Spring 2011

Effect of diet induced weight loss and exercise on hsCReactive protein in sedentary postmenopausal women

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postmenopausal women

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A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Science

Kinesiology

May 2011

Acknowledgements

I would like to thank Dr. Judith Flohr for accepting the position of chairperson for my thesis research. Your support, guidance, and experience throughout the thesis process were extremely valuable.

I would also like to thank Dr. Christopher Womack and Dr. Jeremy Akers for accepting positions on my thesis committee. Your assistance and feedback throughout the duration of my thesis was greatly appreciated.

I would like to thank Christine Nicewonger and Leslie Redmond for their assistance with data collection. Finally, I would also like to thank Ryann Diehl and Caitlin Payne for their assistance with inputting dietary records into Nutritionist Pro software. Your knowledge, professional assistance, and manual assistance have allowed me to complete my thesis timely manner.

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Abstract

Cardiovascular disease (CVD) risk factors, such as increased body fat, dyslipidemia, and high sensitivity C-reactive protein (CRP) levels are intensified in postmenopausal women. CRP has been reported as an independent indicator of risk for cardiovascular events. CRP is affected by multiple factors such as age, race, body mass index (BMI), and physical activity level. The purpose of this study was to compare the effects of an exercise intervention on traditional (BMI, percent body fat, waist circumference, physical activity level) and non-traditional (CRP) risk factors for cardiovascular disease to a dietary intervention program in sedentary, postmenopausal women. Twenty apparently healthy postmenopausal women were randomly assigned to a diet intervention group (DI) or an exercise intervention group (EXI) for a period of 9 weeks. DI participants reported once a week for a body weight measurement and EXI participants were asked to attend 3 exercise sessions per week that were 45-55 minute sessions (cardiovascular and resistance training exercises). There were no significant effects of time or treatment intervention, or a time x treatment interaction on CRP levels ($p=0.077$, $p<0.05$). However, there was a significant change (-4.45 kg) in body weight ($p=0.000$, $p<0.05$) and (-1.1 kg) change in fat free mass ($p=0.041$, $p<0.05$) in the DI group. Body weight, FFM, and waist circumference all decreased significantly in the DI group; however, decreases in these variables did not appear to precipitate significant changes in CRP. It is possible that a greater degree of weight loss (over a longer duration) may be necessary to decrease CRP levels. There was no significance between EXI and dependant variables, including CRP, also suggesting that greater intensity or longer intervention duration may be necessary to affect dependant variables through exercise.

Chapter 1

Introduction

Cardiovascular disease (CVD) is the major cause of death among the U.S. population.[8, 16,
33] With aging, the risk of CVD proportionally increases; however, women are affected by CVD
10 years after men.[25,33] Clinical diagnosis for CVD in women is more challenging than men
because women do not present typical signs of chest pain or ischemia. CVD death rates are 34%
higher in black women than white women due to the higher prevalence of CVD risk factors, such
as diabetes and obesity.[23,33] Risk factors for CVD in women are smoking (leading cause),
hypertension, dyslipidemia, diabetes mellitus, obesity, sedentary lifestyle, and menopause.[6, 25, 33,
44, 46] Hypertension, dyslipidemia, and obesity are risk factors for both men and women; 60% of
white women and 79% of black women over the age of 45 are classified as hypertensive (systolic
blood pressure $\geq$140 mm Hg or diastolic blood pressure $\geq$ 90 mm Hg).[33] Regarding increased
total cholesterol, 55% of women over 55 years old are classified as high ($\geq$ 240 mg/dL). Low
HDL level is a stronger CVD predictor for women than men; as, a 1% increase in HDL is
associated with 3-5% decrease in risk for women.[33, 46] Obesity, especially increased abdominal
adiposity, is a risk factor for CVD in women. The prevalence of obesity has increased over the
past decade and currently one out of three women is classified as obese. Obesity is a parallel
factor to sedentary lifestyle, and studies have shown that moderate physical activity reduces the
risk of CVD by 50% in women. Schenck[46] reported in 2009 that 30% of women are overweight
and 27% are classified as obese, and the risk for CVD in obese women increases 3-fold compared
to lean women.[20, 25, 33]

Postmenopausal women experience similar risk factors as men; however, certain CVD
risk factors are intensified in postmenopausal women.[44, 46] Redistribution of fat from gynoid to
android pattern, increased body fat, increased blood pressure, and dyslipidemia amplify CVD
risk.[4, 41] Menopause decreases the estrogen levels and increases testosterone levels in
postmenopausal women, negatively affecting cardiovascular functions and metabolism resulting

in an increase risk for CVD event, such as dyslipidemia resulting from increased testosterone.[41] A 2-fold increase for CVD occurs with low sex hormone binding globulin or high free androgen index value.[41]

In addition to the serum cholesterol risk factors, abnormal lipoprotein, C-reactive protein (CRP) and interleukin-6 levels are known risk factors for CVD in women.[3, 13, 15, 39, 46] Inflammation markers have been determined to have a significant role in the development of atherosclerosis and CVD, but the relationship between the inflammation process and CVD is complex.[4, 16, 22] In response to tissue inflammation, CRP is stimulated by interleukin-6, interleukin-1, and tumor neurosis factor.[22] This response occurs when activated immune cells in the plaque produce inflammatory cytokines, stimulating the production of substantial amounts of interleukin-6. Interleukin-6, in response, stimulates the production of large amounts of acute-phase reactants (C-reactive protein, serum amyloid A, and fibrinogen) in the liver. Plasma concentration of CRP can increase 100-fold after acute inflammatory stimulus.[5, 11, 42] According to Hansson,[16] cytokines have important biologic effects at all steps; however, their amplification at each step of the cascade makes the measurement of downstream mediators such as CRP particularly useful for clinical diagnosis.

CRP is an inflammatory marker that has been investigated regarding its influence on an individual's risk for CVD. Several investigators have suggested that CRP levels reflect the inflammation status, which is related to the severity of atherosclerosis.[5, 11, 22, 42] CRP is an independent indicator of risk for stroke, peripheral vascular disease, and vascular death among healthy men and women without any known vascular diseases and is a stronger predictor for CV events compared to LDL-C levels. [5, 11, 42, 43] Currently practitioners consider CRP value of less than 2 mg/L as healthy, and individuals who have CRP levels of 5mg/L or greater may be at an increased risk for a cardiac event within six months.[22, 26]

CRP is affected by multiple factors such as age, race, body mass index (BMI), and physical activity level.[1, 3, 4, 9, 13, 14, 17, 18, 22, 28, 49, 52, 53] CRP levels have been shown to increase with

age; therefore, BMI and physical activity level are needed to counteract the natural rise in CRP levels.[22, 28] CRP is highly correlated with BMI and several studies have reported a strong correlation between total fat mass and visceral fat and CRP.[17, 18, 23, 52] Heilbroon[17] and Williams[53] have reported that subjects with a BMI >30 kg/m^2 had elevated CRP levels and CRP levels were found to be 270% higher in individuals in the top quintile of BMI compared to those in the bottom quintile. In addition to BMI, a direct relationship between elevated CRP levels and abdominal fat distribution via waist circumference measurement has been documented.[17, 18, 52] A waist circumference greater than 88 centimeters can be associated with a higher BMI and higher risk for CVD due to increased visceral fat and increase in serum resistin.[18, 51] Finally, increased physical activity levels are related to decreased fat and lower BMI resulting in decreased CPR levels.[1, 3, 14, 29, 49]

A review of current literature indicates that there is an inverse relationship between physical activity and CRP levels. Specifically, investigators have observed the lowest CRP levels in individuals with the highest activity levels.[1,3,10,14] Physical activity is associated with a lower risk of subsequent CVD events; however, physical activity in young adulthood was not a predictor of CVD risk several decades later.[10] There is an inverse relationship between physical activity during middle age and CVD risk; therefore, it is necessary to maintain physical activity levels with aging to minimize a cardiovascular event. Along with an inverse relationship between physical activity level and CRP levels being reported, there is an inverse association between intensity and/or frequency of exercise with CRP levels. CRP levels decrease with more vigorous exercise or more frequent exercise.[1, 3, 49] Cross sectional studies used questionnaires to determine participants' physical activity frequency and intensity, and the 6-minute walk test, treadmill VO_{2max} test, and cycle ergometry VO_{2max} test were used to determine physical activity level.[1, 2, 3, 9, 14, 29, 49] Higher VO_{2max} measurements were associated with lower CRP levels.[3,29] The dose-response relationship utilizes frequency and/or intensity of physical activity to determine the decrease for CVD risk.[37]

Multiple studies have shown that with weight loss and exercise, CRP levels decrease in proportion to weight loss.[30, 38, 40, 45] Increased physical activity promotes decreased weight, body fat percentage, adipose tissue, and BMI resulting in decreased CRP levels.[45] Cross sectional studies used questionnaires to determine participants' physical activity frequency and intensity or participants' complete a submaximal treadmill test to determine VO_{2max}.[3, 31, 40] Intervention studies consisting of aerobic activity 3 days/week through group exercise classes, treadmill, or cycling for 30-60 minutes at 60% VO_{2max} or between 60-80% peak heart rate resulted in a decrease in body weight, percentage body fat, fat mass, fat free mass, visceral adipose tissue, and decrease in CRP levels was observed, however VO_{2max} did not change after weight loss.[30, 38, 45]

The consensus of currently published studies indicates that CRP will decrease as a result of weight loss that is achieved through dietary changes. Dietary interventions that have produced weight loss and decreased CRP values have been as short of one month and as long as 18 months in duration. The majority of diet interventions reviewed consisted of a hypocaloric diet for a period of 12 weeks with obese women as the study population.[10, 14, 35, 36, 49] Previous studies have solely investigated changes of CRP levels resulting from weight loss through dietary alterations or weight loss through exercise. It is unknown if exercise without weight loss alone has an effect on CRP levels in sedentary, postmenopausal women. The purpose of this study was to compare the effects of an exercise intervention versus a dietary intervention on traditional (BMI, percent body fat, waist circumference, physical activity level) and non-traditional (CRP) risk factors for cardiovascular disease in sedentary, post menopausal women.

Chapter II

Review of Literature

Increased CRP levels have been reported as an independent predictor for CVD.[54] Risk factors that affect CRP are age, waist circumference, BMI, race, cholesterol, blood pressure, and physical activity level.[4, 13, 23, 25, 39] Studies have been conducted on the obese, postmenopausal population and older men and women to determine if weight loss through diet, physical activity, and/or physical activity with weight loss affects CRP levels. Weight loss through low calorie diet has been shown to reduce CRP levels in obese, postmenopausal women since the in CRP levels is primarily associated with loss in fat mass during weight loss.[50] Also, physical activity has been associated with decreased CRP levels and reduced risk of CVD. There is an inverse relationship between activity level and CVD risk and CRP level; as activity levels increase, CRP levels decrease in both genders.[3, 10, 49] Along with decreased inflammatory markers, decreased weight, body fat percentage, adipose tissue, and BMI result from increased physical activity.[8, 45] Lifestyles changes, especially physical activity and diet, can promote significant improvements of CVD risk factors, such as decreased CRP levels.

Table 1: Risk Factors for Cardiovascular Disease.

Article	Problem/Question Studied	Participants	Procedures	Findings
Cardiovascular disease in postmenopausal women: myths and reality (Lewis, 2002).	Focus on epidemiology and impact of CVD on women and review risk factors.	Review paper.	Review paper.	Positive lifestyle (physical activity, weight control, healthy diet, and smoking cessation) reduce the prevalence of CVD.
Menopause and cardiovascular disease: the evidence (Romano, 2007).	To discuss menopause of a risk factor for CVD.	Review paper.	Review paper.	Menopause is associated with a significant increase in CVD risk due to detrimental effects of estrogen deficiency.
Risk factor for cardiovascular disease in women (Schenck-Gustafsson, 2009).	To discuss CVD risk factors in women.	Review paper.	Review paper.	CVD is presented later in life for women and are more likely to be suffer with co-morbidities, such as high blood pressure and diabetes.

