Investigation of C-Reactive Protein and Leptin as Biomarkers of Obesity with Potential Clinical Utility

Rachel Ann Friedman

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INVESTIGATION OF C-REACTIVE PROTEIN AND LEPTIN AS BIOMARKERS OF OBESITY WITH POTENTIAL CLINICAL UTILITY

A Thesis
Presented to
The Faculty of the Department of Kinesiology, Recreation, and Sport
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Rachel Ann Friedman
August 2011
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Obesity and its subsequent disease states are major health problems in the United States. In many ways, obesity can be considered a “disease state” itself due to the changes it causes on the body. High-intensity exercise also places acute stress on the body, putting humans in recovery from exercise in a state that may be analogous to a temporary disease state. The purpose of this study was to examine biomarkers associated with obesity (CRP and Leptin) before and after continuous and intermittent bouts of exercise in an obese but otherwise healthy sample vs. a healthy, non-obese sample. This investigation focused on examining the obese sample’s biomarkers at rest compared to those of the healthy group immediately and 1 hour-post exercise. Eighteen male subjects participated, with nine in each group. Each subject performed a VO$_2$ max test and a series of three anaerobic Wingate tests at least one week apart in a cross-over study design. Blood was taken at baseline, immediately-post, and 1-hour post for each exercise mode. A significant difference was noted between groups for CRP at baseline on the VO$_2$ testing day. A significant difference between groups existed in leptin levels at baseline on both testing days. The only significant change was the decrease in leptin from post to 1-hour post for during the VO$_2$ in the obese group. However, both exercise protocols demonstrated various effects on the subjects and groups. Healthy participants were examined individually, and two of them showed possible signs of being at risk for obesity and its subsequent disease states based on post exercise “spikes” in CRP and leptin that
caused the levels of the biomarkers to be closer to those in the obese group at rest.

Another three subjects saw at least two spikes. Thus, a total of five subjects could potentially be “at-risk” based on the assumptions of the present study. These results suggest CRP and Leptin could potentially hold the ability to classify someone in a “pre-obesity state.” Further investigations are warranted based on these initial results and should focus on biomarkers more specific to obesity.
CHAPTER 1

INTRODUCTION

Obesity is defined as a condition of increased adipose tissue mass resulting in an increase in body weight beyond the limits of physical requirement (Sikaris, 2004). Fundamentally, obesity is the result of excess energy intake compared to energy expenditure (Sikaris, 2004). The World Health Organization defines obesity as a body mass index of greater than or equal to 30 kg/m$^2$ (1985).

It is estimated that 32 percent of adults in the US are clinically obese (Ogden, 2006). When examining mortality data from the National Health and Nutrition Examination Survey (NHANES) 2000, death rates were significantly lower for those who had a healthy body mass index when compared with the obese population (Flegal et al. 2005). A study by Alison et al. concluded that the estimated number of annual deaths attributable to obesity among US adults is approximately 325,000 from all subjects and 280,000 from only nonsmokers and never-smokers (1999). Researchers claim that increased mortality attributed to obesity results partly from the increased risk of chronic diseases such as cardiovascular disease, diabetes mellitus, dyslipidemia, and hypertension (Bakhai, 2008). Obesity also promotes a state of low-level systemic chronic inflammation and a prothrombic state, both of which can lead to atherosclerosis and subsequent cardiovascular events (Bakhai, 2008). It has been shown that cardiovascular disease remains a leading cause of death outside of the US as well, accounting for 37% of deaths in the United Kingdom (Deaths by Cause, Sex, and Age, 2004). From examining the literature, it is apparent that obesity is a major health problem in the United States claiming many lives each year.
Obesity is clearly a major issue due to the subsequent disease states with which it is associated. In many ways, obesity is considered a “disease state” itself due to the changes it causes on the body as well as increasing the risk for a large number of additional diseases. Excess fat accumulation is associated with inflammatory changes as well as increased oxidative stress (Musaad and Haynes, 2007). There are a multitude of biomarkers that can be measured in human blood that are indicative of inflammatory changes and/or oxidative stress. Therefore, these biomarkers also possess possible links to obesity. By identifying these biomarkers for oxidative stress and inflammation, the potential exists that they could be used for early prediction of cardiovascular disease (Musaad and Haynes, 2007). Two biomarkers that have been supported by research to have a clear link to obesity are leptin and c-reactive protein (CRP) (Mussad and Haynes, 2007). Due to their potential clinical utility and connection to obesity, these biomarkers possess the potential to aid in classifying seemingly healthy individuals into a “pre-obesity state.”

Leptin is an adipokine released from adipose tissues that plays a large role in the negative feedback loop that controls hunger. Ultimately, leptin aids in the regulation of body weight homeostasis (Thong et al. 2000). Leptin is also found in the hypothalamus of the brain, which is involved in the regulation of body weight (Eikelis et al. 2004). A study by Shutte et al. demonstrated that leptin is closely associated with obesity and risk factors for cardiovascular disease such as increase systolic blood pressure (2005). One mechanism by which leptin can increase cardiovascular disease risk is via increased inflammatory markers, and it plays a role in vascular dysfunction. It may also “tell” one’s body that there is not enough fat, thus, producing more in response, thus, increasing
risk for diseases. Two studies by S. Soderberg demonstrated its direct association with both stroke and acute myocardial infarction (1999). The research clearly supports the idea that leptin has links to the disease state of obesity and other subsequent disease states, thus, unlocking the potential to make future health predictions about these ailments.

C-reactive protein is the most extensively studied inflammatory biomarker (Nguyen et al. 2009). It is a protein produced by hepatocytes in the presence of inflammation due to factors such as infection, injury, or conditions such as obesity. Elevated levels of CRP have been associated with increased inflammation in the coronary arteries, and thus it is marker for increased risk of atherosclerosis and cardiovascular disease (Nguyen et al. 2009). Studies using NHANES III data have shown that levels of CRP are elevated in individuals with a high BMI (Ford 1999, Visser 1999). Nguyen’s study found that with each increase in weight category, the mean CRP level increased significantly. The higher the weight class on the BMI scale (obesity class 1, obesity class 2, etc…), the larger the increase in CRP. The largest amount of CRP concentration was reported in individuals in obesity class three (2009). CRP increases with increased body fat, and the more CRP one possesses, the greater likelihood of many diseases.

There is a great deal of literature on obesity, its associated disease states, and its biomarkers. The obesity epidemic is a very real and serious problem in the United States. Although there is an abundance of research on obesity, many neglect to look at the underlying biomarkers. The possibility exists that we look at the mentioned biomarkers (among others) to ultimately predict one’s risk for obesity before they are even overweight. The reasoning behind this logic is that if obesity is considered a “disease state” where one’s body is being stressed, and exercise is another means of stressing your
body, there could be biochemical similarities in someone who is clinically obese (but otherwise healthy) at rest and a seemingly healthy person who is in recovery from intense continuous or intermittent exercise. If there are similarities in these biomarkers, further research could show that this is a means to predict obesity and other disease states, and could ultimately aid in prevention of these health issues.

Statement of Purpose

Both CRP and Leptin are closely associated with obesity and other subsequent disease states. Considering this, it is already known that being “obese” basically means that one is constantly in what researchers consider a “disease state.” Then, consider a healthy individual who is recovering from an acute bout of aerobic exercise. For that brief recovery period, they are in a state that could be considered analogous to being in a disease state, where their body is trying to return to homeostasis. The idea behind this study is to examine these biomarkers before and after an aerobic bout of exercise in the obese but otherwise healthy population and a healthy population. It will be interesting to see the differences at rest, but that has been explored before. However, no previous studies have compared the biomarkers in a healthy person’s blood when recovering from exercise to an obese person’s biomarkers at rest. If we can find similarities in the two, it is possible to suggest that we could predict obesity or other disease states based on these markers. We could then suggest that even though someone is seemingly thin and healthy, that they are in a “pre-obesity” state. This knowledge, once further investigated, could allow a simple finger prick to do the job of an extensive health exam. It may also be helpful in predicting the likelihood of obesity and other subsequent disease states.
Statement of Hypothesis

RESTING LEVELS

$H_{o1}$: There will be no difference in resting leptin and CRP plasma concentration between those who are obese but otherwise healthy and those who are non-obese healthy.

$H_{a1}$: Resting plasma concentrations of Leptin and CRP will be elevated in obese but otherwise healthy individuals compared to healthy non-obese individuals.

LEPTIN

$H_{o2}$: There will be no notable similarities between the amount of leptin in the obese at rest and the biomarkers of the healthy after an acute aerobic exercise session.

$H_{a2}$: There will be notable similarities in the amount of leptin in the obese at rest and the leptin levels in the healthy person after an acute aerobic exercise session.

CRP

$H_{o3}$: There will be no notable similarities between the amount of CRP in the obese at rest and the biomarkers of the healthy after an acute aerobic exercise session.

$H_{a3}$: There will be notable similarities in the amount of CRP in the obese at rest and the CRP levels in the healthy person after an acute aerobic exercise session.

Definition of Terms

- Biomarkers- A biochemical feature or facet that can be used to measure the progress of disease or the effects of treatment. They are generally found in the blood.
- Adipoctyokines- class of protein that synthesize and secrete numerous enzymes, growth factors, cytokines, and hormones that are involved in overall energy
homeostasis, specifically lipid homeostasis. These are released from adipose tissues.

- Cardiovascular disease- Disease affecting the heart or blood vessels. These include arteriosclerosis, coronary artery disease, heart valve disease, arrhythmia, and heart failure.

- Atherosclerosis- A process of progressive thickening and hardening of the walls of medium-sized and large arteries as a result of fat deposits on their inner lining. Risk factors for atherosclerosis include high levels of "bad" cholesterol, high blood pressure (hypertension), smoking, diabetes and a genetic family history of atherosclerotic disease.

- Dislipidemia- A disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. Dislipidemias may be manifested by elevation of the total cholesterol, the "bad" low-density lipoprotein (LDL) cholesterol and the triglyceride concentrations, and a decrease in the "good" high-density lipoprotein (HDL) cholesterol concentration in the blood.

- Insulin Resistance- Insulin resistance is a condition in which the cells of the body become resistant to the effects of insulin. It is often the result of being diabetic, and the body can’t get glucose into the cell’s properly because of this insulin resistance.

- Inflammation- A basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. Inflammation is now recognized as a type of nonspecific immune response.
• Oxidative Stress- general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by free radicals and peroxides.

• Hypertension- High blood pressure means high tension in the arteries. Arteries are vessels that carry blood from the pumping heart to all the tissues and organs of the body. A blood pressure of 140/90 or above is considered high.

• Myocardial infarction (heart attack)- The death of heart muscle from the sudden blockage of a coronary artery by a blood clot.

• Leptin: protein hormone released from adipose tissues and plays a large role in the negative feedback loop that controls hunger. It often signals the brain that one does not have enough fat, thus, causing more to be produced.

• C-Reactive Protein (CRP): inflammatory biomarker produced in the presence of inflammation due to factors such as infection, injury, or conditions such as obesity.

