ASSOCIATIONS BETWEEN C-REACTIVE PROTEIN, PHYSICAL ACTIVITY, AND OTHER CARDIAC RISK FACTORS IN POSTMENOPAUSAL WOMEN

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Coronary heart disease (CHD) is the single largest killer of American females. Prevalent risk factors that give rise to increasing coronary risk for women include markers of inflammation, such as C-reactive protein (CRP), lipid abnormalities, such as lipoprotein(a), and insulin resistance. Also, rates of CHD are relatively low among premenopausal women but increase sharply with age and the onset of menopause. The primary purpose of this study was to examine the associations between CRP and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease CHD. A cohort of 201 postmenopausal, 52-62 year old women who are enrolled in the Women On the Move through Activity and Nutrition (WOMAN) Study was examined at the baseline evaluation. Spearman correlations revealed a significant inverse relationship between CRP and mean pedometer steps (rho= -0.2441, p=0.0348). Significant positive correlations were identified between CRP and body mass index (rho= 0.3081, p<0.0001), waist circumference (rho= 0.25711, p<0.0002), and triglycerides (rho= 0.1925, p=0.0063). Women taking hormone replacement therapy had significantly higher levels of CRP (p=0.0216) than those women not on hormone therapy. There was no significant relationship found between CRP and intima medial thickness, nor with coronary artery calcium score. The women identified with metabolic syndrome by ATPIII

guidelines had significantly lower HDL-cholesterol levels ($p<0.0001$) and higher total cholesterol ($p=0.0072$), triglycerides ($p<0.0001$), glucose ($p<0.0001$), and insulin ($p=0.0183$) levels. These women also had significantly higher body mass index ($p=0.0210$), systolic blood pressure ($p<0.0001$), carotid intimal medial thickness ($p=0.0378$) and coronary calcium score ($p=0.0125$).

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PREFACE

First, I would like to thank the members of my dissertation committee: Dr's Fred Goss, Andrea Kriska, Robert Robertson, and Betsy Nagle for enhancing the quality of this project. I am grateful for having the opportunity to work with the WOMAN study. A special thanks to a fellow doctoral student, Kelley Pettee, whose knowledge of the WOMAN study and SAS was invaluable.

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1.0 THE PROBLEM

1.1 Introduction

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD). This chapter is separated into the following sections: (1) Rationale, (2) Statement of the Problem, (3) Significance of the Problem, (4) Specific Aims and Hypotheses, and (5) Summary.

1.2 Rationale

Coronary heart disease (CHD) is the single largest killer of American males and females. Although there has been a decreased trend in mortality observed in males, the gap between male and female deaths has increased dramatically (3). The reasons for this gender disparity are not well understood. Most epidemiological studies examining CHD have concentrated primarily on males but suggest that the risk factor profile in men and women differ. Prevalent risk factors that give rise to increasing coronary risk for women include markers of inflammation, such as C-reactive protein (CRP), lipid abnormalities, such as lipoprotein(a), and insulin resistance (106). Also, rates of CHD are relatively low among premenopausal women but increase sharply with age and the onset of menopause.

Coronary heart disease is increasingly being viewed as an inflammatory process. Previous research has suggested that there is a relation between the inflammatory process and atherosclerosis (39,54,81). It is suggested that high sensitivity C-reactive protein, a marker of inflammation, becomes elevated with atherogenesis and therefore can aid in prediction of CHD risk (54). Elevated levels of CRP have been associated with increased risk of cardiovascular disease in apparently healthy individuals (50,73,76). It is well known that low to moderate levels of daily physical activity help reduce CHD by improving many of the major predisposing and conditional risk factors (41). Individuals diagnosed with CHD tend to have a clustering of modifiable risk factors; therefore regular physical activity can contribute to primary prevention through metabolic and circulatory mechanisms. There is also evidence that higher levels of physical activity are associated with lower levels of C-reactive protein (1,25). Although, the physiological mechanism responsible for this pattern is unclear, one could hypothesize that physical activity reduces the risk of CHD by reducing or preventing the inflammatory process.

Previous research examining the relation between physical activity and CRP levels has had limitations. First, many studies lack a reliable measure of physical activity. The larger epidemiological studies utilize self-reported levels of physical activity by multiple methods that make it hard to compare studies. Secondly, some investigations did not control for potentially confounding variables that may have elevated baseline levels of CRP such as not excluding individuals with an inflammatory disease (i.e. rheumatoid arthritis, asthma, bronchitis, emphysema, cancer, or stroke), and the lack of adjusting for obesity among the subjects.

Since most risk factors for CHD are modifiable, lifestyle changes could conceivably decrease the incidence of disease or at least slow the process. However, little is known about the associations between physical activity and these newly emerging risk factors in postmenopausal

females. The conceptual understanding of risk factor modification and the differences in risk between the genders is important from a public health perspective. Since CHD is considered to be in part preventable, it is imperative to educate the public regarding lifestyle modification.

This investigation may provide additional evidence of the role physical activity has in the primary prevention of CHD in postmenopausal women. In addition, this investigation may provide evidence of the relation between the traditional and non-traditional risk factors and the role of hormone replacement therapy in risk prediction. Collectively, this information could change the public health approach to the primary prevention of coronary heart disease in women.

1.3 Statement of the Problem

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD).

1.4 Significance of the Problem

Coronary heart disease is considered a multifactorial disease because a wide array of variables is involved in the etiology of the disease. For this reason, many clinicians find that risk assessment is an important tool to estimate the probability that individuals with certain risk factors will develop CHD. This information may promote prevention and early treatment of the disease. The longitudinal Framingham Heart Study has contributed significantly to cardiovascular research and risk factor analysis (18).

The Framingham Coronary Heart Disease Risk Score (FCRS) was created using a multiple logistic model and has identified risk factors for CHD. These risk factors are categorized as 1) major, 2) predisposing, or 3) conditional (105). The major risk factors include: cigarette smoking, elevated blood pressure, elevated total and low density lipoprotein cholesterol (LDL), low serum high density lipoprotein cholesterol (HDL), the presence of diabetes mellitus, and age (103). The predisposing risk factors include: obesity, physical inactivity, family history of premature CHD, ethnic characteristics, and psychosocial factors. Lastly, conditional risk factors include: elevated serum triglycerides, small LDL-C particle size, elevated serum homocysteine, elevated serum lipoprotein(a), prothrombotic factors, and inflammatory markers (103). These conditional risk factors are associated with an increased risk for CHD yet there is not conclusive evidence of their causative, independent, and quantitative contributions to the disease state (27).

Although widely perceived as an important clinical tool, the Framingham prediction model may not identify all persons at high risk for coronary heart disease. Thus, many practitioners are including non-traditional risk factors, such as CRP, into their global cardiovascular disease risk prediction. C-reactive protein levels have been shown to be significantly associated with the level of coronary heart disease risk as measured by the FCRS in men and women not taking hormone replacement therapy (4). This research suggests that the conjunction of CRP and the FCRS may aid in the overall cardiovascular risk determination.