Article	Problem/Question Studied	Participants	Procedures	Findings
Inflammatory markers and the risk of coronary heart disease in men and women (Pai, 2004).	To investigate inflammatory markers as predictors of CVD.	239 women and 265 men.	Blood samples were collected.	Elevated levels of inflammatory makers, especially CRP, indicate risk of CVD.
Sex hormone levels and risk of cardiovascular events in postmenopausal women (Rexrode, 2003).	To examine the relationship of plasma androgens and estrogens with risk of CVD in postmenopausal women.	28,345 postmenopausal women.	Low dose of aspirin and vitamin E were administered; blood samples were collected to analyze estradiol levels.	Low sex hormone binging globulin or high free androgen index is associated with increased risk of CVD events. Low SHBG increased CVD 2-fold.
Regional fat distribution in women and risk of cardiovascular disease (Williams, 1997).	To determine which fat-distribution variables (arm fat, leg fat, trunk fat, intraabdominal adipose fat, or subcutaneous adipose fat) have independent relations with CVD risk.	224 healthy white women; 138 premenopausal and 86 postmenopausal (17-77 years old).	Cross-sectional design that analyzed body fat via CT (abdominal), UWW (bone density), DEXA (total body), height and weight were measured to nearest 0.5cm. Blood sample (after 12 hour fast) to collect lipid measures and blood pressure was performed.	Intraabdominal adipose fat and trunk fat were positively related to CVD risk factors.
Obesity as an independent risk factor of cardiovascular disease (Hubert, 1983).	To investigate to relationship between the degree of obesity and the incidence of CVD in men and women.	5209 participants (2252 men and 2818 women); 28-62 years old.	Height and weight were measured to calculate BMI, blood pressure, serum cholesterol, blood glucose, and smoking status were all measured.	The degree of obesity in men and women is an important long term predictor of CVD incident.
Association between C-reactive protein, metabolic cardiovascular risks, obesity, and oral contraceptive use in young adults (Williams, 2004).	To determine the relationship between levels of CRP, CV risk factors, and oral contraceptive.	822 men and women; 26 years old.	Cross sectional study, Blood samples were collected, anthropometric measurements and blood pressure were measured.	Independent relationship between CRP and obesity as cofounding risk factors of CVD.
Elevated C-reactive protein levels in overweight and obese adults (Visser, 1999).	To test whether overweight and obesity are associated with inflammation as measured by CRP.	16,616 men and women.	BMI, waist circumference were measured. Blood samples collected to measure CRP.	Higher BMI is associated with elevated CRP levels.
Blood pressure, C-reactive protein, and risk of future cardiovascular events (Blake, 2003).	To determine the relationship between CRP in blood pressure in women and determine whether blood pressure is an independent determinant of CRP levels.	15,215 women over 45 years old.	Blood samples were collected, blood pressure and BMI were measured.	Inconclusive on whether blood pressure is a determinant of CRP levels; however BP and CRP were confirmed as risk factors for CVD.

Article	Problem/Question Studied	Participants	Procedures	Findings
Distribution and correlates of C-reactive protein concentrations among adult US women (Ford, 2004).	To understand the distribution among CRP concentrations among women.	2,205 women >20 years old.	Questionnaires completed. Blood samples were collected.	There was an association between CRP and BMI, waist circumference, cholesterol, and blood pressure.
Race and gender differences in C-reactive protein levels (Khera, 2005).	To determine whether there are race and gender differences in CRP levels.	2,749 white and black men and women (age 30-65 years old).	Weight, heart rate, blood pressure, and BMI were measured. Blood samples were collected after an overnight fast.	Black women have higher CRP levels than white women.
Interrelationships among circulating Interlukin-6, C-reactive protein, and traditional cardiovascular risk factors in women (Bermudez, 2002).	To determine the relationship of IL-6, CRP, and CVD risk factors in healthy women.	340 postmenopausal women (mean age 60).	Cross-sectional study; health history questionnaires were completed, blood samples were analyzed for IL-6 and CRP.	Age, BMI, blood pressure smoking status, and HDL levels are associated with CRP levels.
Physical activity and cardiovascular disease risk in middle-aged and older women (Sesso, 1999).	To investigate whether physical activity during middle and late years alters the risk of CVD.	2,363 female subjects (37-69 years old).	Cross sectional design utilizing questionnaires that reported the number of hours spent walking flights of stairs climbed, city blocks walked, and sports participated weekly. Body weight and BMI were calculated to determine energy expenditure.	Walking may decrease CVD risk by 33%. Overall, there is no association of physical activity and CVD risk in women. The benefit of walking was found in lean women only (BMI < 23).

There are multiple factors that have been shown to affect C-reactive protein (CRP) levels and/or risk of cardiovascular disease (CVD) in women. CRP levels and CVD have a direct relationship given that increased CRP levels have been reported as an independent predictor for CVD.[54] There are risk factors that affect CRP while in effect increase the risk of CVD, such as age, waist circumference, BMI, race, cholesterol, blood pressure, and physical activity level.[4, 13, 23, 25, 39] Non-modifiable risk factors for elevated CRP levels are age and ethnicity. According to Rexrode[14] and Rosano,[44] as women age and menopause occurs, there is a decrease in estrogen levels, decrease in sex hormone binding globulin and increase in free androgen index while augments the risk of CVD. Modifiable risk factors, such as waist circumference, BMI, and physical activity level, affect the risk of CVD in men and women. Distribution of fat alters from

gyniod to android placing more fat in the abdominal area as women age. Williams[54] reported that an increase in intraabdominal adipose fat and trunk fat, measured by waist circumference, enhance the risk of CVD in women. In addition to waist circumference, elevated BMI has is associated with elevated CRP levels and an independent predictor of CVD in both men and women.[4, 13, 20, 25, 39, 52, 54] Regarding race, it has been shown that black women have increased BMI and CRP levels compared to white women, increasing their risk for CVD.[23] Finally, elevated total serum cholesterol in women is associated with elevated CRP levels. Total serum cholesterol is related to increased BMI and increased intraabdominal abdominal fat.[13]

The literature was consistent when observing age, waist circumference, BMI, race, and cholesterol. However, there are inconclusive research regarding blood pressure and physical activity level and their effects on CRP levels and/or CVD risk. Literature has shown that within postmenopausal women, increased blood pressure, specifically systolic blood pressure measurement, increase risk for CVD risk, but there is controversy research if blood pressure is a determinant of CRP level.[4, 46] Bermudez[4] stated that elevated blood pressure is associated with elevated CRP levels, whereas Blake[6] reported that there is inconclusive evidence that blood pressure is a determinant for CRP level. Finally, physical activity and its effects on CRP levels will be discussed in for detail in Table 3, but as a risk factor for postmenopausal women, Lewis[25] reported 80% lower risk for a cardiovascular risk and decreased CPR level if moderate to vigorous activity was performed for 30 minutes most days of the week, while Sesso[47] reported that light activity, such as walking, can reduce the CVD risk by 33% but the relationship was not significant enough to state that physical activity is associated to CVD.

Table 2: Effects of C-Reactive Protein with Weight Loss through Diet

Article	Problem/Question Studied	Participants	Procedures	Findings
The effect of weight loss on C-reactive protein (Selvin, 2007).	To test the hypothesis that weight loss is directly related to a decline in CPR level.	Review paper.	A review of weight loss intervention studies that included lifestyle, diet and/or exercise interventions.	A decline in CRP level is associated with weight loss by way of lifestyle or surgical intervention.

Article	Problem/Question Studied	Participants	Procedures	Findings
C-reactive protein and coronary artery disease: influence of obesity, caloric restriction and weight loss (Heilbronn, 2002).	To determine whether particular dietary macronutrient or micronutrients alter IL-6 and/or CRP level.	Review paper.	Review paper.	CRP is a predictor of acute vascular events because inflammatory penetration expresses IL-6 which stimulates CRP.
Effects of a dietary portfolio of cholesterol-lowering foods vs lovastain on serum lipids and C-reactive protein (Jenkins, 2003).	To determine the dose-response relationship of nuts and their advantage in a low-saturated fat therapeutic diet.	Forty-six healthy, hyperlipidemic adults; 25 men and 21 postmenopausal women.	1 m. intervention; body weight was measured weekly and blood samples were obtained every 2 weeks after a 12-hr fast; 1 of 3 diets were assigned (very low in saturated fat, very low in saturated fat plus lovastatin, high in plant sterols).	All diets resulted in a decrease in C-reactive protein levels; however, the statin diet resulted in the greatest reduction.
Effect of high-fiber diet vs a fiber-supplemented diet on C-reactive protein level (King, 2007).	To examine the reduction in inflammation from a diet supplemented with fiber compared to a diet naturally high in fiber.	35 participants (28 women, 7 men; 18 lean normotensive, 17 obese hypertensive).	Crossover intervention of high-fiber DASH diet or fiber-supplemented diet; photographs were taken for 3 days of each 3 week diet; weekly urine samples, weight measurement, and diet consulting.	Both diets demonstrated a decrease in CRP levels, however, CRP decreased significantly in lean group but no significance in obese group.
Relation between a deit with a high glycemic load and plasma concentration of high-sensitivity C-reactive protein in middle-aged women (Liu, 2002).	To examine whether a high dietary glycemic load was associated with elevated CRP concentration and whether this association was modified by body mass index.	244 apparently healthy women.	Cross sectional design; blood draw was done and a food frequency questionnaire was completed.	Dietary glycemic index load is significantly and positively associated with CRP levels in healthy middle-aged women.
Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentration in obese, healthy women (Heilbronn, 2001)	To determine the effects of dynamic weight loss on CRP in healthy, obese women.	Eighty-three nonsmoking, healthy, obese women.	12 wk intervention (every 4 weeks after a 12-hr fast); weight recorded and blood drawn.	CRP was lowered in proportion to weight loss.
Weight loss reduces C-reactive protein levels in obese postmenopausal women (Tchernof, 2001).	To examine the relations among body fat, fat distribution, and CRP levels.	Twenty-five obese, postmenopausal women.	14 m. intervention (blood was tested after 12-hr fast and 3 days of standardized meals); 1200 kcal/day. One group received a modified fasting supplement.	Losses of fat mass in obese, postmenopausal women were associated with proportional reductions in CRP levels.

Article	Problem/Question Studied	Participants	Procedures	Findings
Diet-induced weight loss is associated with decreases in plasma serum Amyloid A and C-reactive protein independent of dietary macronutrient composition in obese subjects (O'Brien, 2005).	To examine the influence of macronutrient composition of weight loss on levels of CRP.	41 obese women.	3 m. intervention; 1200 kcal/day low-fat diet (AHA step 1) or very low-carbohydrate diet; weight and blood samples were completed for baseline and at 3 months.	There was a greater reduction in CRP with the very low-carbohydrate diet compared to the low-fat diet.
Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women (Noakes, 2005).	To evaluate the effects of a diet with a high ratio of protein to carbohydrate during weight loss on body composition, CVD risk, nutritional status, and markers of bone turnover and renal function in overweight women.	100 obese women.	12 wk intervention; 5600 kJ diet of high-protein or high-carbohydrate; consultation with a dietian every 4 weeks and completed a food intake log; fasting blood samples were obtained at baseline, wk 4,8, and 12; weight was measured every 14 days (after fasting overnight).	Hypocaloric high-protein, low-fat diet resulted in nutritional and metabolic (CRP decreased with weight loss) benefits that are greater than the high-carbohydrate diet.
Long-term effects of high-protein, low-carbohydrate diet on weight control and cardiovascular risk markers in obese hyperinsulemnic subjects (Brinkworth, 2004).	To compare long term compliance and effects of two low fat diets differing in carbohydrates to protein ratio on body composition and biomarkers of CVD risk in obese subjects with hyperinsulinemia.	Fifty-eight obese, nondiabetic subjects with hyperinsulinemia; 13 males and 45 females.	68 wk intervention (12 wk of energy restriction, 4 wk of energy balance, 52 wk without counseling); body weight and blood drawn at baseline, wk 16, and wk 68. Two diets were distributed (standard protein intake or high protein intake).	Both diets were equally as effective in weight loss and decrease in CRP compared to conventional weight loss approaches.
Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial (Nicklas, 2004).	To determine the independent and combined effects of diet-induced weight loss and exercise on the markers of chronic inflammation.	316 sedentary, overweight men and women (>60 years).	18 m. intervention; diet-induced weight loss (decease by 5%), exercise (3 days/week for 1 hour sessions), or diet and exercise; blood samples (after fast), anthropometric measurements.	Weight loss via diet reduced overall inflammation. Exercise training did not have an effect on inflammatory markers.

Article	Problem/Question Studied	Participants	Procedures	Findings
Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women (You, 2004).	To determine whether a hypocaloric diet with and without exercise training is effective in reducing plasma C-reactive protein.	34 healthy, nonsmoking, overweight/obese postmenopausal women.	6 m. intervention; body weight was measured, food records and diet instruction to elicit 0.5-1.0kg/wk were done weekly; diet and exercise group also walked 3d/wk at target heart rate.	CRP decreased with diet and exercise but not diet alone, even though both groups lost similar amounts of weight and adipose tissue.

C-reactive protein is a predictor of acute vascular events due to the stimulation of CRP by interleukin-6 (IL-6) during the inflammation process.[17] Among the population of in obese, postmenopausal women, weight loss has been shown to reduce CRP levels.[34, 48, 50] Tchernof [50] reported that the reduction in CRP levels is primarily associated with loss in fat mass [adipose tissue] during weight loss. There have been numerous studies conducted researching different types of weight loss regimes that have resulted in lower CRP levels from baseline levels.[7, 18, 21, 24, 27, 34, 35, 36, 55]

There are discrepancies among studies regarding diet and exercise versus diet alone interventions. The decrease in CRP level and reduction of weight through diet were proportion.[18] Contrary to Heilbronn,[18] CRP levels did not decrease with weight loss and fat loss [adipose tissue] through diet alone; however CRP levels did decrease with weight loss and fat loss [adipose tissue] through diet and exercise.[55]

Table 3: Effects of C-Reactive Protein through Physical Activity

Article	Problem/Question Studied	Participants	Procedures	Findings
Physical activity decreases cardiovascular disease risk in women (Oguma, 2004).	To review and quantify the dose-response relationship of physical activity in healthy women on CVD outcomes.	Meta-analysis.	Studies reviewed inclusion criteria was data on women, assessed physical activity, and provided information on relative risk of CVD.	Walking 1hour/week was associated with reduced risk of CVD outcome. Physical activity was association with reduced risk of CVD among women in a dose-response relationship.

