 213- Human Physiology Lab- Tutored students in lecture material; prepared lab; demonstrated procedures; maintained inventory of supplies and equipment.

09/2008-05-2011- Linfield College

BIO 212- Anatomy Lab- Tutored students in lecture material; set-up, administer, breakdown of practical exams, prepared cadaver and other lab specimens, maintained inventory of supplies and equipment

09/2010-12/2010- Linfield College

HHPA 440- Physiology of Exercise Lab- Tutored students in lecture material; set-up, administer, breakdown of practical exams; demonstrated testing procedures; grading

Academic/Research Interests:

Community and public health obesity prevention strategies
Psychosocial determinants of health and behavior change
Prevention and treatment of chronic diseases through physical activity

Professional Activities

Organization Membership

American College of Sports Medicine (ACSM)
American Alliance for Health, Physical Education, Recreation, and Dance (AAPEHRD)
National Society of Physical Activity Practitioners in Public Health (NSPAPPH)

Service

ACSM Northwest Executive Board- National Student Representative, 2011-2013
ACSM Student Affairs Committee Chair, 2012-present
ACSM Northwest Annual Meeting Planning Committee, 2012, 2013
Student Policies Faculty Committee, Linfield College, 2011

Awards/Honors:

Presidential Award for Volunteer Service, 2012
PEK Honor Society- Linfield College, 2011
American Legion Volunteer Award, 2008