THE INFLUENCE OF EXERCISE DOSE, EXERCISE INTENSITY, AND WEIGHT LOSS AND CHANGE IN C-REACTIVE PROTEIN IN SEDENTARY OVERWEIGHT WOMEN

by

Patricia K. Tuite

BSN University of Pittsburgh, 1985

MSN University of Pittsburgh, 1992

Submitted to the Graduate Faculty of

School of Education in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2012

UNIVERSITY OF PITTSBURGH

SCHOOL OF EDUCATION

This dissertation was presented

by

Patricia K. Tuite

It was defended on

August 3, 2012

and approved by

Lora Burke, PhD, MPH, RN, FAAN, Professor, Department of Health and Community Systems

Paul Martin Coen, M.Sc, PhD, Assistant Professor, Department of Health and Physical Activity

Richard Henker, PhD, RN, FAAN, Professor, Department of Acute and Tertiary Care

Dissertation Advisor: John M. Jakicic, PhD, Professor/Department Chairperson, Department of Health and Physical Activity

THE INFLUENCE OF EXERCISE DOSE, EXERCISE INTENSITY, AND WEIGHT LOSS AND CHANGE IN C-REACTIVE PROTEIN IN SEDENTARY OVERWEIGHT WOMEN

Patricia K. Tuite, RN, MSN

University of Pittsburgh, 2012

Objective: To examine the effect physical activity included in a weight loss program has on high sensitivity C-reactive protein (hs-CRP) levels in sedentary overweight women.

Design, Setting, and Participants: This study examined the change in hs-CRP in overweight and obese women in response to a 6 month behavioral weight loss program. The parent study was a randomized trial involving 201 sedentary overweight women who participated in a weight control program, with data from 182 subjects available for this secondary analysis.

Methods: Participants were randomly assigned to 1 of 4 exercise groups based on energy expenditure (1000kcal/week or 2000 kcal/week) and intensity (vigorous vs. moderate). Groups included: vigorous intensity/high dose; vigorous intensity/moderate dose; moderate intensity/moderate dose; and moderate intensity/high dose. Participants were prescribed an energy restricted diet consisting of 1200 kcal/day or 1500 kcal/day and daily dietary fat intake between 20%-30% of total energy intake.

Results: There were no statistically significant differences between dose of exercise, moderate vs. high, ($F=0.330$, $p=0.58$) or level of intensity, moderate vs. vigorous ($F=0.118$, $p=0.731$) for change in hs-CRP. However, there was a significant decrease in hs-CRP from baseline to 6 months ($F=25.553$, $p<0.0004$); there was a significant 3 way interaction between energy expenditure, intensity, and Pre/Post differences ($F=4.035$, $p=0.035$), post hoc analysis revealed a

significant decrease in hs-CRP in the moderate/high ($p<0.0004$) and vigorous/moderate groups ($p=004$). The results were unchanged after controlling for the change in body weight, body fatness, or body distribution. The change in hs-CRP was not significantly correlated with the change in body weight, percent body fat, waist circumference, or self-reported physical activity. hs-CRP at 6 months was correlated with 6 month measures of weight, BMI, percent body fat, and fat distribution ($p<0.0004$).

Conclusions: hs-CRP was reduced in overweight and obese women in response to a 6 month weight loss intervention that included a prescribed reduction in energy intake and a prescribed increase in exercise with a significant reduction in the moderate intensity/high dose and vigorous intensity/moderate dose groups. Further research is needed to determine what effect exercise and or weight loss may have on markers of inflammation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to express my thanks and gratitude to the members of my dissertation committee for assisting me in completing this project. First, I would like to thank Dr. John Jakicic, my advisor and dissertation chair. Your patience and guidance helped to make this project possible. I am also very grateful for the use of your serum samples and your assistance to develop my idea and move my project forward. Thank you Dr. Coen, for your expertise in working with inflammatory markers. I truly appreciate the support that you have given me during this project.

I would especially like to thank Dr. Burke for her continued mentorship throughout my career and the support she has given to me. I also truly appreciate her expertise and all she has taught me throughout the years. Thank you Dr. Henker, for your never ending support and assistance with data analysis. I would also like to thank Dr. Tom Zullo for the statistical support he provided. I would finally like to thank my family for all of their love and support during my graduate education.

1.0 INTRODUCTION

Obesity is a major health concern in the United States. Obesity increases one's risk for premature death from all causes by 50 to 100 percent with a projected 300,000 deaths per year[1]. Currently the prevalence of obesity exceeds 30% in most age groups and among both genders[2]. Being overweight is defined as having a body mass index (BMI) between 25 and <30 kg/m^2 and obesity is defined as having a BMI $\geq$30 kg/m^2. The proportion of the population that is overweight or obese has been increasing. In 2003-2004, the overall prevalence of overweight and obesity in both men and women was estimated to be 66.3%[3] increasing to 68% in 2007-2008[4]. Recent examination of prevalence rates associated with obesity demonstrated that prevalence rates did not increase as originally anticipated. Most recent results from the latest National Health and Nutrition Examination Survey (NHANES) demonstrated a stabilizing pattern. In 2009-2010, the age adjusted prevalence rates for men and women were 35.5% and 35.8% respectively[2]. When examining the trends over the past 12 years, Flegal and colleagues[2] found there was no significant increase in obesity among women overall from 1999 to 2010, but there were statistically significant changes among non-Hispanic black women and Mexican-American women. Men demonstrated a significant linear trend over the same time period. When examining the last 2 years of data (2009-2010), there is no significant change in obesity prevalence when compared with the prior 6 years (2003-2008)[2]. The Nurses' Health Study documented a 38 percent increase in obesity within that study cohort over time[5]. Although the

prevalence of obesity has steadily increased, the prevalence previously observed over the past 10 years does not seem to be continuing at the same rate especially for women[2]. Trends in obesity prevalence appear to be leveling off.

Obesity has been identified as an independent risk factor for cardiovascular disease (CVD)[6-10] and is associated with an increase in morbidity and mortality[11]. The Nurse's Health study demonstrated that mortality among obese women was more than twice that of lean women and that there was a 4-fold increase in cardiovascular deaths in women with a BMI > 29 kg/m^2, [12]. The National Health and Nutrition Examination Survey I (NHANES I) found that a higher weight in late middle age was associated with an increase in coronary heart disease (CHD) risk later in life[13], while a BMI > 27 kg/m^2 in middle age was also associated with an increased risk of CHD later in life, but did not pose an increased risk in old age. The difference in risk was due to a weight loss of 10% between middle and old age. Risk of CHD was also increased in individuals at a lower weight who had lost weight and heavier people who gained weight when compared to thinner people with stable weight[13]. Poirier[9] and colleagues reported that structural changes to the heart and alterations in function occur in individuals with excess adipose tissue, even in the absence of comorbidities. Poirier[8] et al. concluded that the effect of obesity on the cardiovascular system predisposes one or is associated with cardiac complications such as CHD, heart failure, and sudden death.

Improving overall fitness and increasing exercise capacity decreases cardiovascular risk factors and may result in up to a 35 percent reduction in CHD[14-15]. The Nurses' Health Study is a large observational study designed to assess the effects of a combination of lifestyle habits on the risk of CHD in women[5,16-18]. Results from the Nurses' Health Study revealed that a woman was able to decrease the incidence of a coronary event by more than 80 percent if she did not smoke,

maintained a healthy weight (BMI < 25 kg/m^2), consumed a healthy diet, participated in moderate to vigorous physical exercise for 30 minutes a day, and consumed a moderate amount of alcohol[5,16]. Over the study's 14-year observation period the incidence of CHD declined by 31% across all age groups demonstrating that primary prevention can reduce the incidence of CHD. The study further demonstrated that the total physical activity score at baseline exhibited a strong inverse relationship with CHD[19].

To be effective, lifestyle modifications must be targeted at the underlying mechanism that contributes to the development of CHD. The atherosclerotic process that leads to CHD has been identified as an inflammatory process[20]. There are also certain biological risk markers that have been associated with CHD. These include an elevation in homocysteine levels, lipoprotein(a), and inflammatory markers such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)[21-24]. C-reactive protein (CRP) is an acute phase protein, produced by hepatocytes and released during an inflammatory process[25]. It is recommended that hs-CRP assays be conducted in metabolically stable individuals, free of obvious inflammatory or infectious conditions. The following hs-CRP values are associated with varying levels of risk that includes: low risk (<1.0 mg/L), average risk (1.0-3.0 mg/L), and high risk (>3.0 mg/L); values higher than 10.0 mg/L are thought to be from other inflammatory conditions[20]. The 2010 guidelines established by the American College of Cardiologists and American Heart Association recommend the use of hs-CRP levels with global risk assessment for asymptomatic men and women identified as intermediate risk[26]. Based on this information, the purpose of this study is to examine the effect that physical activity associated with weight loss has on high sensitivity C-reactive protein (hs-CRP) levels in sedentary overweight women.

1.1 RATIONALE

Obesity is associated with an increase in circulating pro-inflammatory cytokines (interleukin-6, tumor necrosis factor-α) and C-reactive protein (CRP)[27-28]. The adipose tissue acts as an endocrine organ with a high metabolic activity. The adipocytes actively secrete hormones called adipokines which are associated with insulin resistance and atherosclerotic processes[28-29]. Levels of adiponectin, which is an anti-inflammatory cytokine, are decreased in the presence of obesity further leading to the pro-inflammatory state[29]. A reduction in pro-inflammatory markers, such as CRP has been shown to be directly related to weight loss and change in waist circumference or truncal obesity[27-28].

Obesity is a modifiable risk factor associated with CHD and inflammation. Mounting evidence demonstrates that increasing physical activity can impact both CHD risk and the inflammatory process. An extensive body of evidence exists that supports the efficacy of exercise in decreasing the risk of CHD[15-16,19,30-33], as well as research examining the effects of exercise on inflammatory markers in individuals without CHD[32-35]. Also, there is evidence demonstrating the decrease in inflammatory markers related to weight loss[36-40].

Two observational studies assessed the effects of exercise training in stable CHD patients and found a reduction in hs-CRP[41] and a 50% decrease in CRP levels, as well as significant declines in interleukin -1 (IL-1) and IL-6 following 12 weeks of aerobic exercise training in CHD patients[42]. Caulin-Glaser and colleagues[43] were able to reproduce the results of the prior studies and showed that cardiac rehabilitation participation was able to decrease CRP levels even if there was not a decrease in triglycerides, body mass index or weight.

Studies specifically targeting exercise demonstrated mixed findings. Bo, Ciccone, Guidi, Gambino, Durazzo, Gentile, et al.[44] demonstrated that CRP levels are inversely associated with

self-reported exercise after controlling for weight reduction. However, results from clinical trials in which aerobic exercise was supervised for periods of 4 to 6 months showed no decrease in CRP in the presence of any weight loss[45-46]. These new findings are contradictory to much of the cross-sectional and observational studies that showed individuals had reduced CRP levels with an increase in exercise. There is a possibility that neither of these trials were long enough to show a change and that over a longer period of time a decrease in CRP level may be observed.

Physical activity is commonly recommended as a component of a comprehensive weight loss intervention for overweight and obese adults. Physical activity has been shown to result in approximately 1 to 3 kg of additional weight loss when added to weight loss achieved through dietary restriction alone[47]. However, there is limited evidence to suggest that prescribing a higher dose of physical activity, from either an energy expenditure perspective or from an intensity of activity perspective will significantly improve weight loss compared to a moderate dose and moderate intensity of activity. For example, Jakicic et al.[48-49] prescribed an energy restricted diet to overweight and obese adult women, with the women then randomized to one of four physical activity prescriptions that included 1000 kilocalories (kcals) at a moderate intensity, 1000 kcals at a vigorous intensity, 2000 kcals at a moderate intensity, or 2000 kcals at a vigorous intensity. While weight loss was significant in all conditions at 6 months, there was no significant difference in the weight loss across the four exercise prescriptions. However, what is unclear in the literature is whether prescribing physical activity at either a higher dose or a higher intensity within the context of a comprehensive behavior weight loss program will have differential effects on markers of CVD risk, particularly inflammatory markers such as CRP.

Thus, this study focused on a secondary analysis of existing data that included the additional analysis of banked blood samples for CRP in overweight and obese women (see figure 1) and examined the following specific aims.

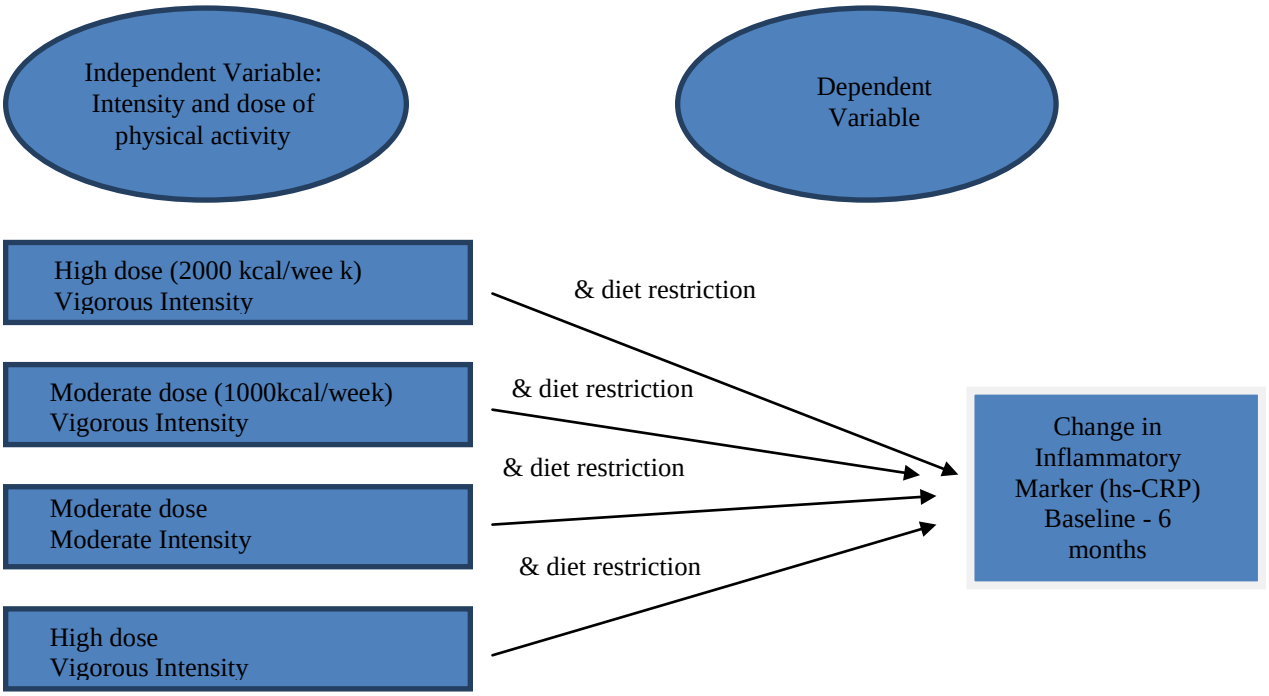


Figure 1: Conceptual framework illustrating an intensity and dose of physical activity intervention on hs-CRP and behavioral weight loss program in sedentary overweight women.

1.2 SPECIFIC AIMS

1. To compare the effect of varying prescribed doses and intensities of physical activity when combined with a prescribed energy restricted diet

on changes in CRP in overweight and obese women over a period of 6 months.

2. To compare the effect of varying prescribed doses and intensities of physical activity when combined with a prescribed energy restricted diet on changes in CRP in overweight and obese women over a period of 6 months after controlling for changes in body weight.