Article	Problem/Question Studied	Participants	Procedures	Findings
Cross-sectional and prospective relationships of Interleukin-6 and C-reactive protein with physical performance in elderly persons (Taaffe, 2000).	To examine the cross-sectional and perspective relationships between markers of inflammation with several measures of physical performance in older persons aged 70 to 79 years.	880 men and women, 65 yr or older.	Blood samples were collected (non-fasting); physical function tests were performed (baseline and 7 yrs later); anthropometric measurements and medications were recorded.	Higher IL-6 and CRP levels in those less active; CRP was related to increased walking speed and grip strength; signature time and chair-rise ability were not associated with changes in CRP.
Past physical activity, current physical activity, and risk of coronary heart disease (Conroy, 2005).	To examine the relationship between physical activity during young adulthood and middle age, and physical activity during each time period and CHD occurring in middle-aged or older.	Cohort study of 37,169 healthy U.S. female health professionals, age 45 yr or older.	10 yr study; subjects completed a mailed baseline questionnaire on sociodemographics, health habits, and medical history; participated in the trial after a 3-month run-in phase; follow-up surveys asking about treatment compliance, risk factors, and endpoints of interest were mailed every 6 months during the first year and annually.	Physical activity at study baseline was associated with a lower risk of subsequent CHD events; however, physical activity in young adulthood was not a predictor of CHD risk several decades later. There is an inverse relationship between physical activity during middle age and CHD risk.
Association between physical activity and markers of inflammation in a healthy elderly population (Geffken, 2001).	To measure the association between self reported physical activity with several markers of inflammation in a population-based elderly cohort.	5,888 participants (male and female); 2,501 diagnosed with clinical CVD and 3,837 did not have CVD.	At baseline, medical history, medication, physical activity level, fasting blood samples, and anthropometric measurements were collected; measurements were obtained 3 yr later.	Physical activity level is associated with significantly lower levels of CRP, independent of known CVD risk factors.
Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults (Abramson, 2002).	To examine the association of physical activity with 3 markers of inflammation in a representative sample of apparently healthy US adults.	3,638 apparently healthy US men and women 40 yr and older.	Cross sectional study; stage I, in-home interview regarding demographics, health status, health behaviors; stage II, conducted within 4 wks of stage I.	A higher frequency of physical activity was associated with lower CRP levels.
Low cardiovascular fitness is associated with elevated C-reactive protein levels in women with Type 2 diabetes (McGavock, 2004).	To examine differences in markers of CVD in women with type 2 diabetes.	28 women with type 2 diabetes but absent of CVD events with low or average cardiorespiratory fitness levels.	Cross sectional study. Physical examination and cycle ergometry test were performed to determine VO2max. Blood samples were collected after an overnight fast along with an EKG.	Low cardiorespiratory fitness is associated with elevated CRP levels.

Article	Problem/Question Studied	Participants	Procedures	Findings
Physical activity, exercise, and inflammatory markers in older adults: Findings from the health, aging, and body composition study (Colbert, 2004).	To examine the association between physical activity and inflammatory markers.	3,075 black and white men and women (70-79 years old).	Cross sectional study. Questionnaires determines physical activity level and blood sample analyzed inflammatory marker levels.	Inflammatory markers are lower in older adults with higher levels of exercise.
Effect of physical activity on serum C-reactive protein (Albert, 2004).	To examine the association between physical activity and CRP levels.	1.732 men and 1.101 women.	Cross sectional study. Blood samples to evaluate CRP and lipids; data on age, smoking status, and diabetic status; physical activity level was self reported.	There is an inverse relationship between physical activity and inflammatory biomarkers.
C-reactive protein is inversely related to fitness in middle-aged subjects (Aronson, 2004).	To examine the relationship between physical fitness and CRP levels in middle-aged subjects.	892 conservative middle aged subjects.	Cross sectional study where body mass index was calculated, blood samples were collected after a 12 hr fast, and fitness level was calculated through a VO_{2max} test using the Bruce protocol.	CRP concentration decreased with increasing levels of fitness and decreased BMI.
Physical activity an d cardiovascular disease risk in middle-aged and older women (Sesso, 1999).	To investigate whether physical activity during middle and late years alters the risk of CVD.	2,363 female subjects (37-69 years old).	Cross sectional study. Questionnaires reported the number of hours spent walking flights of stairs climbed, city blocks walked, and sports participated weekly. Body weight and BMI were calculated to determine energy expenditure.	Walking and/or a lower BMI were inversely related to CVD risk. Overall, there is no association of physical activity and CVD risk in women.

The literature reviewed examining the effects of physical activity on CRP levels were cross-sectional studies. Most of the studies reviewed used both men and women over the age of 40 years old as participants. There was consistent evidence that physical activity was associated with decrease in CRP levels and lower risk of CVD. There is an inverse relationship between activity level and CVD risk and CRP level; as activity levels increase, CRP levels decrease.[3, 10, 49] This relationship was demonstrated through three different methods of analyzing physical activity level. The cross sectional studies that used questionnaires to determine participants' physical

activity level concluded that increased physical activity resulted in lower CRP levels.[1,2,3,9,14] Other protocols used to determine physical activity level were 6-minute walk test, treadmill VO_{2max} test, and cycle ergometer VO_{2max} test. Taffee[49] concluded that increased activity level is associated with lower CPR levels after a 6-minute walk test to men and women over the age of 65 was administered. The Bruce treadmill protocol was utilized to measure the VO_{2max} in middle aged men and women, and the only cross sectional study conducted on postmenopausal women utilized a cycle ergometry test to measure VO_{2max}.[3, 29] Both of the VO_{2max} protocols reported a higher VO_{2max} measurement and was associated with lower CRP levels. It is important to note that physical activity must be maintained though out life to maintain lower CRP levels and a lower risk of developing CVD.[10]

Physical activity has been shown to decrease the risk of CVD in women, but intensity is an important factor .[37, 47] Lewis[25] reported 80% lower risk for a cardiovascular risk and decreased CPR level if moderate to vigorous activity was performed for 30 minutes most days of the week, while Sesso[47] reported that light activity, such as walking, can reduce the CVD risk by 33% but the relationship was not significant enough to state that physical activity is associated to CVD. Interestingly, a meta-analysis by Oguma[37] reported that walking 1 hour/week can decrease CVD risk according to the dose-response relationship.

Table 4: Effects of C-Reactive Protein with Weight Loss through Physical Activity

Article	Problem/Question Studied	Participants	Procedures	Findings
Reduction in C-reactive protein through cardiac rehabilitation and exercise training (Milani, 2004).	To assess the effects of 3-month formal phase II cardiac rehabilitation and exercise training program on high-sensitivity CRP levels in patients with coronary heart disease.	277 patients with CHD (235 completed cardiac rehab program while 42 were the control group and did not receive rehab).	Diet modifications to promote weight loss and exercise intervention were implemented 3 d/wk; blood samples were taken 2-6 weeks after hospital discharge and again 3-6 months following baseline.	Therapeutic lifestyles changes through cardiac rehabilitation can promote significant improvements in cardiac risk factors; favorable effects of CRP through rehabilitation were independent of weight loss.

Article	Problem/Question Studied	Participants	Procedures	Findings
Leisure time physical activity and reduced plasma levels of obesity related inflammatory markers (Pischon, 2003).	To investigate the relationship between physical activity and the obesity related inflammatory marker C-reactive protein.	405 healthy men and 454 healthy women.	Cross-sectional study from 1986 and 1997; Blood samples were requested between 1993-1995; diet was assessed every 4 years; physical activity was determined from a questionnaire in 1994.	Frequent physical activity is associated with lower systemic inflammation and higher insulin sensitivity; in contrast to fat mass, inflammation markers explained little regarding the physical activity and insulin sensitivity relationship.
Prevalence and cardiovascular disease correlates of low cardio-respiratory fitness in adolescents and adults (Carnethon, 2005).	To describe the prevalence of low fitness in the US population aged 12-49 years and to relate low fitness to CVD risk factors.	Cohort study of 3,110 12-19 yr olds and 2,205 20-49 yr olds.	Socioeconomic information was collected via phone, physical examinations and lab measurements were performed in a mobile center, and blood samples were collected locally.	Low cardio-respiratory affects 1 out of 5 persons aged 12-49 resulting in higher BMI; therefore, BMI is inversely related to fitness level.
Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women (Mora, 2006).	To examine the association of physical activity, BMI alone and in combination with cardiovascular markers.	27,158 apparently healthy US women.	Cross sectional study; self reported height and weight and questionnaire for physical activity was completed; blood samples were collected.	Lower levels of physical activity and higher levels of BMI were independently associated with increased inflammatory markers.
Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women (Esposito, 2003).	To determine the effect of lifestyle changes to reduce body weight on markers of systemic vascular inflammatory and insulin resistance.	120 premenopausal obese women (20-46 years).	24 m. intervention; Mediterranean 1300 kcal diet and increased physical activity	Reduction in weight loss was associated with reduction in vascular inflammatory markers and insulin resistance.
Reductions in plasma cytokine levels with weight loss improve insulin sensitivity in overweight and obese postmenopausal women (Ryan, 2004).	To determine whether improvements in insulin sensitivity with weight loss are mediated by changes in inflammation in obese, postmenopausal women.	37 sedentary, overweight/obese postmenopausal women.	6 m. diet & exercise intervention; baseline measurements including anthropometric measurements, fasting blood profile, and graded treadmill test; weekly meetings with a RD, 3 d/wk exercised on treadmills or bikes.	Body weight, percentage body fat, fat mass, fat free mass, and visceral adipose tissue decreased with weight loss, however VO_{2max} did not change after weight loss. Glucose utilization and insulin sensitivity were inversely related to CRP changes.

Article	Problem/Question Studied	Participants	Procedures	Findings
Can exercise training with weight loss lower serum C-reactive protein levels? (Okita, 2004).	To examined the effects of exercise training with weight loss on CRP levels and conventional CVD risks.	199 healthy Japanese women (129 postmenopausal).	2 m intervention; subjects did aerobic exercise 2 d/wk (80 min dance and 30-60 min treadmill); blood samples (after 12 hr fast), anthropometric measurements, muscular strength and CV endurance were tested at baseline and 2 months later.	Body weight significantly decreased and CRP levels were lowered after exercise intervention; adipose tissue is likely a factor modulating CRP levels.

Lifestyles changes, especially physical activity, can promote significant improvements of CVD risk factors, such as decreased CRP levels. Cross sectional and 3-month intervention studies with men and women reported that independent of weight loss, with an increase in physical activity there was a decrease in CRP levels.[30, 40] Along with decreased inflammatory markers, decreased weight, body fat percentage, adipose tissue, and BMI result from increased physical activity.[8,45] Cross sectional with men and women and 6-month intervention with postmenopausal women concluded that there is an inverse relationship between BMI and physical activity level. Intervention studies consisted of postmenopausal women exercising 3 days/week running or cycling for 30-60 minutes at 60% VO_{2max} or between 60-80% peak heart rate.[38, 45] There was an inconsistency within the literature regarding the effect of decreased BMI has on CRP level. According to Okita et al,[38] CRP levels were affected by the decrease in adipose tissue; however, Milani et al[30] reported the decrease in CRP levels were independent of weight loss or decrease in BMI.

Chapter III

Methodology

Participants

Thirty-one women responded to the initial request for study volunteers. Twenty-one

postmenopausal women who were recruited from the James Madison University and

Harrisonburg Rockingham County communities volunteered as participants for this study. The

subjects were reached through email flyers, e-mail, and by word of mouth. Persons who showed

interest in participating were screened to see if they met the minimum criteria for entrance into

the study. The criteria for inclusion included: Apparently healthy (no diagnoses of cardiovascular

disease), female, post-menopausal (no regular menstrual cycle in the past 12 months), and

previously sedentary (no regular exercise (<2 days per week) in the past 6 months). All study

protocols and procedures were approved by the Institutional Review Board.

Overview of Procedures

The protocol was designed to determine the independent effects of weight loss initiated through

dietary changes and fitness (VO_{2max}) without weight loss on CRP levels in post menopausal

women. Some of the measurements were used for other studies and are not related to the research

question being investigated. Pre- and post testing measurements were completed during separate

four sessions.

First Session

During the first pre-test session all test procedures and protocols were explained to the

participants who completed an Informed Consent Form (Appendix I), Health Status

Questionnaire (Appendix II) and PAR-Q (Appendix III). In addition, all participants were given

a Dietary 24-hour Food Recall (Appendix IV) explained by a registered dietitian. The ACSM's

Coronary Artery Disease Risk Factor Thresholds along with the ACSM Risk Stratification were

used to assess the risk for cardiovascular disease. Risk stratification was based upon age, family

history, smoking habits, blood lipid levels, CRP levels (non-traditional risk factor), fasted blood

glucose values, resting blood pressure, body mass index, and physical activity habits. Other

questionnaires completed, but not used for the current study included: the International Physical

Activity Questionnaire (IPAQ) designed to assess the volume of physical activity that the

participant engaged in prior to enrolling in the study, the Pittsburgh Sleep Quality Index (PSQI)

to assess the sleep quality of the participants, the Epworth Sleepiness Scale (ESS) evaluated the

tendency to doze off during daytime activities, the U.S Department of Health and Human

Services Health Behavior Questionnaire to assess lifestyle behaviors, and the Health Related

Quality of Life questionnaire (HRQOL-4) assessed health related quality of life.