Limitations

• Recruited subjects who are “fat but fit”- A BMI of >30kg/m² but with no other health issues or risk factors (insulin resistance, high cholesterol, etc…)

• Only tested men, so can’t generalize the results for everyone

• Some people likely pushed themselves harder than others on both of the tests, so that may have caused some of the data to not be as useful

• The challenge of putting obese people through very strenuous aerobic exercise could have caused them to have to end tests prematurely

• Human error

• Sweat mixed with blood
• Previous exercise training could have skewed the results
• Our subjects did not perform a 12 hr fast before baseline testing (only asked not eat for 4 hours before exercise)
• Limitations associated with individual kits/processes in doing blood work
• Much of the study was based on assumptions
• Time constraints

Delimitations
• There were 9 healthy males between 18-44 who have no preexisting medical conditions or cardiovascular risk factors to make up the “healthy” group
• There were 9 males who are “fat but fit”- meaning they have a BMI over 30kg/m$^2$ but have no other health conditions or risk factors- This will be identified using the ACSM risk stratification questionnaire
• Both groups completed a continuous and intermittent exercise session
• Both groups had BMI calculated
• Both groups had body fat percentage measured via seven-site skinfold protocol
• Subjects were matched for height and fitness level as best as possible
• The aerobic session consisted of a VO$_2$ max test where they ran on a treadmill with increasing speeds and grades until they couldn’t go anymore. They will follow the Bruce Protocol. This test gave us a VO$_2$ max for each subject
• The anaerobic session consisted of three repeated Wingate cycling tests with 2 minutes in between each 30 second bout. We calculated 7.5% of their body weight in kilograms prior to the start of the test. They pedaled as fast as they could for about 10 seconds, and then 7.5% of their body weight was dropped on
the flywheel. They then pedaled as hard as they could for 30 seconds. Peak and mean power output were then able to be calculated.

- The two sessions were separated by at least a week, but not more than 10 days

- Before each test (aerobic and anaerobic) blood was taken to get a resting measurement

- Blood was taken again immediately at the cession of the exercise session and then again one hour post exercise

- The blood was analyzed using ELISA kits and a microtiter plate reader

- With the $V_{O2}$ max test, an oxygen mask was put on the subject and their oxygen, carbon dioxide, and RER levels were monitored using a metabolic cart (True One 2400, Parvomedics True One Metabolic System, Sandy, Utah)
CHAPTER 2

REVIEW OF LITERATURE

Obesity is a worldwide epidemic that has been the foundation of a great deal of research in recent years. This is likely due to the fact that obesity has become a much more significant issue in the past several decades, due to it being linked to a large number of disease states and conditions. A large number of studies have also investigated causes of obesity, and whether it is more related to environmental or genetic influences. With this in mind, biochemists have taken quite an interest in obesity and investigate the biochemical changes as a result of obesity, as well as conditions that could contribute to obesity. They refer to many of the items they investigate as biomarkers. The present study will focus on two biomarkers; c-reactive protein and leptin. These are related to obesity and its subsequent disease states, and they may have the potential to be used as powerful diagnostic tools.

Another recent addition to the literature is the notion of being “fat but fit,” which has garnered a fair amount of attention in the last few years. This is significant to the present study in that half of our subjects will ultimately fall into the criteria of being “fat but fit.

C-Reactive Protein

A review article by Musaad (2007) discusses obesity as a risk factor for cardiovascular diseases as well as its increased prevalence in society. Although the association between obesity and cardiovascular disease risk has been well-established, the mechanisms causing this increased risk are less well-characterized. Inflammation and increased oxidative stress are two potential explanations that could play a major role. Studies have
investigated these two conditions and have found that they both rely on biomarkers. However, validated biomarkers for obesity-related cardiovascular outcomes are still a bit unclear. By finding optimal biomarkers, diagnostic criteria for cardiovascular diseases can be refined in the obese population outside of the ‘traditional’ risk factors to identify early pathologic processes. The objective of this review was to identify potential early biomarkers that increase in response to obesity and are associated with cardiovascular disease. Results of this meta-analysis revealed a large number of obesity-related biomarkers that have potential to correlate with cardiovascular disease risk factors or prediction of subsequent cardiovascular events. Another aim of this review was to assess which biomarkers warrant further investigation and eventual clinical use for predicting cardiovascular disease.

Park conducted a study to explore the relationship between obesity and visceral adiposity with amounts of CRP, IL-6, and TNF-α in the blood (2005). Based on previous research, these three biomarkers appear to play an important role in the development of cardiovascular disease and diabetes mellitus. In certain populations, it has also been noted that CRP levels may very based on ethnic background. Considering that, Asians body mass index is lower than the BMI shown in Western populations, however, they tend to have larger amounts of viscerally located fat. Previous studies had not looked at the relationship between IL-6 and CRP, while also exploring their correlation to body fat distributions in Asian populations. Park had 46 obese and 54 non-obese subjects from ages 20-60. Of their 100 subjects, 70 were females and 30 were males. They were all subjected to a medical evaluation and were excluded for a variety of reasons. All participants had height and weight measurements taken, as well as waist and hip
circumference. Blood was taken and commercial ELISA kits were used to measure serum levels of TNF-α, CRP and IL-6. Body fat mass was measured by bioimpedance analysis and cross-sectional abdominal visceral and subcutaneous adipose tissues were measured by computed tomography. From this, area of total adipose tissue, area of abdominal visceral adipose tissue, area of subcutaneous adipose tissue, and visceral to subcutaneous fat ratios were calculated. Statistical analysis used were student’s t-test, Wilcoxon’s rank sum test, Spearman’s correlation coefficients, and Stepwise multiple regression analysis. The p-value was accepted at <0.05. The median concentrations of CRP, TNF-α, and IL-6 were all significantly higher in the obese group when compared to the non-obese group. CRP, TNF-α and IL-6 concentrations were all significantly correlated with weight, BMI, waist circumference, hip circumference and waist hip ratio. CRP showed the strongest association with all anthropometric measurements. CRP concentration was significantly correlated with BMI, whereas IL-6 concentration was significantly correlated with visceral adipose tissue in these obese subjects. These results may be related to the finding that adipose tissue is a dynamic endocrine organ that secretes a number of factors that contribute to systemic inflammation, which is commonly caused by being obese. Their finding of a significant association between CRP and BMI is in agreement with previous results showing that BMI was the adiposity parameter most strongly correlated with CRP concentration. The previous studies were not performed on Asians, though they elicited the same results as the present study. This study aids in solidifying the notion that CRP and IL-6 have a strong connection with obesity and its subsequent disease states, especially when the excess adipose tissue is located in the midsection. Its practical significance is basically that decreasing obesity and visceral adiposity may prevent the
Nguyen discusses obesity and its link to chronic inflammation, which may be connected to the increased rates of metabolic syndrome, cardiovascular disease, and cancer in obese individuals (2009). With inflammation, certain biomarkers have a tendency to show up in the blood, with C-Reactive Protein (CRP) being the most extensively studied inflammatory biomarker. The purpose of the study was to examine the relationship between obesity class and levels of the inflammatory biomarker CRP. The question they were seeking to answer was if one carries more body fat, will they possess higher levels of CRP as well? Will CRP continue to increase as obesity level rises? Examining CRP more extensively holds the potential to unlock some of the underlying causes of obesity. They also have the potential to become obesity predictors with further research. This study used NHANES data from 1999 to 2004, which is conducted by the National Center for Health Statistics. It provided cross-sectional health and nutrition data for the US population. They used a sample of about 5000 people across 15 counties. It includes an extensive health information interview, a complete physical exam, and extensive laboratory testing, all performed in different testing centers around the country. Additional information was collected during an in-home interview and subsequent medical examination at a mobile exam center. There, blood pressure, height, weight, and lipid profiles were taken. They used standards by the National Heart, Lung, and Blood Institute to define overweight and obesity. Blood was also taken here to get CRP levels, as well as several other inflammatory biomarkers. The final amount of people after some exclusions were made was 13,361. Linear regression models were
used to assess the association between obesity class and CRP levels. They adjusted the regression model for age, race, gender, systolic blood pressure, presence of arthritis, smoking status, and alcohol consumption. The results of the study revealed that there was a positive association between each of the BMI levels and CRP levels. The strongest association between obesity and change in biomarker concentration (CRP) was among individuals in obesity class III. As BMI rose, CRP levels rose as well. Tests revealed that the overall increasing change in CRP concentrations was statistically significant. They also found that individuals with diabetes had higher mean CRP levels than those without diabetes, even when stratified by BMI. Similarly, they found that people with hypertension also had higher mean levels of CRP compared to their non-hypertensive counterparts when stratified with BMI. Their findings suggest that the increasing severity of obesity is associated with increased risk for diabetes. These results also support the idea that inflammation potentially plays a role in developing insulin resistance. Inflammation in those who suffer from obesity is believed to arise primarily in adipose tissue as a result of chronic disruption of metabolic homeostasis, which leads to the activation of inflammatory signaling, thus, increasing inflammatory biomarkers such as CRP.

Lieb studied obesity and its genetic component (2008). It is known that parental obesity increases their offspring’s risk for becoming an obese adult, however, little research has been conducted to see if parental-obesity increases risk for other obesity-associated conditions, such as a proinflammatory or prothrombic state in the absence of offspring obesity. The study incorporates the idea that when obese, one experiences a state of chronic inflammation, thus, inflammatory biomarkers such C-reactive protein
(CRP) are typically elevated. Lieb’s research question is asked if these biomarkers will see increases in offspring who are at high-risk for obesity based on their parents being obese, but who are not actually considered obese. He wondered if their increased risk for obesity causes an altered biomarker profile. Considering that, the purpose of the study was to examine CRP levels in non-obese at-risk (for obesity) offspring and non-obese offspring with no parental obesity and see if there was a significant difference. Lieb’s hypothesis was that the offspring with at least 1 obese parent will have increased levels of CRP. Participants in the study (N=1272) were non-obese (BMI<30kg/m²) offspring who were free of cardiovascular disease and had both biological parents included in the Framingham Heart study done previously. They underwent a physical examination, body composition measurement (via skinfolds), laboratory assessment of cardiovascular risk factors, and a medical history every four years. On these visits, blood was also taken and seven biomarkers that had been previously associated with obesity or obesity-related ailments were analyzed using blood assay kits. However, the main biomarker investigated was CRP. Levels of biomarkers were compared between the offspring and their parents using generalized estimating equation models adjusting for relevant covariates among offspring. Parental obesity was defined as BMI ≥30kg/m² at any time of the course of their life as determined by examinations during the Framingham Heart Study. Parental obesity was used as a categorical variable with 0 meaning no obese parents, 2 meaning 2 obese parents, and 1 representing one obese parent. The group with 0 obese parents served as the referent group. For predicting offspring obesity, clinical covariates and parental obesity, clinical covariates and biomarkers, and clinical covariates, parental obesity, and any biomarker related to both parental obesity in the
primary analysis and offspring BMI. Lieb found that CRP demonstrated a nonlinear association with parental obesity status. Offspring with two obese parents displayed higher CRP levels than the reference group. Offspring with a single obese parent did not have higher CRP levels than the reference group. They found no significant difference between the mother or father being the obese parent. The other systemic biomarkers did not vary according to parental obesity. These findings indicate that parental obesity may create a risk for an inflammatory state, thus increasing CRP levels, even in non-obese offspring. Overall, the key finding was simply that when an offspring has two obese parents, they are likely to have elevated CRP levels even if they are not overweight/obese. Their biomarker profile is altered. This is consistent with previous literature in saying that parents transmit a susceptibility predisposing their kids to systemic inflammation that can be detected even in the absence or before the development of obesity.

Visser explored how adipose tissue has been shown to play an active role in one’s metabolism(1999). Adipose tissue is estimated to produce about 25% of the IL-6 in human systems. Studies have shown that IL-6 may induce low-grade systemic inflammation in people with excess body fat. When inflammation occurs, C-reactive protein levels typically become elevated. High levels of CRP have been linked to predicting future risk of coronary heart disease. The aim of this study was to test whether overweight and obesity are associated with low-grade systemic inflammation as measured by CRP concentration. This study included 16616 adults who took the NHANES III survey and fit their inclusion criteria. Body weight and height were measured using standard procedures, and BMI was then calculated. They also took
measurements and found their waist-hip ratio, another method of determining obesity. These individuals also had blood taken and assays were used to measure serum CRP levels. They then divided the subjects into two groups based on CRP concentration; undetectable (less than 22 mg/dL) and elevated (greater than 22 mg/dL). They found that with increasing BMI, the prevalence of elevated CRP level increased in both men and women. However, they found that with increasing BMI, the prevalence of clinically raised CRP level increased among women only. Obese men were 2.13 times more likely and obese women were 6.21 times more likely to have elevated CRP levels when compared with their normal-weight counterparts. The higher prevalence in this category for women could be attributed to the fact that women are more likely to be extremely obese (BMI over 35). Overall, they found that a positive association between body mass index and CRP levels and it remained statistically significant when adjusted for age, smoking status, and waist-to-hip ratio.