The increased incidence of cardiovascular disease in women observed after menopause may be due to an estrogen deficiency. Therefore, in the past women were being treated with estrogen replacement not only to decrease the symptoms of menopause but to also prevent cardiovascular disease. However, this philosophy of treating women with hormone replacement

therapy (HRT) to prevent cardiovascular disease has been refuted based upon the results of clinical trials, specifically the Women's Health Initiative (WHI). According to an examination of national data on annual trends in hormone therapy in the United States, 42% of women aged 50-74 years were taking HRT in 2001. Following the release of WHI in 2002, a decline in HRT was observed with only 28% of women in the same age group using hormone therapy (34). The new guidelines recommend against routine hormone therapy use for chronic conditions and current users have been advised to taper doses toward discontinuation (64, 92).

Past research has suggested that hormone therapy can improve the overall lipid profile of postmenopausal women (30, 56, 80, 55). Beneficial changes including a decrease in LDL-cholesterol, and an increase in HDL-cholesterol have been observed. Hormone therapy, however, has also been shown to increase triglyceride levels. Triglycerides have been identified as an independent risk factor for CVD among women. Despite the improvements of the lipid profile, hormone therapy has not been shown to be cardio-protective and in some cases HRT has been linked with an increased risk of heart disease. Therefore, it is imperative to explore other strategies to decrease CHD risk in postmenopausal women.

The association between cardiorespiratory fitness and CRP, and the effect of regular physical exercise training on CRP has been examined previously (20, 51). The cardiorespiratory fitness level of a sample of men from the Aerobics Center Longitudinal Study was determined with a maximal treadmill test and CRP values were examined across the fitness levels (20). Statistical adjustments were made for body weight and within weight categories. The researchers found that cardiorespiratory fitness levels were inversely related to CRP values. This relation was independent of body weight.

LaMonte et. al (2002) assessed cardiorespiratory fitness, CHD risk factors, and CRP levels in a tri-ethnic (Caucasian, African, and Native American) sampling of women. Significant correlations ($p<0.05$) were observed between CRP and fitness ($r= -0.25$), BMI ($r=0.25$), waist girth ($r=0.21$), insulin ($r=0.26$), and triglycerides ($r=0.27$). The women who participated in this study were middle-aged, overweight, and had relatively low CHD risk factors. The team of investigators concluded that aerobic fitness may reduce the risk of CHD in this population in part due to anti-inflammatory mechanisms (51).

A few intervention studies have examined the direct effect of aerobic exercise training on CRP levels (61,91). Mattusch, et al. (2000) found that nine months of extreme cardiovascular endurance training significantly decreased CRP levels ($p<0.05$) in male marathon runners. Fourteen, apparently healthy, moderately trained men participated in formal training sessions 3-4 days per week. The subjects ran continuously for 50 minutes at an intensity previously determined to be 75% of the speed at which their blood lactate concentration reached 4mmol/L. The researchers suggested that the decreased CRP levels observed in the runners may be due to the suppression of the inflammatory reaction (61).

Another study conducted by Smith, et al. (1999), investigated the impact of a six-month exercise program on serum levels of CRP. The 43 subjects (25 women and 18 men) included in this investigation had initial CRP levels which placed them at higher risk of developing ischemic CHD (91). The subjects exercised at a hospital based wellness center where they completed a supervised and individually tailored exercise program. On average, the subjects exercised for 2.5 hours per week completing a program that consisted of cardiovascular training, weight lifting, and flexibility exercises. The results of this investigation showed a clinically significant 35% decrease in CRP ($p=0.12$).

Therefore, since a relationship has been shown linking the direct measurement of cardiorespiratory fitness and CRP, we can make the assumption that regular leisure-time physical activity as reported by questionnaire should have the same effect on CRP. Self-reported physical activity and CRP has been studied previously with varied conclusions. More research looking specifically at postmenopausal women is needed.

1.5 Specific Aims

The specific aims of this study were to:

1. Examine the relation between CRP and physical activity in postmenopausal women.
2. To identify the factors that are related to CRP in postmenopausal women by using linear regression models with CRP value as the dependent variable and with physical activity and other cardiovascular risk factors as the independent variables.
3. Determine the association between CRP levels and subclinical measures including coronary artery calcium score and carotid artery intimal thickness, as well as other cardiovascular risk factors in postmenopausal women.
4. To determine the factors that are related to the coronary artery calcium score (CACS) in postmenopausal women by using a stepwise multiple regression model with CACS as the dependent variable and with physical activity and other cardiovascular risk factors as the independent variables.
5. Identify subjects with characteristics of metabolic syndrome and determine if there are differences in CRP, CACS, carotid artery intimal thickness, and physical activity among women without metabolic syndrome.

1.6 Summary

Coronary heart disease remains the largest cause of death among women and will continue to kill in large numbers as obesity coupled with a sedentary lifestyle become more common. With this trend, prevalence of other elevated coronary risk factors may increase resulting in more individuals presenting with a clustering of risk factors. Physical activity is critically important as it has been linked to a reduction in most major modifiable, predisposing and conditional coronary risk factors and thereby can ultimately aid in the reduction of the presence or severity of CHD.

Clinicians have utilized the Framingham CHD risk profile score as a method of estimating the risk of future coronary events in both men and women. Although an important clinical tool, not all individuals at high CHD risk are identified. To improve this risk prediction, some other novel cardiovascular risk factors, such as CRP, have been considered as adjuncts to the primary prevention model.

The emergence of CRP, a marker of inflammation, as a possible risk factor for cardiovascular disease has intrigued researchers. While CRP has not been widely studied, the growing body of evidence suggests that there is an inverse relation between CRP levels with physical activity as measured by self-report. However, there is less compelling evidence of the association in postmenopausal women. Therefore, the primary objective of this study will be to investigate the relation between physical activity and resting CRP levels in postmenopausal women.

2.0 THE REVIEW OF RELATED LITERATURE

2.1 Introduction

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD). This chapter is separated into the following sections: (1) Women and CHD, (2) Risk Factors, (3) The Menopausal Transition (4) Hormone Therapy and Cardiovascular Disease, (5) Inflammation in Atherogenesis, (6) CRP and Cardiovascular Risk Prediction, (7) Physical Activity Assessment, (8) Physical Activity and Postmenopausal Women, (9) Physical Activity and CRP, (10) Cardiorespiratory Fitness and CRP (11) Subclinical Measurements, and (12) Summary.

2.2 Women and CHD

Cardiovascular disease is the leading cause of death among both men and women and claims more lives each year than the next five leading causes of death combined (3). A common myth is that heart disease is a "man's disease". However, the fact remains that cardiovascular diseases are devastating to women, too. According to the American Heart Association (2002), cardiovascular diseases claim the lives of more than half a million females every year; about a death a minute. Typically for females, CHD occurs later in life as compared to men and a

women's risk dramatically increases after the onset of menopause. Theoretically, the protective effect of estrogen acts to reduce cardiac risk by dilating the coronary arteries and decreasing platelet aggregation and fibrogen levels (12). Also, estrogen protects by raising HDL cholesterol levels and lowering LDL cholesterol levels. As the female hormones decrease after menopause, the risk of coronary artery disease increases.