Second Session

The second session included a venous blood draw, measurement of resting blood pressure, and

body composition assessment via dual-energy x-ray absorptiometry (DEXA Hologic, Bedford,

Massachusetts). After an overnight fast, blood obtained from the anticubital vein and was stored

at -80°C until serum was analyzed for CRP. Serum CRP levels were determined using an

Enzyme Linked Immunoassay (ELISA) kit (BioCheck Inc, Foster City, California). Blood lipid

values (total cholesterol, HDL cholesterol, triglycerides) were measured and determined using

dry slide technology (Johnson and Johnson Vitro DT 6011, Rochester, New York). LDL

cholesterol values were obtained using the Friedewald equation. Blood lipid values were not

included for analysis in the current study. Height was measured to the nearest 0.25 cm and body

weight was measured to the nearest 0.25 pounds to calculate BMI (kg/m^2). Also, waist

circumference was measured at least twice to the nearest centimeter using a tension-controlled

tape measure according to procedures as specified in the *ACSM Guidelines for Exercise Testing

and Prescription* (eighth edition, 2009).

Third Session

The third session included a graded walking test on a treadmill (Quinton Club Track 612, Bothell,

Washington) (Appendix V) to determine cardiovascular endurance (maximal oxygen

consumption VO$_{2max}$). Oxygen consumption, heart rate, and rating of perceived exertion were

monitored throughout the test. Metabolic measures were calculated using open circuit spirometry via a computerized metabolic cart (Sensormedics Vmax 229, Yorba Linda, California). At the end of the first stage, workload was increased by increasing the elevation (grade) on the treadmill every three minutes. The protocol continued in the same manner for three minute stages until the participant reached volitional fatigue, their age-predicted maximal heart rate, asks to stop, or experiences adverse signs or symptoms (ACSM, 2010).

Fourth Session

The fourth pre-test session consisted of muscular strength, muscular endurance, and flexibility measurement used for a larger project. Muscular strength of the upper and lower body was measured by completing a 5 repetition maximum (RM) on the chest press and leg press machines (Cybex, Medway, Massachusetts). Participants completed a number of submaximal repetitions as a warm up. A weight that was within 50-70% of perceived capacity for 5 repetitions was selected, and the participant completed 5 repetitions at this weight. If 5 repetitions were successfully completed, weight increased following a 3 to 5 minute rest period and 5 more repetitions were attempted. This protocol continued until the participant could no longer complete 5 repetitions at a selected weight. Muscular endurance was measured by following ACSM push-up and curl-up test procedures. For the push-up test, participants assumed the modified "knee push-up" position, with legs together, lower leg in contact with the mat and toes pointed, back straight and hands shoulder width apart. The participant performed as many modified "knee push-ups" as possible by straightening the elbows and then lowering the body until the chin touched the mat. For the curl-up test, participants assumed a supine position on a mat with the knees at 90-degree angles. Arms were at the side with palms facing down and fingers touching a piece of masking tape. A second piece of tape was placed 10 centimeters in front of the first piece. A metronome was set to 50 beats per minute, and the participant performed curl ups in time to the metronome, lifting the shoulder blades off the mat to touch the second piece of tape. The number of curl-ups performed without pausing, or a maximum of 25,

was recorded as the score. Muscular flexibility was assessed using the V-Sit and Reach test.

Participants sat without shoes with the soles of the feet flat against a sit-and-reach box

(flexometer) at the 26-centimeter mark. Participants slowly reached forward as far as possible,

keeping the legs straight and both hands overlapped, and holding this position for approximately

2 seconds. The most distant point reached with the fingertips was recorded.

Interventions

Weight Loss: Dietary Intervention (DI)

Participants were randomly assigned to a hypocaloric dietary intervention (DI) for 9 weeks.

Participants reported to the James Madison University Human Performance Laboratory once a

week for a body weight measurement. The study's goal for DI participants was an 8-10% weight

loss from baseline weight. Assessment of dietary and beverage intake by 24-hour Food Recall

and dietary guidance administered by a registered dietician occurred pre-intervention, week six of

intervention, and post-intervention.

Maximal oxygen consumption (VO$_{2max}$): Exercise Intervention (EXI)

Participants randomly assigned to an exercise intervention (EXI) reported to James Madison

University Crawford Fitness Center 3 days per week for 45-55 minute sessions. The study's goal

for the EXI participants was to increase cardiovascular endurance from baseline VO$_{2max}$.

Cardiovascular training was initially set for each participant to train at 60% of VO$_{2max}$ and

gradually progress to training at 75-80% of max by week 9 of the intervention; participants had

the option of walking, cycling, elliptical, or rowing. Resistance training consisted of a circuit of

upper and lower body exercises designed to improve muscular strength and endurance.

Participants trained at an intensity of that allowed each individual to perform between 8-15

repetitions (60-70% of 1RM) per set. The regimen began with one set of each exercise and

progressed to 2 sets of each exercise by week 9 of the intervention. Resistance exercises were

performed on Cybex equipment (Cybex, Medway, Massachusetts). Upper body exercises

included chest press, bicep curl, triceps extension, and latissimus pull down. Lower body

exercises will include leg extension, leg curl, leg press, and calf raises. Two additional resistance exercises were added to increase muscular strength and endurance of the core. Exercise sessions also gradually increased in time. Sessions started at 30 minutes and progressed to 60 minutes per session (30 minutes performing cardiovascular exercise and 30 minutes of resistance exercise).

Statistical Analysis

Differences in CRP levels and dependent variables (weight, BMI, fat mass, body fat percent, waist circumference, and VO_{2max}) within the DI and EXI were compared by repeated measures analysis of variance. Post-hoc paired t-tests were performed on variables that demonstrated significance between interventions to determine significance in change (pre-post). Descriptive statistics were calculated for each treatment and measurement. SPSS statistical software package (SPSS, Somers, New York) was used for all data analysis.

Chapter IV

<u>Manuscript</u>

Effect of diet induced weight loss and exercise on hsCReactive Protein in sedentary

postmenopausal women

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ABSTRACT

Background: Cardiovascular disease (CVD) risk factors that are intensified in postmenopausal women include body fat, blood pressure, dyslipidemia, and high sensitivity C-reactive protein (CRP) levels. CRP has been reported as an independent indicator of risk for cardiovascular events. CRP is affected by multiple factors such as age, race, body mass index (BMI), and physical activity level. The purpose of this study was to examine whether weight loss or exercise without weight loss had an impact on CRP levels. **Methods and Results**: Twenty apparently healthy postmenopausal women were randomly assigned to a diet intervention group (DI) or an exercise intervention group (EXI) for a period of 9 weeks. DI participants reported once a week for a body weight measurement and EXI participants were asked to attend three 45-60 minute sessions per week (cardiovascular and resistance training exercises). There were no significant differences effects of time or treatment on CRP levels (p=0.077). However, there was a significant change (-4.45 kg) in body weight (p=0.000, p< 0.05) and (-1.1 kg) change in fat free mass (p=0.041, p<0.05) in the DI group. Our study found that there was no significance in the change in CRP within the DI or EXI group. **Conclusions**: Body weight, FFM, and waist circumference all decreased significantly in the DI group; however, decreases in these variables did not appear to precipitate significant changes in CRP. It is possible that a greater degree of weight loss (over a longer duration) may be necessary to decrease CRP levels. There was no significance between EXI and dependant variables, including CRP, also suggesting that greater intensity or longer intervention duration may be necessary to affect dependant variables through exercise.

Key Words: cardiovascular disease, diet, exercise.

INTRODUCTION

Cardiovascular disease (CVD) is the major cause of death among the U.S. population.[8, 16, 33] With aging, the risk of CVD proportionally increases; however, women are affected by CVD 10 years after men.[25,33] Risk factors for CVD in women are smoking (leading cause), hypertension, dyslipidemia, diabetes mellitus, obesity, sedentary lifestyle, and menopause.[6, 25, 33, 44, 46] Increased abdominal adiposity, associated with obesity, is a risk factor for CVD in women; the prevalence of obesity has increased over the past decade and currently one out of three women is classified as obese. Obesity is a parallel factor to sedentary lifestyle, and studies have shown that moderate physical activity reduces the risk of CVD by 50% in women. Schenck[46] reported in 2009 that 30% of women are overweight and 27% are classified as obese, and the risk for CVD in obese women increases 3-fold compared to lean women.[20, 25, 33] Menopause decreases the estrogen levels and increases testosterone levels in postmenopausal women negatively affecting cardiovascular functions and metabolism resulting in an increase risk for CVD event, such as dyslipidemia resulting from increased testosterone.[25]

In addition to the blood lipids, abnormal lipoprotein, C-reactive protein (CRP) and interleukin-6 levels are known risk factors for CVD in women.[3, 13, 15, 39, 46] Inflammation markers have been determined to have a significant role in the development of atherosclerosis and CVD, but the relationship between the inflammation process and CVD is complex.[4, 16, 22] In response to tissue inflammation, CRP is stimulated by interleukin-6, interleukin-1, and tumor neurosis factor.[22] This response occurs when activated immune cells in the plaque produce inflammatory cytokines, stimulating the production of substantial amounts of interleukin-6. Interleukin-6, in response, stimulates the production of large amounts of acute-phase reactants (C-reactive protein, serum amyloid A, and fibrinogen) in the liver. Plasma concentration of CRP can increase 100-fold after acute inflammatory stimulus.[5, 11, 42] According to Hansson,[16] cytokines have important biologic effects at all steps; however, their amplification at each step of the cascade makes the measurement of downstream mediators such as CRP particularly useful for clinical diagnosis.

CRP is an independent indicator of risk for stroke, peripheral vascular disease, and vascular death among healthy men and women without any known vascular diseases and is a stronger predictor for CV events compared to LDL-C levels. [5, 11, 42, 43] Currently practitioners consider CRP value of less than 2 mg/L as healthy, and individuals who have CRP levels of 5mg/L or greater may be at an increased risk for a cardiac event within six months.[22, 26]

CRP is affected by multiple factors such as age, race, body mass index (BMI), and physical activity level.[1, 3, 4, 9, 13, 14, 17, 18, 22, 28, 49, 52, 53] CRP levels have been shown to increase with age; therefore, BMI and physical activity level have to counteract the natural rise in CPR levels.[22, 28] CRP is highly correlated with BMI and several studies have reported a strong correlation between total fat mass and visceral fat and CRP.[17, 18, 23, 52] Heilbroon[17] and Williams[53] have reported that subjects with a BMI >30 kg/m^2 had elevated CRP levels and CRP levels were 270% higher in individuals in the top quintile of BMI compared to those in the bottom quintile. In addition to BMI, a direct relationship between elevated CRP levels and abdominal fat distribution via waist circumference measurement has been documented.[17, 18, 52] A waist circumference greater than 88 centimeters indicates higher BMI values and higher risk for CVD due to increased visceral fat and increase in serum resistin.[18, 51] When comparing race, more black women are considered obese and have higher CRP levels than white women.[23] Finally, CRP levels have been shown to have an inverse relationship with physical activity level.[29] Increased physical activity levels are related to decreased fat and lower BMI resulting in decreased CPR levels.[1, 3, 14, 29, 49]

It has been observed that the lowest CRP levels in individuals with the highest activity levels.[1,3,10,14] Physical activity is associated with a lower risk of subsequent CVD events; however, physical activity in young adulthood was not a predictor of CVD risk several decades later.[10] Along with an inverse relationship between physical activity level and CRP, there is an inverse association between intensity and/or frequency of exercise with CRP levels. CRP levels decrease with more vigorous exercise or more frequent exercise.[1, 3, 49] Multiple studies have

shown that with weight loss and exercise, CRP levels decrease in proportion to weight loss.[30, 38, 40, 45] Increased physical activity promotes decreased weight, body fat percentage, adipose tissue, and BMI resulting in decreased CRP levels.[45] Intervention studies consisting of aerobic activity 3 days/week through group exercise classes, treadmill, or cycling for 30-60 minutes at 60% VO_{2max} or between 60-80% peak heart rate resulted in a decrease in body weight, percentage body fat, fat mass, fat free mass, visceral adipose tissue, and decrease in CRP levels, however VO_{2max} did not change after weight loss during an exercise intervention.[30, 38, 45]

Research has shown that CRP decreases with weight loss through diet alone. The majority of diet interventions reviewed consisted of a 1200 kcal/day diet for a period of 12 weeks with obese women as the study population.[10, 14, 49] The diet intervention protocols consisted of 1200 kcal/week.[35, 36, 50] Previous studies have solely investigated changes of CRP levels resulting from weight loss through dietary alterations or weight loss through exercise. It is unknown if weight loss or exercise status alone have an effect on CRP levels in sedentary, postmenopausal women. The purpose of this study was to compare the effects of an exercise intervention versus a dietary intervention on traditional (BMI, percent body fat, waist circumference, physical activity level) and non-traditional (CRP) risk factors for cardiovascular disease in sedentary, post menopausal women.