C-reactive protein has been shown to rise dramatically in response to infection, inflammation, and injury (Ford, 1999). The aim of Ford’s study was to examine the association between C-reactive protein and BMI and diabetes among a large population. The study had 16,573 participants, all of which were older than 20 years old. Participants came in for an examination session and a fasting blood sample was taken from each. Height and weight measurements were also taken, and BMI was then calculated. People with underlying conditions were eliminated from the study. Ford found a very strong correlation between elevated c-reactive protein levels and body mass index. The study also determined that CRP was lowest among those who did not have diabetes, somewhat
higher in those who had impaired fasting glucose, and were highest among those with diagnosed diabetes.

Based on the literature, it becomes clear there is a positive association between BMI and CRP. Considering this, it has been used in some clinical settings as a diagnostic tool. In recent years, it has been investigated as a way to predict a higher risk for future cardiovascular disease. C-Reactive Protein is the most extensively studied inflammatory biomarker and is often produced in response to being obese. Elevated levels of CRP have been associated with increased inflammation in the arteries, therefore, it increases the risk of atherosclerosis and cardiovascular disease. A genetic link has also been shown between CRP levels in parents and children. This information is unique because it is one of the few studies that examines at CRP levels in nonobese individuals and testing to see if they have more of it, based on genetics and a possible increased risk for developing obesity. This is a very similar idea to what I want to investigate in my study. Previous research is saying that these nonobese offspring have an increased risk for developing obesity based on their parents and higher levels of CRP (Lieb, 2008). CRP is clearly a good biomarker to use in possibly predicting one’s risk for becoming obese.

Thong and Hudson conducted a studying dealing with leptin (2000). The purpose of their study was to examine the effects of diet and exercise on plasma leptin levels in moderately obese men in the presence or absence of weight loss. Fifty-two sedentary males with upper body obesity participated in this study. The study took place over a 12 week period. They were all put on a diet of 55-60% carbohydrates, 15-20% protein, and 20-25% fat. They were also asked to keep detailed food records. VO\(_2\) max was assessed with a graded exercise test on a treadmill. The exercise program consisted of brisk
walking or jogging on a treadmill everyday for 12 weeks. The duration of the session was determined by how long it took to burn 700kcal at an intensity no more than 75% of their respective VO$_2$ max. Energy expenditure was determined by heart rate. Blood samples were taken before and after the 12 week program. Results demonstrated significant reductions in plasma leptin after weight loss from dieting and exercising. This reduction in circulating leptin was associated with a reduction in subcutaneous adipose tissue. This study helps solidify the idea that leptin is a biomarker very closely linked with obesity, which is why I are including it in my study.

Leptin is involved in the regulation of body weight and metabolism, according to a study by Soderberg (1999). The aim of this case-reference study was to examine the extent to which leptin alone, or in combination with other risk factors, may be an independent risk marker for first ever myocardial infarction in a region with a high incidence of cardiovascular disease. Population-based surveys were performed in 1986, 1990, and 1994 in the northernmost counties in Sweden with a total population of 510,000. The age group was both women and men between ages 25-64 and were selected by stratified randomization for age and sex. Participants in the survey were asked to give a fasting blood sample. The people that had donated blood and completed the survey were kept track of and all cases of an acute myocardial infarction were to be reported. Subjects also had weight, height, cholesterol, and blood pressure measurements taken to match subjects in order to keep from skewing results. Once the study was complete, 62 men qualified for being included in the data for the study after having a first time acute myocardial infarction. The results demonstrated that patients who had an acute myocardial infarction had a higher BMI and blood pressure and were more often smokers.
than their matched referents. Nonsmoking acute myocardial infarction cases had significantly higher leptin levels. High levels of leptin were associated with high BMI, high blood pressure, and high levels on insulin. They concluded that circulating levels of leptin were found to be significantly associated with established cardiovascular risk factors such as elevated blood pressure and obesity. High leptin levels were discussed to be a predictor for the future risk of acute myocardial infarction after being adjusted for a number of cardiovascular risk factors.

High leptin levels are often observed in human obesity and suspected to play a role in obesity-related hypertension (Shutte, 2005). The aim of the study was to examine effects of leptin on cardiovascular function in African women. Schutte hypothesized that leptin would be directly associated with blood pressure and decreased arterial compliance, and that leptin levels would be significantly higher in hypertensive overweight or obese African women compared to overweight or obese African women with normal blood pressure levels. Ninety-eight Africans were included in this case–control study. They were divided into lean groups as follows: lean with normal blood pressure, overweight/obese with normal blood pressure, and overweight/obese with hypertension. The Finometer apparatus was used to obtain an elaborate cardiovascular profile. Serum leptin and insulin levels were determined by a blood sample. Additional various anthropometric measures were also obtained. The results showed that leptin levels were elevated in the overweight/obese normal blood pressure and hypertensive groups compared to the lean with normal blood pressure group, but were similar in the overweight/obese non-hypertensive and hypertensive groups. After adjusting for obesity, insulin resistance and age, a direct positive correlation was shown between leptin and
systolic blood pressure. Additionally, leptin also correlated negatively with arterial compliance. The less arterial compliance one has, the less stretch can be produced in the arteries, causing a possible increase in blood pressure.

Leptin has many links to obesity and myocardial infarction risk, therefore, it is a very useful biomarker in the present study. The Schutte study is unique in that it relates to what I will be investigating more directly than many of the other previously mentioned articles in the way that it differentiates between people who are overweight/obese but have high blood pressure and those who are overweight/obese and have normal blood pressure. The obese subjects to be used in our study will also have a normal blood pressure. However, in this study, this group still had correlations to elevated leptin, which is what we were expecting to find.

In addition to examining leptin and its association to obesity and disease states, the present study also aims to see what will happen to leptin after an acute exercise session. In a study by Fisher (2001), the effect that an acute exercise session had on serum leptin levels was measured. Eight young, lean, nonsmoking male subjects participated in the study. On two occasions, separated by 1-2 weeks, participants came to the lab early in the morning after fasting the night before. They were given a 5 minute warm up, then cycled at a power output equivalent to 85% of their VO2 max for 5 minutes, which was followed by a 3 min recovery at 50% of their max. This was repeated 3 more times (a total of 4 work periods and 4 recovery periods). Blood samples were taken before, after each 5 min work period, immediately after the last recovery period, and during 60, 90, 120, 240 min of recovery. Immunoassay kits were used to measure leptin concentrations. They found that serum leptin levels decreased immediately after
exercise and returned to baseline by 240 min post-exercise. It was also noted in the study that this is contrary to what many other studies have found. Many studies suggest that exercise has no acute effect on leptin.

Sikaris discusses how obesity is the excessive accumulation of adipose tissue leading to an increase of body weight beyond the limits of physical requirement (2004). The article also discusses how widespread of a problem obesity is becoming, even in children. With the variations of obesity numbers varying from country to country, it does become obvious there are some environmental influences. At the same time, a great deal of research has shown a strong genetic link to obesity. For example, twin studies have shown important (up to 75%) genetic explanation to BMI. This is relevant because if carrying extra weight is mostly genetic, then can one predict the likelihood of becoming fat? This is a question that can possibly be answered by looking at some of the biomarkers mentioned in the remainder of the review. The first biomarker Sikaris discusses is leptin. Leptin is secreted from adipocytes into circulation, and obesity is usually accompanied by high leptin levels. Fat mass is the primary determinant of serum leptin levels in humans, with a higher fat mass resulting in higher amounts of leptin.

Musaad also looked at studies involving Leptin (2007). Leptin has been shown to have a close association with obesity as well as risk factors for cardiovascular disease. One such cardiovascular disease risk factor that has been shown to correlate with leptin is increased systolic blood pressure. One mechanism by which leptin can increase risk for cardiovascular disease is via increased production of inflammatory makers, such as CRP. It also plays a role in vascular dysfunction. A link between leptin and cardiovascular disease has been directly demonstrated in its independent association with stroke and
myocardial infarction. Leptin is a biomarker that Musaad suggest has “good clinical utility.” Overall, this review found that biomarkers associated with obesity may prove very useful for early identification of susceptible individuals.

Fontana of Washington University conducted a study exploring fat adipokine secretion is associated with systemic inflammation in obese humans (2007). Excessive visceral fat has been associated with insulin resistance and diabetes. Along with this, waist circumference, which correlates with visceral fat mass, has been recommended as a clinical marker to identify patients at increased risk for metabolic diseases with large waist circumference being one of the criteria used to diagnose the metabolic syndrome. However, the mechanisms responsible for the relationship between visceral fat and metabolic abnormalities are not known, and it is not clear whether visceral fat is simply associated with or actually causes metabolic disease. Visceral fat could cause metabolic abnormalities by secreting inflammatory adipokines, such as interleukin (IL-6), tumor necrosis factor-α (TNF-α), and leptin, which have been shown to induce insulin resistance and diabetes. The purpose of this study was to evaluate the relative contribution of inflammatory adipokines (IL-6, TNF-α, and leptin) from visceral fat in insulin-resistant subjects with abdominal obesity. They also posed the question, “Will there be more adipokines in the portal vein or the peripheral artery?” They had 25 subjects with class III upper-body obesity (average BMI was 54.7 and average waist circumference 150cm). They had 6 males and 19 females with an average age of 42 years take part in the study. All participants were previously scheduled to undergo gastric bypass surgery and had a history of type II diabetes or high homeostasis model (HOMA) assessment score. All surgeries were performed in the morning after overnight fasting. Blood samples were
obtained during the surgery simultaneously from the radial artery and hepatic portal vein before gastric stapling or intestinal resectioning was initiated. Commercial ELISA kits were used to measure CRP, and Leptin concentration was measured with immunoassay kits. Statistical analysis used included Student’s Paired T-tests, Wilcoxon two-sample test for variables, and Pearson correlation coefficients. They concluded that mean plasma insulin concentration was more than twofold greater in the portal vein than in the peripheral artery. Plasma leptin was about 20% lower in the portal vein than the peripheral artery. They also noted that portal vein IL-6 was correlated directly to arterial CRP concentration. These results suggest that visceral fat is an important source of biomarker production in obese people, as well as provide the first evidence of a potential mechanistic link between visceral fat mass and systemic inflammation in human subjects.

Fat but Fit

Blair deals with the “Fat but Fit” notion, which is a topic that he has been the key researcher on from the time it emerged in research (1989). The purpose of this study was to report all-cause mortality in relation to physical fitness categories. There were a total of 13334 participants, 10224 men and 3120 women in this study. There were given a full medical exam at the Cooper Institute it Dallas, TX and asked to run on a treadmill at 85% of their age-predicted max heart rate. If they could not complete this task, they were excluded from the study under the assumption that there may be an underlying condition. Physical Fitness level was then found using a treadmill max test. The follow-up was approximately eight years later for each participant. There were 240 deaths in men and 43 deaths in women. Lower mortality rates were found in higher fitness levels for cancer of numerous sites and cardiovascular disease. Higher levels of physical fitness appeared to
decrease all-cause mortality rates primarily due to the lowered risk of cardiovascular disease and cancer. Blair found his results to not be confounded by age or other risk factors, such as obesity. With that being said, this supports Blair’s idea about being “fat but fit.” He argues that one can be overweight, obese in fact, yet still be physically fit, and therefore, have less of a chance of dying from cardiovascular disease and cancer.