Cardiovascular disease is progressive in nature and most diagnosed individuals will present with symptoms and a clustering of risk factors. Frighteningly, according to the AHA (2002), 50% of men and 63% of women who died suddenly from CHD had no previous symptoms. Thus, individual awareness of symptomology, family history, and cardiovascular screening is important to identify this disease early. Cardiovascular disease is the leading cause of death and an important contributor to morbidity and disability among women. This disease is largely preventable, and the epidemiology of this disease suggests that aggressive risk reduction including counseling about lifestyle on an ongoing process throughout a women's life offers profound benefits. Thus, it is important for physicians and health educators to be aware of gender differences in prevention of CHD and the interpretation of risk factors.

2.3 Risk Factors

Risk factors for CHD in women are well documented (62) and previous research has shown that early risk reduction may prevent CHD. However, there are alarming trends in the prevalence and management of risk factors in women (62). The prevalence of obesity is increasing, and women are more likely than men to not participate in regular sustained physical activity. Smoking rates are declining less for women than men (87). Approximately 22% of women aged 20 years and older have elevated serum cholesterol levels $\geq$ 240mg/dL (87). Also,

30% of females aged 35-44 years have elevated blood pressure readings of at least 140/90 mmHg (19).

Traditional risk factors for developing coronary artery disease in women can be categorized as those that are modifiable and those not modifiable. The modifiable risk factors include: elevated LDL-C, HDL-C < 40 mg/dL, smoking, hypertension, body mass index, diet, and exercise. Those risk factors determined to not be modifiable include: current age, family history, diabetes mellitus/insulin resistance, and age at menopause.

The most common risk factor for CHD in women is a sedentary lifestyle. National statistics show that 43% of females 18 years and older report no leisure-time physical activity with rates of physical inactivity the highest among minority women (87). Only 20% of females over the age of 18 routinely engage in vigorous physical activity that promotes the development and maintenance of cardiorespiratory fitness (87). Physical inactivity has been directly linked to higher rates of obesity and myocardial infarction (94). The Nurses Health Study (1997) suggests that modest exercise consisting of 30-45 minutes of walking, three times weekly is strongly associated with a 50% reduction in the risk of myocardial infarction in women (21). A regular exercise program has also been shown to have a positive impact on other independent risk factors such as hypertension, obesity, and diabetes management.

Obesity, another independent CHD risk factor is associated with diabetes, hyperlipidemia, and hypertension. The risk of CHD increases with increasing weight. Current statistics in the United States identify that 63% of women between the ages of 35-44 years are overweight based upon a BMI $\geq$25 and 34% of women the same age are obese with a BMI >30 (19). Also, previous research has identified that the location of the adiposity stores may increase

one's risk. Central obesity, quantified as a waist-to-hip ratio greater that 0.8, is associated with a disproportionate increase in coronary risk (12).

The development of Type II Diabetes Mellitus is the most important risk factor for coronary disease in women. Typically, CHD presents at an older age in women than men, however, the presence of diabetes mellitus in women wipes out this gender-protective effect. Also, in women with diabetes mellitus, coronary disease is more likely to be fatal (8).

The Adult Treatment Panel (ATP III) has devised guidelines for the treatment of hyperlipidemia (63). The risk factors identified for treatment by the new ATP III guidelines include elevated low-density lipoprotein cholesterol (LDL-C), cigarette smoking, hypertension, and high-density lipoprotein (HDL-C) less than 40 mg/dL. The primary therapy recommended by ATP III is the lowering of LDL-C regardless of gender by lifestyle management and statin drug therapy. Women with CHD should be treated with therapy focusing on lifestyle changes between the ages of 20 to 45 years who have LDL-C levels above 130 mg/dL. The appropriate goal recommended is based upon a risk assessment of the individual. An LDL-C level of less than 100 mg/dL is recommended for people with known CHD. Individuals with two or more cardiac risk factors should have an LDL-C < 130 mg/dL. In the presence of one risk factor the goal is to have LDL-C < 160 md/dL. For asymptomatic women without any risk factors, it is recommended that treatment should not be started unless the LDL-C is over 190 mg/dL.

A secondary therapy recommendation by the ATP III guidelines is identification of the "metabolic syndrome", which is more common in females than males and is characterized by the presence of abdominal obesity, dyslipidemia, hypertension, and insulin resistance. According to the ATP III guidelines, a woman would be classified as having metabolic syndrome if they exhibited any three or more of the following criteria: 1) waist circumference >88 cm, 2) serum

triglycerides ≥ 1.7 mmol/L, 3) blood pressure ≥130/85 mmHg, 4) HDL cholesterol <1.3 mmol/L, and 5) serum glucose ≥6.1 mmol/L (32). Since most instances of premature CHD occur in women who have a clustering of risk factors as seen in the "metabolic syndrome", these individuals should be targeted for more intensive cardiovascular risk reduction.

One criticism of the ATP III guidelines is that there is no direct consideration of the importance of HDL-C or triglycerides for the prediction of heart disease in women despite the research based evidence that both were shown to be better independent predictors of mortality from CHD (9). Since the lipid profile is not the only factor associated with the etiology of CHD, additive tools to help identify cardiovascular risk are needed in women. According to Blake and Ridker (2001), lipid levels and other conventional risk factors explain only 50% of coronary events (14). Ultrasensitive-CRP, a marker of inflammation, should be obtained in women who do not closely meet ATP III guidelines but who may still have increased risk for CHD (90).

2.4 The Menopausal Transition

One determination of menopause can be determined by the woman's menstrual history and can be defined as the absence of menses for 12 consecutive months (16). It has been established that with the onset of menopause the risk of developing CHD increases presumably due the estrogen deficiency. The relative importances of factors which influence the increased CHD risk are still unknown. Substantial components of this increased risk are the lipid alterations seen with estrogen deficiency. Other factors that change with menopause such as body fat distribution, insulin action, fibrinolysis, and arterial wall changes may also influence cardiovascular risk. Overall, it is a combination of these factors which contribute to an increased

prevalence of metabolic syndrome in postmenopausal women compared with premenopausal women (67).

The distribution of body fat tends to change as a woman enters into menopause with an increased central distribution of fat. Increased intraabdominal fat accumulation has emerged as a cardiovascular risk factor independent of overall obesity (40). Previous research has shown that abdominal adiposity increases during the menopause transition independent of the effect of age and total body adiposity (69, 108, 13). Lipid abnormalities are associated with increased abdominal adiposity, thus increasing the CHD risk. High amounts of intraabdominal fat are associated with increased insulin resistance, free fatty acid levels, and decreased adoponectin. These factors together lead to hypertriglyceridemia and increased hepatic lipase activity which further lead to lipid abnormalities. Overall, compared to premenopausal women, postmenopausal women have higher total cholesterol, LDL cholesterol, triglycerides, and lipoprotein(a) levels and lower HDL levels (38, 15, 53).