3. To compare the effect of varying prescribed doses and intensities of physical activity when combined with a prescribed energy restricted diet on changes in CRP in overweight and obese women over a period of 6 months after controlling for changes in body fatness.

4. To explore the association of self-reported physical activity dose and intensity of physical activity when combined with a prescribed energy restricted diet on changes in CRP in overweight and obese women over a period of 6 months.

5. To explore the association of self-reported physical activity dose and intensities of physical activity when combined with a prescribed energy restricted diet on changes in CRP in overweight and obese women over a period of 6 months after controlling for changes in body weight.

6. To explore the association of self-reported physical activity when combined with a prescribed energy restricted diet on changes in CRP in overweight and obese women over a period of 6 months after controlling for changes in body fatness.

2.0 SECOND CHAPTER

2.1 INTRODUCTION

This study focused on a secondary analysis of existing data that included the additional analysis of banked blood samples for hs-CRP in overweight and obese women in response to a 6 month behavioral weight loss program. The parent study used a combination of exercise and reduced caloric intake to promote weight loss. Women were randomized to one of four groups, this secondary analysis will enable the researcher to compare four different doses of exercise (varying intensity and energy expenditure) on hs-CRP in sedentary overweight women. The women were also instructed to reduce their caloric intake and dietary fat intake. Determining the amount of weight loss necessary to decrease inflammation will provide health care professionals with the information necessary to counsel individuals on ways to promote a healthier lifestyle. Inflammation is associated with the development of CHD. Decreasing levels of inflammation may result in a decrease in CHD risk, thus promoting participation in healthier lifestyle habits may decrease CHD risk in sedentary overweight women. There is a significant body of evidence that demonstrates that both obesity and a lack of physical activity are both risk factors for CHD and that decreasing obesity decreases inflammation that is associated with atherosclerosis. This literature review discussed the results and limitations of these prior studies providing a basis for the current study.

2.2 OBESITY PREVELANCE

The prevalence of obesity has increased over prior years within the United States and has reached epidemic proportions. In 2010, the Centers for Disease Control reported that approximately 72.5 million adults were obese within the United States[50]. The most recent data from National Health and Nutrition Examination Survey (NHANES) identifies the current prevalence of obesity to be greater than 30 percent in most age groups and among both genders[2]. NHANES data has shown statistically significant increases in obesity in all age groups for both genders except men between the ages of 40-59 from 1999-2000[4]; however, the most recent data demonstrated that prevalence rates have stabilized some and did not increase as much as originally anticipated[2]. Data from 1994-1998 and 1999-2000 showed the prevalence of obesity increasing by 7.1 and 8.1 percentage points for men and women respectively[4,51]. Based on earlier data, researchers presumed that prevalence rates would continue to increase by 6 to 7 percentage points between 1999-2000 and 2008-2009, but after examining the NHANES data from 1999-2000 and 2007-2008, there was only an increase of 4.7 percentage points for men and 2.1 percentage points for women[4]. These data reflect more positive news than originally anticipated. Based on current data, overall obesity prevalence is 33.8%; 32.2% and 35.5% for men and women respectively. The corresponding prevalence rates for both overweight and obesity (BMI≥ 25) were 68% overall, and 72.2% for men and 64.1% for women [4]. Further examination of the trends over the last 12 years, demonstrated no significant increases in obesity among women overall from 1999 to 2010, but did show statistically significant changes among non-Hispanic black women and Mexican-American women[2]. Healthy People 2010 set a goal for all states to reach a prevalence rate of 15% for obesity, however no state met that goal as of 2009[50].

The increasing prevalence of obesity has been clearly identified. There are many other concerns associated with weight gain as well. Obesity has been identified as a risk factor for CVD[6-8,10-11]. Obesity is also associated with increasing medical costs within the United States. It is estimated that 147 billion dollars per year is spent on medical costs associated with obesity; this accounts for approximately 1,429 dollars per year for each obese individual as compared to individuals of normal weight[52]. Based on these data, the *Surgeon General's Vision for Healthy and Fit Nation 2010* recommends a comprehensive approach to addressing increasing prevalence for obesity. The Surgeon General recommends addressing both nutrition and physical activity, working across multiple settings and changing policy and environments to affect individual behaviors[50]. Improving physical activity plays a major role in improving health and decreasing weight.

2.3 OBESITY AND INFLAMMATION

Cottam et al.[28] identified a strong association between obesity and inflammation depicting obesity as a chronic inflammatory state. White adipose tissue serves as a major endocrine and secretory organ. Adipocytes produce and secrete several proteins referred to as adipokines that act as hormones and play important roles as contributors to the inflammatory and atherosclerotic processes[29,53-54]. Leptin, TNF-α, IL-6, angiotensinogen and plasminogen activator inhibitor-1 are all examples of pro-inflammatory adipokines[29]. Elevated levels of these pro-inflammatory hormones have been noted in obesity resulting in a state of chronic inflammation[28-29,55]. IL-6 stimulates the formation of CRP in the liver[23,54], thus obesity results in the increase in IL-6

leading to an increase in CRP. Bastard et al.[56] examined CRP and IL-6 levels in 13 fasting obese men and women and found a strong correlation between both CRP and IL-6 levels and adipose tissue. Visser and colleagues[57] also noted that higher BMIs were also associated with higher CRP concentrations. Data from 16,616 men and non-pregnant women over the age of 17 from the Third National Health and Nutrition Examination Survey (NHANES III) were analyzed. Elevated CRP levels were more likely noted in individuals with BMIs > 27 kg/m^2. Waist to hip ratio was also positively associated with both elevated and clinically raised CRP levels in this population, independent of BMI[57]. Mohamed-Ali and colleagues[58] also identified that IL-6 was released by subcutaneous adipose tissue. The presence of pro-inflammatory cytokines in overweight and obese individuals results in an increase in CRP levels suggesting that overweight and obese individuals experience chronic low grade systemic inflammation[59]. There is evidence demonstrating that a reduction in fat mass correlates with a decrease in serum levels of many of the pro-inflammatory adipokines[29,60]. Review of the literature performed by Cottam and associates[28] further determined that weight loss resulted in a reduction of these markers and thus may lead to an improvement in the comorbidities associated with obesity such as CHD.

2.4 CHD IN WOMEN

More than 200,000 women die each year from CHD[61-62]. CHD results in approximately a death a minute, and is the leading cause of death in women greater than 65 years of age[12,61]. In 2005, one out of every six female deaths was associated with CHD[61].

Recent trends have shown an improvement in these statistics. Between 1980 and 2002, death rates from CHD among men and women greater than 65 years of age decreased by 52% and 49% in men and women, respectively. However, the decrease varied depending on age. Although, the death rate among women 35 to 55 years old decreased during the 1980's and 1990's, the change was not sufficient to reach statistical significance, and more recently (2000-2002) the death rate increased slightly (1.5%)[63]. Of more concern, the CHD death rate in younger women, aged 35-44 years, increased by an average of 1.3% annually from 1997-2002[63]. This change highlights the need to identify women at high risk and implement strategies to prevent CHD earlier than previously viewed as necessary.

Prior studies have identified evidence-based therapies successful in changing behavior to alter risk factors[64]. Two large cohort studies the Framingham Heart Study and the Nurses' Health Study have provided significant observational data on risk factors. These results prompted many researchers to examine altering behavior to decrease CHD risk.

As previously mentioned the Nurses' Health Study reported the effects of a combination of lifestyle practices on the risk of CHD in women[5,16-18]. Results from the Nurses' Health Study revealed that changing one's lifestyle and adopting more healthy habits, specifically increasing physical activity and maintaining a healthy weight, resulted in a reduced incidence of coronary events by more than 80 percent[5,16]. The incidence of CHD declined by 31% across all age

groups over the 14-year observation period demonstrating that a woman is able to decrease her CHD-associated risk profile.

Some CHD risk factors are modifiable such as: age, ethnicity, and family history. Smoking, dyslipidemia, hypertension, a sedentary lifestyle and obesity are proven risk factors for CHD that are modifiable[10,65]. Extensive data exist demonstrating that alteration of these risk factors decreases the incidence of CHD[10,65].

Smoking cessation has been identified as an important goal in the prevention of CHD. The Nurse's Health Study clearly demonstrated a decrease in coronary events through reported changes in lifestyle including smoking cessation. The risk of CHD declined across all age groups, and these results were consistent with a decrease in smoking by 41%[5]. Maintaining a blood pressure < 120/80 mm Hg is also optimal for the prevention of CHD and can be accomplished through weight control, increasing physical activity, using alcohol in moderation, restricting sodium intake, and increasing the intake of fruits, vegetables, and low-fat dairy products[10,65]. Dyslipidemia is controlled through lifestyle interventions such as increasing physical activity and maintaining a healthy diet and adjunctive pharmacotherapy as indicated[10,65].

Being overweight is a known and modifiable risk factor for CHD[10]. The prevalence rates for both overweight and obesity (BMI ≥ 25) increased in 2007 -2008 data to 68% overall, and 72.2% for men and 64.1% for women[4]. In 2009-2010 the prevalence rate for men was 35.5% and 35.8% for women[2]. The Nurses' Health Study documented a 38 percent increase in obesity, which appeared to slow the decline in CHD incidence noted in this study[5]. Notably, mortality among obese women was more than twice that of lean women and there was a 4-fold increase in cardiovascular deaths in women with a BMI > 29 kg/m^2 [13]. The National Health and Nutrition Examination Survey I (NHANES I) found that a heavier weight in late middle age was

associated with an increase in CHD risk later in life[14], while a BMI > 27 kg/m^2 in middle age was also associated with an increased risk of CHD later in life, but did not pose an increased risk in old age. The difference in risk was due to a weight difference of 10% between middle and old age. Risk of CHD was also increased in thinner people who had lost weight and heavier people who gained weight when compared to thinner people with stable weight[14].

While concerning, there are evidence-based strategies that can decrease risk. Improving overall fitness and increasing exercise have been shown to result in up to a 35 percent reduction in CHD[14-15]. Manson and colleagues[19] found that the total physical activity score at baseline exhibited a strong inverse relationship with CHD. The more activity a woman participated in, the lower her risk for the development of CHD. There is also evidence that increasing physical activity and decreasing weight may result in a decrease in other risk factors associated with CHD at the mechanistic level, e.g. inflammation.

2.5 INFLAMMATORY PROCESS

To be effective, lifestyle modifications must be targeted at the underlying mechanism that results in CHD. The atherosclerotic process that leads to CHD has been identified as an inflammatory process[20]. There are also certain biological risk markers that have been associated with CHD. These include an elevation in homocysteine levels, lipoprotein(a), and inflammatory markers such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis

factor α (TNF-α)[21-24] . The presence of these inflammatory markers serves as predictors of higher risk for CHD[21,23].

Atherosclerosis has been described as an inflammatory disease initiated by vascular injury that occurs from oxidized LDL and reactive oxygen species[66]. In addition, the process is associated with the development and rupture of atherosclerotic plaque. During the acute phase response that occurs as a result of the inflammation, CRP and other markers of inflammation are released from the hepatocytes[25].

Various risk factors that promote the development of atherosclerosis have been identified e.g. cigarette smoking, hypertension, hypercholesteremia, and hyperglycemia. These risk factors give rise to noxious stimuli, which in turn elicits an inflammatory response[20] . Inflammation in the blood, either within the vessel or in the circulating blood volume, initiates the release of primary pro-inflammatory cytokines such as TNF-α and interleukin –1 (IL-1).

These primary cytokines directly elicit the production of adhesion molecules, procoagulants, and other mediators from endothelial cells. They also stimulate the production of IL-6, which serves as a messenger cytokine[67]. The interleukins are "biochemical messengers produced predominately by macrophages and lymphocytes in response to their recognition of a pathogen or stimulation by other products of inflammation"[68(p.196)]. Pro-inflammatory cytokines produced mainly by macrophages include IL-1 and TNF-α. IL-6 is a messenger cytokine, whose presence is crucial for leukocyte and endothelial cell activation. It also promotes the production of hepatic acute-phase reactants, including CRP[23]. C-reactive protein (CRP) is an acute phase protein produced by the liver in response to inflammatory cytokines such as IL-1 and IL-6[69]. C-reactive protein may contribute to atherogenesis by causing the expression of cell adhesion molecules (CAMs) such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion

molecule-1 (VCAM-1), and selectins on the endothelial cells, thus mediating leukocyte adhesion, which is an early step in the formation of plaque[70]. Thus, these markers can provide a picture of the inflammatory status of the individual. CRP may play a direct role in the development and progression of atherosclerosis[70].

As early as 1930, C-reactive protein (CRP) was identified as occurring in response to the inflammatory process[25]. Levels of CRP increase in response to trauma, infection and inflammation[71]. In individuals without inflammation, the hs-CRP level is usually below 1 μg/ml, but in patients experiencing infection, autoimmune disease or cancer, the levels can be as high as 100 μg/ml. For patients with cardiac disease the predictive power lies in the range between 1 and 5 μg/ml hs-CRP[72]. Monitoring inflammatory markers can help to identify vulnerable individuals earlier and thus focus risk-reducing interventions on these individuals. Thus addressing an individual's lifestyle habits, e.g. regular physical activity or weight loss, may result in decreasing inflammation and decreasing the risk for CHD[14-15,30-31].

2.6 THE EFFECTS OF PHYSICAL ACTIVITY

A report from the Surgeon General on physical activity and health states that more than 60% of American adults are not regularly physically active, and 25% are not physically active at all[73]. The same survey also identified that 54% of men and 66% of women age 75 years and older do not engage in any leisure-time physical activity[73]. In 2004, data from a government conducted

survey in all 50 states found that approximately 21% of men and 26% of women reported no leisure-time physical activity[74]. This is the lowest reported prevalence over the last 10 years. These latest data show improvement in prevalence rates, but still approximately one out of four individuals is not participating in any physical activity. The highest level of leisure-time physical inactivity is among adults ≥ 70 years of age, with women being more physically inactive than men[74-75]. When examining the incidence and prevalence of various chronic disorders the values are not all that different from the general population. The prevalence of regular leisure time physical activity in individuals with cardiovascular disease is 30.1% overall; in males it is 31.4% and in females 29%[62].

Improving exercise capacity and fitness predicts more favorable outcomes in all-cause mortality[76-77]. By improving overall fitness and increasing exercise capacity one can decrease cardiovascular risk factors, which may result in up to a 35 percent reduction in CHD[15,78]. Pate et.al[79] reported on numerous epidemiologic studies and controlled experimental investigations that demonstrated that physically active adults developed and maintained higher levels of physical fitness. The improved physical fitness provides protective effects and improved risk factors for several chronic diseases including CHD, diabetes, and stroke. Brown, Burton, and Rowan[80] reviewed 10 years of literature and found a significant reduction in risk (28 to 58%) in cardiovascular outcomes. They concluded that there is strong evidence for the role of physical activity in the primary prevention of cardiovascular disease as well as other chronic diseases[80]. The American Heart Association[81] and the American College of Sports Medicine[81] recommend that every adult should participate in a minimum of 150 minutes of moderate-intensity exercise during the week or a minimum of 20 minutes of vigorous intensity exercise three times per week to receive the associated health benefits. An individual can also combine both moderate and

vigorous intensity activity for the same benefits[81]. Increasing the amount of activity above these recommendations will provide additional health benefits.

Yu and colleagues[82] reported that in the Caerphilly study, 1,975 men between the ages of 49 and 64 years of age did not show a decrease in CHD risk with leisure activity. Only exercise classified as heavy or vigorous was associated with reduced risk of premature death from CHD. The intensity of physical activity reported by the subjects was classified as light or moderate intensity. Thirty percent of the population reported no vigorous activity at all[82]. This study focused on leisure activity and activity undertaken during work in an all male cohort. The participants did not participate in an activity that was not within their usual routine. One can conclude that an individual must increase his usual activity level to receive the cardioprotective benefits.