The purpose of Dubose’s study was to look at the influence of aerobic fitness and BMI on the metabolic syndrome score in children (2007). Metabolic syndrome occurs when one has a number of specific cardiovascular disease risk factors, including high blood pressure, dyslipidemia, insulin resistance, and central obesity. A total of 375 children (193 girls and 182 boys) between the ages of 7 and 9 years old were included in the study. They were categorized as being normal weight, at risk for overweight, and overweight on the basis of BMI and aerobic fitness. The aerobic fitness test was a submaximal physical working capacity test on a cycle ergometer. Participants were placed into six BMI fitness categories. High-density lipoprotein cholesterol and triglyceride levels, insulin resistance measurements, mean arterial pressure, and waist circumference were used to create a metabolic syndrome score. They found that both BMI and fitness levels were associated with the metabolic syndrome score. In general, the metabolic syndrome score increased across groups. The normal-weight, high-fit group had the lowest metabolic syndrome score and the overweight, unfit group had the highest metabolic syndrome score. Children who were at risk for becoming overweight and had high fitness had a lower metabolic syndrome score compared with those at-risk-for-overweight less-fit children. Their score was similar to that of the less-fit, normal-weight children. To elaborate, a high fitness level resulted in a lower metabolic syndrome score.
in overweight children compared with overweight children with low fitness. They then concluded that high fitness levels modified the impact that BMI had on the metabolic syndrome score in children. Increasing a child's fitness level could be one method for reducing the risk of obesity-related diseases or health problems. These findings, along with the decrease of the metabolic syndrome score with obesity level as a result of being aerobically fit, support the idea of being "fat but fit".

Whitaker’s study talked about being obese as a child leads to an increased risk of being an obese adult, however, at the time the article was written, little was known about how parental obesity affects the chances of a child becoming obese (1997). The aim of this study was to explore obesity risk in young adulthood associated with both childhood obesity and obesity in one or both parents. This was a retrospective cohort study, where they used height and weight measurements from the outpatient medical records of a cohort of young adults and their parents were part of a health organization in Washington State. They identified 1333 members born between 1965 and 1970 to follow who had at least one outpatient visit each after the age of 21 years. The majority of subjects had come to the organization for most of their lives for routine care and check-ups. Of the subjects, 854 met inclusion criteria for the study that included: at least one weight measurement at the age of 21 or older, at least one height measurement at 18 years or older for men and 16 years for women, no chronic disease or condition that could affect weight, and birth at a gestational age of 9 months or more. The parents of all 854 subjects’ records were at the health center. The study defined childhood obesity according adiposity indexes by height and weight (BMI). They used intervals of their ages while growing up to create average BMIs. They followed a similar procedure for
calculating the BMI of the parents. The authors found that at every age interval, both obese and non obese children were at greater risk for becoming obese adults if at least one parent was obese. The effect or parental obesity was most pronounced among obese or and non obese children under 10 years of age. They found an even stronger likelihood of adult onset of obesity if both parents were obese.

This is another interesting study in that it looks at the genetic link of becoming obese. It demonstrates a clear relationship between having an obese parent or obese parents and one’s risk for becoming an obese adult. Although it does not look at any reasons or links between this relationship, it does garner further research because it ultimately asks the question of “why/how does it increase one’s risk?” This question could possibly be answered by looking at some biomarkers of obesity.

Dubose conducted a study dealing with aerobic fitness and metabolic syndrome in normal weight, at risk-for-overweight, and overweight children (2007). There is excess body weight in the youth population and it is positively correlated to metabolic syndrome. Numerous studies are mentioned to demonstrate this theory. In both children and adults, high fitness levels show better metabolic syndrome profiles across the board, even in overweight or obese individuals. Studies have also demonstrated that youth who are fit at an early age, tend to stay more fit throughout their lifetime. In addition to this idea, studies have shown that better fitness levels lead to decreased risk of chronic diseases. The purpose of Dubose’s study was to examine independent and combined influences of BMI and aerobic fitness levels on metabolic syndrome in children. They investigated whether metabolic syndrome score varied among normal weight, at-risk-for-overweight, and overweight children by aerobic fitness level. The authors hypothesized
that aerobic fitness would attenuate the metabolic syndrome score within BMI categories. The subjects in the study were part of a three year physical activity intervention called Physical Activity Across the Curriculum. They also included a subsample of kids from 22 other elementary schools. The child had to be free of insulin-dependent diabetes, cardiovascular disease, or any other disease impairing their ability to participate in physical activity. There were 449 kids initially entered in the study, although only 375 ended up being included due to other confounding factors or incomplete tests. There were equal numbers of boys and girls, and 27% of the sample was a race other than non-Hispanic white. Baseline testing included: height, weight, circumference measures, skinfold measures, resting blood pressure, fasting blood draw, aerobic fitness and academic achievement tests, and physical activity and nutrition surveys. The main measures of interest were height, weight, waste circumference, blood pressure, blood chemistry, and aerobic fitness. The authors used a physical working capacity 170 cycle ergometer test to assess aerobic fitness. They basically completed a serious of stages until their heart rate was greater than 85% of their heart rate reserve or until they could not longer hold a cadence of 60bpm. They basically created a metabolic score for children, deriving it by standardizing the individual metabolic syndrome variables and then regressed them by age, gender, and race. The standardized remaining numbers (z-scores) were summed to create a continuous metabolic syndrome score. The variables chosen were waist circumference, mean arterial pressure, HOMA, HDL, and triglyceride levels. HDL was multiplied by -1 since it has the reverse affect on metabolic syndrome. Statistical analyses used throughout the testing were independent t-tests, Pearson’s correlations, analysis of covariance, and posthoc comparisons using tukey least-
significant differences. Dubose’s study found that metabolic syndrome has obvious relationships with both fatness and fitness. There were significant differences in the metabolic syndrome score between BMI groups, with normal weight having the lowest score and overweight having the highest score. They also found 35% of children in the normal weight group possessed low fitness, whereas 30% of the at-risk-for-overweight and overweight groups possessed high fitness. In general, the metabolic syndrome score increased across groups, with the normal weight, high-fit group having the lowest metabolic syndrome score and the overweight, unfit group possessing the highest score.

Also, the metabolic syndrome score was lower in children in the high-fitness group for all three BMI classes when compared with their low fitness counterparts. To elaborate, a high fitness level resulted in a lower metabolic syndrome score in overweight children compared with overweight children with low fitness. The authors then concluded that high fitness levels modified the impact that BMI had on the metabolic syndrome score in children. Increasing a child's fitness level could be one method for reducing the risk of obesity-related diseases or health problems. Considering that, one of the key points to be made with these results is that they further support the idea of being “fat but fit” and having a reduced risk of chronic disease compared to those who are not fat, but not fit either.

The “fat but fit” idea is relevant to the present study because we do have a population of subjects who could easily fall into this category. They were be clinically obese, but will be otherwise healthy and capable of completing our tests and possibly showing that they are fit. They will have no other cardiovascular risk factors which means they are likely still pretty healthy. They may perform very well as our tests of
physical fitness. Their risk for cardiovascular disease and cancer could then be less than a seemingly healthy subject (who is thin) but does poorly on our fitness tests. Also, these obese people may not have some of the mentioned biomarkers in the blood because they are more fit, while a thin person who is at “risk” of becoming obese may have some of these biomarkers show up in their blood. If we find some biomarkers linked to risk factors in the blood of the healthy population, especially those who perform poorly, and we do not find them in the blood of the obese population, specifically the ones who show to have a higher level of fitness, it could support Blair’s idea of “fat but fit.”

Continuous vs. Intermittent Exercise

A study by Bloomer looked at biomarkers of oxidative stress in response to an aerobic vs. anaerobic exercise protocol (2005). Although this is not exactly the same as the present study, the aerobic protocol would be considered continuous while the anaerobic protocol could be considered intermittent. The purpose of the study was to compare oxidative modification of blood biomarkers in the 24 hours following aerobic and anaerobic exercise using similar muscle groups. Ten cross-trained men between the ages of 18 and 35 participated. They were all regularly participating in both anaerobic and aerobic activities (no less than 3 days a week). To start, each subject completed a VO$_{2\text{max}}$ test on the cycle ergometer. On separate occasions, they also performed a 1-RM test on the bent knee squat exercise using free weights. They were given 6-8 attempts with 3 minutes between attempts. This 1-RM was then used to calculate the weight during the submaximal protocol. Within two weeks, subjects returned to the lab. They took part in an overnight fast and asked not to exercise for the 48 hours prior to entering the lab. Blood was then taken at rest before testing (Pre). In random order, subjects
performed aerobic (continuous) and anaerobic (intermittent) exercise protocols in a cross study design. The aerobic test consisted of subjects cycling warming up for 6 minutes, with 2 minutes spend at 40, 50, and 60% of their VO$_2$\text{\textsubscript{max}}. The workload was then increased to 70% of their VO$_2$\text{\textsubscript{max}} and was adjusted every 5 minutes as necessary to stay at 70% for entire 30 minute test period. A blood sample was taken immediately after the 30 minute session. For the anaerobic test, subjects started by warming up with 5 minutes of stationary cycling and with 3 warm-up sets of the squat exercise for 5-6 reps each, with 90-120 seconds between sets, at a weight equal to 40, 50, and 60% of their previously recorded 1-RM. The weight was then increased to 70% of their 1-RM and it was left at this weight for the entire 30 minute session. Each set was performed to the point of momentary muscular failure (which was 5-12 reps) depending on the subject and degree of fatigue as the protocol progressed. They rested for 90-120 seconds in between sets. Their blood was also taken at the cessation of the 30 minute period. Blood was also taken for both groups 1 hour, 6 hours, and 24 hours post exercise. The results of the study suggest that 30 minutes of aerobic and anaerobic exercise performed can increase certain biomarkers of oxidative stress in blood. However, they have different effects on these oxidative stress biomarkers and result in a different magnitude of oxidation based on the biomarker being looked it. They also found that protein oxidation was greater following anaerobic exercise. This study demonstrates that it is likely differences will be noted in the body’s physiological response to different modes of exercise. It can be expected that if the physiological responses are different, there may also be changes in the blood profile of these subjects.
A study by Drust compared physiological responses in soccer-specific intermittent and continuous exercise sessions (2005). They devised a protocol on a treadmill that represented work rates similar to those performed on the soccer field. They then compared the physiological responses between intermittent and continuous bouts of exercise performed at the same average speed for 45 minutes. This study was a cross-over design, so both groups came back another day and performed the other protocol. They measured oxygen consumption, heart rate, rectal temperature, sweat production, minute ventilation, and rating of perceived exertion. The authors found that there was no significant differences in oxygen consumption, heart rate, rectal temperature, and sweat production between the two types of exercise. However, average minute ventilation was greater during intermittent exercise, as was rating of perceived exertion. Overall, this study demonstrated that the demands of continuous and intermittent exercise are similar as far as what they are asking the body to do. This study shows that it’s possible not to see much difference in the blood biomarker profile between the two modes of exercise being performed, especially since both protocols in the present study are to be considered “maximal effort.”

Both of the previous studies are relevant to the present study in that it is looking at differences in biomarkers before and after continuous vs. intermittent exercise, as well as differences in these markers between the obese (but healthy) and non-obese. The previous research suggests that in the same subjects, I may see some differences in biomarkers depending on the mode of exercise. Although the biomarkers being investigated are not the same ones looked at in the studies mentioned, it is still a fair assumption that they may show differences as well depending on the type of training.
CHAPTER 3

METHODOLOGY

Subjects

The desired subjects for this study consisted of two different groups of males between the ages of 18 and 44, making them low risk according to ACSM guidelines. All subjects filled out an informed consent document (See Appendix A). The first group (N=9, ages 19-31) was comprised of healthy, non-obese males BMI (<30kg/m²) with none of the following existing cardiovascular risk factors: high blood pressure, smoking, dislipidemia, or diabetes. Exclusion criteria included being an athlete or completely sedentary. The second group (N=9) consisted of males (ages 19-36) who are clinically obese (BMI>30kg/m²), but otherwise healthy according to American College of Sports Medicine Guidelines. (See Appendix B). This means that although they are obese, they do not have high blood pressure, diabetes, dislipidemia, and are not a smoker. Additional exclusion criteria for either group was that subjects could not have had a recent, major change in body weight (lost or gained more than 20 pounds in the last 6 months). They were also asked if they had none, one, or two parents that are overweight/obese (See Appendix B). They also had a body fat percentage of greater than 25% to ensure the excess weight is from adipose tissue (See Appendix C). Subjects were recruited from the WKU student and faculty population, as well as the Bowling Green community. Subject’s descriptive statistics can be found in Appendix D, table 3.1.