2.5 Hormone Therapy and Cardiovascular Disease

Menopause is associated with increased levels of total serum cholesterol, LDL cholesterol and triglycerides, as well as a decrease in HDL cholesterol levels which worsen the overall cardiovascular risk profile. It is hypothesized that a major reason for these changes is a result of the fluctuation in hormones, especially the deficiency in estrogen. Thus, the use of exogenous hormone therapy could be considered to be justified to improve the cardiovascular risk profile. Generally, estrogen replacement has been shown to decrease total cholesterol, LDL cholesterol while increasing HDL cholesterol and triglycerides. Except for the increased

triglyceride levels, these changes are considered positive and may help decrease the overall risk for cardiovascular disease.

Earlier observational studies supported the beneficial relationship between hormone therapy use and prevention of CVD (29, 31, 28, 78, 93, 104). A meta-analysis of epidemiological studies suggested that hormone therapy users had an overall 40-50% reduction of CVD risk when compared to non-hormone therapy users (30). However, recent results from randomized clinical trials have failed to validate the proposed cardio-protective benefits of hormone therapy.

The secondary prevention randomized clinical trials demonstrate that hormone therapy does not slow the progression of coronary disease for women with known heart disease. The Heart and Estrogen/progestin Replacement Study (HERS) is a randomized, double-blind, placebo-controlled study which investigated the effect of HRT in secondary prevention of cardiovascular disease (36). This investigation followed 2,763 elderly postmenopausal women with documented heart disease for a mean 4.1 years. The results of this investigation showed no significant difference in the overall rates of cardiovascular events between the placebo and HRT group. However, there was a significant increase in cardiovascular events observed during the first year of hormone treatment, with a RH of 1.52 (95% CI, 1.01-2.29) suggesting that caution be taken when initiating HRT in elderly women with cardiovascular disease (36).

The Estrogen Replacement and Atherosclerosis trial, a randomized, double-blind, placebo-controlled clinical trial, also investigated the effects of HRT on the progression of coronary atherosclerosis in women (33). A sampling of 309 postmenopausal women with verified coronary artery disease at baseline were randomized into an estrogen, estrogen plus medroxyprogesterone acetate, or placebo group and were followed for three years. Significant

reductions in LDL-cholesterol levels were found in both of the hormone therapy groups as compared to placebo. There was a 9.4% decrease ($p=0.02$) for the estrogen group, and 16.5% ($p<0.001$) reduction for the estrogen plus medroxyprogesterone acetate group. Also, significant $p<0.01$ increases in HDL cholesterol levels were observed; 18.8% and 14.2% respectively. However, overall there was no significant difference observed in either treatment group on the progression of coronary atherosclerosis.

The results of this investigation were most recently verified by the Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial (WELL-HART) (35) and The Women's Angiographic Vitamin and Estrogen (WAVE) trial (100). Thus, the overwhelming evidence suggests that women with existing heart disease should not use estrogen replacement with an expectation of cardiovascular benefit.

In addition to the secondary prevention trials, the primary CVD prevention randomized clinical trials also demonstrate that HRT does not prevent cardiovascular events in apparently healthy post-menopausal women. The Women's Health Initiative (WHI) is a primary CVD prevention trial which investigated strategies that could potentially decrease CVD, breast and colorectal cancer, and bone fracture risk in postmenopausal women (82). A very large sample of 161, 809 post menopausal women aged 50 to 79 years were randomized into one of the study's three separate arms: 1) low-fat dietary pattern, calcium and vitamin D supplementation, 2) combined estrogen and progestin hormone therapy, and 3) estrogen only hormone therapy.

In the combined estrogen plus progestin hormone therapy arm of the WHI, participants ($N=16,608$) were randomly assigned to receive conjugated equine estrogens (0.625 mg daily) plus medroxyprogesterone acetate (2.5 mg daily) or placebo. The primary outcome was defined as acute myocardial infarct (MI) requiring overnight hospitalization, silent MI determined from

electrocardiogram, or CHD death. In 1999, two years after the trial began; the data safety monitoring board (DSMB) observed small, early adverse effects in cardiovascular outcomes, however none of the disease specific boundaries had been crossed and the trial continued. In 2002, the DSMB observed that the adverse effects in cardiovascular diseases persisted and the boundary for breast cancer incidence had been crossed. As a result of the evidence for breast cancer harm, and the increase in cardiovascular disease outcomes, the combined estrogen and progestin arm was terminated early after a mean follow-up of 5.2 years (planned duration was 8.5 years) (82).

The final results with regard to estrogen plus progestin and CHD from the WHI was published in 2003 (59). Overall, the researchers concluded that estrogen plus progestin HRT should not be prescribed for the prevention of cardiovascular disease since the therapy had no cardio-protective effect and might slightly increase the risk of coronary events. Compared to the placebo group, women taking the combined HRT had a 24% increased risk for CHD with a hazard ratio of 1.24 (95% CI, 1.00-1.54). Subgroup analyses were conducted to determine whether any demographic or clinical characteristics put the women at higher risk for CHD. Overall, only the subgroup of women with higher baseline LDL cholesterol levels had evidence of a pattern of hazard ratios for CHD that were different from patterns found among all women (59).

The estrogen only arm of the WHI investigated the role of 0.625 mg/d of conjugated equine estrogen in preventing chronic diseases in 10,739 postmenopausal women with prior hysterectomy (6). As with the estrogen and progestin arm of the WHI, the primary outcome of this investigation was defined as acute myocardial infarct (MI) requiring overnight hospitalization, silent MI determined from electrocardiogram, or CHD death. In compliance

with the DSMB recommendations, the participants in the estrogen alone trial were informed of the early increases in rates of heart disease, strokes, and blood clots observed in the women taking hormone pills. In 2002, the DSMB reported that since no increase in breast cancer rates had been observed at that point in the women taking only estrogen, the estrogen only arm of the WHI could continue. The DSMB continued to closely monitor the data and after a review in 2004, the National Institutes of Health (NIH) decided to stop the intervention phase of the trial. The NIH concluded that estrogen therapy did not appear to affect the risk of heart disease, but did increase the risk of stroke (6). Overall, due to the findings of WHI, the current guidelines for hormone therapy have changed and routine HRT is not recommended for treatment/prevention of chronic conditions including cardiovascular disease.

Hormone therapy has been shown to impact a number of cardiovascular and metabolic risk factors. The woman randomly assigned to hormone therapy in the estrogen and progestin arm of the WHI, had significantly ($p<0.05$) greater reductions in the total cholesterol, LDL-cholesterol, glucose and insulin levels as compared with the placebo group (59). Also, the HRT group had significant increases in HDL-cholesterol, and trigylcerides. There were also significant differences in blood pressure. Systolic blood pressure at year one was 1mmHg higher among women receiving hormones than those receiving placebo. Diastolic blood pressure did not differ between groups. Body weight and waist circumference at follow-up were slightly lower among women in the HRT group (59).