Manson, Hu, Rich-Edwards, et al.[17] examined the association between scores for physical activity, walking, and vigorous exercise and the risk of CHD in a cohort of 72,488 females enrolled in the Nurses' Health Study. Findings from this study were validated by the findings from the Women's Health Initiative. Data demonstrated that walking and vigorous exercise were associated with a decrease in CHD risk[17]. The Women's Health Initiative Observational Study enrolled 73,743 postmenopausal women to examine total physical activity score, walking, vigorous exercise, and hours spent sitting or sleeping as predictors of cardiovascular events[19]. They found that the total physical activity score at baseline exhibited a strong inverse relationship with CHD. The more activity a woman participated in, the lower her risk for the development of disease. Women, who exercised for 2.5 hours per week, by either walking or through vigorous exercise, had decreased their risk for CHD by 30 percent[19]. This is consistent with the guidelines established by the Centers for Disease Control (CDC) and the American

College of Sports Medicine. Risk was decreased further if the women participated in both vigorous activity and walking than either one alone. Cardiovascular risk was decreased less in women who spent more time sitting or lying than those who moved around more[19]. In summary, their findings indicated that both walking and vigorous exercise were associated with a decrease in cardiovascular events among postmenopausal women, regardless of race or ethnic group, age, and BMI.

Lee and colleagues[83] examined the relationship between physical activity, specifically walking, and the risk of CHD among women. The Women's Health Study recruited a cohort of 39,372 healthy female health professionals aged 45 years or older. Data indicated that at least one hour of walking per week predicted a lower risk for CHD. This study indicated that even light-to-moderate activity is associated with lower CHD rates in women[83]. Women that participated in 600 to1499 kcal/week had a significant decrease in CHD risk factors. Increasing the amount of energy expenditure did not add additional risk reduction. When women in the highest level of physical activity were compared with women in the lowest category, data showed a lower risk of CHD among the women who were most active[83].

2.7 EXERCISE AND INFLAMMATION

Numerous studies outline the benefits of exercise on the risk of CHD, but there are few studies that examined the changes in cardiac inflammatory markers and the subsequent risk of CHD.

Bassuk and Manson[32] determined that increased physical activity reduced cardiovascular risk independently of weight regulation. Thirty minutes of moderate-intensity activity lowered blood pressure, improved glucose tolerance and insulin sensitivity, and improved lipid levels, suggesting that the cardioprotective effects of exercise may be a result of the influence on inflammation and homeostasis. Bassuk and Manson[33] also linked moderate-intensity exercise to reduced levels of the inflammatory marker CRP in both men and women. Reductions in plasma fibrinogen levels and platelet aggregation and elevation in plasma tissue plasminogen activity were identified in men who participated in moderate-intensity exercise[33], although these relationships have not yet been identified in women. Nicklas et al.[34] found no significant effect of exercise on the CRP concentrations in older, overweight and obese, sedentary men and women, although CRP levels decreased more in men than women. These results differed from those identified by Bassuk & Manson[33]. Nicklas[34] did recommend that further studies look at different modes and intensities of exercise on inflammation.

Gielen[84] (2005) described the anti-inflammatory effects of exercise. Two observational studies assessed the effects of exercise training on stable coronary artery disease (CAD) patients and found a reduction in hs-CRP[41] and a 50% decrease in CRP levels, as well as significant declines in IL-1 and IL-6 after aerobic exercise training in CAD patients[42]. Milani and colleagues[41] analyzed the effects of a cardiac rehabilitation program on hs-CRP in 277 patients and found a significant reduction in hs-CRP after a 3-month intervention. The reduction in hs-CRP was noted in patients who both gained and lost weight, implying that adipose tissue is not the only source of pro-inflammatory cytokines[41]. Colbert and colleagues[35] examined cross sectional data to determine the association between physical activity and inflammatory markers.

They concluded that higher levels of exercise were associated with lower levels of CRP, IL-6 and TNF-α..

Caulin-Glaser and colleagues[43] were able to reproduce the results of the prior studies and show that cardiac rehabilitation was able to decrease CRP levels even if there was not a decrease in triglycerides, body mass index or weight. Regardless of other cardiac risk factors, for example metabolic syndrome, CRP levels remained reduced after exercise in a cardiac rehabilitation program. These results are reassuring because the results were achieved in a "real world" setting recruiting patients as they were referred into the program; patients weren't chosen because they were likely to be successful; and the study shows that underrepresented groups such as women and the elderly benefit equally well from the intervention[84].

In a recent prospective randomized trial, exercise training was compared with percutaneous angioplasty in 101 stable CAD patients[85]. The patients were either randomized to 1 year of aerobic training or interventional therapy. After 12 months of training, hs-CRP levels were reduced by 36% in the exercise group. Hambrecht[85] and colleagues were able to demonstrate that exercise alone is sufficient to decrease CRP levels resulting in a reduced risk of ischemic events.

Prior studies within the cardiac rehabilitation patient population displayed a decrease in CRP levels with the exercise prescribed within the programs. Based on that evidence and those noted in other clinical trials[86] Gielen[84] hypothesized that the following 4 potential mechanisms are responsible for decreasing CRP levels: 1) reduction in central adiposity decreases adipocyte-derived IL-6 and TNF-α; 2) improvement of endothelial dysfunction lowers endothelial IL-1 and IL-6 release; 3) reduced monocyte activation with simultaneous cytokine production; and 4) decreased cytokine production in other tissues, such as skeletal muscle.

As mentioned earlier in this paper, there is a developing body of evidence in the cardiac rehabilitation population demonstrating a decrease in CRP with exercise[41-43]. Milani and colleagues[41] demonstrated a decrease in hs-CRP levels with exercise regardless of weight change. Hambrecht et al.[85] also noted a decrease in hs-CRP in patients only receiving an exercise intervention. These results are promising in terms of the response of exercise on the inflammatory process. When researchers examined the effects of exercise on CRP in healthy individuals the results were not as favorable; results yielded no change in CRP with exercise[87-88]. Campbell, et al.[87] found no intervention effects on CRP in both men and women who participated in a moderate to vigorous aerobic exercise intervention that were without serious medical conditions, but lived a sedentary lifestyle. The participants were randomized to either the intervention group or to the usual lifestyle group. The intervention included aerobic exercise for 60 minutes per day, 6 days per week for 12 months. The participants exercised at 60-85% of maximal heart rate. Usual diet was maintained throughout the trial. The exercise intervention resulted in an increase in cardiopulmonary fitness, but no significant change in CRP. The participants did not experience a significant weight loss during this intervention[87].

Church and colleagues[45] completed a randomized control trial designed specifically to measure CRP. The objective of the study was to determine if an aerobic intervention without a dietary component would decrease CRP in patients with an elevated CRP levels[45]. They recruited 162 sedentary men and women with elevated CRP levels (between 2.0 mg/L and 10 mg/L) and no history of serious medical illnesses. The participants were randomized to either the no exercise control group or the exercise training group. The exercise dose was set at 16 kcal/kg body weight divided among three to five sessions per week completed in an exercise laboratory 60%-80% VO_{2max}[45]. Participants did not experience weight loss during this intervention. Thus,

researchers determined that exercise training without weight loss resulted in no change in CRP levels among participants. Researchers did note a 12% increase in fitness levels among those in the exercise group. Stewart and colleagues[46] completed a randomized control trial examining four different exercise interventions, none of the interventions resulted in a decrease in CRP levels. The participants that completed the most exercise (12kcal/kg/week) presented with the greatest weight loss, this weight loss was associated with a decrease in CRP levels. Based on these findings the researchers concluded that weight loss not exercise is associated with a reduction in CRP levels[46] .

Two other randomized control trials utilizing exercise as an intervention resulted in a reduction in CRP levels among participants. Lakka and colleagues completed the HERITAGE family study. The study was a 20 week exercise intervention study in five universities in the United States and Canada[89]. The intervention included an exercise training program consisting of three sessions per week on cycle ergometers in an exercise laboratory. Participants initially exercised for 30 minutes at 55% of their maximal heart rate, after 2 weeks, the exercise sessions were increased to 50 minutes at 75% of maximal heart rate. That level was sustained for the last six weeks of the study[89]. A total of 652 sedentary men and women were included in the analysis. The participants were stratified into three groups related to baseline CRP levels: low (CRP < 1.0 mg/L), moderate (CRP levels 1.0 mg/L – 3.0 mg/L) or high (CRP levels > 3.0 mg/L). Results indicated a reduction in CRP levels in individuals with high baseline CRP levels (> 3.0 mg/L) after adjusting for changes in body weight, glucose, insulin, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol levels, triglycerides, systolic and diastolic blood pressure, and maximal oxygen uptake[89].

Campbell and colleagues[90] examined the effect of a 12 month moderate intensity aerobic intervention on CRP in a subset of 115 postmenopausal women recruited for another study. The women were randomized into either the exercise group or the control group which consisted of 45 minutes of stretching per week. Randomization was stratified by BMI to ensure equal number of both obese and lighter participants per group (BMI < 27.5 kg/m^2 or ≥ 27.5 kg/m^2)[91]. The participants exercised for 45 minutes at 60%-75% of maximal heart rate. During the first 3 months, the participants attended 3 supervised sessions and exercised 2 days at home; for months 4-12 participants attended at least 1 supervised exercise session and completed exercising at home for the other 4 days. Over the 12 month period, those in the intervention group averaged 3.8 days of exercise per week for 166 minutes/week. Participants in the intervention group increased aerobic fitness, decreased body weight, and decreased percent body fat when compared with the control group[90]. Results demonstrated a decrease in CRP levels in the exercise group among sedentary post-menopausal women with BMI ≥ 27.5 kg/m^2.

A cross-sectional design examined 152 overweight/obese postmenopausal women who were sedentary and free of chronic or inflammatory diseases[91]. Researchers compared total energy expenditure (TEE), physical activity energy expenditure (PAEE) and the resting energy expenditure (REE). Results demonstrated that hs-CRP was positively associated with REE and that women with the highest tertile of PAEE (1233-1319 kcal/day) had the lowest concentrations of hs-CRP when compared to the lowest tertile of PAEE (553-621 kcal/day) after adjusting for fat mass[91].

The above studies demonstrated conflicting results related to exercise and CRP levels. When participants were stratified according to BMI or level of baseline CRP, there was a significant decrease in CRP levels. Although, Church et al.[45] recruited participants with mean

BMIs of approximately 31 kg/m^2, and mean baseline CRP levels of 4.9 mg/L, and the researchers still found no change in CRP levels in the intervention group. These results conflict with the results from the Campbell et al.[90] and Lakka et al.[89]. Investigating the role that weight loss plays in the decrease in the CRP may yield differing results. Lavoie et al.[91] also demonstrated a decrease in CRP with higher levels of physical activity, but this was a cross sectional design not a randomized trial.

2.8 WEIGHT LOSS AND INFLAMMATION

2.8.1 Surgical Interventions

Selvin et al.[92] completed a systematic review analyzing the relationship between CRP and weight loss. The reviewed spanned 40 years and included 33 studies, 28 studies involving a lifestyle intervention and 5 involving surgical intervention. The surgical studies demonstrated a mean change in weight of negative 33.1 kg with a decrease in CRP level of 4.5 mg/L. The studies utilizing a life style change as the intervention, the mean weight loss across studies was 6.2 kg with a mean decrease in CRP levels of 0.9 mg/L[92]. After analyzing all of the studies, the correlation between mean baseline weight and mean CRP level was 0.76 (weighted Pearson correlation (r), and when examining the regression slope the overall change was 0.13 mg/L decline in CRP for each 1 kg of weight loss (weighted r=0.85)[92]. On average, they found that the

largest change in weight was associated with the greatest decrease in CRP levels, thus concluding that weight loss may be effective in decreasing markers of inflammation.

As illustrated in the systematic review by Selvin[92], gastric bypass surgery was successful in demonstrating a decrease in CRP levels. A study completed by Ramalho, Guimarães, Gil, Neves, Guimarães and Delgado[93] enrolled 32 morbidly obese females undergoing laparoscopic gastric banding surgery. At baseline, the mean weight and BMI was 114.3 ±11.6 kg and 43.4 ±4.4 kg/m^2, respectively; 18 months after surgery, weight and BMI were 80.5± 16.0 kg and 30.5± 6.1 kg/m^2, resulting in a 63.2% excess body weight loss. One month after surgery, CRP levels began to decline resulting in significant decrease in CRP levels at 18 months post surgery[93].

2.8.2 Dietary Interventions

Two additional studies utilized a low caloric diet to promote weight loss and analyzed the effect on CRP levels[94-95] Solá and colleagues[94] compared 67 morbidly obese patients with 67 healthy subjects. The intervention group received a very low caloric diet for four weeks followed by a low caloric diet for two months. The participants were able to obtain a moderate weight loss (19.6%), but there was no decrease in CRP noted[94].

Belza et al.[95]enrolled 33 obese patients into a 20 week controlled dietary intervention. The intervention was divided into four sections: a low energy liquid diet (3.4 MJ/day) for the first 8 weeks, participants reduced body weight by 8 percent followed by a 4 week maintenance program. During the next 4 weeks, participants followed a low energy liquid diet of 4.2 MJ/day. Participants were allowed to supplement with 750 kJ/day of free food choice items during the second weight loss period. The second weight loss period was followed by another 4 weeks of a

weight maintenance period. There was no change in hs-CRP after the first 8 weeks of the diet, but there was a 35% reduction in hs-CRP after the second weight loss period[95]. The researchers concluded that a reduction of fat mass may be responsible for the decrease in inflammatory marker.

An additional study demonstrated conflicting results with the two low energy diets presented above. Tchernof, Nolan, Sites, Ades, and Poehlman[37] enrolled 61 obese postmenopausal women into a weight loss protocol. The subjects consumed a 1200 kcal/day American Heart Association Step 2 diet for approximately 13.9±2.6 months prior to metabolic testing. Reductions in both body weight (average weight loss – 14.5±6.2 kg) and total body fat mass were associated with a decrease in plasma CRP levels[37]. These results are not consistent with the prior studies, but the diet was followed for a longer period of time in this particular study.

2.8.3 Diet and Exercise Combinations

Various studies have shown a decrease in inflammatory markers associated with weight loss[34,38,96-97]. Nicklas and colleagues[34] completed an 18 month randomized control trial examining the effects of diet, exercise and a combination of diet and exercise among older, sedentary, overweight or obese men and women. They concluded that diet-induced weight loss had more of an effect on inflammatory markers than exercise alone. Esposito et.al.[38] randomized 120 pre-menopausal obese women in Italy to one of two groups to determine if changes in one's lifestyle habits can reduce body weight and inflammatory markers over a two-year time period. Serum concentrations of CRP were significantly reduced in the intervention

group. They concluded that a multidisciplinary program that reduced body weight in obese women through lifestyle changes (diet and exercise) did reduce inflammatory markers and insulin resistance. Giannopoulou and colleagues[39] found little change in inflammatory cytokines (hs-CRP, TNF-α) with diet and exercise in older women with type 2 diabetes, thus concluding that dramatic weight loss or clinical intervention is needed. The sample size in that particular study was very small with only 33 women.