PARTICIPANTS AND METHODS

Participants

Thirty-one women responded to the initial request for study volunteers. Twenty-one postmenopausal women who were recruited from the James Madison University and Harrisonburg Rockingham County communities volunteered as participants for this study. The subjects were reached through email flyers, e-mail, and by word of mouth. Persons who showed interest in participating were screened to see if they met the minimum criteria for entrance into the study. The criteria for inclusion included: Apparently healthy (no diagnoses of cardiovascular

disease), female, post-menopausal (no regular menstrual cycle in the past 12 months), and previously sedentary (no regular exercise (<2 days per week) in the past 6 months). All study protocols and procedures were approved by the Institutional Review Board.

Overview of Procedures

The protocol was designed to determine the independent effects of weight loss initiated through dietary changes and fitness (VO_{2max}) without weight loss on CRP levels in post menopausal women. Some of the measurements were used for other studies and are not related to the research question being investigated. Pre- and post testing measurements were completed during separate four sessions.

First Session

During the first pre-test session all test procedures and protocols were explained to the participants who completed an Informed Consent Form (Appendix I), Health Status Questionnaire (Appendix II) and PAR-Q (Appendix III). In addition, all participants were given a Dietary 24-hour Food Recall (Appendix IV) explained by a registered dietitian. The ACSM's Coronary Artery Disease Risk Factor Thresholds along with the ACSM Risk Stratification were used to assess the risk for cardiovascular disease. Risk stratification was based upon age, family history, smoking habits, blood lipid levels, CRP levels (non-traditional risk factor), fasted blood glucose values, resting blood pressure, body mass index, and physical activity habits. Other questionnaires completed, but not used for the current study included: the International Physical Activity Questionnaire (IPAQ) designed to assess the volume of physical activity that the participant engaged in prior to enrolling in the study, the Pittsburgh Sleep Quality Index (PSQI) to assess the sleep quality of the participants, the Epworth Sleepiness Scale (ESS) evaluated the tendency to doze off during daytime activities, the U.S Department of Health and Human Services Health Behavior Questionnaire to assess lifestyle behaviors, and the Health Related Quality of Life questionnaire (HRQOL-4) assessed health related quality of life.

Second Session

The second session included a venous blood draw, measurement of resting blood pressure, and body composition assessment via dual-energy x-ray absorptiometry (DEXA Hologic, Bedford, Massachusetts). After an overnight fast, blood obtained from the anticubital vein and was stored at -80°C until serum was analyzed for CRP. Serum CRP levels were determined using an Enzyme Linked Immunoassay (ELISA) kit (BioCheck Inc, Foster City, California). Blood lipid values (total cholesterol, HDL cholesterol, triglycerides) were measured and determined using dry slide technology (Johnson and Johnson Vitro DT 6011, Rochester, New York). LDL cholesterol values were obtained using the Friedewald equation. Blood lipid values were not included for analysis in the current study. Height was measured to the nearest 0.25 cm and body weight was measured to the nearest 0.25 pounds to calculate BMI (kg/m^2). Also, waist circumference was measured at least twice to the nearest centimeter using a tension-controlled tape measure according to procedures as specified in the *ACSM Guidelines for Exercise Testing and Prescription* (eighth edition, 2009).

Third Session

The third session included a graded walking test on a treadmill (Quinton Club Track 612, Bothell, Washington) (Appendix V) to determine cardiovascular endurance (maximal oxygen consumption VO$_{2max}$). Oxygen consumption, heart rate, and rating of perceived exertion were monitored throughout the test. Metabolic measures were calculated using open circuit spirometry via a computerized metabolic cart (Sensormedics Vmax 229, Yorba Linda, California). At the end of the first stage, workload was increased by increasing the elevation (grade) on the treadmill every three minutes. The protocol continued in the same manner for three minute stages until the participant reached volitional fatigue, their age-predicted maximal heart rate, asks to stop, or experiences adverse signs or symptoms (ACSM, 2010).

Fourth Session

The fourth pre-test session consisted of muscular strength, muscular endurance, and flexibility measurement used for a larger project. Muscular strength of the upper and lower body was measured by completing a 5 repetition maximum (RM) on the chest press and leg press machines (Cybex, Medway, Massachusetts). Participants completed a number of submaximal repetitions as a warm up. A weight that was within 50-70% of perceived capacity for 5 repetitions was selected, and the participant completed 5 repetitions at this weight. If 5 repetitions were successfully completed, weight increased following a 3 to 5 minute rest period and 5 more repetitions were attempted. This protocol continued until the participant could no longer complete 5 repetitions at a selected weight. Muscular endurance was measured by following ACSM push-up and curl-up test procedures. For the push-up test, participants assumed the modified "knee push-up" position, with legs together, lower leg in contact with the mat and toes pointed, back straight and hands shoulder width apart. The participant performed as many modified "knee push-ups" as possible by straightening the elbows and then lowering the body until the chin touched the mat. For the curl-up test, participants assumed a supine position on a mat with the knees at 90-degree angles. Arms were at the side with palms facing down and fingers touching a piece of masking tape. A second piece of tape was placed 10 centimeters in front of the first piece. A metronome was set to 50 beats per minute, and the participant performed curl ups in time to the metronome, lifting the shoulder blades off the mat to touch the second piece of tape. The number of curl-ups performed without pausing, or a maximum of 25, was recorded as the score. Muscular flexibility was assessed using the V-Sit and Reach test. Participants sat without shoes with the soles of the feet flat against a sit-and-reach box (flexometer) at the 26-centimeter mark. Participants slowly reached forward as far as possible, keeping the legs straight and both hands overlapped, and holding this position for approximately 2 seconds. The most distant point reached with the fingertips was recorded.

Interventions

Weight Loss: Dietary Intervention (DI)

Participants were randomly assigned to a hypocaloric dietary intervention (DI) for 9 weeks. Participants reported to the James Madison University Human Performance Laboratory once a week for a body weight measurement. The study's goal for DI participants was an 8-10% weight loss from baseline weight. Assessment of dietary and beverage intake by 24-hour Food Recall and dietary guidance administered by a registered dietician occurred pre-intervention, week six of intervention, and post-intervention.

Maximal oxygen consumption (VO_{2max}): Exercise Intervention (EXI)

Participants randomly assigned to an exercise intervention (EXI) reported to James Madison University Crawford Fitness Center 3 days per week for 45-55 minute sessions. The study's goal for the EXI participants was to increase cardiovascular endurance from baseline VO_{2max}. Cardiovascular training was initially set for each participant to train at 60% of VO_{2max} and gradually progress to training at 75-80% of max by week 9 of the intervention; participants had the option of walking, cycling, elliptical, or rowing. Resistance training consisted of a circuit of upper and lower body exercises designed to improve muscular strength and endurance. Participants trained at an intensity of that allowed each individual to perform between 8-15 repetitions (60-70% of 1RM) per set. The regimen began with one set of each exercise and progressed to 2 sets of each exercise by week 9 of the intervention. Resistance exercises were performed on Cybex equipment (Cybex, Medway, Massachusetts). Upper body exercises included chest press, bicep curl, triceps extension, and latissimus pull down. Lower body exercises will include leg extension, leg curl, leg press, and calf raises. Two additional resistance exercises were added to increase muscular strength and endurance of the core. Exercise sessions also gradually increased in time. Sessions started at 30 minutes and progressed to 60 minutes per session (30 minutes performing cardiovascular exercise and 30 minutes of resistance exercise).

Statistical Analysis

Differences in CRP levels and dependent variables (weight, BMI, fat mass, body fat percent, waist circumference, and VO_{2max}) within the DI and EXI were compared by repeated measures analysis of variance. Post-hoc paired t-tests were performed on variables that demonstrated significance between interventions to determine significance in change (pre-post). Descriptive statistics were calculated for each treatment and measurement. SPSS statistical software package (SPSS, Somers, New York) was used for all data analysis.

RESULTS

Thirty-one women responded to the study's advertisement and twenty-one women agreed to be participants in the study and completed pre-testing. Twenty sedentary, postmenopausal women (48-67 years; DI group 56 ± 4.35 and EXI group 59.8 ± 5.88 years) completed the study. One woman completed pre-testing but discontinued the study prior to the intervention phase. Ten women were randomly assigned to the diet intervention group and 10 women were randomly assigned to the exercise intervention group. Table 5 illustrates the change in dependant variables pre and post intervention.

Table 5: Differences in pre-measurements and post-measurements.

Measurement	DI Pre	DI Post	DI Δ	EXI Pre	EXI Post	EXI Δ
CRP (mg/L)	3.69 ± 2.01	2.83 ± 2.84	-0.86 ± 1.09	2.27 ± 1.97	3.07 ± 2.57	0.79 ± 1.09
Weight (kg)*	83.4 ± 17.9	79.7 ± 17.6	-4.4 ± 2.3	66.9 ± 10.3	66.5 ± 10.8	-0.3 ± 1.0
Fat Free Mass (kg)*	46.6 ± 6.4	45.5 ± 6.3	-1.0 ± 1.7	40.3 ± 4.2	40.5 ± 4.7	0.1 ± 0.5
BF %	42.7 ± 5.1	41.0 ± 5.8	-1.7 ± 1.5	38.1 ± 6.8	37.4 ± 7.0	-0.6 ± 0.8
BMI (kg/m^2)*	31.7 ± 6.6	30.1 ± 6.6	-1.6 ± 0.8	26.1 ± 4.4	26.2 ± 4.7	0.3 ± 0.8
WC (cm)*	89.3 ± 11.4	86.1 ± 11.9	-3.2 ± 2.5	81.1 ± 7.6	81.1 ± 8.6	-0.0 ± 1.9
VO_{2max} (L/min)	2.02 ± 0.30	1.99 ± 0.20	-0.02 ± 0.18	1.81 ± 0.23	1.86 ± 0.26	0.04 ± 0.12
VO_{2max} (mL/kg/min)	24.93 ± 5.87	26.19 ± 6.65	1.26 ± 2.17	27.77 ± 4.89	28.59 ± 5.30	0.82 ± 1.94

All values reported as mean ± standard deviation.
*p<0.05

There was no significant effect of time (p=0.971) or treatment (p=0.717) on CRP level. There were statistical differences in baseline weight (p=0.019, p<0.05) and baseline FFM (p=0.018, p<0.05) between the DI and EXI groups.

Figure 2 provides pre and post measurements of weight and FFM variables. There was significant time x treatment effect for both weight and FFM (p<0.05). The DI group experienced a significant decrease in weight (-4.4 ± 2.3 kg). Although there was a time x treatment effect, FFM did not change from pre- to post-testing for either the DI o EXI group (p=0.076 and p=0.369, respectively) after performing paired t-tests. Figures 3 and 4 provide pre- and post-measurements of BMI and waist circumference variables. There was a time x treatment effect for BMI (p=0.000) and waist circumference (p=0.005). The change in BMI was -1.6 ± 0.8 kg/m^2 (p=0.000) and the change in waist circumference was -3.2 ± 2.5 cm (p=0.003) within the DI group. There was no difference in BMI or waist circumference in the EXI group. No difference in body fat percentage (p=0.072) was found within groups because the objective of the EXI group was to maintain weight; these aforementioned findings were expected.