2.6 Inflammation in Atherogenesis

There is compelling evidence that inflammation and underlying cellular and molecular mechanisms have a role in atherogenesis (54). Much research has been conducted to determine the role of inflammation in atherogenesis. There is evidence that inflammation aids in the lipid accumulation in the artery wall and that inflammatory processes promote the initiation and evolution of atheroma, and triggers the thrombus that causes most acute complications of atherosclerosis (54).

Libby, Ridker, and Maseri (2002) concisely outline the impact of inflammation in all stages of atherosclerosis. Blood leukocytes, mediators of host defenses and inflammation, are present in the atherosclerotic lesions. In the early stages of atherosclerosis, an inflammation of the endothelial layer of the artery allows blood leukocytes to adhere better than in the normal, un-inflamed endothelium. The leukocytes now adhered to the endothelium, penetrate into the intima of the artery. Within the arterial wall, these cells initiate a local inflammatory response that aids in advancing the atherosclerosis lesion. Ultimately, the intimal lesion is rendered weak and becomes susceptible to rupture. The inflammatory process triggers the potent procoagulant tissue factor, which in turn triggers the thrombus that can cause acute myocardial infarction. Thus, there seems to be a direct link between arterial inflammation and thrombosis (54).

Research has also revealed other triggers for inflammation in atherogenesis. The oxidation of lipoproteins such as low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) may also result in inflammation (54). The oxidation hypothesis states the LDL retained in the intima undergoes oxidative modification that causes the newly modified lipids to induce the expression of inflammation mediators in macrophages and vascular wall cells (102). Similarly, the VLDL molecules can also undergo oxidative modification (54). Hypertension, a

significant risk factor for CHD, also has inflammatory characteristics that may contribute to atherosclerosis. Angiotension II, a vasoconstrictor, has also been linked to increased intimal inflammation (43). Diabetes mellitus is also considered a trigger for inflammation in atherogenesis. Hyperglycemia can lead to modification of macromolecules, which can augment inflammatory pathways in vascular endothelial cells (84). Obesity has multiple roles in triggering the inflammation associated with atherogenesis. Not only does obesity increase the risk of insulin resistance and diabetes, but it also contributes to dyslipidemia; all which may cause inflammation (54). Obesity directly increases inflammation due to adipose tissue synthesizing proinflammatory cytokines such as interleukin-6, a primary hepatic stimulant for CRP production (107). Lastly, chronic infection may increase inflammatory cytokines that may accelerate the evolution of remote atherosclerotic lesions (22).

2.7 CRP and Cardiovascular Risk Prediction

Currently, there is compiling evidence that using inflammatory markers such as CRP in coronary risk prediction may increase the accuracy in detecting patients at high risk for subsequent coronary occlusion. C-reactive protein is an acute phase reactant, which is a marker for underlying systemic inflammation. Several large prospective studies of apparently healthy adults have shown an association between elevated CRP levels and the risk of coronary events (48, 75, 76, 79), stroke (83), and peripheral vascular disease (74), independent of traditional risk factors. Thus, CRP testing conducted during primary prevention in combination with lipid screening may improve the global assessment of cardiovascular risk (73).

A strong association between CRP and cardiovascular risk has been shown in men. However, the relation of CRP and cardiovascular risk in women is not well established. C-

reactive protein and other inflammatory markers were collected in a cohort of apparently healthy post-menopausal women participating in the Women's Health Study (76). Twelve measurements including four inflammatory markers and eight lipid parameters were collected at baseline and study participants were followed for a mean of three years. For each woman who had a confirmed cardiovascular event during follow-up, two control subjects were matched for both age and smoking status. Overall, 122 case subjects and 244 control subjects were selected for analysis. C-reactive protein and the other markers of inflammation were significantly elevated at baseline in those women who subsequently developed a cardiovascular event. Also observed in the post-event cases, was a significant elevation in total cholesterol, LDL cholesterol, and the ratio of total to HDL cholesterol.

Interestingly, the researchers found that of the twelve markers measured, CRP was the strongest univariate predictor of risk with an odds ratio of 4.4 for the highest quartile compared with the lowest quartile; 95% CI [2.2-8.9], $p<0.001$ (76). After adjusting the risk model for BMI, hypertension, diabetes, and parental history of premature CHD, CRP ($p<0.02$) and the ratio of total cholesterol to HDL cholesterol ($p<0.02$) were found to be independent risk predictors. The researchers concluded that the addition of inflammation markers to classic lipid testing clinically provided a significantly better risk prediction model than just lipid testing alone ($p<0.001$). Clinically, the most interesting finding was involving the sub-grouping of women with "safe" levels of LDL cholesterol, classified by NCEP as <130 dL. In this investigation, women within this LDL-group who had elevated baseline levels of CRP and the other inflammatory markers were also found to be at increased risk for future cardiovascular events. The relative risk of 4.1 was observed for women in the highest quartile as compared to the lowest quartiles; 95% CI [1.7-11.3], $p<0.002$.

This study provides some important clinical implications. First, the addition of screening for CRP in primary prevention may better identify patients who would benefit from moderate risk reduction. Secondly, it is important to recognize that a lipid profile within established "goal" guidelines may not be enough to protect from CHD. There remains a need for a comprehensive risk prediction model that includes lipid parameters, markers of inflammation, and other lifestyle risk factors.

Similar results were reported by the research team led by Ridker, 2002 utilizing a much larger cohort of the Women's Health Study (77). Baseline CRP and LDL cholesterol levels were obtained and the women were followed for a mean of eight years. With the large sample size of 27,939 women, the researchers were able to calculate survival curves associated with CRP and to compare the relationship between CRP and LDL cholesterol to predict coronary events. Also, due to the large sample, the researchers were able to separately examine those women who were nonusers and users of hormone-replacement therapy.

The results of this study showed that both baseline values of CRP and LDL cholesterol had a strong linear relationship with the incidence of coronary events. However, CRP and LDL were minimally correlated ($r=0.08$) to each other. The relative risk of first cardiovascular events according to increasing quintiles of CRP were 1.4, 1.6, 2.0, and 2.3, as compared to those in the lowest quintile ($p<0.001$) and according to LDL cholesterol were 0.9, 1.1, 1.3, and 1.5, respectfully ($p<0.001$). Thus, the researchers concluded that CRP was a stronger predictor than LDL cholesterol for cardiovascular events.

Similarly to the study conducted by Ridker in 2000, an overwhelming amount of cardiovascular events occurred in women at "goal" levels of LDL cholesterol. In this large sample, 77% of the coronary events occurred in women with LDL levels below 160 dL and 46%

occurred in women whose LDL was below 130 dL (77). The results of this larger study again illustrate the need for CRP levels to be considered with the entire lipid panel for better overall risk stratification.