Prior to testing, the study was be approved by the Western Kentucky University Human Studies Review Board (HS111-12), and all participants provided informed consent.
Protocol

Prior to day one of testing, each subject completed a risk stratification form to determine if they met inclusion criteria (See Appendix A). Upon arrival to the lab, their height and weight measurements were taken. Based on results, BMI was then calculated immediately to ensure inclusion criteria were met. Body composition was then measured using seven-site method with skinfold calipers (Lange Skinfold Caliper, BETA technology, Santa Cruz, CA). Each measurement was taken twice per site, and provided that the measurements were within 2 cm, a third measurement was not taken. The average of the two measurements was calculated and the body fat percentage was determined based on body density. Jackson and Pollock’s protocol was followed for the seven site skinfold measurements and to determine body density (1978). The Siri equation was then used to determine percent body fat based on body density (1961). The principal investigator administered all skinfold measurements to prevent interpersonal variability.

On their first visit to the Exercise Physiology Lab, the subjects performed either a maximal aerobic test to exhaustion (VO$_2$ max test) or a series of Wingate Anaerobic Cycling Tests. On the second visit, which was at least one week later, they returned and performed whichever test they did not perform on the first visit. Half of the subjects performed the VO$_2$ test first and the other half performed the Wingate test first in this counterbalanced study design. For the VO$_2$ max test, a mouth piece attached by tubing to the metabolic cart was placed in their mouth and a nose clip was used to keep all air from entering or escaping through the nose. The air entering and exiting via the mouth was analyzed by metabolic cart (True One 2400, Parvomedics True One Metabolic System, Sandy, Utah), thus allowing oxygen, carbon dioxide, and RER levels to be carefully
monitored throughout the test. Just prior to beginning the test, 600 microliters of blood was obtained by finger pricking via lancets (Capijet Safety Lancet, Turumo Medical Corporation, Somerset, New Jersey). Once a tiny incision was made by the lancet, blood was then drawn into a test tube (Multivette 600, Sarstedt, Numbrecht, Germany). Subjects were then put on the treadmill in order to start the Bruce protocol treadmill test (Quintin TM55, Cardiac Science, Bothell, Washington). No warm-up was included considering the first 3 minute stage of the protocol can be considered warm-up for all of the participating subjects. The Bruce protocol is shown in detail in appendix E, figure 3.1. The test ended when the subject satisfied two of the following criteria: heart rate within 10 beats of age-predicated max HR (220-age), RER >1.10, RPE≥20 (Borg scale), or volitional fatigue. At any point, the test was ended if the subject requested to stop or decided to stop and straddle the treadmill. The highest VO$_2$ level recorded during the test was considered their VO$_2$ max. At the cessation of the max test, subjects were asked to be seated and 600 microliters of blood was once again obtained. Blood was taken a final time one hour after the exercise bout had ended.

For the Wingate Anaerobic test, the subjects followed the same protocol before and after the exercise bout. Once blood was drawn, the Monark leg ergometer (Monark 828E cycle ergometer, Monark Exercise-AB, VANSBRO, Sweden) was adjusted for the individual. They were encouraged to have a slight bend in the knee when sitting on the bike with their foot in the stirrup at its lowest position. The standard Wingate protocol was followed for each bout. They were asked to cycle as fast as possible for approximately five seconds. Once they started pedaling at a maximal rate, the weight (which was 7.5% of their body weight in kg), was placed on the flywheel. Once the
weight was added, the time was started. The subjects were then asked to continue to pedal as fast as they could for a 30 second time period. Every five seconds their RPM’s were recorded. At the end of the 30 seconds, they were notified, and then allotted a two-minute rest period in which they could sit, walk around, or continue to pedal slowly. After two minutes, the 30 second test was repeated. After the second thirty second test, they were given another two minute rest period. They were then asked to do a third, 30 second cycle test (Appendix F, figure 3.2). Immediately after the third test and one hour after the completion of the test, 600 microliters of blood will be taken.

After each blood sample was obtained, it was centrifuged for 5 minutes (Myfuge, Benchmark Scientific Inc, Edison, New Jersey). Plasma was then removed via pipettes, placed in labeled test tubes, and stored in the freezer (Ultra Low Freezer, So-Low, Cincinnati, Ohio) for later use.

The blood work was done according to specific protocols that accompanied each of the kits. The following are the steps required for each kit.

**CRP Assay Method**

All reagents were removed from the refrigerator and allowed to reach room temperature. Each lyophilized standard was reconstituted with 1.0 ml of deionized water, mixed gently, and allowed to stand for 20 minutes. Each sample was diluted 100 fold by adding 1 µl of plasma to 100 µl CRP dilutent. Then, 10 µl of CRP standards and samples were added into appropriate wells, all of which were done in duplicate. Next, 100 µl of CRP enzyme conjugate reagent was added to each well. The plate was then mixed gently for 30 seconds and incubated at room temperature for 45 minutes. The incubated mixture was then removed by being flicked over a waste container. It was then rinsed with
deionized water and flick into the waste container five times. The plate was then struck sharply onto clean, dry, paper towels to remove all residual water droplets. At this point, 100 µl of TMB solution was added into each well, mixed gently for five seconds, and incubated at room temperature for 20 minutes. At the end of the 20 minutes, the reaction was stopped by adding 100 µl of stop solution to each well. The wells were mixed gently for 30 seconds and once they changed from blue to yellow, absorbance was read with a microtiter well reader (ELx800, Biotek Instruments, Vienna, VA) at 450nm within 15 minutes. Once read, a standard curve was created and the amounts of CRP in each sample were calculated.

Leptin Assay Method

In preparation, the 5x assay wash buffer was diluted to 1x by adding 40 mL of the 5x assay wash buffer to 160mL of deionized water. The biotin-labeled mouse anti-human leptin antibody was diluted 400x by adding 10mL of 1x diluent buffer and the streptavidin-HRP was diluted 200x by adding 10mL of 1x diluent buffer before use. For each sample, 90 µl of assay wash buffer was added to 10 µl of subject’s plasma.

Once prepared correctly, 100 µl of each standard and sample was added to the appropriate wells, all of which were ran in duplicate. They were then allowed to incubate for 1 hour at room temperature with gentle shaking (Vortex Genie Model G-560, Scientific Industries Inc, Bohemia, NY). Each well was then aspirated and washed by adding 200 µl of 1X assay wash buffer. This process was repeated for a total of three washes. The complete removal of liquid was done by inverting the plate against, dry paper towels. Then, 100 µl of diluted biotin-labeled mouse leptin antibody was added to each well and the plate was incubated at room temperature for 1 hour. Each well was then
aspirated and washed by adding 200 µl of 1X assay wash buffer. This process was repeated for a total of three washes. The complete removal of liquid was done by inverting the plate against, dry paper towels. Next, 100 µl of diluted streptavidin-HRP conjugate was added to each well and incubated at room temperature for 45 minutes. The aspiration/wash procedure was then performed for a third time. At this point, 100 µl of substrate was added to each well and incubated for 10 minutes. Then, 50 µl of stop solution was added to the wells, turning the color of them from blue to yellow. The optical density was then determined by reading them on a microplate reader (ELx800, Biotek Instruments, Vienna, VA) at 450nm within 30 minutes.

Statistical Analysis
Descriptive data for anthropometric and physiological variables were calculated as mean ± standard deviation (SD). All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 18.0, Chicago, Ill., USA). Statistical significance was set at an alpha level of $p \leq 0.05$ for all analyses.

An analysis of variance (ANOVA) with repeated measures was used to determine differences between the resting serum levels of each biomarker and the serum measurements immediately after and an hour after exercise. The repeated measures analysis compared the resting, immediate-post, and 1 hour post measurements of each biomarker. Post hoc analysis for multiple comparisons were analyzed using post hoc procedure. The amounts of leptin and CRP in the blood pre-testing, post-testing, and one hour post testing for each day of testing was analyzed with a 2x3 (group X time) ANOVA with repeated measures on the last factor (time). This also examined effects between groups (overweight vs. healthy). This was performed for both leptin and CRP. Due to high variability and spread of the numbers, the obese group’s mean CRP and
leptin levels at baseline were compared to each individual healthy subject’s CRP and leptin levels post and 1-hour post exercise by using a one sample t-test (p<.05). This addressed the question of “Are there healthy subjects who could be at a higher risk for becoming overweight based on their post-exercises responses to these obesity-related biomarkers?” This was done for both the wingate (intermittent) and VO2 max tests (continuous) in order to answer the primary research question. Also, Pearson Product Correlation Coefficients were used to determine if there was a correlation between baseline levels of CRP (PRE) and measurements of body fat for both groups.
CHAPTER 4

RESULTS

CRP

Using Pearson Product Correlation Coefficients, there was a significant positive correlation between baseline levels of CRP (PRE) and measurements of body fat for both groups. On the VO\textsubscript{2} max test day, body mass index \((r=.563, p=.018)\), body fat percentage \((r=.567, p=.018)\), and body weight \((r=.533, p=.027)\) were all correlated to resting levels of CRP. Refer to Appendix G-Figure 4.1, Appendix H-Figure 4.2, and Appendix I-Figure 4.3, respectively. On the Wingate testing day, there was also a significant correlation between baseline measurements of CRP and body mass index \((r=.557, p=.022)\), body fat percentage \((r=.584, p=.014)\), and body weight \((r=.593, p=.012)\). Refer to Appendix J-Figure 4.4, Appendix K-Figure 4.5, and Appendix L-Figure 4.6, respectively.

On the VO\textsubscript{2} max testing day, the healthy group’s baseline mean CRP levels were \(0.49\pm0.27\) mg/dl. Immediately following the test, they decreased to \(0.45\pm0.25\)mg/dl, and increased to higher than baseline at \(0.52\pm0.42\) mg/dl one-hour following the end of the max test. In the obese group, the mean CRP levels started at \(0.94\pm0.54\)mg/dl, increased to \(1.18\pm0.72\)mg/dl immediately after the VO\textsubscript{2} max test, and decreased to near baseline at \(0.97\pm0.60\)mg/dl 1 hour following the end of the exercise bout. A mixed analysis of variance (group X time) was performed with repeated measures on the second factor (time), and no significance was noted for main effect or interaction \(F(2,30=.987)\). However, in response the first hypothesis, a paired sample \(t\)-test was performed, revealing a significant
difference between the healthy and obese group’s baseline levels (PRE) of CRP. See Appendix S, Table 4.1 and Figure Appendix U, Figure 4.13.

For the repeated Wingate trial, the healthy group’s baseline mean CRP levels were .52±.26 mg/dl. Immediately following the test, they increased to .57±.48mg/dl, and an hour post they decreased to below baseline at .48±.22 mg/dl. In the obese group, the mean CRP levels started at .91±.44mg/dl, dropped slightly to .83±.36mg/dl immediately after the VO₂ max test, and returned to baseline at .90±.49mg/dl 1 hour following the end of the exercise session. A mixed analysis of variance (group X time) was performed with repeated measures on the second factor (time), and no significance was noted for main effect or interaction $F(2,30=.057)$. Differences are evident, however, none of them are considered statistically significant. See Appendix T, Table 4.2 and Appendix U, Figure 4.13.