There was no difference (p<0.05) in VO_{2max} (mL/kg/min) in either of the intervention groups. The objective of the DI group was to maintain fitness level; therefore in the lack of change in VO_{2max} was expected. The participants in the EXI group were asked to report 3 days per week (27 sessions total) to complete a cardiovascular and resistance training circuit; participants attended 24.6 ± 2.59 sessions during the intervention phase. Unfortunately, the aim of the EXI group was to increase fitness and this goal was not achieved.
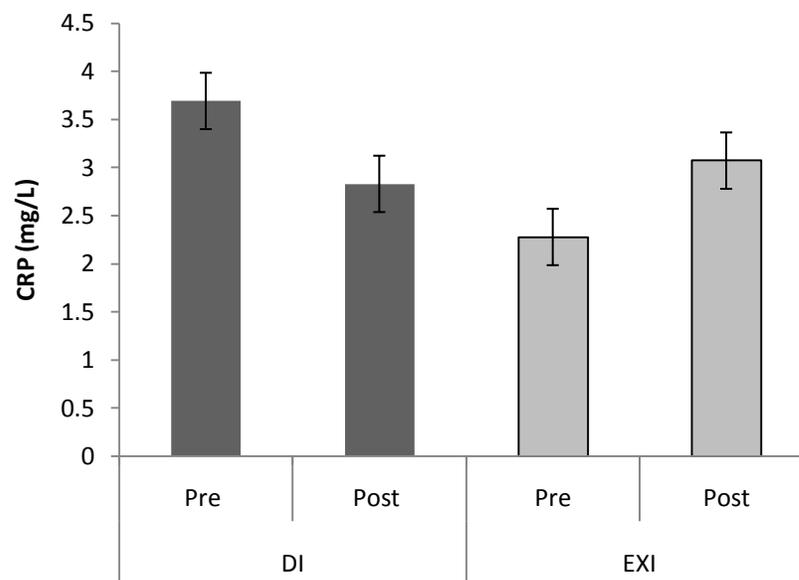
Figure 1: Changes in CRP pre and post intervention.
DI   = Dietary Intervention
EXI = Exercise Intervention
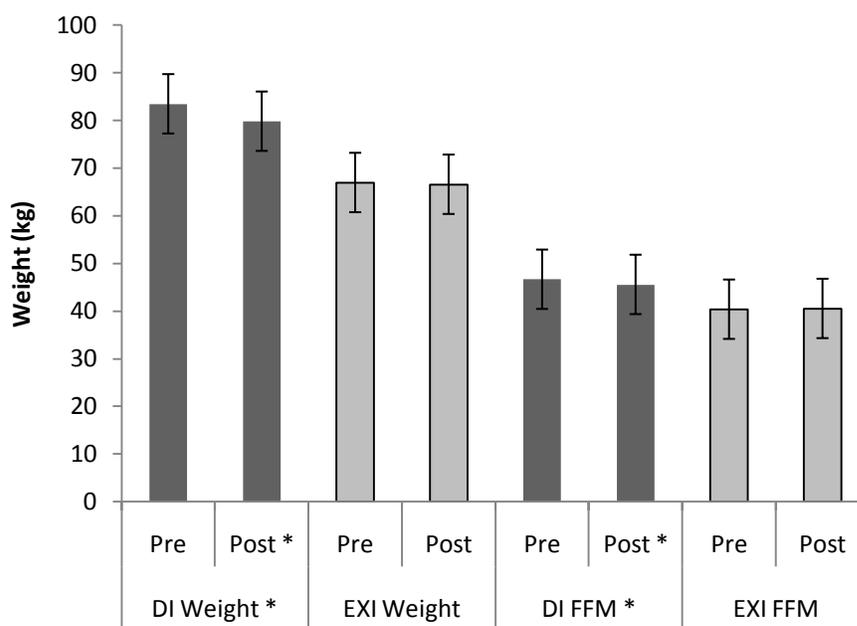
Figure 2: Pre and post measurements of body weight and fat free mass.
* $p < 0.05$
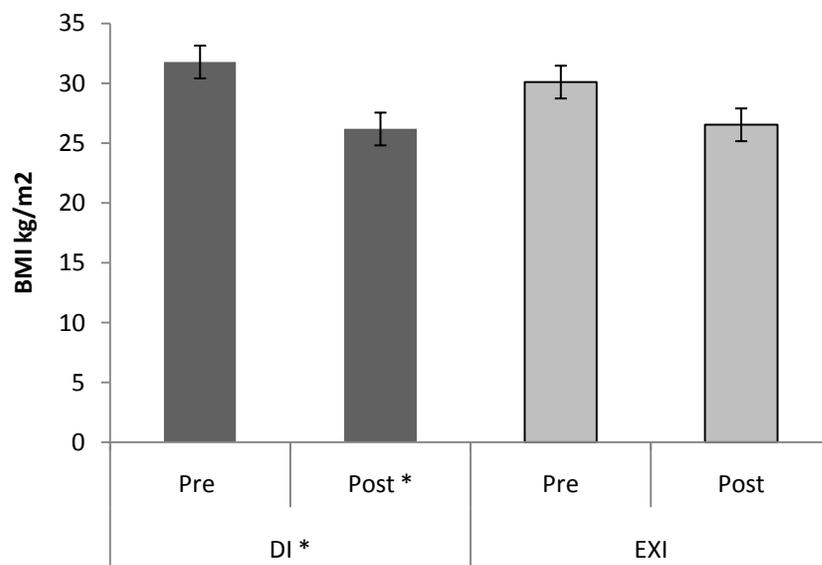
Figure 3: Pre and post measurements of body mass index.
* p < 0.05
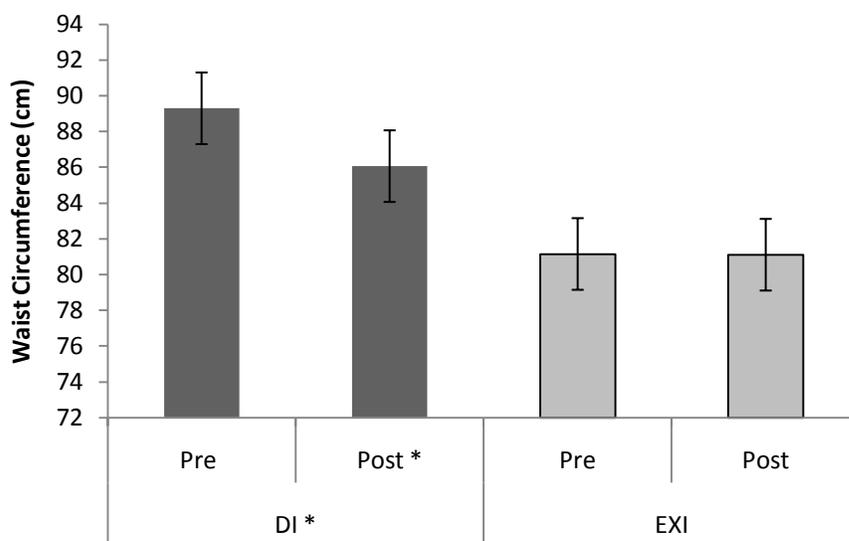
Figure 4: Pre and post measurements of waist circumference.
* p < 0.05

DISCUSSION

The purpose of this study was to compare the effects of an exercise intervention versus a dietary intervention on traditional (BMI, waist circumference) and a non-traditional (CRP) risk factor for cardiovascular disease in sedentary, postmenopausal women. Previous research has established that CRP is a predictor for cardiovascular disease (CVD) in postmenopausal women.[4,]

[25, 41, 44, 53] In addition, obesity has been determined to be a risk factor for increased risk for CVD and potentially precipitating elevated CRP.[13, 20, 54] Previous studies have reported that body weights between 92-130 kg and/or a BMI between 25-46 kg/m^2 were associated with CRP levels above 3 mg/L.[12, 18, 19, 23, 32, 39, 50, 51, 54] Individuals with a CRP level less than 2 mg/L are classified as healthy, above 3 mg/L are considered at high risk for cardiovascular events, and above 5 mg/L may be at an increased risk for a cardiac event within six months.[22, 26] Our study found that there was no change in CRP with either intervention.

Even though CRP levels did not significantly change, there was a significant difference in body weight and fat free mass in the DI group. It is possible that the participants' baseline CRP levels were classified as "at moderate risk" and that the weight loss was not great enough to elicit a significant decrease in CRP levels. It has been reported that with a greater reduction in weight loss there is a greater reduction in CRP level; Heilbronn[17, 18] reported a 26% decrease in CRP level with a 7.9 kg decrease after 12 weeks and Tchernof[50] stated a 32% decrease in CRP with a weight loss of 15.6 kg after a 14 week intervention. Thus a greater degree of weight loss (over a longer duration) may be necessary to decrease CRP levels. The BMI of participants in the DI group were classified as obese class I, but CRP levels were not classified as "at risk". Therefore, an elevated BMI may be needed to observe a greater change in CRP levels. Nicklas[34] reported that there was a significant difference in CRP among the diet-induced weight loss group; however, the baseline mean BMI was 34 kg/m^2 and mean CRP was 6.0 g/mL. The current study's baseline measurements of body weight, BMI, and CRP were lower than participants in the Nicklas[34] study.

The relationship between physical activity and CRP is not well defined. Previous studies investigating physical activity level and CRP have been primarily cross-sectional studies and the consensus appears to be that higher physical activity level are associated with a lower BMIs which results in lower CRP level.[1, 2, 3, 29, 47] To our knowledge there are no previous studies that have reported that an increase in physical activity levels of individuals with elevated BMIs results

in lower CRP levels, without concomitant changes in body weight or body fat. The investigators have not been able to distinguish the independent effects of body weight (body fat) or fitness on CRP. In the current study, physical activity only had minimal effects on the dependant measurement of body fat percentage and fat free mass due to maintenance of body weight. Decreases in body fat percentage and increases in fat free mass from weight loss through exercise are generally associated with decreases in CRP levels.[8,39] Past research regarding weight loss through exercise has concluded that there is an inverse relationship with between physical activity and BMI.[8, 31, 45] Currently, there is controversy in the literature concerning the effect that weight loss [decrease in adipose tissue] due to physical activity has on CRP level. Okita and colleagues[38] showed that CRP levels are affected by the decrease in adipose tissue from weight loss due to physical activity, while other studies reported that independent of weight loss, with an increase in physical activity there was a decrease in CRP levels.[30, 40] The findings of the current study are unique in that the exercise intervention was designed to increase fitness without weight loss in an attempt to determine the independent effect of fitness on CRP levels. The exercise program was not effective in increasing the participants VO_{2max}. There were varied adaptations to the exercise intervention. According to ACSM, a VO_{2max} value of 28.7 mL/kg/min is classified as "fair" for women between 50-60 years old. Out of the 10 women in the EXI group, 5 were classified below fair (poor) and 5 were classified as fair, good, or excellent pre-intervention. Post-intervention, 6 women increased their VO_{2max} from the EXI group. VO_{2max} did not change in the DI group regardless of the significant change in body weight; however, the objective of the DI group was to lose weight and not increase fitness. The majority of earlier investigations that have examined the relationship between physical activity and CRP have reported that low cardiorespiratory fitness is associated with elevated CRP levels. [1, 2, 3,29, 47] The current study's findings can be compared to the findings in the Nicklas[34] study in which participants were assigned to a 3 month exercise intervention that consisted of cardiovascular endurance (50-75% of heart rate reserve) and resistance training regimen for 3 days per week for 45 minutes. The authors of previously

published studies have reported that there was no significant differences in CRP levels after an exercise intervention.[34] In attempting to explain the lack on change in the CRP level in the EXI group, it may be a function of the low baseline values we measured in the study population prior to the intervention. The CRP levels measured in the current study population were not classified as "at risk". Also, it may be possible that the intensity or duration of the exercise protocol was not enough to elicit change in VO_{2max} and CRP, or the modified treadmill protocol did not effectively measure VO_{2max} in this population of sedentary, postmenopausal women considering their chief complaint of being uncomfortable during the test. Many participants stated they had a dry mouth from the mouthpiece; this uncomfortable (or unfamiliar) sensation may have made the participants stop the treadmill test prior to reaching a maximal effort. From this inconsistent information, it can be stated a physical activity intervention without weight loss has an inconclusive result on CRP levels without an adequate overload to elicit a significant increase in VO_{2max}.

Additional variables that were measured included BMI, body fat percentage, fat free mass, and waist circumference. Previous studies concluded that a lower BMI is correlated with lower CRP levels.[18, 19, 32, 34, 50] Studies that examined weight loss through physical activity have concluded that a 2-5 kg/m^2 decrease in BMI, a 2-3% decrease in body fat percentage, a 1-3 kg decrease in fat free mass, and 3-4% decrease in waist circumference were associated with a decrease in CRP level.[19, 45, 50, 52, 55] With the significant changes in BMI (decrease 1.7 ± 0.8 kg/m^2) and waist circumference (decrease 3.2 ± 2.5 cm) in the DI, it was not a great enough change to elicit a significant difference in CRP level. Since the participants had an "at risk" BMI but not an "at risk" waist circumference or CRP level, it is possible that along with a BMI over 30 kg/m^2, waist circumference should be greater than 88 cm and CRP levels need to be above 3 mg/L to observe a significant change.

Limitations

The findings presented above should be interpreted with caution in view of the limitations of the current study. A greater number of participants may have resulted in the observance of larger differences in dependant measures between the two intervention groups. Another limitation was the differences in the baseline measurements between the DI and EXI groups. Although all participants were categorized as sedentary their BMI values ranged from healthy to obese. Also, participants were volunteers that did not smoke and may have had a physical activity history prior to the past 12 months; the pre-intervention profiles may not have permitted for a statistical difference in CRP. Perhaps more importantly, the sequence of baseline and post-testing sessions could have influenced CRP levels; some participants performed the VO_{2max} or muscular fitness tests less than 24 hours prior to the fasted blood draw. By performing exercise within 48 hours of the blood draw this may have a negatively influenced the CRP levels. Finally, the compliance of the DI participants with the initial weight loss goal of 8-10% from baseline weight was a limitation; of the ten participant in the DI group, two women succeeded in achieving this goal, while four other women achieved a weight loss between 5-6%.

Conclusion

Our study found that there was no significance in the change in CRP within the DI or EXI group. The objective of decreasing weight was achieved because there was a significant difference in body weight, fat free mass, BMI, and waist circumference within the DI group. These differences were not great enough to have a significant difference in CRP level though. The objective of increasing VO_{2max} was not achieved within the EXI group. More research is needed to determine if an increase in fitness level without weight loss has an impact on CRP levels.

Recommendations for Future

The first recommendation for future studies would be to recruit a greater number of participants to form larger intervention groups and/or have a control group. The second recommendation would be to have inclusion criteria in which participants are within overweight/obese category for

BMI and are at poor to very poor levels of cardiovascular endurance (VO$_{2max}$). Finally, a physical activity intervention study with a control group will enable an investigation of CRP levels with increased physical activity level with weight maintenance will clarify the relationship.