Two additional studies demonstrated decreases in CRP levels with weight loss of approximately five to seven percent[96-97]. Christiansen et al[96], compared exercise training versus a diet-induced weight loss program in 79 obese healthy men and women. The participants were randomized to an exercise group, a low calorie diet group, or a combination of diet and exercise. Participants in both the low calorie diet and combined diet and exercise group lost about twice as much weight as the participants in the exercise only group. A reduction in CRP levels was associated with the increased weight loss[96]. The Look Ahead study, a large randomized control trial of individuals with type 2 diabetes examined a subset of individuals enrolled in the study to evaluate a life style intervention that targeted a seven percent weight loss among the participants[97]. Participants were randomized to a control group that received diabetes support and education or the intervention group. The intervention group began an exercise regimen of moderate-intensity exercise, which was increased to 175 minutes/week and was placed on a reduced calorie diet consisting of decrease in saturated fat intake and changes in macro nutrient composition to improve glycemic control. After one year, participants in the intervention group had a seven percent weight loss resulting in a significant decrease in hs-CRP.

2.9 SUMMARY

Obesity is a major health concern within the United States. Albeit, prevalence rates have stabilized and have not increased over the last couple of years, obesity continues to be a risk factor for many disease states. Obesity is a major risk factor for CHD and CHD is the leading cause of death among both men and women. The increase in adipose tissue in the obese state results in the secretion of pro-inflammatory adipokines such as: leptin, TNF-α, and IL-6. IL-6 stimulates the release of CRP resulting in an inflammatory state. Elevated CRP levels have been identified in states of chronic inflammation. Researchers have identified that physical activity may play a role in decreasing CRP levels. There is a significant body of literature demonstrating that increasing physical activity decreases CHD risk[17,19,80,82-83] and recommendations from the American College of Sports Medicine and the American Heart Association suggest participating in at least 150 minutes of moderate intensity exercise or 75 minutes of vigorous intensity exercises per week will result in an improved state of health[81]. Numerous cross sectional studies have demonstrated an inverse relationship between physical activity and CRP levels, whereas exercise intervention studies have shown conflicting results[32-34,86]. Two randomized control trials utilizing exercise as the intervention resulted in no significant reduction in CRP levels among the participants[45-46], whereas two other trials did show a decrease in CRP levels[89-90]. There were changes in CRP levels noted in patients receiving cardiac rehabilitation[41-43]. Reductions in CRP levels were also noted in individuals who are obese or those who displayed higher levels of inflammation (CRP levels > 3.0 mg/L)[89].

Weight loss has been identified in the reductions of CRP levels. Significant weight loss from bariatric surgery has resulted in a decrease in CRP levels[92] whereas additional trials

demonstrated conflicting results related to dietary interventions such as low calorie diets. Interventions that incorporated both diet and exercise resulting in weight loss appeared to reduce CRP levels[38-39]. It also appears that the greater the weight loss the greater the decrease in CRP level.

There is a significant body of literature examining weight loss and exercise in relation to decreasing CRP levels. Based on these results, exercise alone does not appear to decrease CRP levels in overweight and obese healthy individuals, although decreases in CRP levels were noted in the cardiac rehabilitation patient population, patients with osteoarthritis, and those with type 2 diabetes. A decrease in CRP levels was also noted in participants of weight loss studies and in studies that combined weight loss and exercise. Most of the studies used a consistent form of exercise, most often of moderate intensity. What was not clear is whether or not varying the intensity of the exercise along with the amount of energy expended during the exercise affected CRP levels. Literature does not demonstrate whether or not altering the prescribed dose of exercise in combination with an energy restricted diet resulted in a greater decrease in CRP levels. Thus, this study conducted a secondary analysis of existing data that included the additional analysis of banked blood samples for hs-CRP in overweight and obese women in response to a 6- month behavioral weight loss program to determine if intensity or amount of energy expended during exercise resulted in a decrease in CRP levels signifying a decrease in inflammation.

3.0 METHODS

Physical activity, in combination with an energy restricted diet, is commonly recommended as a component of a comprehensive weight loss intervention for overweight and obese adults. Physical activity has been shown to result in approximately 1 to 3 kg of additional weight loss when added to weight loss achieved through dietary restriction alone[47]. However, what is unclear in the literature is whether prescribing physical activity at either a higher dose or a higher intensity within the context of a comprehensive behavior weight loss program had differential effects on markers of CVD risk, particularly inflammatory markers such as high sensitivity c-reactive protein (hs-CRP). Thus, this study conducted a secondary analysis of existing data that included the additional analysis of banked blood samples for hs-CRP in overweight and obese women in response to a 6 month behavioral weight loss program.

This study involved a secondary analysis of 6 month data from a previously published clinical trial[48-49]. In addition, stored blood samples were analyzed for hs-CRP to address the specific aims of this study. The methods and procedures are described below.

3.1 METHOD FROM THE PARENT STUDY

3.1.1 Subjects

Two hundred and four sedentary overweight or obese females were assessed for eligibility for this study, with 201 randomized into 1 of 4 groups as described below. For the purpose of this study only subjects who provided blood samples were included in the analysis. Thus, 182 subjects were included in this secondary analysis.

Eligibility criteria included being female with a baseline age between 21 to 45 years and with a baseline BMI between 27.0 and <40 kg/m^2. At baseline individuals were determined to be ineligible based on the following criteria:

1. History of myocardial infarction
2. Taking medication that would alter the heart rate response during exercise (e.g., beta blockade)
3. Taking medications that would affect metabolism or weight loss (e.g. thyroid medication)
4. Currently receiving treatment for psychological conditions (e.g., depression, anxiety, etc.)
5. Currently pregnant or recently pregnant within the previous six months
6. Currently diagnosed with any medical condition that could affect metabolism or body weight (e.g. diabetes, hypothyroidism, etc.)

7. Currently experiencing any condition that would affect one's ability to perform exercise.
8. Participating in regular exercise of at least 20 minutes per day on 3 or more days of the week during the prior 6 months.

3.1.2 Recruitment, Initial Screening, and Informed Consent

Subjects were recruited through newspaper advertisements that were approved by the local Institutional Review Board. Individuals responding to these advertisements were instructed to call the investigators by telephone to obtain further information about the study. Upon receipt of a telephone call the staff provided a brief description of the study, with individuals interested in participation after hearing this description answering questions to determine initial eligibility based on the criteria listed above. Individuals who appeared to be eligible based on this initial telephone screening were invited to an orientation session where the study was explained in greater detail, components of informed consent were explained, and the individual was given the opportunity to ask additional questions of the investigators.

Prior to undergoing any experimental procedures for this study written informed consent was obtained from the potential subject, and the individual completed both a detailed medical history and a physical activity readiness questionnaire to confirm that no conditions were present that would exclude participation. Moreover, prior to undergoing any experimental procedures, individuals also provided clearance from their personal physician stating that it was safe to participate in a weight loss intervention that included a reduced energy intake diet and exercise.

3.1.3 Research Design and Randomization

Following baseline assessments to confirm eligibility and to collect additional study-related data, eligible individuals were randomly assigned to one of four weight loss intervention conditions. These weight loss interventions included the following:

1. Weight loss that included an energy restricted diet and exercise prescribed to progress to a moderate dose (1,000 kcal/wk) and moderate intensity (MOD/MOD).
2. Weight loss that included an energy restricted diet and exercise prescribed to progress to a moderate dose (1,000 kcal/wk) and vigorous intensity (MOD/VIG).
3. Weight loss that included an energy restricted diet and exercise prescribed to progress to a high dose (2,000 kcal/wk) and moderate intensity (HIGH/MOD).
4. Weight loss that included an energy restricted diet and exercise prescribed to progress to a high dose (2,000 kcal/wk) and vigorous intensity (HIGH/VIG).

The details of each of these interventions are described below.

3.1.4 Behavioral Weight Loss Intervention

For the purpose of this study only the initial 6 months of the intervention and data collection were included. Therefore, the 6 month weight loss intervention that was implemented in the parent study is described below and has previously been published (Jakicic et al. 2003).

Intervention Sessions: Subjects in all intervention conditions were instructed to attend weekly weight loss group session. These groups were closed to only those participants randomly assigned to a particular intervention condition (MOD/MOD, MOD/VIG, HIGH/MOD,

HIGH/VIG). These intervention sessions focused on behavioral strategies to reduce energy intake and to increase exercise consistent with the intervention protocol. Sessions were led by a variety of professionals that included health psychologist, nutritionists, and exercise physiologists. These sessions were scheduled weekly during the six month intervention period. If a group session was missed, a brief individual make-up session was offered to allow the content to be shared with the subject. Body weight was measured at each of these intervention sessions to determine responsiveness of the subject Subjects unable to attend either the group session or the make-up session were mailed intervention materials that may have been distributed to the other subjects who were able to attend the schedule session.

Diet Intervention: The identical dietary intervention was provided to all subjects regardless of randomized intervention assignment (MOD/MOD, MOD/VIG, HIGH/MOD, HIGH/VIG). This included prescribing subjects to consume between 1200 and 1500 kcal per day, and to reduce their dietary fat intake to between 20 and 30 percent of their total energy intake. Meal plans were provided to facilitate adoption and compliance with these dietary recommendations. In addition, subjects self-monitored their dietary intake in a food diary that was returned to the intervention staff at each intervention session, with the intervention staff reviewing these diaries and providing written feedback relative to self-reported eating behavior.

Exercise Intervention: The exercise prescription differed between the randomized intervention conditions (MOD/MOD, MOD/VIG, HIGH/MOD, HIGH/VIG), with these exercise interventions varying by estimated energy expenditure (1000 kcal/wk versus 2000 kcal/wk) and exercise intensity (moderate versus vigorous). The targeted estimated energy expenditure of the exercise was converted to minutes per week based on the differences in exercise intensity that were prescribed. A summary of the exercise prescription is shown in Table 1, with the exercise

for each intervention condition described briefly below. To facilitate compliance, subjects were instructed to exercise in bouts that were at least 10 minutes in duration. Because exercise was not supervised on site, motorized treadmills were placed in the homes of all subjects to facilitate compliance with the prescribed exercise prescription, and the weekly intervention sessions addressed strategies to promote exercise adherence.

MOD/MOD: Subjects in the MOD/MOD treatment group were instructed to exercise 5 days per week, and to progress from 20 to 40 minutes of exercise per day by the 9th week of the program (approximately 1000 calories per week). The exercise intensity was prescribed at 50-65% of age-predicted maximal heart rate, and subjects were taught the proper technique of palpating their heart rate and use of the Borg 15-point Rating of Perceived Exertion (RPE) Scale to regulate exercise intensity.

MOD/VIG: Subjects in the MOD/VIG treatment group were instructed to exercise 5 days per week, and to progress to 30 minutes of exercise per day (approximately 1000 calories per week). The exercise intensity was prescribed at 70-85% of age-predicted maximal heart rate, and subjects were taught the proper technique of palpating their heart rate and use of the Borg 15-point Rating of Perceived Exertion (RPE) Scale to regulate exercise intensity.

HIGH/MOD: Subjects in the HIGH/MOD treatment group were instructed to exercise 5 days per week, and to progress from 20 to 60 minutes of exercise per day by the 17th week of the program (approximately 2000 calories per week). The exercise intensity was prescribed at 50-65% of age-predicted maximal heart rate, and subjects were taught the proper technique of palpating their heart rate and use of the Borg 15-point Rating of Perceived Exertion (RPE) Scale to regulate exercise intensity.

HIGH/VIG: Subjects in the HIGH/VIG treatment group were instructed to exercise 5 days per week, and to progress from 20 to 40 minutes of exercise per day by the 9th week of the program (approximately 2000 calories per week). The exercise intensity was prescribed at 70-85% of age-predicted maximal heart rate, and subjects were taught the proper technique of palpating their heart rate and use of the Borg 15-point Rating of Perceived Exertion (RPE) Scale to regulate exercise intensity.

Table 1: Exercise Prescription for the Four Treatment Conditions[48]

	Exercise Intervention Groups			
	MOD/MOD	MOD/VIG	HIGH/MOD	HIGH/VIG
Frequency	5 days/week	5 days/week	5 days/week	5 days/week
Intensity (%HRmax)				
• Weeks 1-8	50-65%	50-65%	50-65%	50-65%
• Weeks 9-16	50-65%	60-75%	50-65%	60-75%
• Weeks 16-24	50-65%	70-85%	50-65%	70-85%
• Weeks 25-104	50-65%	70-85%	50-65%	70-85%
Duration (min/day)				
• Weeks 1-4	20 min/day	20 min/day	20 min/day	20 min/day
• Weeks 5-8	30 min/day	30 min/day	30 min/day	30 min/day
• Weeks 9-12	40 min/day	30 min/day	40 min/day	40 min/day
• Weeks 13-16	40 min/day	30 min/day	50 min/day	40 min/day
• Weeks 17-20	40 min/day	30 min/day	60 min/day	40 min/day
• Weeks 21-24	40 min/day	30 min/day	60 min/day	40 min/day
• Weeks 25-104	40 min/day	30 min/day	60 min/day	40 min/day

3.1.5 Assessment Procedures

Within the parent study that is providing data to address the specific aims as described in Chapter 1, outcome data were collected at baseline and following the 6 month weight loss intervention. The assessments included measurements of demographic characteristics (e.g., race/ethnicity, age, etc.), height, weight, body mass index (BMI), body composition, body fat distribution, dietary intake, and physical activity. The investigator on this current project was provided a de-identified dataset containing these outcome measures. In addition, the investigator on this current project was provided a de-identified sample of stored serum that was analyzed for hs-CRP to address the primary aims of this investigation. The specific procedures used to assess the outcomes measures are described below.

Height, Weight, and BMI: Height was measured to the nearest 0.25 inches using a wall-mounted stadiometer with shoes of the subject removed. Weight was measured to the nearest 0.25 pounds on a balance-beam scale with the subject clothed in a light-weight hospital gown. BMI was computed as kg/m^2, where kg represents body weight converted to kilograms and m^2 represents height converted to meters and then squared.

Body Composition: Body composition was assessed using bioelectrical impedance. The equation proposed by Segal et al.[98] was used to compute lean body mass (LBM), with percent body fat computed as: Percent Body Fat = [(weight – LBM)/weight]*100.

Body Fat Distribution: Body fat distribution was assessed using anthropometry. Waist circumference was measured in duplicate, with measures differing by $\leq$1.0 cm, at the level of the umbilicus using a Gulick measurement tape. Hip circumference was measured in duplicate, with measures differing by $\leq$1.0 cm, at the widest protuberance of the buttocks using a Gulick

measurement tape. Waist-to-hip (WHR) was computed as the waist measurement divided by the hip measurement.

Dietary Intake: Dietary intake was measured using the Block Food Frequency Questionnaire (FFQ).[99-100]. This questionnaire was used to assess the usual frequency of consumption of specific foods and typical portion sizes. The FFQ was used to obtain information on mean daily energy intake and macronutrient composition (carbohydrate, protein, fat)[99-100].

Physical Activity: Self-reported physical activity was assessed using the Paffenbarger Physical Activity Questionnaire[101]. The Paffenbarger questionnaire was used to determine energy expenditure during participation in leisure-time physical activity. Participants were queried on daily average number of flights of stairs walked up, average daily walking performed for the purpose of exercise, and any sport, recreational, or fitness activities the subject engaged in over the previous week typical week. Activity was converted to energy expenditure based on metabolic equivalents (METs) provided in the compendium of physical activity[99]. The Paffenbarger questionnaire has been shown to provide an acceptable level of validity and reliability[102].

Serum Samples: Serum samples were collected at baseline and 6 months. These samples were collected in the morning with subjects instructed to fast and abstain from moderate-to-vigorous physical activity for a period of 12-hours prior to blood collection. Compliance with these instructions was confirmed by questioning the subject prior to blood collection. Blood was drawn into evacuated tubes containing EDTA, sodium heparin, or SST clot activator. Baseline and 6-month samples have been stored in a -70° C freezer. hs-CRP will be measured by a solid-phase, chemiluminescent immunometric assay (Immulite 2000 High-Sensitivity CRP; Diagnostics Products Corporation, Los Angeles, CA). All blood samples were

processed at the Heinz Nutrition Laboratory in the Graduate School of Public Health at the University of Pittsburgh.