Based upon the recommendations from the previous mentioned studies, a cross-sectional survey was conducted comparing CRP levels with calculated 10-year Framingham Coronary Heart Disease Risk Scores (FCRS) of 1666 men and women free of cardiovascular disease (4). The researchers found that CRP levels significantly correlated with the 10-year FCRS in women not taking hormone replacement therapy and men. The data showed a positive trend between increasing CRP levels and progressively higher FCRS with the use of the total cholesterol ($p_{trend}<$ 0.01) and LDL cholesterol ($p_{trend}<0.01$) scoring algorithms. However, when CRP was correlated with each individual component of the FCRS, no significant correlation was observed. Overall, the results of this investigation support the hypothesis that CRP and the FCRS may reflect different aspects of coronary risk; therefore the addition of CRP in the overall risk determination may be useful.

2.8 Physical Activity Assessment

Among the body of research investigating cardiovascular health, physical activity patterns and cardiorespiratory fitness levels are critically important. Physical activity can be defined as voluntary movement produced by skeletal muscles which results in energy expenditure (17). The term physical activity includes a vast spectrum of activities; at the lower end are general activities of daily living and at the other end are moderate to high intensity activities (44). Therefore, housework, gardening, occupational, and leisure activity may all be considered types of physical activity. The term exercise differs from physical activity in that it is

planned, structured, and consists of repetitive movements with the purpose to improve or to maintain one or more components of physical fitness (17). Physical fitness is defined as an overall set of attributes including cardiorespiratory fitness and muscular strength, that an individual possesses or can achieve that relates to the ability to participate in exercise or physical activity (17).

The overall definition of physical activity is broad and allows for much interpretation; therefore clouding the ability to compare methods of assessment. Therefore, it is imperative to select a subjective measurement tool such as the questionnaire carefully so that it captures the information on the types of physical activities that encompass the greatest proportion of energy expenditure in the study population (44). To accurately assess physical activity levels, the assessment tool should capture the entire spectrum of activity.

According to a meta-analysis conducted by Williams, 2001, there is a wide array of research investigations which have looked at self-reported physical activity and cardiovascular disease (101). However, among these epidemiological studies, the method of collecting information on physical activity has varied widely making interstudy comparisons almost impossible. The physical activity questionnaires used differ by recall period, type of activity recorded, and whether the physical activity is ranked by total expenditure or intensity (101).

Physical activity can be assessed using either subjective or objective measures. Self-reported questionnaires are widely used to subjectively assess physical activity. Questionnaires can vary in their complexity, time frame, and activity type (68). The questionnaire may ask a single item, or be comprised of a more complex history. They may require the respondent to recall activity within the past day, week, month, or even over a lifetime. The questionnaires which recall activity done over a long time frame, such as 1 year, are more likely to reflect the

usual activity patterns of the individual. Comparatively, the questionnaires with a short time frame are less likely to be vulnerable to recall bias and the physical activity measured is more easily validated with objective tools. Lastly, the physical activity type measured may include domains such as school/occupational, sports/leisure, or housework/child or elderly care.

A popular objective measurement tool of physical activity is the pedometer. Pedometers are small, inexpensive devices that measure ambulatory activity in terms of steps taken (11). Each step is registered when the vertical forces of foot-strike cause movement of a spring-suspended lever arm. Pedometers have been shown to be a reliable tool when walking is the primary type of activity (10). Pedometers do have some limitations. First, any vertical force through the hip area can trigger the device. Therefore, the pedometer may falsely capture movement not associated with physical activity. Secondly, pedometers do not provide researchers with information relating to activity type, duration, or intensity. Lastly, pedometers do not capture activity done with the upper body alone and have been shown to not be accurate when the gait speed is slow (11).

2.9 Physical Activity and Postmenopausal Women

Physical activity has been shown to be a negative risk factor for CHD in women. Evidence from previous research shows that individuals who participate in regular physical activity have a reduced risk of developing CHD (86, 96). However, more than 41% of postmenopausal women are physically inactive during their leisure time (88).

Many prospective studies investigating physical activity and cardiovascular disease in postmenopausal women have found an inverse relationship (49, 60, 59, 52). Both moderate and vigorous physical activity has been associated with substantial reductions in the incidence of

cardiovascular events. In these investigations, physical activity was assessed using varying subjective questionnaires which makes interstudy comparison more difficult. Overall, based upon the evidence, low to high levels of physical activity has been shown to reduce cardiovascular risk in this population of women.

2.10 Physical Activity and CRP

Research investigations have shown inverse associations between physical activity levels and CRP in an apparently healthy adult population (25, 5). However, other research investigations suggest that the relationship between CRP and physical activity is not as strong as the impact of body mass index and/or body fat is on CRP levels (71, 98). Lastly, the association between physical activity and CRP in postmenopausal women has not been widely studied (95, 57).

An investigation conducted by Ford, 2002 explored the association between CRP and physical activity in a large (N=13,748) representative sample of the U.S. population (25). This sample included individuals who participated in the National Health and Nutrition Examination Survey III (NHANES III). The results of this investigation support the hypothesis that physical activity may reduce inflammation (25). The researchers found that the amount of leisure-time physical activity was inversely associated with baseline serum CRP levels ($p<0.001$). Even after adjustments were made for confounding variables including BMI and waist-hip ratio, a significant relationship between physical activity and CRP remained.

The inverse association between physical activity and CRP was verified in an investigation conducted by Albert, 2004 (5). The sample included 1,732 men and 1,101 women who participated in the Pravastatin Inflammation/CRP Evaluation (PRINCE) study. The inverse

relationship between self-reported physical activity and CRP after adjustment for confounders was only apparent for the men (p=0.007) and not for the women (p=0.38). The investigators hypothesized that this gender difference may be an effect of the subject size and also due to the women reporting lower amounts of physical activity.

Two other recent investigations failed to confirm this inverse relationship between physical activity and CRP (71, 98). In both of these investigations, BMI not physical activity was significantly related to CRP levels. The absence of an affect of physical activity on CRP in these two investigations could be due to smaller sample sizes and also a large proportion of the samples being sedentary.

The relationship between CRP and physical activity in postmenopausal women has also been investigated (95, 57). Findings from these studies suggest that physically active postmenopausal women had lower CRP levels, regardless of hormone therapy status, when compared to their less active counterparts. Manns et al., (2003) designed a study to determine if increased participation in physical activity was associated with a reduction in CRP levels independent of hormone therapy and body fat in postmenopausal women. One hundred thirty-three women between the ages of 50 and 78 were included in the study sample. Fifty of the 133 women were not hormone therapy users, 51 took estrogen plus progestin, and the remaining 32 used estrogen only. Physical activity was assessed using a Stanford 7 Day Physical Activity Recall. The median CRP levels for women on hormone therapy (with or without progestin) was 3.5 – 5.5 times higher than those not taking hormone therapy (median CRP level was highest among those women taking unopposed estrogen). Increased participation in physical activity reduced CRP levels regardless of hormone therapy use. However, as hypothesized, this

association was dependent upon lower amounts of body fat that were observed in the active women (57).