Chapter V

Summary

C-reactive protein is an inflammatory marker that is an independent indicator of risk for stroke, cardiovascular disease, and vascular death among postmenopausal women without any known vascular diseases and may be a stronger predictor for CV events compared to LDL-C levels.[5, 11, 42, 43] Risk factors for CRP in women are age, smoking (leading cause), hypertension, dyslipidemia, obesity, sedentary lifestyle, and menopause.[6, 25, 33, 44, 46] CRP levels have been shown to increase with age; therefore, controlling BMI and physical activity level may help to minimize the age dependent rise in CPR levels.[22, 28] CRP is highly correlated with BMI; and several studies have reported a strong correlation between total fat mass and visceral fat and CRP.[17, 18, 23, 52] Increased abdominal adiposity, associated with obesity, is a risk factor for CVD in women; the prevalence of obesity has increased over the past decade and currently one out of three women is classified as obese. Obesity is a parallel factor to sedentary lifestyle, and studies have shown that moderate physical activity reduces the risk of CVD by 50% in women. Schenck[46] reported in 2009 that 30% of women are overweight and 27% are classified as obese, and the risk for CVD in obese women increases 3-fold compared to lean women.[20, 25, 33] Menopause decreases the estrogen levels and increases testosterone levels in postmenopausal women negatively affecting cardiovascular functions and metabolism resulting in an increase risk for CVD event, such as dyslipidemia resulting from increased testosterone. Currently practitioners consider CRP value of less than 2 mg/L as healthy, and individuals who have CRP levels of 5mg/L or greater may be at an increased risk for a cardiac event within six months.[22, 26]

Studies have been conducted on the obese, postmenopausal population and older men and women to determine if weight loss through diet, physical activity, and/or physical activity with weight loss affects CRP levels. Lifestyles changes, especially physical activity and diet, can promote significant improvements of CVD risk factors, such as decreased CRP levels. Weight loss through low calorie diet has been shown to reduce CRP levels in obese, postmenopausal

women since the in CRP levels is primarily associated with loss in fat mass during weight loss.[50] There is fairly consistent evidence that physical activity is associated with decrease in CRP levels and lower risk of CVD. There is an inverse relationship between activity level and CVD risk and CRP level; as activity levels increase, CRP levels decrease.[3, 10, 49] Also, CRP levels decrease with more vigorous exercise or more frequent exercise.[1, 3, 49] Along with decreased inflammatory markers, decreased weight, body fat percentage, adipose tissue, and BMI result from increased physical activity.[8, 45] Previous studies have solely investigated changes of CRP levels resulting from weight loss through dietary alterations or weight loss through exercise. It is unknown if weight loss or exercise status alone have an effect on CRP levels in sedentary, postmenopausal women. The purpose of this study was to compare the effects of an exercise intervention versus a dietary intervention on traditional (BMI, percent body fat, waist circumference, physical activity level) and non-traditional (CRP) risk factors for cardiovascular disease in sedentary, post menopausal women.

Thirty-one women responded to the initial request for study volunteers. Twenty-one postmenopausal women who were recruited from the James Madison University and Harrisonburg Rockingham County communities volunteered as participants for this study. Persons who showed interest in participating were screened to see if they met the minimum criteria for entrance into the study. The criteria for inclusion included: Apparently healthy (no diagnoses of cardiovascular disease), female, post-menopausal (no regular menstrual cycle in the past 12 months), and previously sedentary (no regular exercise (<2 days per week) in the past 6 months). Participation in the study was entirely voluntary and withdrawal at anytime was allowed without any consequences. All study protocols and procedures were approved by the Institutional Review Board. Pre and post testing measurements were completed during separate four sessions. During the first pre-test session all test procedures and protocols were explained to the participants who completed an Informed Consent Form, Health Status Questionnaire, and PARQ. In addition, all participants were given a Dietary 24-hour Food Recall supervised by a registered

dietitian. After an overnight fast, venous blood draw, measurement of resting blood pressure, and body composition assessment via dual-energy x-ray absorptiometry. Blood obtained from the anticubital vein was analyzed for CRP. The third session included a graded walking test on a treadmill to determine cardiovascular endurance (maximal oxygen consumption VO_{2max}). Oxygen consumption, heart rate and rating, of perceived exertion (RPE) were monitored throughout the test. The fourth pre-test session consisted of muscular strength, muscular endurance, and flexibility measurement used for a larger project.

The protocol in this study was designed to determine the independent effects of weight loss initiated through dietary changes and fitness (VO_{2max}) without weight loss on CRP levels in postmenopausal women. Participants were randomly assigned to a diet intervention (DI) or exercise intervention (EXI) group. The objective of the DI was to produce an 8-10% weight loss during the 9 week intervention through a 1,200 kcal/day diet. Participants reported once a week for a body weight measurement, and assessment of dietary and beverage intake by 24-hour Food Recall and dietary guidance occurred pre-intervention, week six of intervention, and post-intervention. The objective of the EXI group was to increase cardiovascular fitness. Participants in the EXI group were asked to report 3 days per week for 45-55 minute sessions. Cardiovascular training was initially set for each participant to train at 60% of VO_{2max} and gradually progress to training at 75-80% of max by week 9 of the intervention. Resistance training consisted of a circuit of upper and lower body exercises designed to improve muscular strength and endurance. Participants trained at an intensity of that allowed each individual to perform between 8-15 repetitions (60-70% of 1RM) per set. Sessions started at 30 minutes and progressed to 60 minutes per session.

There was no significant effect of time ($p=0.971$) or treatment ($p=0.717$) on CRP level. There were statistical differences in baseline weight ($p=0.019$, $p<0.05$) and baseline FFM ($p=0.018$, $p<0.05$) between the DI and EXI groups.

There was significant difference with weight within (p=0.000) and FFM (p=0.04) and between (p=0.031 and p=0.03) groups following the intervention. The DI group experienced significant difference in change (Δ) in weight -4.4 ± 2.3 kg (p=0.000). Although significance was found in FFM within groups, no significance was found in the DI o EXI group (p=0.076 and p=0.369, respectively) after performing a paired t-test. Figure 2 provides pre and post measurements of weight and FFM variables. There was significant difference in BMI (p=0.000) and waist circumference (p=0.005), but there was not a difference between interventions (p=0.090) and (p=0.161), respectively. The change in BMI was -1.6 ± 0.8 kg/m^2 (p=0.000) and the change in waist circumference was -3.2 ± 2.5 cm (p=0.003) within the DI group. There was no difference in BMI or waist circumference in the EXI group. Figures 3 and 4 provide pre and post measurements of BMI and waist circumference variables. No difference in body fat percentage (p=0.072) was found within groups. The objective of the EXI group was to maintain weight; therefore, having no significance in body weight, BMI, or waist circumference is a positive result.

Regarding fitness level, there was no difference in VO$_{2max}$ (mL/kg/min) within (p=0.639) or between (p=0.312) intervention groups. The objective of the DI group was to maintain fitness level; therefore no significant difference in VO$_{2max}$ is a positive result. Unfortunately, the aim of the EXI group was to increase fitness and this goal was not achieved.

Even though significance was not found in CRP levels, there was a significant difference in body weight and fat free mass in the DI group. It is possible that the participants' baseline CRP levels were classified as "at moderate risk" and that the weight loss was not great enough to elicit a significant decrease in CRP levels. It has been reported that with a greater reduction in weight loss there is a greater reduction in CRP level; Heilbronn[17, 18] reported a 26% decrease in CRP level with a 7.9 kg decrease after 12 weeks and Tchernof [50] stated a 32% decrease in CRP with a weight loss of 15.6 kg after a 14 week intervention. Thus a greater degree of weight loss (over a longer duration) may be necessary to decrease CRP levels. The BMI of participants in the

DI group were classified as obese class I, but CRP levels were not classified as "at risk". Therefore, an elevated BMI may be needed to observe a greater change in CRP levels. The current study's baseline measurements of body weight, BMI, and CRP were lower than participants in the Nicklas[34] study. Nicklas[34] reported that there was a significant difference in CRP among the diet-induced weight loss group; however, the baseline mean BMI was 34 kg/m^2 and mean CRP was 6.0 g/mL.

To our knowledge there are no previous studies that have reported that an increase in physical activity levels of individuals with elevated BMIs results in lower CRP levels, without concomitant changed in body weight or body fat. In the current study, physical activity did not elicit a significant change in body fat percentage and fat free mass due to maintenance of body weight. Decreases in body fat percentage and increases in fat free mass from weight loss through exercise are generally associated with decreases in CRP levels.[8,39] The majority of previously published studies that have investigated the relationship between physical activity and CRP have reported that low cardiorespiratory fitness is associated with elevated CRP levels.[1, 2, 3,29, 47] The current study's findings can be compared to the findings in the Nicklas[34] study in which participants were assigned to a 3 month exercise intervention that consisted of cardiovascular endurance (50-75% of heart rate reserve) and resistance training regimen for 3 days per week for 45 minutes.

Our study found that there was no significance in the change in CRP within the DI or EXI group. The objective of decreasing weight was achieved because there was a significant difference in body weight, fat free mass, BMI, and waist circumference within the DI group. However, these changes were not great enough to precipitate a significant difference in CRP, which may be related to the initial low levels demonstrated in the study population. The objective of increasing VO$_{2max}$ was not achieved within the EXI group and thus it is not surprising that CRP did not change in addition to the fact that again the levels in the group were not considered "at risk" or elevated. More research, through intervention studies, is needed to

determine if there is an impact on CRP levels with an increase in fitness level without weight

loss.

Appendix I

James Madison University
Departments of Kinesiology and Health Sciences
Consent for Investigative Procedure
(Informed Consent)

You are being asked to participate in a research study Women Inflammation Atherosclerosis and Thrombosis (WAIAT) conducted by Drs. Judith A. Flohr, Jeremy Akers, Nicholas Luden, R. Theresa Prodoehl and Christopher J. Womack from the Departments of Kinesiology and Health Sciences at James Madison University. The purpose of this study is to determine the effects of diet induced weight loss and exercise on traditional (blood pressure, blood lipids, BMI, blood glucose) and nontraditional risk factors (markers of chronic inflammation hsCRP, IL-6, fibrinolysis profile (blood clotting potential), endothelial (lining/wall of blood vessel function) function, and sleep quality) for cardiovascular disease (CVD) by using a randomized controlled intervention trial in post menopausal women.

Research Procedures:
The study will consist of various supervised lab tests and questionnaires to measure the physical fitness, health status, lifestyle behaviors, body composition, blood lipids and blood glucose, and risk for cardiovascular disease in women.

Note: In the event you discover that any of the following information is not clear, please ask one of the investigators to explain immediately.

In this study I understand I will be randomly assigned to one of the following intervention groups
Exercise Intervention (Training Protocol) Participants in this group will follow the program described below

> Frequency- Three (3-5) days per week, 45-55 minutes/session (Participants will be asked to complete 3 to5 supervised sessions per week.
> Intensity- Cardiovascular training will initially be set so that each participant is training at 60% of her VO_{2max} (maximal oxygen consumption) and gradually progress to training at 75-80% of max by week 10 of the intervention. Resistance training- participants will perform a circuit of upper and lower body exercises designed to improve muscular strength and endurance. Participants will train at an intensity of that allows each individual to perform between 8-15 repetitions (60-70% of 1 Repetition Maximum (1RM) per set. The program will start with one set of each exercise and progress to 2 sets of each exercise by week 10 of the intervention.
> Time- Exercise sessions will start at 30 minutes and progress so that by week 10 of the intervention each participant is spending approximately 20 minutes performing cardiovascular exercise and 20 minutes of resistance exercise during each exercise session for a total of 40 minutes per session.
> Type-Cardiovascular- Participants will have the option of walking, cycling, elliptical or rowing as modes of cardiovascular training. Resistance exercises will be performed on selectorized equipment. Upper body exercises will include chest press, bicep curl, triceps extension and lat pull down. Lower body exercises will include leg extension, leg curl, leg press and calf raises. Two additional resistance exercises will be added to increase muscular strength and endurance of the core (i.e. abdomen and back).
> Maintenance of weight- Participants in the EXI group will be asked to maintain baseline body weight. Dietary 24-hr Food Recalls will be given to assess dietary and beverage intake.

Dietary Intervention Participants in this group will be asked to come to the Human Performance Laboratory (Godwin Hall 209) once a week for a body weight measure, assessment of dietary and beverage intake by 24-hr Food Intake Recall, and dietary guidance.

In this study I understand the following measurements and tests will be taken:

Blood Pressure. Blood pressure measurements will be taken using a sphygmomanometer and stethoscope. A blood pressure cuff will be placed over the brachial artery of the right arm, slightly above the elbow.

Blood Draws: A blood sample (10 milliliters, 2 teaspoons) will be obtained from each participant via venapuncture of the antecubital vein during the test sessions #2 and #5. The following procedures will be followed to minimize the transfer of blood-borne pathogens: investigators will wear latex gloves at all times during blood sampling and testing. For each venous blood draw, the following measurements will be taken: Blood lipids (total cholesterol (TCHOL), low density lipoprotein cholesterol (LDL-C) high density lipoprotein cholesterol (HDL-C), triglycerides (TRIG), Hematocrit (packed cell volume), Hemoglobin (Hb), markers of inflammation (High sensitivity C-Reactive Protein (hsCRP),Interleukin 6 (IL-6)), Blood clotting factors (Tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI), vonWillenbrand factor) and blood glucose.