3.2 STATISTICAL ANALYSIS PLAN

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 20. Statistical significance will be set at $P<0.05$. Data was examined for normality prior to analysis, with data determined to not be normally distributed analyzed using non-parametric statistical techniques.

Descriptive baseline data were expressed as mean ± standard deviation for continuous variables, or expressed as frequencies for categorical variables. The randomized intervention groups were compared on baseline demographic variables using one-way analysis of variance (ANOVA) for continuous variables or chi-square analysis for categorical variables. The data analysis plan for each of the specific aims of this study was performed as described below.

1. To compare whether there is an effect of exercise intervention on change in hs-CRP, a two-factor (Group X Time) ANOVA was performed. Group was defined based on randomization assignment (MOD/MOD, MOD/VIG, HIGH/MOD, HIGH/VIG) and time defined as baseline (0 months) or 6 months.
2. To compare whether change in weight influences the effect of exercise intervention on change in hs-CRP, analysis of covariance (ANCOVA) was performed controlling for change in body weight.

3. To compare whether change in body fatness influences the effect of exercise intervention on change in hs-CRP, analysis of covariance (ANCOVA) was performed controlling for either change in percent body fat or change in body fat distribution.
4. To examine the association between change in self-reported physical activity and change in hs-CRP, correlation coefficients were computed.
5. To examine if the association between change in self-reported physical activity and change in hs-CRP is influenced by change in body weight, partial correlation coefficients were computed controlling for change in body weight.
6. To examine if the association between change in self-reported physical activity and change in hs-CRP is influenced by change in body fatness, partial correlation coefficients were computed controlling for either change in percent body fat or body fat distribution.

3.3 POWER ANALYSIS

A power analysis was conducted based on the primary aim of this study, which was to examine the difference in hs-CRP between the four exercise conditions that were implemented in the parent study. Based on a fixed effects ANOVA, the power analysis indicated that 40 participants in each of the 4 groups would provide 80% power to detect a near modest effect size for interaction between intensity and duration of exercise (f=.26) at a significance level of 0.05. Based on the retention in each of the intervention conditions for this study (MOD/MOD,

MOD/VIG, HIGH/MOD, HIGH/VIG), there is sufficient sample to detect this effect size in this proposed study.

4.0 RESULTS

The purpose of this study was to complete a secondary analysis of existing data to examine the effect of four different prescribed doses of exercise (varying intensity and energy expenditure), combined with a prescribed reduction in calorie intake, on change in hs-CRP in overweight and obese sedentary women. Moreover, this study examined whether a change in weight or body fatness influenced the observed effect of exercise on hs-CRP.

4.1 SUBJECTS CHARACTERISTICS

The sample consisted of 182 subjects who provided a measure of hs-CRP from the original 201 subjects included in the parent study[48-49]. As designed in the parent study, subjects were randomized into one of four exercise groups. Table 2 provides a summary of the demographic variables, serum values, and measures of weight, body fatness, fat distribution, and amount of activity participation for the total sample and stratified by weight category.

Table 2: Demographic Characteristics for Total Subjects by Treatment Group

	Total n= (182) (Mean±SD)	HIGH/VIG (n=49) (Mean±SD)	HIGH/MOD (n=42) (Mean±SD)	MOD/MOD (n=45) (Mean±SD)	MOD/VIG (n=46) (Mean±SD)	P value*
Age (yrs)	37.5±5.6	38.9±5.5	37.7±5.0	37.1±6.1	36.3±5.7	0.13
Height (cm)	163.5±6.4	163.4±6.5	163.8±7.5	163.1±5.7	163.5±5.9	0.97
Baseline weight (kg)	87.5±13.3	87.7±10.9	86.8±14.4	87.2±13.1	88.2±15.0	0.97
BMI (kg/m^2)	32.7±4.2	32.9±3.9	32.2±4.0	32.7±7.0	32.9±4.7	0.87
Waist circumference (cm)	99.3±11.7	100.5±10.5	100.1±13.4	96.3±11.2	100.2±11.8	0.27
Body Fatness (%)	41.4±5.0	41.4±4.0	41.2±4.0	41.7±5.0	41.3±6.0	0.97
Total Cholesterol (mg/dL)	193.4±32.1	197.1±6.9	187.5±30.8	196.1±37.2	194±33.4	0.51
High density lipoprotein (mg/dL)	52.5±12.7	53.1±12.0	50.1±10.6	54.7±15.7	52±11.8	0.40
Low density lipoprotein (mg/dL)	118.9±29.2	120.9±26.6	114.8±28.8	121.4±32.6	118.4±29.5	0.71
Triglyceride (mg/dL)	115.8±64.6	115.8±55.9	113.3±48.0	115.4±86.3	118.7±64.5	0.99
Glucose (mg/dL)	102.6±9.0	101.7±8.3	101.4±10.1	102.2±10.1	104.9±10.3	0.30
Insulin (mcU/mL)	18.1±8.6	18.3±9.2	17.2±7.6	17.3±9.3	19.2±8.4	0.65
hsCRP mg/L	5.5±4.9	5.5±5.0	5.9±6.4	5.2±4.3	5.4±3.9	0.93
Physical Activity Energy Expenditure (kcal/week)	533±721.0	458±862.0	545±592.0	482±605.0	652±772.0	0.57
	N (%)	N (%)	N (%)	N (%)	N (%)	
Ethnicity						
American Indian or Alaskan Native	1(0.5)	1(0.5)	0	0	0	.02**
Black	14(8)	3(2)	2(1)	8(4)	1(0.5)	
Hispanic, Latino, Portuguese, or Cape Verdean	16(9)	1(0.5)	1(0.5)	6(3)	8(4)	
Native Hawaiian or Pacific Islander	1(0.5)	0	1(0.5)	0	0	
White	147(81)	43(24)	38(21)	30(16)	36(20)	
Other	2(1)	0	0	1(0.5)	1(0.5)	
Employment Status						
Working	166(91)	42(23)	39(21)	41(23)	44(24)	.56**
Not working	2(1)	1(0.5)	1(0.5)	0	0	
Employment/ Job						
Professional, administrative, executive	74(41)	19(1)	16(9)	18(10)	21(12)	
Clerical work, administrative support, sales or technician	66(36)	15(8)	22(12)	13(7)	16(9)	.28**
Crafts, trade, factory worker, service, or labor	11(6)	4(2)	1(0.5)	4(2)	2(1)	
Other	15(8)	5(3)	0	5(3)	5(3)	
Not Working						
Homemaker	13(7)	5(3)	2(1)	3(2)	3(2)	
Student	3(2)	1(0.5)	1(0.5)	0	1(0.5)	
Currently not Employed	2(1)	0	0	2(1)	0	.33**
Marital Status						
Married	115(63)	33(18)	30(16)	24(13)	28(15)	.38**
Separated	5(3)	3(2)	0	1(0.5)	1(0.5)	
Divorced	14(8)	3(2)	3(2)	6(3)	2(1)	
Widowed	3(2)	0	1(0.5)	0	2(1)	
Never Married	45(25)	10(5)	8(4)	14(8)	13(7)	
Education						
High School	29(16)	11(6)	6(3)	6(3)	6(3)	.91**
Vocational Training	9(5)	1(0.5)	4(2)	3(2)	1(0.5)	
Some College (<4yrs)	72(40)	17(9)	17(9)	18(10)	0	
College/University Degree	48(26)	13(7)	10(5)	12(7)	13(7)	
Graduate or Professional education	24(13)	7(4)	5(3)	6(3)	6(3)	

*p-values are based on statistical tests comparing the 4 intervention groups; ** p-value for Pearson Chi-Square analysis

The sample included women who were sedentary, overweight or obese (BMI: 32.7± 4.4 kg/m^2; percent of body fat 41.4% ± 5.0) prior to initiating the intervention, and were 37.5±5.6 years of age. The majority of the sample reported themselves as white (81%), married (63%), and working (91%) in either a professional (41%) or clerical work (36%) position. The majority of subjects (79%) reported themselves as having some college education, with 39% completing a 4 year degree or graduate or professional education. The mean hs-CRP for all subjects is 5.5± 4.9 mg/L. C-reactive protein levels > 3.0 mg/L are considered to place one at a higher risk for CHD when combined with other risk factors[20].

Analysis of variance revealed no differences between the intervention groups for baseline age, weight, BMI, waist circumference, body fatness, serum levels or baseline activity. Chi-square analysis revealed no difference between groups for marital status, employment status, and educational status. There was a significant difference in category of ethnicity between the groups ($p<0.023$). However, these results have to be tempered by the fact that greater than 20% of the cells have expected frequencies less than 5, a violation of one of the assumptions of the Chi-Square analysis.

Figure 2 illustrates the mean change in physical activity energy expenditure (PAEE) and energy intake (kcal/day) from baseline to 6 months. There was a significant decrease in energy intake (kcal/day) for all groups ($p<0.0004$), with the range of the mean change being 661 kcals/day to 745 kcals/day. There was not a significant difference between the groups for the change in energy intake from baseline to 6 months ($p=0.92$). There was a significant increase in PAEE for all groups ($p<0.0004$), the range of the mean change being 914 kcals/week to 1770 kcals/week. There was not a significant difference between the groups for the change in PAEE from baseline to 6 months ($p=0.13$).

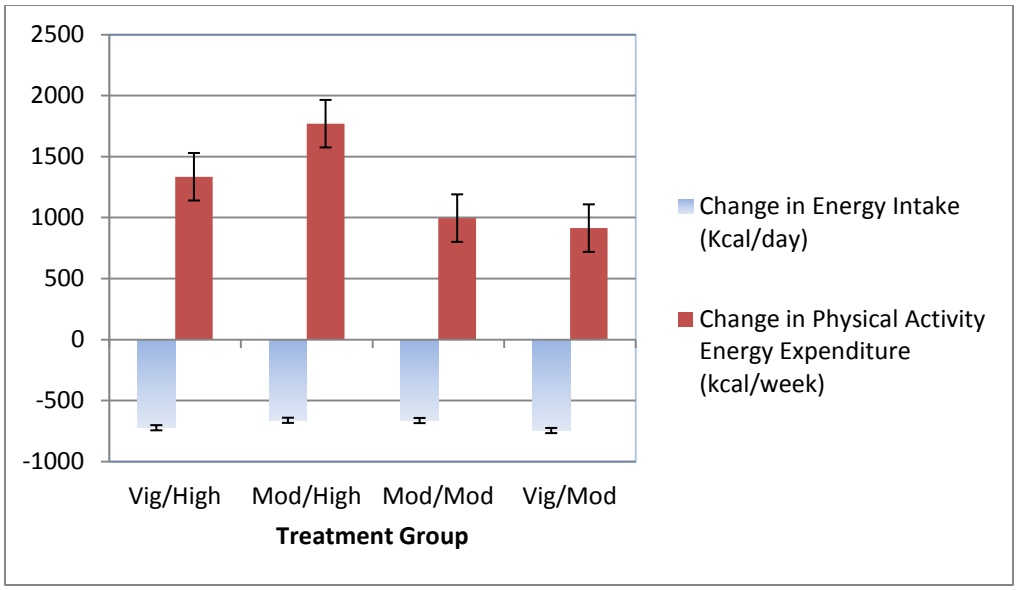


Figure 2: Change in Energy Intake (kcal/day) and Physical Activity Energy Expenditure (kcal/week) Between Treatment Groups from Baseline to 6 Months

Participants exercised at either a moderate or vigorous intensity. There was a significant exercise intensity effect ($p<0.001$)[48]. The rating of perceived exertion was higher in the vigorous intensity/high dose and vigorous intensity/moderate dose groups than the moderate intensity/high dose and moderate intensity/moderate dose groups. Mean scores based on the BORG 15-point RPE scale for each group are as follows: HIGH/VIG 12.7 ± 1.1, MOD/VIG 12.8 ± 1.2, HIGH/MOD 12.0 ± 1.4 and MOD/MOD 11.6 ± 2.0[48]. There was also a significant effect for heart rate, a significant exercise intensity effect ($p=0.002$). Results for heart rate were as follows: HIGH/VIG 127.4 ±10.8 beats per minute, MOD/VIG 128.7 ± 10.3 beats per minute, HIGH/MOD 117.0 ± 11.8 beats per minute, and MOD/MOD 120.2 ± 13.0 beats per minute[48].

4.2 COMPARISON OF THE EFFECT OF EXERCISE BY TREATMENT GROUP ON HS-CRP

The effect of the exercise intervention on change in hs-CRP was examined using a two-factor (Group x Time) analysis of variance with repeated measures. Analysis of variance revealed that there were no statistically significant differences for hs-CRP between level of energy expenditure, moderate vs. high, ($F=0.330$, $p=0.58$) or level of intensity, moderate vs vigorous ($F=0.118$, $p=0.731$). There was a statistically significant difference between Pre and Post values of hs-CRP ($F=25.553$, $p<0.0004$), see Table 3. This finding however, must be interpreted in light of the fact that there was also a statistically significant 3 way interaction between energy expenditure, intensity, and Pre/Post differences ($F=4.035$, $p=0.035$), see Table 4. A Least Square-Differences post-hoc analysis of the 3 way interaction showed that the change in hs-CRP from baseline to 6 months was statistically significant for the HIGH/MOD ($p<0.0004$) and the MOD/VIG ($p=0.004$) groups, with a trend towards statistical significance for the MOD/MOD ($p=0.063$) group. There change in hs-CRP was not statistically significant for the HIGH/VIG ($p=0.278$) group. (Figure 3 and Table 3)

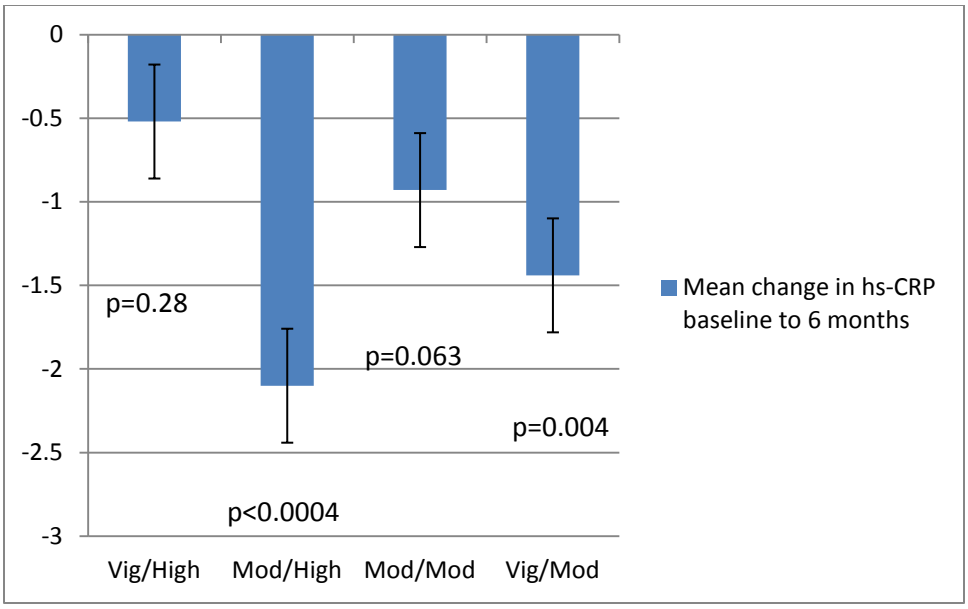


Figure 3: Mean change in hs-CRP Baseline to 6 Months

Table 3: Change in hs-CRP Values from Baseline to 6 Months

Treatment Group	hs-CRP (mg/L) Baseline	hs-CRP (mg/L) 6 Months	Change in hs-CRP Baseline to 6 months	Time Effect		Treatment Effect		Time X Treatment Effect	
	Mean± Standard Deviation	Mean ± Standard Deviation	Mean ± Standard Deviation	F value	P-value	F value	P-Value	F value	P-value
High/Vig (N=49)	5.54±4.98	5.02±4.61	0.52±2.38	25.553	0.0004	0.216	0.885	1.905	0.13
High/Mod (N=42)	5.85±6.39	3.75±5.36	2.10±4.86						
Mod/Mod (N=45)	5.16±4.25	4.23±3.9	0.93±2.54						
Mod/Vig (N=46)	5.37±3.85	3.93±2.65	1.44±3.12						
Total (N=182)	5.47±4.89	4.26±4.22							

Table 4: Interaction Between Change in hs-CRP, PAEE, & Intensity of Exercise

Outcome Variable	HIGH/VIG (N=49) (Mean±SD)	HIGH/MOD (N=42) (Mean±SD)	MOD/MOD (N=45) (Mean±SD)	MOD/VIG (N=46) (Mean±SD)	Time Effect (BL to 6M)		Time X PAEE		Time X Intensity		Time, PAEE& Intensity	
					F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
hs-CRP baseline	5.54±4.98	5.85±6.39	5.16±4.25	5.37±3.85	5.553	0004	.65	80	.188	28	.525	035
hs-CRP 6 mos.	5.02±4.61	3.75±5.36	4.23±3.9	3.93±2.65								
Change from baseline	-0.52±2.38	-2.10±4.86	-0.93±2.54	-1.44±3.12								
p-value for the change from baseline	0.28	<0.0004	0.063	0.004								

4.3 INFLUENCE OF CHANGE IN WEIGHT ON THE EFFECT OF THE EXERCISE INTERVENTION ON CHANGE IN CRP

The mean percent change in weight for the subjects from baseline to six months is illustrated in Figure 4. There was a significant decrease in weight from baseline to 6 months ($p<0.0004$). There was no statistically significant differences between the four different treatment groups for percent weight change ($p=0.16$).