Investigators also examined the hypothesis that hormone replacement therapy related increases in CRP would either be blunted or absent in those postmenopausal women who regularly exercised (95). The sample studied (N=114) included 39 physically active and 75 sedentary postmenopausal women. Sixty-five women were users of hormone therapy and 49 were nonusers. Plasma CRP levels were ~75% higher ($p<0.01$) in the sedentary group who were hormone therapy users when compared to sedentary non-users. There was no significant difference in CRP levels between hormone therapy groups for the physically active women. Additionally, plasma CRP levels in the physically active hormone therapy users and non-users were 68% and 64% lower than their sedentary counterparts. The researchers concluded that since the elevation in CRP due to hormone replacement therapy observed in sedentary women is absent in women who engage in regular physical activity, physical activity may prevent the increase in CRP due to hormone replacement therapy (95).

2.11 Cardiorespiratory Fitness and CRP

Only a few studies have examined the association of cardiorespiratory fitness and CRP (20, 51). Church et al. (2002) assessed cardiorespiratory fitness with a maximal treadmill test and examined the prevalence of elevated CRP values in a sample of 722 men from the Aerobics Center Longitudinal Study (20). The subjects were also screened for many cardiovascular disease risk factors including body mass index, blood pressure, fasting glucose, and lipid levels. Each variable was analyzed by CRP quartiles and by fitness quintiles. There was a statically significant positive association between CRP levels and BMI ($p<0.0001$), systolic blood pressure

($p<0.0001$), diastolic blood pressure ($p<0.0001$), fasting glucose ($p= 0.001$), and triglycerides ($p<0.0001$). Also, a significant inverse relationship was observed between CRP and fitness ($p<0.0001$), and HDL cholesterol ($p<0.0001$).

The secondary analysis revealed that CRP levels were significantly different across fitness quintiles ($p<0.001$). Subjects within the lower fitness group had higher CRP values than those in the higher fitness quintile. This association was independent of body composition and fat distribution, as assessed by BMI, percent body fat, and waist girth. The authors suggest that since there remained a strong association between fitness and CRP concentration after accounting for the possible confounding factor of BMI, individuals may be able to decrease CRP levels by increasing fitness even without substantial weight loss (20).

This association between cardiorespiratory fitness and CRP levels was also examined in a sampling of apparently healthy (44 African Americans, 45 Native Americans, and 46 Caucasian) women (51). A maximal treadmill exercise test was administered to determine cardiorespiratory fitness. Other variables collected included: BMI, resting blood pressure, fasting insulin and glucose, lipid profile, apolipoprotein B, and homocysteine. The researchers analyzed CRP concentrations across categories of race, fitness, and BMI.

Overall, among the entire cohort, CRP correlated significantly with fitness ($r= -0.25$), BMI ($r= 0.25$), waist girth ($r= 0.21$), insulin ($r= 0.26$), and triglyceride ($r= 0.27$). The authors also reported significant correlations between CRP and race ($p=0.002$), fitness ($p=0.002$), BMI ($p=0.0007$), and waist girth ($p=0.004$). C-reactive protein levels were lowest among the Caucasian women and highest among the African American women. An inverse relationship was observed between CRP and fitness. Linear, positive trends existed across BMI and waist girth. This investigation of women provided similar results as the Church, 2002 investigation of

men. Although there is limited research investigating CRP and cardiorespiratory fitness, preliminary evidence suggests that exercise training which increases cardiorespiratory fitness may reduce CRP levels and therefore CHD risk.

2.12 Subclinical Measurements

Electron beam computerized tomography (EBCT) is a non-invasive scanning technique that allows both detection and quantification of coronary artery calcium. This test may be beneficial in the early diagnosis of CHD since more coronary calcium means more coronary atherosclerosis, suggesting a greater likelihood of future cardiovascular problems. Under the assumption that calcification precedes manifestation of coronary artery disease, this radiologic appearance of calcium may be used to assess CHD risk.

Briefly, the EBCT technique involves taking 30 to 40 axial images of the myocardium. The tomographic slices have a fixed slice thickness of 3 mm, and the entire coronary tree is imaged during a single 20-30 second breath hold. The rapid acquisition at 100 msec allows accurate visualization of calcium deposits in the coronary tree. Since calcium attenuates the x-ray beam, computed tomography is extremely sensitive in detecting vascular calcification (26). The coronary calcium score is most often calculated using the Agatston scoring method: the calcium area (square millimeters) is multiplied by a modifier that adjusts for peak calcium density for each lesion (2). Several commercial software packages have incorporated this scoring protocol and are used to quantify the coronary arterial calcification.

Coronary calcium scores have been found to be predictive of CHD in women (42, 89, 70). Outcome data was collected from a large cohort (N=5635) of asymptomatic males and females (36%) for a mean of 37 $\pm$ 13 months (42). At baseline, EBCT images were taken and

coronary calcium scores were calculated. During follow-up, a variety of endpoints including cardiovascular mortality, myocardial infarction, and revascularization procedures were recorded. Overall, 51% of the women had visible coronary artery calcium at baseline. In women, the occurrence of all events was significantly associated with the presence of coronary artery calcium ($p=0.037$). The findings of Kondos et al, 2003 was verified by the results of a longitudinal investigation which followed a large cohort (N=10,377) of asymptomatic men and women (40%) for an average of 5 ± 3.5 years (89). In both genders, coronary artery calcium was an independent predictor of death ($p<0.001$) and the risk increased proportionally to the baseline calcium scores by EBCT.

Coronary calcification as measured by EBCT has been shown to be associated with traditional risk factors of coronary artery disease in women. The Healthy Women Study (HWS) investigated the relationship between cardiovascular risk factors measured premenopausally and coronary calcification measured 11 years later (47). At the follow-up visit, 63% of the women had no calcification in the coronary arteries as measured by EBCT. The investigators did find relationships between conventional risk factors and coronary calcification scores. There were very strong associations between coronary calcification and LDL cholesterol ($p=0.0001$), HDL cholesterol ($p= 0.0001$), and HDL_2 cholesterol ($p=0.0000$). The women with an LDL-C <100 mg/dL had no calcification. Also, women with a lower HDL-C had a much higher prevalence of coronary calcification. Other risk factors found to be significantly related to coronary calcium scores were: systolic blood pressure, 2-hour blood glucose levels, trigylcerides, apoB, BMI, and cigarette smoking. This was the first study to examine the relationship of risk factors measured premenopausally to the extent of subclinical disease by EBCT among postmenopausal women.

The association of traditional and nontraditional cardiovascular risk factors with coronary artery calcification was further examined in a cohort of the Atherosclerosis Risk in Communities (ARIC) Study (24). Coronary artery calcium was measured by EBCT in 360 individuals free of known coronary artery disease. The coronary calcium scores were related to other risk factors measured approximately 12 years prior. Most of the traditional risk factors were associated with the coronary calcium score after adjustment for age and gender. Coronary calcium scores were associated positively ($p<0.05$) with total and LDL-cholesterol, triglycerides, waist/hip ratio, pack/years of smoking, diabetes prevalence, and hypertension. There also was a significant negative association with HDL-cholesterol. Interestingly, the researchers did not find significant associations between the coronary calcium score and sports physical activity, insulin, nor with the inflammation marker CRP. In this investigation, only the traditional cardiovascular risk factors were associated with coronary calcification and no evidence was found for the inclusion of the "non-traditional" risk factors in the overall CHD risk assessment model.