Based on these data, it can also be noted that the baseline CRP levels are significantly higher in the obese group on the VO₂ testing day, and although not demonstrated statistically, there is a distinction between the baseline measurements between groups on the Wingate day as well.

Due to large variability, each healthy subject was individually investigated to determine those who may be at risk for becoming obese. See Appendix V Figure 4.14 and Appendix W, Figure 4.15. This addresses the issue of trying to identify if there are any healthy subjects who could be at a higher risk for becoming overweight based on their post-exercises responses to this inflammatory marker. This was done for both the Wingate (intermittent) and VO₂ max test (continuous).
Using Pearson Product Correlation Coefficients, there was a significant correlation between baseline levels of leptin and measures of body fatness for both groups. Before the VO₂ max test, there was a significant correlation between serum Leptin levels and body mass index (r=.632, p=.005), as well as body weight (r=.575, p=.013). Refer to Appendix M, figure 4.7 and Appendix O, figure 4.9. There was not a significant correlation between leptin level and body fat percentage (r=.459, p=.055). Refer to Appendix N, figure 4.8. On the Wingate testing day, there was also a significant correlation between baseline measurements of Leptin and body mass index (r=.624, p=.006), body fat percentage (r=.484, p=.042), and body weight (r=.579, p=.012). Refer to Appendix P-Figure 4.10, Appendix Q-Figure 4.11, and Appendix R-Figure 4.12, respectively.

For the VO₂ max test, the healthy group’s baseline mean leptin level was 5.97±5.88 ng/ml. Immediately following the test, they increased to 6.64±6.51 ng/ml, and an hour post they slightly decreased to 6.29±5.45 ng/ml. In the obese group, the mean leptin levels started at 19.80±14.74 ng/ml, increased to 23.66±16.67 ng/ml immediately after the VO₂ max test, and returned to baseline at 19.75±15.79 ng/ml 1 hour following the end of the exercise bout. See Appendix X, Table 4.3 and Appendix Z, Figure 4.16. A mixed analysis of variance (group X time) with repeated measures on the second factor (time) was used and a main effect was found F(2,32 = 3.903, p=.021). Post-hoc analysis confirmed significant differences between healthy and obese groups between pre, post, and 1-hour post measurements. No interaction was found between variables was found.
For the repeated Wingate trial, the healthy group’s baseline mean serum leptin levels were 5.39±4.24 ng/ml. Immediately following the test, they increased to 6.98±5.72ng/ml, and an hour post they decreased to near baseline levels at 5.59±3.99ng/ml one-hour following the end of the three cycle tests. In the obese group, the mean leptin levels started at 18.68±13.29ng/dl, climbed slightly to 20.30±12.13ng/ml immediately after the Wingate tests, and returned to baseline at 18.96±10.31ng/ml 1 hour following the end of the exercise session. Changes are evident, however, none of them are considered statistically significant. A mixed analysis of variance (group X time) with repeated measures on the second factor (time), and no significance was noted for main effect or interaction F(2,32=.800). See Appendix Y, Table 4.4 and Appendix Z, table 4.16.

Due to large variability, each healthy subject was individually investigated to determine those who may be at risk for becoming obese. See Appendix AA, Figure 4.17 and Appendix BB, Figure 4.18. This addresses the issue of trying to identify if there are any healthy subjects who could be at a higher risk for becoming overweight based on their post-exercises responses to this inflammatory marker. This was done for both the Wingate (intermittent) and VO₂ max test (continuous).

\[ VO₂ \text{ vs. Wingate} \]

In comparing the VO₂ (continuous) and the Wingate (intermittent) protocols, there was no significant main effect F(2,26=.005) or interaction F(2,26=.433) for CRP levels in the healthy group. For CRP levels in the obese group, a significant interaction F(2,32=3.41) was demonstrated, while there was not a significant main effect F(2,32=.942) shown. Post hoc analysis indicated a significant difference between CRP
post and CRP 1-hour post exercise in the obese group on the VO$_2$ testing day (p=.037).

Appendix S, Table 4.1 and Figure Appendix U, Figure 4.13.

In comparing the VO$_2$ (continuous) and the Wingate (intermittent) protocols, there was no significant main effect $F(2,32=1.149)$ or interaction $F(2,32=.261)$ for Leptin levels in the healthy group. Likewise, no significant main effect $F(2,32= 2.30)$ or interaction $F(2,32=.467)$ was reported for the obese group.
CHAPTER 5
DISCUSSION

The present study was designed to examine baseline, post, and 1 hour post measurements of CRP and leptin in the blood of those who were healthy and those who were obese but otherwise healthy. The primary purpose was to compare the biomarkers in a healthy person’s blood when recovering from exercise to an obese person’s biomarkers at rest. It was hypothesized that obese subjects would have higher baseline measures in both markers, and that the healthy group may show some similarities to the obese group’s elevated resting numbers in the post-exercise and 1-hour post exercise measurements. Overall findings suggest that obese subjects generally have higher levels of CRP and leptin before, after, and 1 hour after intense exercise. However, when examining individuals, some healthy subjects demonstrated spikes in these markers, suggesting that they could be at risk for obesity and its related disease states. Also, the Wingate and VO$_2$ tests demonstrated differing effects on the two groups, although notable, none of these changes were too dramatic with only one decrease considered statistically significant.

On both of the testing days, CRP levels differed between the healthy and obese groups at baseline. On the VO$_2$ testing day, this difference was considered statistically significant. This confirms the initial hypothesis that resting levels of CRP would be higher in those who are considered clinically obese, based on the fact that this biomarker has been tightly linked to chronic inflammation and obesity. This finding is consistent with the findings of Park (2005) who demonstrated that CRP is significantly correlated with BMI. In the present study, BMI was significantly higher in the obese group, with all subjects in that group having a BMI over 30kg/m$^2$. Baseline CRP levels were also
significantly higher in this group, thus, confirming Park’s findings. Our results are also in line with those of Nguyen, who noted that as weight class went up, so did CRP levels (2009). Overall, the present study’s baseline differences are consistently in line with previous literature that connects obesity and CRP (Lieb 2008, Visser 1999, Ford 1999).

In addition to CRP’s links to obesity, it becomes indirectly linked to other disease states and chronic conditions associated with obesity. These include cardiovascular disease, diabetes, hypertension, and dislipidemia. CRP has also recently shown great clinical utility in assessing progress of chronic inflammatory conditions (Mussad, 2007).

However, the key problem in using it to assess cardiovascular disease and other conditions is that it is not specific to obesity, which produces the need for studying biomarkers linked more exclusively to obesity. The use of these biomarkers could eventually be very useful in predicting likelihood of becoming obese or suffering from any of obesity’s subsequent disease states, especially in certain subpopulations.

When examining the results of the CRP tests, it is notable that some differences appear to exist between the two groups. Although there is no significant main effect or interaction effect, the results still show that regardless of the time blood was drawn, CRP levels were higher in those who were obese. A statistically significant difference was demonstrated on the Wingate testing day when comparing the PRE levels in both groups, thus, confirming the fact that CRP levels vary according to body weight, with the obese group having noticeably higher levels throughout testing. It is noteworthy that CRP levels above 1.0 mg/dl is considered “elevated” in a clinical setting. Therefore, both of the mean resting measurements for the obese group were very near to this level. Additionally, many obese subjects had levels well over 1.0 mg/dl. It is interesting to note that with the
VO₂ testing, the obese groups numbers increased, then back near baseline after 1 hour. This fall back to baseline from post to 1 hour post was statistically significant, showing that exercise had noticeable effects on CRP levels in these subjects. During the Wingate testing, they increased post-exercise, and then returned to baseline 1 hour later. Although most of these climbs and drops in CRP levels were not statistically significant, it is interesting to note that the healthy population’s responses were the exact opposite. The amount of serum CRP increased following the Wingate, then decreased to near baseline an hour post. With the VO₂ test, their serum CRP levels fell post-exercise and returned to near baseline levels 1 hour later. It can then be noted that the only slight changes were in the immediate post exercise measurement. The pre and 1-hour post measurements were very similar to each other in all cases.

When comparing the two protocols, the Wingate appeared to have a more aggressive effect on the healthy group’s CRP levels, and the VO₂ appeared to have a more aggressive effect on the obese group’s. The small spikes or drops in CRP levels immediately after exercise may demonstrate differences in the effect that continuous and intermittent exercise has on different populations. Since CRP is an inflammatory marker, it is possible it rises as muscles become fatigued, thus, damaged and possibly inflamed. This could help explain these findings in that the obese group seemed to induce more damage during the Wingate trials. This is logical in that they were actually more able to “slack off” in this stage of the experiment. There was no cadence or set speed they had to reach, and many of the obese subjects nearly came to a dead stop when pedaling during the test. This could be attributed to the higher amount of weight on the fly wheel (since it is a percentage of their body weight), or it also could be that they were able to not try as
hard on this test. On the treadmill test, however, the speed and grade kept changing and they had no choice but to maintain their effort until they were at exhaustion or a max. Based strictly on observation, it can be noted that the effort produced for the obese group was higher on the VO$_2$ test than the Wingate. In the healthy group, the changes from pre, post, and 1 hr post were slightly smaller, and the response was the opposite. Some of these subjects were recreational runners and worked out on a more regular basis. Therefore, the running (treadmill test) may have been a more familiar and comfortable modality for them, thus, possibly inducing less inflammation and CRP. For future study, measuring RPE could be beneficial in assessing this issue. Another theory for this could be the way that the groups responded to continuous or intermittent activities are different. A more continuous activity may be seemingly easier for the healthy group, who based on having higher VO$_2$ maxes, should have enhanced cardiorespiratory endurance. The intermittent activity however, which uses more of an anaerobic energy system, may have caused them more stress. In the obese group, the continuous activity without breaks was perhaps more difficult for them, thus, causing the spike of CRP after exercise. Based on previous literature by Bloomer, both intermittent and continuous activities can increase certain biomarkers of oxidative stress in blood (2005). This is consistent with our findings in that both types of exercise demonstrated some changes in these markers. However, it appears that it varies from person to person, likely depending on their abilities, training programs, and exercise modalities.

The general trend was for the healthy groups CRP and Leptin levels to be below obese groups in the pre, post, and 1 hour post measurements. The healthy subjects that showed increases in either biomarker after exercise are the ones of most interest to this
particular study. The purpose of the present study was to assess those individuals who may be “at risk” based on their responses to exercise. The subjects’ data were analyzed individually to see if any had spikes of CRP after the exercise bouts. Another consideration when examining this portion of the data is parental obesity. Subjects were asked to report if they had at least one overweight or obese parent, and of the 9 healthy subjects, three of them (subjects 1, 4, and 7) had 1 obese parent. For the VO\textsubscript{2} test, subjects 4, 5, 6, and 7 all had noticeably increased levels of CRP after the exercise bout (either in post or 1-hour post) when compared to baseline. Of the four subjects that had spikes post or 1-hour post exercise in this case (4, 5, 6, and 7), 2 of them had at least 1 obese parent (4 and 7). This is relevant in that research has shown obesity has a genetic component. Leib found that parental obesity increases the risk for becoming obese or having obesity related conditions, even in the absence of obesity (2008). He also found that higher CRP levels were observed in offspring that were not obese, but had obese parents. Subjects 4 and 7 had heightened CRP levels post-exercise, and based on their parents, are a higher risk for becoming overweight. When analyzing the Wingate test data, subjects 1, 2, 5, and 8 produced higher levels of CRP after exercise. Subject 1 is the third subject who reported having at least one overweight parent, which is again interesting in that parental obesity has a tendency to be passed on to their offspring.

Much like CRP, serum baseline levels of leptin were also significantly higher in the obese group on one of the testing days. This is consistent with the initial hypothesis, as well as in line with previous literature. Soderberg and colleagues concluded that high levels of leptin were positively associated with BMI (1999). Shutte drew similar conclusions finding leptin levels were clearly elevated in subjects who were overweight
or obese (2005). Muassad pointed out that leptin can also increase cardiovascular disease risk by increasing production of inflammatory biomarkers, such as CRP (2007). The fact that both of these biomarkers increased according to BMI class and body fat percentage falls directly in line with previous literature.