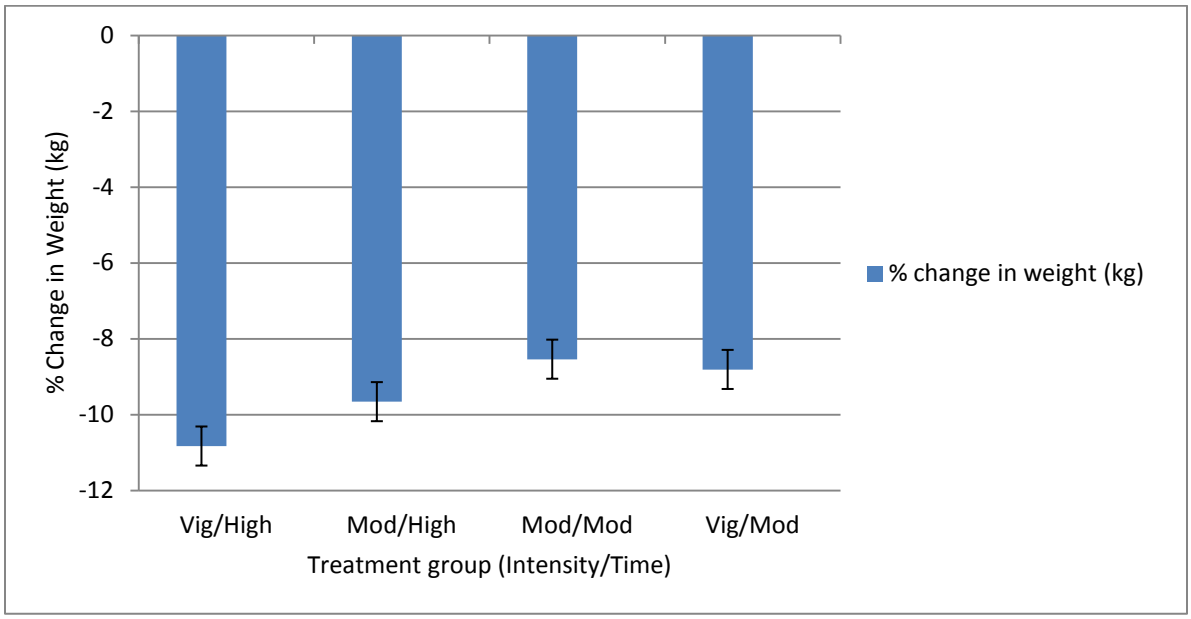


Figure 4: Mean % Change in Weight from Baseline to 6 Months

An analysis of covariance was completed to examine if the change in weight influenced the effect of the exercise intervention on change in hs-CRP. When controlling for weight change

the reduction in hs-CRP remained statistically significant (p=0.039). Moreover, after controlling for weight change, the three-way interaction (time x prescribed energy expenditure x exercise intensity) remained statistically significant (p=0.033) (Table 5).

Table 5: Effect of Exercise on hs-CRP Controlling for Change in Body Weight

Outcome Variable	HIGH/VIG (N=49) (Mean±SD)	HIGH/MOD (N=42) (Mean±SD)	MOD/MOD (N=45) (Mean±SD)	MOD/VIG (N=46) (Mean±SD)	Time Effect (BL to 6M)		Time X PAEE		Time X Intensity		Time, PAEE& Intensity	
					F value	P value	F value	P value	F value	P value	F value	P value
hs-CRP baseline	5.54±4.98	5.85±6.39	5.16±4.25	5.37±3.85	.338	039	037	85	.262	26	.609	033
hs-CRP 6 mos.	5.02±4.61	3.75±5.36	4.23±3.9	3.93±2.65								

4.4 INFLUENCE OF CHANGE IN PERCENT BODY FAT OR CHANGE IN BODY FAT DISTRIBUTION ON THE EFFECT OF THE EXERCISE INTERVENTION ON CHANGE IN CRP

The change in percent body fat is illustrated in Figure 5. There was a significant decrease in percent body fat from baseline to 6 months ($p<0.0004$); however, there was no significant difference between the groups for the change in percent body fat ($p=0.303$). There was a significant decrease in waist circumference, ($p<0.0004$) (see Figure 6), and the reduction in waist circumference was significantly different between the intervention groups ($p=0.02$). A Scheffe post-hoc analysis showed a significantly greater reduction in waist circumference in HIGH/MOD compared to MOD/MOD ($p=0.048$) (see Figure 6).

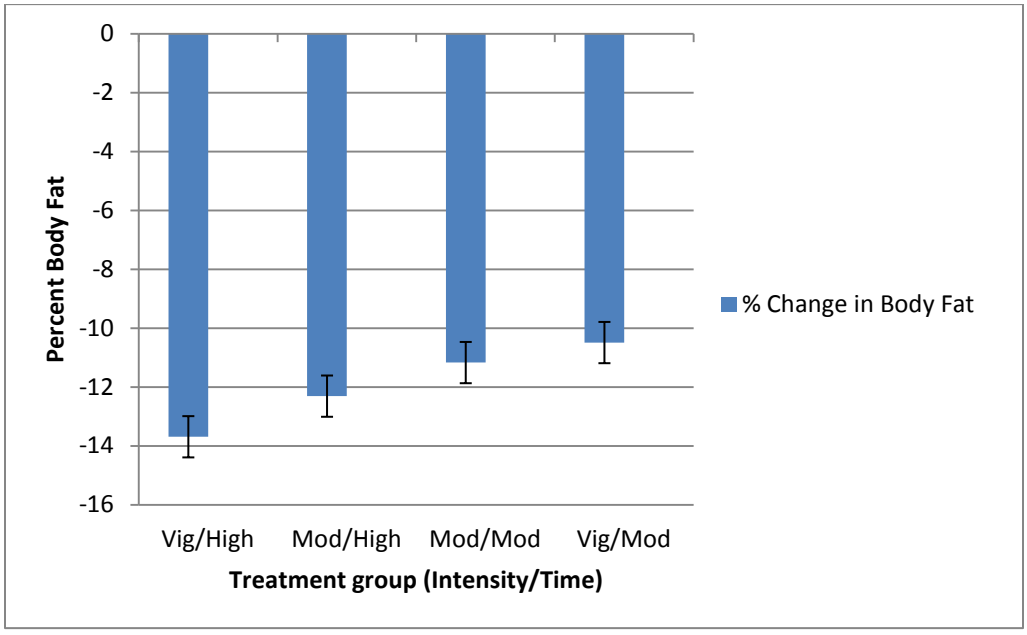
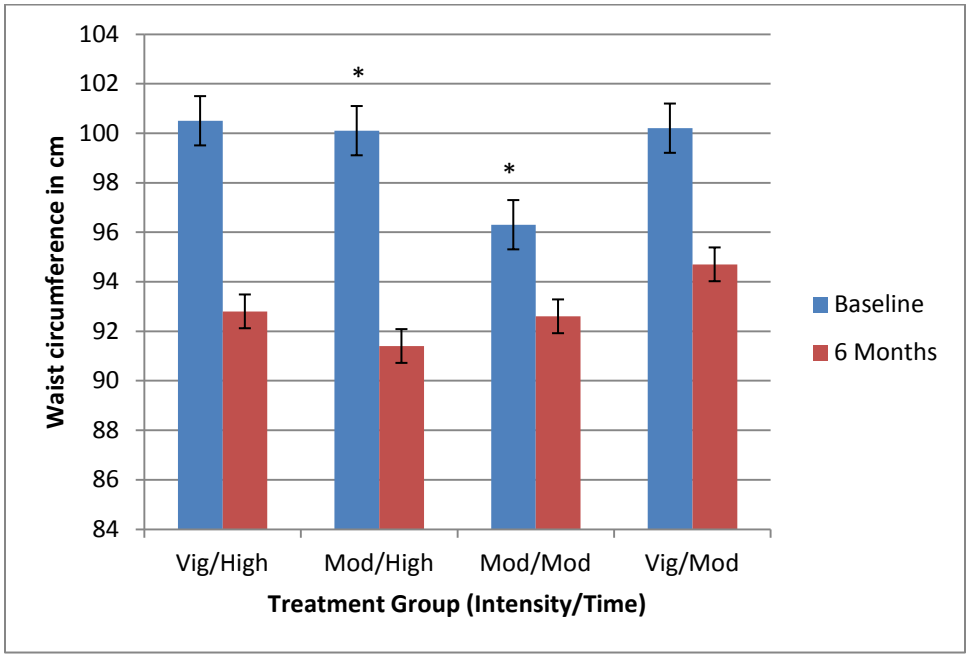


Figure 5: Mean % Change in Body Fat from Baseline to 6 Months



* p=0.048

Figure 6: Mean Change in Waist Circumference Baseline to 6 Months

An analysis of covariance was completed to examine if either a change in body fat or a change in fat distribution influenced the effect on change in hs-CRP. When controlling for body fat and a change in body fat distribution, the reduction in hs-CRP remained statistically significant ($p=0.018$ and $p<0.0004$ respectively). Moreover, the 3-way interaction (time x prescribed energy expenditure x exercise intensity) for change in hs-CRP remained statistically significant (see Table 6 and Table 7).

Table 6: Effect of Exercise on hs-CRP Controlling for Change in Percent Body Fat

Outcome Variable	HIGH/VIG (N=49) Mean±SD	HIGH/MOD (N=42) Mean±SD	MOD/MOD (N=45) Mean±SD	MOD/VIG (N=46) Mean±SD	Time Effect (BL to 6M)		Time X PAEE		Time X Intensity		Time, PAEE & Intensity	
					F value	P value	F value	P value	F value	P value	F value	P value
hs-CRP baseline	5.54±4.98	5.85±6.39	5.16±4.25	5.37±3.85	5.682	0.02	0.001	0.66	0.870	0.352	3.923	0.049
hs-CRP 6 mos.	5.02±4.61	3.75±5.36	4.23±3.9	3.93±2.65								

Table 7: Effect of Exercise on hs-CRP Controlling for Change in Body Fat Distribution (Waist Circumference)

Outcome Variable	HIGH/VIG(N=49) Mean±SD	HIGH/MOD (N=42) Mean±SD	MOD/MOD (N=45) Mean±SD	MOD/VIG (N=46) Mean±SD	Time Effect (BL to 6M)		Time X PAEE		Time X Intensity		Time, PAEE & Intensity	
					F value	P value	F value	P value	F value	P value	F value	P value
hs-CRP baseline	5.54±4.98	5.85±6.39	5.16±4.25	5.37±3.85	13.651	0.0004	.25	.86	.206	.28	.315	.039
hs-CRP 6 mos.	5.02±4.61	3.75±5.36	4.23±3.9	3.93±2.65								

4.5 CORRELATIONS BETWEEN SELF-REPORTED PHYSICAL ACTIVITY AND CHANGE IN HS-CRP

Self-reported physical activity was not correlated with change in hs-CRP. There was no influence from the change in body weight, percent body fat, or waist circumference on the association between hs-CRP and self-reported physical activity (Table 8). Similar results were found when only those subjects with a baseline hs-CRP >3.0 mg/L were examined (Table 9). High sensitivity CRP at six months was correlated with weight, BMI, % body fat and fat distribution (waist circumference). There was no correlation with physical activity or when controlling for body weight, BMI, percent body fatness, and waist circumference (Table 10).

Table 8: Pearson Correlation Coefficients Between 6 month Change in hs-CRP and Change in Self-Reported Physical Activity, Weight, Body Fatness, and Waist Circumference

	Change in hs-CRP	P-Value
Change in weight (kg)	r = 0.023	0.76
Change in BMI (kg/m^2)	r =0.038	0.61
Change in % body fat	r =0.021	0.783
Change in waist circumference (cm)	r = 0.044	0.56
Change in physical activity (kcal/week)	r = -0.007	0.93
Change in PAEE controlling for change in % body fat	r = -0.003	0.97
Change in PAEE controlling for change in fat distribution	r = -0.010	0.90
Change in PAEE controlling for change in weight	r = -0.010	0.89

Table 9: Pearson Correlation Coefficients Between 6 Month Change in hs-CRP and Change in Self-Reported Physical Activity, Weight, Body Fatness, Waist Circumference for Individuals with CRP >3.0 mg/L (N=114)

	Change in hs-CRP	P-Value
Change in weight (kg)	-0.044	0.640
Change in BMI (kg/m^2)	-0.054	0.569
Change in % body fat	-0.075	0.429
Change in waist circumference (cm)	0.057	0.549
Change in physical activity (kcal/week)	-0.003	0.979
Change in PAEE controlling for change in % body fat	-0.004	0.966
Change in PAEE controlling for change in fat distribution	-0.004	0.968
Change in PAEE controlling for change in weight	-0.008	0.936

Table 10: Pearson Correlation Coefficient Between hs-CRP and Self-Reported Physical Activity, Weight, Body Fatness, and Waist Circumference

	hs-CRP measured at 6 months	P-Value
Weight (kg) at 6 months	r = 0.293	p<0.0004
BMI (kg/m^2) at 6 months	r =0.416	p<0.0004
% Body Fat at 6 months	r =0.417	p<0.0004
Waist circumference at 6 months	r = 0.304	p<0.0004
Physical Activity (kcal/week) at 6 months	r = -0.075	0.31
Physical Activity at 6 months controlling for weight (kg) at 6 months	r = -0.030	0.69
Physical Activity at 6 months controlling for BMI at 6 months	r = -0.001	0.97
Physical Activity at 6 months controlling for % body fat at 6 months	r = -.035	0.65
Physical Activity at 6 months controlling for waist circumference at 6 months	r = 0.001	0.99

5.0 DISCUSSION

This study was a secondary analysis of existing data to examine the influence of physical activity, when included within a comprehensive behavioral weight loss program, on change in hs-CRP in overweight and obese women over an intervention period of 6 months. The parent study was a randomized trial that used a combination of exercise and reduced caloric intake to promote weight loss. The exercise intervention included a program based on intensity (moderate or vigorous) and prescribed energy expenditure (either 1000 kcal or 2000 kcal/week). The women were also instructed to reduce their caloric intake and dietary fat intake.