Health Status Health status will be determined via questionnaires and ACSM's Risk Stratification for Cardiovascular Disease Risk (ACSM, 2010). Assessment of a woman's risk for cardiovascular disease will be made using the ACSM's Coronary Artery Disease Risk Factor Thresholds along with the ACSM Risk Stratification. Risk stratification is based upon age, family history, smoking habits, blood lipid levels, and fasted blood glucose values, resting blood pressure, body mass index and physical activity habits.

Health Related Quality of Life (HRQOL-4) –Health related quality of life status will be assessed using a 4 item questionnaire developed by the Centers for Disease Prevention and Control. The CDC's HRQOL-4, also referred to as the Healthy Days Core Module, will assess when the participant has felt that her physical or mental health was not good in the previous 30 days

Physical Fitness – A series of physical fitness tests will be administered. The components of fitness to be measured include cardiovascular endurance, muscular strength and endurance, and muscular flexibility.

a. *Cardiovascular endurance*. Cardiovascular endurance will be assessed through a walking test performed on a treadmill. Oxygen consumption, blood pressure, heart rate and rating of perceived exertion (RPE) will be monitored throughout the test. The workload will be increased gradually by raising the elevation (grade) on the treadmill every three minutes. The test will continue in the same manner every three minutes until the participant reaches volitional (voluntary) fatigue, their age-predicted maximal heart rate or asks to stop.

b. *Muscular strength*. Upper body strength will be assessed using the chest press machine. You will be asked to perform five repetitions on the chest press; the weight will be increased until you can no longer complete five repetitions or at the point you wish to stop the test. Lower body strength will be assessed using the leg press machine and will follow the same steps and the bench press test. A minimal level of discomfort is associated with the completion of the muscular strength tests due to muscle soreness, which may be felt 24-72 hours after the completion of the test.

c. *Muscular endurance*. Muscular endurance will be measured by following ACSM (2010) push-up and curl-up test procedures. For the push-up test, participants assume the modified "knee push-up" position, with legs together, lower leg in contact with the mat and toes pointed, back straight and hands shoulder width apart. The participant performs as many modified "knee push-ups" as possible by straightening the elbows and then lowering the body until the chin touches the mat. The maximal number of push-ups performed correctly and without rest recorded as the score (ACSM, 2010). For the curl-up test, participants will assume a supine (face up) position on a mat with the knees at 90-degree angles. Arms will be at the side with palms facing down and fingers touching a piece of masking tape. A second piece of tape is placed 10 centimeters in front of the first piece. A metronome is set to 50 beats per minute, and the participant does curl ups in time to the metronome, lifting the shoulder blades off the mat to touch the second piece of tape. The number of curl-ups performed without pausing, or a maximum or 25, is recorded as the score (ACSM 2010).

d. *Muscular flexibility*. Muscular flexibility will be assessed using the V-Sit and Reach test. Participants will sit without shoes with the soles of the feet flat against a sit-and-reach box (flexometer) at the 26-centimeter mark. Participants will slowly reach forward as far as possible, keeping the legs straight and both hands overlapped, and holding this position for approximately 2 seconds.

Lifestyle Behaviors

    a. *Exercise Behavior* – The volume of physical activity that the participant engaged in prior to enrolling in the study will be estimated using the International Physical Activity Questionnaire (IPAQ). The questionnaire consists of 5 activity domains, asked independently, in which the participant reports about the time spend being physically active in the last 7 days.

    b. Dietary Behavior –Dietary 24-hr Food Recalls will be taken from study participants to assess dietary intake including beverage consumption. Participants in both groups will be given proper dietary interventions by a Registered Dietitian. Two-dimensional food diagrams will be provided to assist participants in portion size determination. All food and beverage records will be reviewed for accuracy and completeness and analyzed using the NutritionistPro nutrition analysis software.

    c. *Sleep Quality* –Participants will complete the Pittsburgh Sleep Quality Index (PSQI) which has 19 self-rated questions about sleep over the past month

    d. Daytime Sleepiness-Participants will complete the Epworth Sleepiness Scale (ESS) which has 8 self-rated questions about tendency to doze during daytime.

    e. *Other Lifestyle Behaviors* – Additional lifestyle behaviors will be assessed using the U.S Department of Health and Human Services Health Behavior Questionnaire. The other behaviors being assessed include smoking status, use of alcohol and drugs, and stress.

Body Weight and Composition. Height and body weight of all participants will be measured to calculate body mass index BMI (kgs/m^2). Dual-energy x-ray absorptiometry (DEXA) will be used to estimate body composition data through the whole body scan. Assessment will take place using the Hologic® DELPHI-W. The participant will be asked to lie on their back, positioned on the machine according to protocol. They will be asked to lie completely still, while breathing normally, and closing their eyes while the scan absorptiometry is in progress. The whole body scan lasts approximately 6 minutes. The DEXA analysis provides the most accurate method available to assess body composition. According to the manufacture's specifications (i.e., Hologic Inc.), whole body DXA analysis exposes participants to 1.5 mrem of radiation. The exposure to radiation during a single chest x-ray (i.e., 5 mrem) is more than 3 times greater than radiation from DEXA. Also, background radiation from DEXA is about equal to the amount of radiation one experiences during a flight from New York to London. Please note that the effects of the DEXA scan are cumulative depending on your prior exposure to radiation. If you have questions regarding your risk from the scan please consult with the investigators.

Time Required:

Total time to complete the test sessions is estimated to be: Test session #1 (Orientation session, 1 hour), Test session #2 and #4 (1 hour each), and test sessions #3 and 5 (1 hour each). You will be allowed to rest as long as necessary between exercise testing stations. Participants in the Exercise Group will be asked to attend 3-4 exercise sessions (45-55 minutes in duration) for 10 weeks. Participants in the Dietary Intervention will be asked to come to the Human Performance Lab in Godwin once a week (30 minutes) for a body weight measure, assessment of dietary and beverage intake by 24-hr Food Intake Recall, and dietary guidance.

Risks:

There is minimal risk associated with exercise testing in individuals who are "low risk" according to guidelines established by the American College of Sports Medicine. There is a minimal level of discomfort that may be experienced during the exercise testing, which includes muscle soreness and fatigue. Muscle soreness may be felt 24-48 hours following the testing.

The risks of venipuncture blood sampling include possible mild bruising and the risk of transfer of blood-borne pathogens. This risk is considered to be minimal, and all safety precautions for handing blood samples will be followed according to Occupational Health and Safety Administration (OSHA) protocols.

Benefits:

You will receive the results from your individual fitness testing, including a rating of how you compare to females within your age category. You will also receive information regarding your current risk factors for cardiovascular disease, Type II diabetes, osteoporosis and cancer.

Confidentiality:
All data and results will be kept confidential. Participants will be assigned an identification code. At no time will a participant's name be identified with individual data. All data will be secured in a locked cabinet in a locked office. Upon completion of the study, all information that matches up individual respondents with their answers or fitness test results will be destroyed.

Participation and Withdrawal:
Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Reporting Procedures:
Participants will be provided health and fitness tests data at the completion of each test. The findings of the study will be presented at regional and national organizations conferences and submitted for publication in professional journals

Questions about the Study:
If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

Dr. Judith A. Flohr, Ph.D
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540-568-3448

Questions about Your Rights as a Research Subject
Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Giving of Consent
I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

My initials below signify that I agree to participate in the test for the phase(s) I have indicated above.

_____
Name of Participant (Printed)

_____ _____
Name of Participant (Signed)   Date

_____
Name of Witness (Printed)

_____ _____
Name of Witness (Signed)   Date

Appendix II

James Madison University
Department of Kinesiology
Women Atherosclerosis Inflammation and Thrombosis (WAIAT)
Health Status Questionnaire

Instructions: Complete each question accurately. All information provided is **confidential.**
Part I Demographic Information

1. _____   _____
        ID# _____   Date

2. _____   _____
        Legal Name   Nickname

3. _____   _____
        Mailing Address (Local Address)   Local Phone

   _____   _____

4. _____   _____
        Permanent Address (Home)   Phone

   _____   _____

5. Gender (circle one)   Male   Female

6. Date of Birth   _____
                            Month/ Day/ Year

Part II Medical History
7. Circle any that died of heart attack before age 50   Father   Mother   Brother   Sister   Grandparent

8. Date of Last medical exam: _____   Last physical fitness test: _____

9. Circle operations you have had: Back   Heart   Kidney   Eyes   Joint   Neck   Ears   Hernia

                                     Lung   Other   _____

10. Please circle any of the following for which you have been diagnosed of treated by a physician or
health professional:

Alcoholism	Diabetes	Kidney problems
Anemia (sickle cell)	Emphysema	Mental Illness
Anemia (other)	Epilepsy	Neck Strain
Asthma	Eye Problems	Obesity
Back Strain	Gout	Phlebitis
Bleeding trait	Hearing Loss	Rheumatoid arthritis
Bronchitis, chronic	Heart Problem	Stroke
Cancer	High Blood Pressure	Thyroid problem
Cirrhosis, liver	Hypoglycemia	Ulcer
Concussion	Hyperlipidemia	Other _____
Congenital defect	Infectious Mononucleosis	

11. Circle all medications taken in the last six months:

Blood thinner   Epilepsy medication   Nitroglycerin
Diabetic pill   Heart-rhythm medication   Other _____
Digitalis   High-blood pressure medication
Diuretic   Insulin

12. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

5 = Very often   4 = Fairly often   3 = Sometimes   2 = Infrequently   1 = practically never

a. cough up blood   f. chest pain
  1 2 3 4 5   1 2 3 4 5

b. abdominal pain   g. swollen joints
  1 2 3 4 5   1 2 3 4 5

c. low back pain   h. feels faint
  1 2 3 4 5   1 2 3 4 5

d. leg pain   i. Dizziness
  1 2 3 4 5   1 2 3 4 5

e. arm or shoulder pain   j. breathless on slight exertion
  1 2 3 4 5   1 2 3 4 5

Part III Health Related Behavior

13. Do you smoke?   Yes   No

14. If you are a smoker, indicate the number smoked per day:

Cigarettes: 40 or more   20-39   10-19   1-9
Cigars or pipes only: 5 or more or any inhaled   less than 5, none inhaled

15. Do you exercise regularly?   Yes   No

16. How many times in a week do you spend at least 20 minutes in moderate to strenuous exercise?

    1   2   3   4   5   6   7   days per week

17. Can you walk 4 miles briskly without fatigue?   Yes   No

18. Can you jog 3 miles continuously at a moderate pace without discomfort?   Yes   No

19. Weight now: _____ lb.   One year ago: _____ lb.   Age 21: _____ lb.

Source: Howley and Franks. (2007). *Health Fitness Instructor's Handbook*. Health Status Questionnaire. Champaign: Human Kinetics.

Appendix III

James Madison University
Department of Kinesiology
**Women Atherosclerosis Inflammation and Thrombosis (WAIAT)
Physical Activity Readiness Questionnaire**

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of <u>any other reason</u> why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____   DATE_____

SIGNATURE OF PARENT _____   WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix IV

James Madison University
Department of Kinesiology
Women Atherosclerosis Inflammation and Thrombosis (WAIAT)
Food Intake Record

Name:_____   **Date:**_____   **Day: 1 2 3** (*circle one*)
Notes:_____

Time	Location	DETAILED Description (Be as detailed as possible in describing the food item. For example not just chicken, but how it's cooked (fried, grilled, baked, etc), and include any sauces or dressings put on any food item. Remember beverages too.)	Amount EATEN (*be specific*)	User Notes

Appendix V

James Madison University
Department of Kinesiology
Women Atherosclerosis Inflammation and Thrombosis (WAIAT)
Maximal Oxygen Consumption Data

Study ID # _____   Date _____ Time of Day
_____

Height _____(in) _____(cm) Weight _____(lb) _____(kg) Date of Birth
_____

Age _____   Resting HR _____bpm   Resting BP
_____/_____mmHg

Modified Balke Protocol

Time (min)	Speed (mph)	Grade (%)	Heart Rate	VO$_2$ L/min	VCO$_2$ L/min	RPE	Comments
WU 1	2-3						
WU 2	2-3	0%					
WU 3	2-3						
WU 4	2-3						
WU 5	2-3						
E 1	3.0	3%					
E 2	3.0						
E3	3.0	6%					
E4	3.0						
E5	3.0	9%					
E6	3.0						
E7	3.0	12%					
E8	3.0						
E9	3.0	15%					
E 10	3.0						
E 11	3.3	15%					
E 12	3.3						
E 13	3.6	15%					
E 14	3.6						
E 15	3.9	15%					
E 16	3.9						
CD 1	3.0						
CD 2	3.0						
CD 3	3.0	0%					
CD 4	3.0						
CD 5	3.0						

VO$_2$ Max(L/min): _____ VO$_2$ Max(mL/min):_____RER:
_____

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