When analyzing leptin levels, both groups experienced a very slight (not significant) increase post-exercise, and a return to baseline one hour later. There was no difference between groups in the leptin response to exercise modality. Much like CRP, leptin levels were examined on an individual basis to determine those who may be at risk for obesity and its subsequent disease states. In examining the VO$_2$ test, subjects 5, 6, and 9 had elevated leptin levels post exercise. It can be noted here that subject six also saw a spike in CRP after the VO2 test. This not only makes them of greater interest based on their risk for obesity, but it also helps to possibly rule out the fact that CRP was raised for additional reasons than acute inflammation. When examining the Wingate, subjects 4, 5, 7, 9 had elevated levels of leptin after exercise (either post, 1 hr post, or both). Four and seven were two of the subjects noted in looking at CRP. They both had at least one overweight/obese parent. Subjects 5 and 9 had elevated leptin responses with both modes of exercise, thus pointing to possibly being at an increased risk for obesity and its disease states. At closer examination, subject 5 had elevated responses post-exercise in all four measurements (crp-$vo_2$, crp-$w$, leptin-$vo_2$, leptin-$w$). Subject 9 (who is actually number 8 in the CRP results due to the removal of an outlier), had elevated responses in three of the four. Those are the two subjects, based on the assumptions made in the present study, could be at the highest risk for becoming overweight based on their biomarkers. Along with them, 1, 2, 4 and 7 garner further investigation.
With several healthy individuals standing out as possibly being at increased risk for becoming overweight, it leads one to wonder if any of these healthy subjects are actually have greater risk than some currently obese subjects for carrying excess weight later in life and/or developing obesity related disease states such as cardiovascular disease, hypertension, diabetes, dislipidemia, etc. Could it be possible that some of the obese subjects may actually be “healthier” in a long-term sense? Although the average VO$_2$ maxes were higher in the healthy group, is it possible that several obese subjects were actually more fit than several healthy subjects? A possible example is subject 3 in the obese group. He possessed no risk factors for cardiovascular disease except for that he had a BMI over 30, similarly to all subjects in this group. However, his VO$_2$ max was 58.2 ml/kg/min, which was well over the mean VO$_2$ max for the healthy group (54.66 ml/kg/min). In fact, the highest VO$_2$ max of all 18 subjects was 60.2 ml/kg/min, therefore, he was not far from having the highest cardiorespiratory endurance of even the healthy group. This is relevant to the “fat but fit” notion previously mentioned in that although he was bigger than many of the healthy subjects, he was fitter (Blair 1989). Additionally, he did not see post-exercise spikes in these biomarkers, so perhaps he is actually at less risk than some of his healthy counterparts for developing obesity related conditions later in life. The “fat but fit” notion is connected with a great deal of literature examining the genetic component of obesity. A study by Whitaker explores the genetic link of becoming obese, finding a very clear connection (1997). It demonstrates a relationship between having an obese parent or obese parents and one’s risk for becoming an obese adult. Although it does not look at any reasons or links between this relationship, it does garner further research because it ultimately asks the question of
“why/how does it increase one’s risk?” This question could at some point, with more research, could possibly be answered by looking at leptin, CRP, and other biomarkers of obesity.

When comparing the two protocols, the Wingate appeared to have a more aggressive effect on the healthy group, and the opposite was true for the obese group. Based on investigating individual subjects, many had different responses to different modes of exercise. This is likely based on individual’s ability as well as previous training status. Some subjects may have been more accustomed and/or more comfortable with particular modes of exercise. Based on previous literature by Drust, continuous and intermittent activities often produce similar physiological responses (2005). Drust’s study shows that it is possible not to see much difference in the blood biomarker profile between the two modes of exercise being performed. This is especially relevant to the present study since both protocols in the present study are to be considered “maximal effort.” Our findings are consistent with this idea in that the only significant change in either biomarker noted was for CRP between the post and 1 hour post for the VO\textsubscript{2 max} test. Thus, showing that the other responses were statistically insignificant and, therefore, similar to each other.

In the present study, numerous limitations were noted. The first limitation is that much of the present study was based upon assumption. The idea that the obese subjects are in a “chronic disease state” and the healthy subjects post-exercise are in a “temporary disease state” is based on quality logic; however, there is still a great deal of assumption going into the backbone theme of this study. Another major limitation was that before most serum leptin or CRP tests, individuals are required to do a 12 hour fast the night
prior. In the current study, they were not asked to fast. However, subjects were asked not
to eat 4 hours before coming into the lab, thus it was considered, but we did not directly
control for it. A longer fast could not really be a requirement considering they were
doing maximal effort protocols on both days they came in; therefore, some fuel was
necessary in order to perform. Technically, the present study did not have a “fasting”
baseline blood sample. However, the post and 1 hour post measurements can be
considered more valid in the sense that much like fasting, the exercise protocol performed
was an activity resulting in a calorie deficit. Another notable limitation we did not control
for exercise between sessions. Subjects were asked not to exercise 48 hours upon arrival,
but the other 5 days in between the sessions were not controlled. This could be limiting in
that some subjects possibly had lingering effects from a workout 3 days before they came
in, however, this is not very likely considering most soreness, etc. is alleviated after 48
hours. Also, individuals’ motivation and effort can not be directly controlled.

Additionally, time constraints were another major limitation. Based on a power analysis
performed, 15 subjects per group would have been ideal. However, having only one
semester to complete this project, only 9 per group were obtained. Recruiting willing
obese subjects also was difficult. If the number of participants had been higher, thus
providing appropriate statistical power, this may have yielded more significant findings.

An additional and important limitation is in examining CRP as a biomarker of obesity.
CRP is an inflammatory biomarker linked tightly with chronic inflammation and obesity.
However, it could be elevated in subjects for other, more acute reasons. This makes it
difficult to “trust” any CRP-related findings in that they could be attributed to other
causes or conditions. Musaad explains that CRP had recently shown great clinical utility
in diagnosing inflammatory conditions (2007). However, the fact that it is not specific to obesity alone makes it difficult to study.

Due to the fact that the present study is extremely novel, and nothing similar has been done before, the need for future study is not only vital, but multidirectional. First, if the study is repeated, it is recommended to measure RPE for both exercise tests. This would give a better idea of the effort being put forth in both activity sessions. Also, examining a 24-hour post measurement would be interesting to see how long some of the biomarkers stayed elevated. For some subjects, they had already come back to baseline 1-hour post. However, some spiked an hour after, so specifically for them, seeing what happened 24 hours later would possibly help make some better observations. Perhaps some of these healthy subjects saw these “spikes” 24 hours later when the delayed onset muscle soreness was in full effect. This could demonstrate that perhaps the CRP was elevated for other reasons, such as exercise-related inflammatory causes. Another route this research could take is analyzing the same subjects longitudinally. Take the present study for example, and have these same subjects are tested every 6 months for 20 years. During the visit, they would have weight, body mass index, body composition, and blood pressure measurements taken. Perhaps some of the men we identified as being “at risk” would have gained weight and would now be considered overweight or obese. It is possible that some of them may have developed high blood pressure or other obesity related conditions. If this could be noted, then the results of the present study could be somewhat substantiated in that our “predictions” seemed to have some authority. A final and important consideration for future research rests in selection of biomarkers. Exploring biomarkers more specific to obesity may be better than ones like CRP that
have other explanations behind their appearance in blood. Musaad reports that there is clinical need for biomarkers that are specific to obesity and that can predict risk for cardiovascular disease (2007). Future study should seek these biomarkers. However, if ones such as CRP are evaluated, the additional factors that affect its presence in the blood should be controlled.

Overall, a great deal was investigated and analyzed over the course of the present study. A major baseline finding is that the obese group had higher levels of CRP and leptin in resting conditions. After the VO$_2$ test, the obese group’s CRP levels increased, while the healthy group’s decreased, and both groups returned to baseline at 1 hour post. After the Wingate testing, the two groups basically switched their responses. In looking at leptin on both testing days, both groups saw a slight increase immediately after exercise and numbers returned to baseline 1-hour post. However, significant differences between the groups were noted on all measurements on the VO$_2$ testing day. In looking at subjects on a more individual basis, subjects 5 and 9 stood out as possible “at-risk” participants. Subjects 1, 4, and 7 also had notable responses, as well as being the offspring of parental obesity, thus, putting them in position to be studied more extensively as well. These results, however interesting, need further investigation and validation with future study. The novel basis of the present study adds to its unique nature, but also calls for more research on this hot topic in today’s society. Obesity has been recently called an “epidemic,” and this study is one tiny step in the direction of finding solutions.
Appendix A
INFORMED CONSENT DOCUMENT

Project Title: INVESTIGATION OF C-REACTIVE PROTEIN AND LEPTIN AS BIOMARKERS OF OBESITY WITH POTENTIAL CLINICAL UTILITY

Investigator: Rachel Friedman, Department of Kinesiology, Recreation, and Sport, (618)604-2712.

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigator will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask him/her any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have. If you then decide to participate in the project, please sign on the last page of this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:** The main purpose of this project is to compare biomarkers of those who are clinically obese (BMI ≥30kg/m²) to those who have a healthy (BMI<30). The four biomarkers that will be studied are leptin, tumor necrosis factor-α, C-reactive protein, and interleukin-6. All four of these can be found in the blood, and they are each associated with obesity and other disease states related to obesity. The idea behind the study is to see if there are similarities in the obese group’s blood at rest and the healthy group’s blood after the exercise bout, because in both situations the body is in a “disease state” and/or is “stressed.” If there are similarities, it could be possible to do a simple finger prick in place of an extensive diagnostic exam. The results could indicate a way to “predict” obesity and its subsequent disease states.

2. **Explanation of Procedures:** On your first visit to the Exercise Physiology Lab, you will perform either a maximal treadmill test to exhaustion or a series of cycling tests. On the second visit, at least one week later, you will come in and perform whichever test you did not perform on your first visit. For the treadmill test, a mask will be placed over your nose and mouth so that expired air can be measured. Prior to the start of the test, blood will be drawn by finger pricking. You will then be put on the treadmill (with the mask still on your face) and given a three minute warm-up period at a comfortable speed. You will then begin the Bruce protocol treadmill test (a standard test that slowly increases in speed and incline every stage). The test will end when you satisfy two of the criteria indicating you have reached a maximal effort. At any point, the test will also end if you request to stop. As soon as the test is complete, blood will again be taken via the needle prick method. At the end of the recovery period (1-hour), blood will once again be drawn via the finger prick method.

For the Wingate Anaerobic test, they will follow the same protocol before and after the exercise bout. You will then be asked to sit comfortably on the cycle. The standard Wingate protocol will then be followed for each bout. You will be asked to cycle as fast as you can for a matter of seconds. Once you start peddling at a maximal rate, the weight (which was 7.5% of your body weight in kg), will be placed on the flywheel. Once the weight is added, the time will be started. You will be asked to continue to peddle as fast as you can for a 30 second time period. At the end of the 30 seconds, you will be notified, and will then be allotted a two-minute rest period in which you can sit, walk around, or continue to peddle slowly. After two minutes, the 30 second test will be repeated. After the second thirty second test, you will be given another two minute rest period. You will then be asked to do a third, 30 second cycle test. Immediately after the test, blood will be drawn. It will be drawn a final time 1-hour after completed the Wingate cycle tests.