Obesity is associated with increased levels of pro-inflammatory markers such as IL-6 and TNF-α[27-28] and a decrease in the anti-inflammatory cytokine, adiponectin[29] resulting in a pro-inflammatory state. C-reactive protein is an acute phase protein produced by the liver in response to the inflammatory marker, IL-6[69] and may be associated with atherosclerotic plaque formation[70]. Thus, obesity is considered a risk factor for CHD[6-10] and is associated with an increase in morbidity and mortality[11].

A significant body of literature examining weight loss and exercise in relation to decreasing CRP levels exists[32-40]. Those existing studies did not examine the effect of various intensities of exercise or the effect of varying the amount of energy expended on CRP levels.

This study examined whether altering the prescribed dose of exercise in combination with an energy restricted diet resulted in a greater decrease in CRP levels.

Results from this study indicated that hs-CRP decreased over the six month intervention period (see Table 3), with weight loss induced by the combination of increased energy expenditure from exercise and a decrease in energy intake. Selvin et al.[92] completed a review of the literature to examine the association between change in weight and change in CRP. Results were favorable suggesting that weight loss may be an effective non-pharmacologic strategy for decreasing CRP levels. Selvin and colleagues found on average that the largest change in weight was associated with the greatest decrease in CRP levels[92]. In contrast, the current study did not show a significant association with magnitude of change in weight, BMI, percent body fat, or waist circumference and change in hs-CRP (See Table 8). However, the current study did show a significant association between absolute weight at 6 months and hs-CRP at 6 months (see Table 10), suggesting that excess body weight may contribute to higher levels of hs-CRP, which may increase risk for cardiovascular disease.

Esposito[38] et al. completed a single blind randomized trial examining the effects of a low-energy Mediterranean-style diet versus a control group. One hundred and twenty pre-menopausal women age 20-46 years without a history of diabetes, hypertension, or hyperlipidemia participated in the study. After two years, BMI decreased more in the intervention group with a decrease in mean weight of 14 kg and a decrease in CRP levels of 1.1 mg/L. The magnitude of the decrease in CRP was correlated with the magnitude of the decrease in BMI. These findings are in contrast to data from the current study that did not show a significant association between the magnitudes of weight loss or loss of body fat and the change in hs-CRP (see Table 8).

Giannopoulou et al.[39] randomized 33 obese sedentary women with Type 2 diabetes to 1 of 3 groups: diet, exercise, or combined diet and exercise group. Weight loss was 4.6 ± 1.4 kg weight loss (5.2% decrease in body fat), 1.7± 0.8 kg (4.7% decrease in body fat), and 5.4 ± 1.3kg (5.4% decrease in body fat) in these three intervention group, respectively. There was also a significant decrease in CRP levels (approximately 15%) in all three groups with no significant differences between the groups[39]. Thus, these results do not appear to show an association between the magnitude of weight loss and change in CRP, which is similar to the results of the current study that also shown no significant association between the magnitude of weight loss and change in hs-CRP (see Table 8). In contrast to the findings of Giannopoulou et al.[39], Nicklas[34] completed a randomized control trial consisting of control group, diet only group, and diet and exercise (combination aerobic exercise and resistance training) group and only reported a significant decrease in CRP at 18 months with a diet induced-weight loss intervention that resulted in a decrease in weight from baseline of 5.7%. The weight loss of 4.4% resulting from diet plus exercise and the weight loss of a 2.6% decrease resulting from exercise alone did not result in a significant reduction in CRP from baseline. These differences in findings between studies warrant further investigation.

This study also showed that when coupled with a reduction in energy intake to induce weight loss, there may be a differential effect of exercise on hs-CRP that is dependent on the intensity and volume of the exercise performed (see Table 4). We found a statistically significant decrease of 2.10 mg/L, in the group of women who were prescribed exercise at a moderate intensity and progressed to 2000 kcal/week, and the group prescribed exercise at a vigorous intensity and progressed to 1000 kcal/week (-1.44 mg/L). The smallest reduction in hs-CRP was observed in the group prescribed to exercise at a vigorous intensity and to expended

2000 kcal/week, (decrease of 0.52 mg/L), and the moderate intensity/ moderate energy expenditure had a 0.93 mg/L reduction in hs-CRP. However, based on correlational analysis, this study did not show a significant association between change in physical activity from baseline to 6 months and change in hs-CRP (see Table 8). These results are not consistent with cross-sectional studies showing that higher levels of physical activity (>180 min/week[35] and expending > 7kcal/min[103]) were associated with lower levels of CRP.

Results from prior studies have shown conflicting results for the effect of exercise on change in CRP. Bassuk and Manson found that CRP levels were decreased in both men and women who participated in moderate intensity exercise[33]. Milani et al.[41], Goldhammer et al.[42], and Caulin-Glaser et al.[43] also demonstrated a decrease in CRP levels in their cardiac rehabilitation patient population. Hambrecht[85] and colleagues completed a randomized trial in stable CAD patients and demonstrated a decrease in CRP levels. These studies showed that increasing exercise during a cardiac rehabilitation program is successful in decreasing levels of CRP.

Campbell et al.[87] and Church et al.[45] did not find significant changes in CRP in healthy individuals participating in an aerobic exercise intervention. Both of these trials controlled for weight loss and individuals were instructed to maintain the same diet throughout the trial preventing weight loss from occurring. In contrast, Stewart et al.[46] completed a randomized control trial examining 3 different doses of aerobic exercise with energy expenditure of 4, 8, or 12 kcal/kg/week. There was no significant change in CRP among the exercise groups, but change in weight was significantly associated with change in CRP. The group with the greatest weight loss (5.9 ± 3 kg) had a significant decrease in CRP[46]. This suggests that for exercise to

result in significant reductions in CRP, the exercise may need to result in a significant decrease in body weight.

Unfortunately, the current study did not include a group that reduced weight only through a reduction in energy intake without exercise, or exercise groups that were not also prescribed a reduction in energy intake. Therefore, it is difficult to make direct comparison to these prior exercise studies to determine the magnitude of change in hs-CRP that resulted from exercise in the current study. However, examination of the association between change in physical activity and change in hs-CRP after controlling for either change in body weight or change in other measures of body composition showed no significant association between physical activity and hs-CRP in this current study.

The differences across studies with regard to the effect of exercise on CRP may be a result of the volume or intensity of exercise resulting in an increase in inflammation, as measured by CRP, or a blunting of a decrease in CRP as some have hypothesized with weight loss. For example, Taylor et al.[104] et al. found that CRP levels in trained athletes were approximately 13.9 mg/L, which is a level that is above acceptable clinical levels, (> 10 mg/L signifies an inflammatory state)[20]. The authors concluded that the elevated CRP may have been a result of the response to chronic strenuous exercise. Moreover, after competing in a 160 km triathlon, CRP levels were increased by 300% 24 hours after the race. Okita et al.[105] enrolled healthy Japanese women into a 2 month exercise program to promote weight loss. The exercise consisted of 80 minutes of a dance program completed twice per week followed by an additional 30-60 minutes of additional treadmill or cycling exercises at 60-80% maximum heart rate. This program resulted in a mean weight loss of 3 kg and a significant reduction in CRP (0.22 mg/L). However, the investigators reported that the decrease in CRP was not proportionally associated

with weight reduction with the quartile of participants with the largest weight reduction not having a significant decrease in CRP levels, whereas those with a moderate amount of weight loss showing a significant decreases in CRP[105]. The investigators hypothesized that the strenuous exercise may have blunted the decrease in CRP that was observed with weight loss. By comparison, in the current study, hs-CRP was not decreased significantly in the intervention group prescribed exercise at a more vigorous intensity (70-85% of maximal heart rate) and for a higher energy expenditure (2000 kcal/wk); however, it was decreased significantly in the group prescribed exercise at a more vigorous intensity while at a energy expenditure (1000 kcal/wk). This may suggest that it is not necessarily the intensity of the exercise that influences changes in hs-CRP with weight loss, but rather the influence of both intensity and duration. This observation warrants further investigation.

5.1 LIMITATION AND FUTURE DIRECTIONS

This study was a secondary analysis of a prior study with the focus of implementing various intensities and doses of exercise to promote weight loss. There are several limitations to this study that may have influenced the outcomes, which also provides insight into areas to consider for future research.

1. The sample was limited to the subjects who provided serum samples at baseline and at 6 months. Whether results would be different with a longer intervention

period is unclear. Therefore, future studies should consider extending beyond a period of 6 months.

2. This study did not include a no treatment control group, a diet only comparison group, or exercise groups that did not include a change in prescribed energy intake. Therefore, this study cannot determine the independent effect of energy restriction, energy expenditure from exercise, or weight loss on the observed changes in hs-CRP. Future studies should consider the optimal control and comparison groups to allow for a more thorough understand of weight loss, energy intake, and energy expenditure on hs-CRP.

3. There are a variety of over the counter and prescription medications that could influence hs-CRP. Data on medication usage is unavailable for this study. Thus, future studies should collected data on medication usage by the participants to control for any effect that these may have on change in hs-CRP.

4. This study was limited to women, which limits the generalizability of these results to men. Future studies should consider designs with sufficient samples of men and women to examine gender comparisons for changes in hs-CRP results from weight loss, exercise, and dietary changes.

5. The sample for this study consisted of individuals who primarily self-identified as Caucasian. This limits the generalizability of these results to other race/ethnic groups, which should be examined in future studies.

6. The mean age of the participants in this study was 37.5 ± 5.6 years. Whether similar results would be observed in individuals who are younger or older is unclear and should be the focus of future research.

7. The measures of physical activity were based on self-report using a questionnaire, which may introduce error into this assessment. Future studies should include objective assessment of physical activity.
8. Participants were instructed to refrain from any physical activity 12 hours prior to blood collection. Aside from self-report, this study was unable to objectively confirm that participants were compliant with this directive. There is evidence that shows that both the intensity and duration of exercise results in an increased production of IL-6 [106-109]. Future studies should recommend a longer time period for abstention of exercise prior to blood draws.
9. The study examined hs-CRP as a marker of inflammation. It is important to note that there are other markers of inflammation such as IL-6 and TNF-α that may respond differently to exercise, dietary change, or weight loss. Future examination of additional markers of inflammation in future studies is recommended.

5.2 CONCLUSIONS

The results of this study showed that hs-CRP was reduced in overweight and obese women in response to a 6 month weight loss intervention that included a prescribed reduction in energy intake and a prescribed increase in exercise. Moreover, this study found that varying doses or intensities of exercise prescribed within the context of this weight loss intervention differentially influenced the observed reduction in hs-CRP. Whether there is an optimal dose or intensity of exercise that should be prescribed to elicit weight loss while also reducing markers of

inflammation in overweight and obese adults cannot be determined from this study. Moreover, the added influence of exercise when combined with a reduction in energy intake on hs-CRP cannot be determine from this study, and it appears that there are mixed results published in the literature on the influence of exercise on CRP. Thus, further research is needed to identify the effect that exercise may have on markers of inflammation, whether there is an optimal dose or intensity of exercise that will precipitate changes in these markers of inflammation, and the combine and independent influence of exercise and weight loss on markers of inflammation in overweight and obese adults.

BIBLIOGRAPHY

1. The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity. (2001).Available at http://www.surgeongeneral.gov/topics/obesity/calltoaction/fact_consequences.html . Accessibility verified September 27, 2009.

2. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA.* 2012; 307(5): 491-497.

3. Ogden DL, Carroll MD, Curtin, LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA.* 2006; 295(13): 1549-1555.

4. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA.* 2010; 303(3): 235-241.

5. Hu FB, Stampfer MJ, Manson JE, et al. Trends in the incidence of coronary heart disease and changes in diet and lifestyle in women. *The New England Journal of Medicine*. 2000; 343(8): 530-537.

6. Sowers JR. Obesity as a cardiovascular risk factor. *American Journal of Medicine.* 2003; 115(8A): 37S-41S.

7. Klein S, Burke L, Bray G, et al. Clinical implications of obesity with specific focus on cardiovascular disease. A statement for professionals from the American Heart Association council on nutrition, physical activity, and metabolism. Endorsed by the American college of cardiology foundation. *Circulation.* 2004; 110(18): 2952-2967.

8. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of with loss. An update of the 2007 American Heart Association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism. *Circulation.* 2006; 113(6): 898-918.

9. Poirier P, Martin J, Marceau P, Biron S, Marceau S. Impact of bariatric surgery on cardiac structure, function, and clinical manifestations in morbid obesity. *Expert Revue in Cardiovascular Therapy.* 2004; 2(2): 193-201.

10. Mosca L, Banka CL, Benjamin EJ, et al. Evidence-based guidelines for cardiovascular disease prevention in women: 2007 update. *Circulation.* 2007; 115(11): 1481-1501.

11. Kokkinos P, Myers J. Exercise and physical activity clinical outcomes and applications. *Circulation.* 2010; 122(16): 1637-1648.

12. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics – 2012 update a report from the American Heart Association. *Circulation.* 2012; 125(1):e2-e220.

13. Brochu M, Poehlman ET, Ades PA. Obesity, body fat distribution, and coronary artery disease. *Journal of Cardiopulmonary Rehabilitation.* 2000; 20(24):96-108.

14. Harris, TB, Launer, LJ, Madans J, Feldman JJ. Cohort study of effect of being overweight and change in weight on risk of coronary heart disease in old age. *British Medical Journal.* 1997; 314(7097): 1791-1794.

15. Thompson PD, Buchner D, Piña IL, et al. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. A statement from the council on clinical cardiology (subcommittee on exercise, rehabilitation, and prevention) and the council on nutrition, physical activity, and metabolism (subcommittee on physical activity). *Circulation.* 2003; 107(24): 3109-3116.

16. Stampfer MJ, Hu FB, Manson JE, Rimm, E.B, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. *New England Journal of Medicine.* 2000; 343(1): 16-22.

17. Manson JE, Hu FB, Rich-Edwards JW, et al. A prospective Study of walking as compared with vigorous exercise in the prevention of coronary heart disease. *The New England Journal of Medicine.* 1999;341(9): 650-658.

18. Hu FB, Stampfer MJ, Solomon C, et al. Physical activity and risk for cardiovascular events in diabetic women. *Annals of Internal Medicine.* 2001;134(2): 96-105.

19. Manson JE, Greenland P, LaCroix AZ, et.al. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. *New England Journal of Medicine.* 2002; 347(10): 716-725.

20. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease application to clinical and public health practice a statement for healthcare professionals from the centers for disease control and prevention and the American heart association. *Circulation.* 2003; 107(3): 499-511.

21. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine.* 2000; 342(12): 836-843.

22. Danesh J, Phil D, Wheeler J, et.al. C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. *New England Journal of Medicine*. 2004; 350(14):1387-97.

23. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes. Part I: introduction and cytokines. *Circulation.* 2006; 113(12): e72-e75.

24. Kritchevsky SB, Cesari M, Pahor M. Inflammatory markers and cardiovascular health in older adults. *Cardiovascular Research.* 2005; 66(2): 265-275.

25. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine.* 1999; 340(6): 448-454.

26. Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guidelines for assessment of cardiovascular risk in asymptomatic adults. *Journal of American College of Cardiology.* 2010; 56(25): e50-103.

27. Kopelman P. Health risks associated with overweight and obesity. *Obesity Reviews.* 2007; 8 (Suppl. 1): 13-17.

28. Cottam DR, Mattar SG, Barinas-Mitchell E, et al. The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. *Obesity Surgery.* 2004; 14(5): 589-600.

29. Vendrell J, Broch M, Vilarrasa N, Molina A, Gomez JM, Gutierrez C. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obesity research.* 2004; 12(6): 962-971.

30. Dubnov G, Brzezinski A, Berry EM. Weight control and the management of obesity after menopause: the role of physical activity. *Maturits.* 2003; 44(2): 89-101.

31. Welty FK, Stuart E, O'Meara M, Huddleston J. Effect of addition of exercise to therapeutic lifestyle changes diet in enabling women and men with coronary heart disease to reach adult treatment panel III Low-density lipoprotein cholesterol goal without lowering high-density lipoprotein cholesterol. *The American Journal of Cardiology.* 2002; 89(10): 1201-1204.

32. Bassuk SS, Manson JE. Physical activity and cardiovascular disease prevention in women: how much is good enough? *Exercise and Sport Sciences Review.* 2003; 31(4): 176-181.

33. Bassuk SS, Manson JE. Physical activity and the prevention of cardiovascular disease. *Current Atherosclerosis Reports.* 2003; 5(4): 299-307.

34. Nicklas BJ, Ambrosius W, Messier SP, et al. Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *American Journal of Clinical Nutrition.* 2004; 79(4): 544-51.

35. Colbert LH, Visser M, Simonsick EM, et al. Physical activity, exercise, and inflammatory markers in older adults: findings from the health, aging, and body composition study.

36. Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. *Arteriosclerosis Thrombosis & Vascular Biology.* 2003; 23(6): 1042-1047.

37. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces c-reactive protein levels in obese postmenopausal women. *Circulation.* 2002;105(5): 564-69.

38. Esposito K, Pontillo A, Di Palo C et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women a randomized trial. *JAMA.* 2003; 289(14): 1799-1804.

39. Giannopoulou I, Fernall B, Carhart R et al. Effects of diet and/or exercise on the adipocytokine and inflammatory cytokine levels of postmenopausal women with type 2 diabetes. *Metabolic Clinical & Experimental.* 2005; 54(7): 866-875.

40. Belalcazar LM, Reboussin DM, Haffner SM et al. A 1-year lifestyle intervention for weight loss in individuals with type 2 diabetes reduces high c-reactive protein levels and identifies metabolic predictors of change. *Diabetes Care.* 2010; 33(11): 2297-2303.

41. Milani VM, Lavie CJ, Mehra MR. Reduction in c-reactive protein through cardiac rehabilitation and exercise training. *Journal of the American College of Cardiology.* 2004; 43(6): 1056-1061.

42. Goldhammer E, Tanchilevitch A, Maor I, Beniamini Y, Rosenchein U, Sagiv M. Exercise training modulates cytokines activity in coronary heart disease patients. *International Journal of Cardiology.* 2005; 100(1): 93-99.

43. Caulin-Glaser T, Falko J, Hindman L, La Londe M, Snow R. Cardiac rehabilitation is associated with an improvement in c-reactive protein levels in both men and women with cardiovascular disease. *Journal of Cardiopulmonary Rehabilitation.* 2005; 25(6): 332-336.

44. Bo S, Ciccone G, Guidi S, Gambino R, Durazzo M, Gentile L. Diet or exercise: what is more effective in preventing or reducing metabolic alterations? *European Journal of Endocrinology.* 2008; 159(6): 685-691.

45. Church TS, Earnest CP, Thompson AM, et al. Exercise without weight loss doe not reduce c-reactive protein: the inflame study. *Medicine & Science in Sports & Exercise.* 2010; 42(4): 708-716.

46. Stewart LK, Earnest CP, Blair SN, Church TS. Effects of different doses of physical activity on c-reactive protein among women. *Medicine & Science in Sports & Exercise.* 2010; 42(4): 701-707.

47. Donnelly JE, Blair, SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine & Science in Sports & Exercise.* 2009; 41(2): 459-471.

48. Jakicic JM, Marcus BH, Gallagher KI, Napolitano M, Lang W. Effect of exercise duration and intensity on weight loss in overweight, sedentary women a randomized trial. *JAMA.* 2003; 290(10): 1323-1330.

49. Jakicic JM, Marcus BH, Lang W, Janney C. Effect of exercise on 24-month weight loss maintenance in overweight women. *Archives of Internal Medicine.* 2008; 168(14): 1550-1559.

50. Sherry, B, Blanck, HM, Galuska DA, Pan L, Dietz WH. Vital signs: specific obesity relevance among adults- United States, 2009. *MMWR.* 2010; 59(August 3).

51. Flegal, KM, Carroll, MD, Kuczmarski, RJ, Johnson, CL. Overweight and obesity in the United States; prevalence and trends, 1960-1994. *International Journal of Obesity and Related Metabolic Disorders.* 1998; *22*(1): 39-47.

52. Finkelstein, EA, Trogdon, JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Affairs.* 2009; 28(5): w822-w831.

53. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *British Journal of Nutrition.* 2004; 92(3): 347-355.

54. Berg AH Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circulation Research.* 2005; 96(9): 939-949.

55. Das UN. Is obesity an inflammatory condition? *Nutrition.* 2001; 17(11/12): 953-966.

56. Bastard J, Jardel C, Delattre J, Hainque B, Bruckert E, Oberlin F. Evidence for a link between adipose tissue interleukin-6 content and serum c-reactive protein concentrations in obese subjects. *Circulation.* 1999; 99(16): 2219-2222.

57. Visser M, Bouter LM, McQuillan GM, Werner MH, Harris TB. Elevated c-reactive protein levels in overweight and obese adults. *JAMA.* 1999; 282(22): 2131-2135.

58. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in Vivo. *Journal of clinical Endocrinology & Metabolism.* 1997; 82(12): 4196-4200.

59. Yudkin JS, Stehouwer A, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arteriosclerosis Thrombosis & Vascular Biology.* 1999; 19(4): 972-978.

60. Cottam DR, Schaefer PA, Shaftan GW, Velcu L, Anguss LDG. Effect of surgically-induced weight loss on leukocyte indicators of chronic inflammation in morbid obesity. *Obesity Surgery.* 2002; 12(3): 335-342.

61. Lloyd-Jones D, Adams R, Carnethon M, et al. Heart disease and stroke statistics 2009 update. A report from the American Heart Association statistics committee and stroke statistics subcommittee. *Circulation.* 2009; 119(3), e21-e181.

62. Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics-2006 update. A report from the American heart association statistics committee and stroke statistics subcommittee. *Circulation.* 2006; 113(6): e85-e151.

63. Ford ES, Capewell S. Coronary heart disease mortality among young adults in the U.S. from 1980 through 2002: concealed leveling of mortality rates. *Journal of American College of Cardiology.* 2007; 50(22): 2128-2132.

64. Ford ES, Ajani UA, Croft JB, et al. Explaining the decrease in U.S. deaths from coronary heart disease, 1980-2000. *New England Journal of Medicine.* 2007; 356(23): 2388-2398.

65. Schroetter SA, Peck SD. Women's risk of heart disease: promoting awareness and prevention - a primary care approach. *Medsurg Nursing.* 2008;17(2):107-113.

66. Ross R. Atherosclerosis: an inflammatory disease. *New England Journal of Medicine.* 1999; 340(2): 115-126.

67. Libby P, Ridker PM. Novel inflammatory markers of coronary risk. Theory versus practice. *Circulation* . 1999; 100(11): 1148-1150.

68. Trask BC, Rote NS, Huether SE. Innate immunity: inflammation. In KL McCance, SE Huether eds. *Pathophysiology the Biologic Basis for Disease in Adults and Children* 5th ed. St. Louis: Elsevier Mosby; 2006: 175-206.

69. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes part II: acute-phase reactants and biomarkers of endothelial cell activation. *Circulation.* 2006; 113(7): e152-e155.

70. Adams DH, Shaw S. Leukocyte-endothelial interactions and regulation of leukocyte migration. *Lancet.* 1994; 343(8901): 831-836.

71. Du Clos T. Function of C-reactive Protein. *Annals of Medicine*. 2000; 32(4): 274-278.

72. Lavie CJ, Milani RV. Effects of cardiac rehabilitation, exercise training, and weight reduction on exercise capacity, coronary risk factors, behavioral characteristics, and quality of life in obese coronary patients. *The American Journal of Cardiology*. 1997;79(4): 397-401.

73. U. S. Department of Health and Human Services. A report of the Surgeon General: Physical activity and health: The link between physical activity and morbidity and mortality. Washington, D. C.: Author. 1998; Available at: http://www.cdc.gov/nccdphp/sgr/pdf/mm.pdf . Accessibility verified July 12, 2006.

74. Trends in leisure-time physical inactivity by age, sex, & race/ethnicity --- United States, 1994-2004. *MMWR*. 2005; *54*(39): 991-994.

75. Shay CM, Ning H, Allen NB, et al. Status of Cardiovascular Health in US Adults Prevalence estimates from the National Health and Nutrition Examination Surveys (NHANES) 2003-2008. *Circulation.* 2012; 125(1): 45-56.

76. Blair SN, Brodney S. Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues. *Medicine & Science in Sports & Exercise.* 1999; 31(Suppl. 1): S646-662.

77. Blair SN, Kohl III HW, Barlow CE, Paffenbarger Jr. RS, Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality: a prospective study of healthy and unhealthy men. *JAMA.* 1995; 273(14): 1093-1098.

78. Edwards, T. Lifestyle influences and coronary artery disease prevention. *Physician Assistant.* 2001; 25(8): 19-21,24-27.

79. Pate RR, Pratt M, Blair SN, et al. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA.* 1995; 273(5): 402-407.

80. Brown WJ, Burton NW, Rowan PJ. Updating the evidence on physical activity & health in women. *American Journal of Preventative Medicine.* 2007; 33(5): 404-411.

81. Haskell WL, Lee IM, Pate RR, et al. Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation.* 2007;116(9):1081-1093.

82. Yu S, Yarnell JWG, Sweetnam PM, Murray L. What level of physical activity protects against premature cardiovascular death? The Caerphilly Study. *Heart.* 2003; 89(5): 502-506.

83. Lee IM, Rexrode KM, Cook NR, Manson JE, Buring JE. Physical activity and coronary heart disease in women. Is "no pain, no gain" passé? *JAMA.* 2001; 285(11): 1447-1454.

84. Gielen S, Walther C, Schuler G, & Hambrecht R. (2005). Anti-inflammatory effects of physical exercise. A new mechanism to explain the benefits of cardiac rehabilitation? *Journal of Cardiopulmonary Rehabilitation, 25,* 339-342.

85. Hambrecht R, Walther C, Mobius-Winkler S, et al. Percutaneous coronary angioplasty compared with exercise training in patients with stable coronary artery disease: a randomized trial. *Circulation.* 2004; 109(11): 1371-1378.

86. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *Journal of American College of Cardiology.* 2005; 45(10): 1563-1569.

87. Campbell KL, Campbell PT, Ulrich CM, et al. No reduction in c-reactive protein following a 12-month randomized controlled trial of exercise in men and women. *Cancer Epidemiology Biomarkers Prevention.* 2008; 17(7): 1714-1718.

88. Arsenault BJ, Côté M, Cartier A, et al. Effect of exercise training on cardiometabolic risk markers among sedentary, but metabolically healthy overweight or obese post-menopausal women with elevated blood pressure. A*therosclerosis.* 2009; 207(2): 530-533.

89. Lakka TA, Lakka H, Rankinen T, et al. Effect of exercise training on plasma levels of c-reactive protein in healthy adults: the HERITAGE family study. *European Heart Journal.* 2005; 26(19): 2018-2025.

90. Campbell PT, Campbell KL, Wener MH, et al. A yearlong exercise intervention decreases CRP among obese postmenopausal women. *Medicine & Science in Sports & Exercise.* 2009; 41(8): 1533-1539.

91. Lavoie ME, Rabasa-Lhoret R, Doucet É, et al. Association between physical activity energy expenditure and inflammatory markers in sedentary overweight and obese women. *International Journal of Obesity.* 2010; 34(9): 1387-1395.

92. Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on c-reactive protein a systematic review. *Archives of Internal Medicine.* 2007; 167(1): 31-39.

93. Ramalho R, Guimarães C, Gil C, Neves C, Guimarães JT, Delgado L. Morbid obesity and inflammation: a prospective study after adjustable gastric banding surgery. *Obesity Surgery.* 2009; 19(7): 915-920.

94. Solá E, Jover A, Lópe-Ruiz A, et al. Parameters of inflammation in morbid obesity: lack of effect of moderate weight loss. *Obesity Surgery.* 2009; 19(5): 571-576.

95. Belza A, Toubro S, Stender S, Astrup A. Effect of diet-induced energy deficit and body fat reduction on high-sensitive CRP and other inflammatory markers in obese subjects. *International Journal of Obesity.* 2009; 33(4): 456-464.

96. Christiansen T, Paulsen SK, Bruun JM, Pedersen ST, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12 week randomized intervention study. *American Journal of Physiology, Endocrinology & Metabolism.* 2010; 298(4): E824-E831.

97. Belalcazar LM, Reboussin DM, Haffner SM, et al. A 1-year lifestyle intervention for weight loss in individuals with type 2 diabetes reduces high c-reactive protein levels and identifies metabolic predictors of change. *Diabetes Care.* 2010; *33*(11): 2297-2303.

98. Segal KR, Gutin B, Presta E, Wang J, Van Itallie TB. Estimation of human body composition by electrical impedance methods: a comparative study. *Journal of Applied Physiology.* 1985;58(5):1565-1571.

99. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. *Journal of Clinical Epidemiology.* 1990; 43(12): 1327-1335.

100. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires. *American Journal of Epidemiology.* 2001; 154(12): 1089-1099.

101. Paffenbarger RS, Hyde RT, Wing AL, Hsieh CC. Physical activity, all-cause mortality and longevity of college alumni. *New England Journal of Medicine.* 1986; 314(10): 605-613.

102. Pereira MA, Fitzergerald SJ, Gregg EW, et al. A collection of physical activity questionnaires for health related research. *Medicine & Science Sports & Exercise.* 1997;29(Supple 6): S1-S205.

103. Panagiotakos DB, Pitsavos C, Chrysohoou C, et al. The associations between leisure-time physical activity and inflammatory and coagulation markers related to cardiovascular disease: the ATTICA Study. *Preventive Medicine.* 2005;40:432-437.

104. Taylor C, Rogers G, Goodman C et al. hematologic iron-related & acute phase protein responses to sustained strenuous exercise. *Journal of Applied physiology.* 1987: 62; 464-469.

105. Okita, K. Nishijimi, H, Murakami, T. et al. Can exercise training with weight loss lower CRP levels. *Arteriosclerosis Thrombosis and Vascular Biology*. 2004:24(10); 1868-1873.

106. Pederson BK, Steensberg A, Schjerling P. Muscle-derived interleukin-6: possible biological effects. *Journal of Physiology*. 2001:536(2); 329-337.

107. Pederson BK, Fischer CP. Physiological roles of muscle-derived interleukin-6 in response to exercise. *Current Opinion in Clinical Nutrition and Metabolic Care.* 2007:10; 265-271.

108. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB Journal*. 2002:16; 1335-1347.

109. Mendham AE, Donges CE, Liberts EA, Duffield R. Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *European Journal of Applied Physiology.* 2011:111; 1035-1045.