3. **Discomfort and Risks:** The main risks associated with this study are in performing the maximal effort exercise. The treadmill test is an incremental run to exhaustion that maximally stresses the cardiorespiratory system. The increased myocardial demand of maximal intensity exercise may precipitate cardiovascular events in individuals with heart disease. However, the American College of Sports Medicine has stated that the risk of death during or immediately after an exercise test is less than or equal to 0.01%,
while the risk of an acute myocardial infarction is less than or equal to 0.04%. Data from these surveys included a wide variety of healthy and diseased individuals. As you have been screened to be a healthy “low risk” individual, the risks are estimated to be considerably lower. Another risk would be muscle soreness from the intense efforts required for the exercises. A final risk would be slight soreness on the finger from being pricked.

4. **Benefits:** The direct benefit to you is the information you will receive regarding aerobic fitness level. General benefits from the research are that the knowledge generated could become very useful in assessing risk for obesity and other disease states, as well as diagnosing these conditions.

5. **Confidentiality:** You will be assigned a numerically coded identification number. Your data will be stored on computer disks, which will be locked in the primary investigators office (Smith Stadium 1039).

6. **Refusal/Withdrawal:**

Refusal to participate in this study will have no effect on any future services you may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

_You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks._

__________________________________________  ________________
Signature of Participant  Date

__________________________________________  ________________
Witness  Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT

THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY

THE WESTERN KENTUCKY UNIVERSITY INSTITUTIONAL REVIEW BOARD

Paul Mooney, Human Protections Administrator

TELEPHONE: (270) 745-4652
Appendix B
ACSM Initial Risk Stratification Questionnaire

Please provide accurate information for all requested items. Ask a staff member to assist you if you need clarification of any item.

Name: ______________________________________

Date of Birth: _____________________________

Write a Y for all statements that are true, write a N for all statements that are false, and write a U for all statements that are unknown.

Cardiovascular Risk Factors

_____ You have a first-degree relative who had a heart attack or coronary revascularization or sudden death before age 55 (father or brother) or age 65 (mother or sister).

_____ You smoke cigarettes or you quit smoking cigarettes within the last 6 months.

_____ Your systolic blood pressure is ≥ 140 or your diastolic blood pressure is ≥ 90 mmHg or you take blood pressure medication.

_____ Your LDL-cholesterol is ≥ 130 mg/dl (if LDL not known: your total cholesterol is ≥ 200 mg/dl) or your HDL-cholesterol is < 40 mg/dl or you take lipid lowering medication.

_____ You are sedentary (i.e. you get less than 30 minutes/day of moderate intensity physical activity on most days and you do not participate in a regular exercise program).

_____ Your HDL-cholesterol is ≥ 60 mg/dl

Symptoms

_____ You experience pain or discomfort in the chest, neck, or arms.

_____ You experience shortness of breath at rest or with mild exertion.

_____ You experience dizziness or have had episodes of blackouts.

_____ You have swelling of the ankles

_____ You experience shortness of breath with change of posture or while sleeping

_____ You experience episodes of rapid heart beats or skipped heart beats.

_____ You experience pain or cramping sensations in your legs when walking.

_____ You experience fatigue or shortness of breath with unusual activities.

Medical History

List all other notable health problems, injuries, or conditions

You have or have had:

_____ Heart murmur  _____ heart attack

_____ heart surgery  _____ a lung disease

_____ a metabolic disease (diabetes, thyroid disorder, kidney or liver disease)

List names/doses/frequency of all medications taken (if not taking any medications, write “NONE”)

ACSM initial risk stratification (circle classification): LOW MODERATE HIGH

Have you lost or gained more than 20 pounds in the last 6 months?  Y or N
Are you an “elite” level athlete?  Y or N
How many (if any) of your biological parents are overweight/obese?  0  1  2
Skinfold Test Evaluation

Athlete Name: _________________________________ Gender: __________ Age: __________

Sport: _________________________________

Strength and Conditioning Coach: _______________________________

### Skinfold Measurements

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triceps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscapular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midaxillary</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Suprailiac</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Thigh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sum of Skinfolds: _______ mm

Calculations: (Jackson-Pollock)

Body Density: ________________  Percent body fat: __________
Table 3.1

Descriptive data (Means ±SD)

<table>
<thead>
<tr>
<th>Descriptive</th>
<th>Healthy</th>
<th>Obese</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>21.6±3.9</td>
<td>24.8±5.4</td>
<td>p=.168</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.77±0.07</td>
<td>1.78±0.08</td>
<td>p=.852</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>73.44±8.40</td>
<td>110.03±13.92</td>
<td>p=.000</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>23.31±2.58</td>
<td>34.63±2.54</td>
<td>p=.000</td>
</tr>
<tr>
<td>Body Fat % *</td>
<td>13.18±4.34</td>
<td>28.96±2.03</td>
<td>p=.000</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)*</td>
<td>54.66±6.7</td>
<td>41.74±8.6</td>
<td>p=.003</td>
</tr>
</tbody>
</table>

*Denotes statistical significance between groups (p<0.05)
Appendix E
Figure 3.1

Bruce Treadmill Protocol

<table>
<thead>
<tr>
<th>Stage</th>
<th>Speed</th>
<th>Grade</th>
<th>Dur.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.7 mph</td>
<td>10 %</td>
<td>3 min</td>
</tr>
<tr>
<td>II</td>
<td>2.5 mph</td>
<td>12 %</td>
<td>3 min</td>
</tr>
<tr>
<td>III</td>
<td>3.4 mph</td>
<td>14 %</td>
<td>3 min</td>
</tr>
<tr>
<td>IV</td>
<td>4.2 mph</td>
<td>16 %</td>
<td>3 min</td>
</tr>
<tr>
<td>V</td>
<td>5.0 mph</td>
<td>18 %</td>
<td>3 min</td>
</tr>
<tr>
<td>VI</td>
<td>5.5 mph</td>
<td>20 %</td>
<td>3 min</td>
</tr>
</tbody>
</table>

Arnell, 2006.
Appendix F
Figure 3.2

Repeated Wingate Anaerobic Protocol

<table>
<thead>
<tr>
<th>30 min</th>
<th>30s</th>
<th>2 min</th>
<th>30s</th>
<th>2 min</th>
<th>30s</th>
<th>1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMR</td>
<td>WAT</td>
<td>Rest</td>
<td>WAT</td>
<td>Rest</td>
<td>WAT</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

WAT= Wingate Anaerobic Test
Figure 4.1

Correlation between BMI and resting CRP Levels on VO₂ max testing day
Appendix H
Figure 4.2

*Correlation between body fat percentage and resting CRP levels on VO2 Max testing day*
Appendix I
Figure 4.3

Correlation between body weight and resting CRP levels on VO$_2$ max testing day
Appendix J
Figure 4.4

Correlation between body mass index and resting CRP values on Wingate testing day
Figure 4.5

Correlation between body fat percentage and resting CRP levels on Wingate testing day
Figure 4.6

Correlation between body weight and resting CRP levels on Wingate testing day

R² Linear = 0.351
Appendix M
Figure 4.7

Correlation between body mass index and resting Leptin levels for VO$_2$ Max testing day
Appendix N
Figure 4.8

Correlation between body fat percentage and resting Leptin levels on VO₂ Max testing day
Appendix O
Figure 4.9

Correlation between body weight and resting Leptin levels on VO$_2$ Max testing day
Appendix P
Figure 4.10

Correlation between body mass index and resting Leptin levels on Wingate testing day
Appendix Q
Figure 4.11

*Correlation between body fat percentage and resting Leptin levels on Wingate testing day*
Appendix R
Figure 4.12

Correlation between body weight and resting Leptin levels on Wingate testing day
Table 4.1

Mean CRP levels (mg/dl) for VO2 max testing

<table>
<thead>
<tr>
<th></th>
<th>PRE*</th>
<th>POST</th>
<th>1-HOUR POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>.49±.27</td>
<td>.45±.25</td>
<td>.52±.42</td>
</tr>
<tr>
<td>Obese</td>
<td>.94±.54</td>
<td>1.18±.72</td>
<td>**.97±.60</td>
</tr>
</tbody>
</table>

Note: *indicates significant difference between healthy and overweight group at baseline (p<0.05). ** Indicates a significant decrease in CRP levels from POST to 1-HOUR POST.
Appendix T
Table 4.2

*Mean CRP levels (mg/dl) for series of Wingate cycle tests*

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>POST</th>
<th>1-HOUR POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>.52±.26</td>
<td>.57±.48</td>
<td>.48±.22</td>
</tr>
<tr>
<td>Obese</td>
<td>.91±.44</td>
<td>.83±.36</td>
<td>.90±.49</td>
</tr>
</tbody>
</table>

Note: Pre levels are not considered statistically significant from each other, however, numbers are similar to those seen on VO2 testing day.
Figure 4.13

*Comparison of Healthy vs. Obese Mean CRP Levels Based on Exercise Mode*

Note: CRP levels in both the healthy and obese groups pre, post, and 1 hour post for the VO\(_2\)max(continuous) test as well as Wingate test (intermittent). * Indicates significant drop in CRP levels from POST to 1-HOUR POST.
Appendix V
Figure 4.14

*Healthy Individual Subject’s Pre, Post, and 1-Hour Post CRP Levels for the VO$_2$ Testing Day*

Note: Comparisons here can be used to determine healthy individuals with a possibly elevated risk for becoming obese. There are only 8 subjects because the 8$^{th}$ of 9 subjects was removed as an outlier with clinically high (>4.0 mg/dl) levels of CRP. Line at .94 denotes mean CRP levels at rest for the obese group.
Figure 4.15

*Healthy Individual Subject’s Pre, Post, and 1-Hour post CRP Levels for the Wingate Test Session*

Note: Comparisons here can be used to determine healthy individuals with a possibly elevated risk for becoming obese. There are only 8 subjects because the 8th of 9 subjects was removed as an outlier with clinically high (>4.0 mg/dl) levels of CRP. Line at .91 denotes mean CRP levels at rest for the obese group.
Table 4.3

Mean Leptin levels (ng/ml) for VO\textsubscript{2} max testing

<table>
<thead>
<tr>
<th></th>
<th>PRE*</th>
<th>POST*</th>
<th>1-HOUR POST*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>5.97±5.88</td>
<td>6.64±6.51</td>
<td>6.29±5.45</td>
</tr>
<tr>
<td>Obese</td>
<td>19.80±14.74</td>
<td>23.66±16.67</td>
<td>19.75±15.79</td>
</tr>
</tbody>
</table>

Note: * Indicates a significant difference between the healthy and obese groups at pre, post, and 1-hour post measurements.
Appendix Y
Table 4.4

*Mean Leptin levels (ng/ml) for Wingate Testing Day*

<table>
<thead>
<tr>
<th></th>
<th>*PRE</th>
<th>POST</th>
<th>1-HOUR POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>5.39±4.24</td>
<td>6.98±5.72</td>
<td>5.59±3.99</td>
</tr>
<tr>
<td>Obese</td>
<td>18.68±13.29</td>
<td>20.30±12.13</td>
<td>18.96±10.31</td>
</tr>
</tbody>
</table>

Note: *indicates significant difference between healthy and overweight group at baseline (p<0.05).
Figure 4.16

*Leptin levels in both the healthy and obese groups pre, post, and 1 hour post for the VO_{2\text{max}}(continuous) test as well as Wingate test (intermittent)*
Figure 4.17

*Healthy Individual Subject’s Leptin Levels on VO2 Testing Day*

Note: Comparisons here can be used to determine healthy individuals with a possibly elevated risk for becoming obese. Line at 19.80 denotes mean Leptin levels at rest for the obese group.
Appendix BB
Figure 4.18

Healthy individual subject’s pre, post, and 1-hour post CRP levels for the Wingate test session

Note: Comparisons here can be used to determine healthy individuals with a possibly elevated risk for becoming obese. Line at 18.68 denotes mean Leptin levels for the obese group at rest, which is being used for comparison.
Allison DB PhD; Kevin R. Fontaine, PhD; JoAnn E. Manson, MD, PhD; June Stevens, PhD; Theodore B. VanItallie, MD. Annual Deaths Attributable to Obesity in the United States. JAMA. 282:1530-1538. 1999.