The relationship between CRP and coronary calcification has yet to be strongly determined. Some previous research investigations have shown a correlation (99, 65) while others have not found any relationship (72, 37) between the marker of inflammation and coronary calcification. Most recently, the St. Francis Heart Study compared the prognostic accuracy of EBCT scanning of the coronary arteries with that of coronary disease risk factors and CRP (7). A large sample of 4,903 asymptomatic men and women were observed over a period of 4.3 years. This investigation added to the literature supporting the prognostic value of coronary calcification to atherosclerotic cardiovascular disease events. Those subjects which had an event, had statistically higher baseline coronary calcium scores than those without events ($p<0.0001$). The results of this investigation also yielded an association between most standard

risk factors with CRP and the coronary calcium score. The coronary calcium score and CRP together were weakly correlated ($r=0.06$, $p=0.01$). However, in a multivariate regression model after adjustment for standard risk factors, CRP levels no longer predicted the coronary calcium score ($p=0.22$). Lastly, the researchers found that the calcium score predicted coronary artery disease events more accurately than the Framingham risk index ($p=0.0006$). In combination with the Framingham risk score, the addition of the calcium score enhanced overall risk stratification ($p<0.0001$). Therefore, it was concluded that the coronary calcium score may enhance risk stratification by identifying individuals with greater risk who were originally classified as low-risk.

The relation of physical activity to the coronary artery calcium score has also been examined (23). A cross-sectional investigation of 779 asymptomatic men and women who had multiple metabolic risk factors was conducted with the primary purpose of examining whether an increasing degree of physical activity would be inversely associated to the prevalence of coronary artery calcium. Study participants were categorized into 3 groups based upon their past year leisure time physical activity: sedentary (no PA), moderate-duration PA (<30minutes, 1-2 times/week), and long-duration PA ($\geq$30 minutes, $\geq$3 times/week). Overall, 37% of the participants were sedentary, 26% engaged in moderate-duration, and 37% in long-duration. A relationship between physical activity and coronary calcification was apparent in this investigation. Advanced coronary artery calcium was prevalent in 26% of the sedentary group, 24% of the moderate-duration group, and 16% of the individuals in the long-duration group ($p<0.05$). Thus, long-duration physical activity had an independent inverse association with coronary artery calcium.

2.13 Summary

Cardiovascular disease is the leading cause of death and an important contributor to morbidity and disability among women. This disease is largely preventable, and epidemiological research suggests that aggressive risk reduction including counseling about lifestyle should be an ongoing process throughout a women's life. Frighteningly, according to the AHA (2002), 63% of women who died suddenly from CVD had no previous symptoms. Thus, individual awareness of symptomology, family history, and early cardiovascular screening is important to identify this disease early.

Conventional risk factors for CHD in women are well documented and include obesity, smoking, physical inactivity, dyslipidemia, hypertension, and diabetes mellitus. Commonly identified in women is metabolic syndrome which is characterized by the presence of abdominal obesity, dyslipidemia, hypertension, and insulin resistance. Since most instances of premature CHD occur in women who have a clustering of risk factors, these individuals should be targeted for more intensive cardiovascular risk reduction. Unfortunately, lipid levels and other conventional risk factors explain only 50% of coronary events. Therefore, it has been suggested that ultrasensitive CRP, a marker of inflammation, should be obtained in women who may have increased CHD risk but a normal lipid profile.

The onset of menopause increases the risk of developing CHD presumably due the estrogen deficiency. The relative importances of factors which influence the increased CHD risk are still unknown. Substantial components of this increased risk are the lipid alterations seen with estrogen deficiency. Other factors that change with menopause such as body fat distribution, insulin action, fibrinolysis, and arterial wall changes may also influence cardiovascular risk. Earlier observational studies supported the beneficial relationship between

hormone therapy use and prevention of CVD. A meta-analysis of epidemiological studies suggested that hormone therapy users had an overall 40-50% reduction of CVD risk when compared to non-hormone therapy users. However, recent results from randomized clinical trials have failed to validate the proposed cardio-protective benefits of hormone therapy.

There is evidence that inflammation aids in the lipid accumulation in the artery wall. In addition, these inflammatory processes promote the initiation and evolution of atheroma, and trigger the thrombus that causes most acute complications of atherosclerosis. Within primary prevention, utilizing inflammatory markers such as CRP in coronary risk prediction may increase the accuracy in detecting high-risk patients for subsequent coronary occlusion. Previous research demonstrates a positive linear association between CRP and cardiovascular risk in men while studies investigating women are less numerous. There is compelling evidence that a comprehensive risk prediction model that includes lipid parameters, markers of inflammation, and other lifestyle risk factors together may provide the best CHD risk screening outcome.

Both moderate and vigorous physical activity has been associated with substantial reductions in the incidence of cardiovascular events in postmenopausal women. Inverse associations between physical activity levels and CRP have been observed in apparently healthy adult populations. However, other research investigations suggest that the relationship between CRP and physical activity is not as strong as the impact of body mass index and/or body fat is on CRP levels. Lastly, the association between physical activity and CRP in postmenopausal women has not been widely studied.

Electron beam computerized tomography (EBCT) is a non-invasive scanning technique that allows both detection and quantification of coronary artery calcium. Coronary calcification as measured by EBCT has been shown to be associated with traditional risk factors of coronary

artery disease in women. The relationship between CRP and coronary calcification has yet to be strongly determined.

3.0 PROCEDURES

3.1 Introduction

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD). This chapter is separated into the following sections: (1) Selection of Subjects, (2) Collection of Data, (3) Treatment of Data, and (4) Summary.

3.2 Selection of Subjects

A cohort of 201 postmenopausal, 52-62 year old women who are enrolled in the Women On the Move through Activity and Nutrition (WOMAN) Study was examined at the baseline evaluation. The WOMAN study is a 5-year randomized clinical trial of primary cardiovascular disease prevention in Pittsburgh, Pennsylvania. The primary purpose of this study is to test whether an aggressive non-pharmacological intervention among postmenopausal women will modify or reduce measures of subclinical cardiovascular disease. Currently, this longitudinal study is collecting the 30 and 42-month data. The women were recruited in the community through mailings, and contacts with physician offices in the Pittsburgh area. Initially, to be eligible for participation, the subjects had to be currently taking hormone replacement therapy

(HRT). However, due to the publication of the negative effects of HRT in the Women's Health Initiative (WHI), the eligibility criteria changed and women who were not HRT users were eligible to participate as well. Therefore, there are currently women taking HRT and those not taking HRT in the study.

Other inclusion criteria for enrollment into the WOMAN study included a waist circumference ≥80 centimeters, a LDL cholesterol level between 100-160 mg/dL, and the physical ability to participate in a walking intervention. Individuals were excluded based upon self-report if they had known heart disease, or diabetes mellitus, were currently on lipid lowering drug therapy, or were being treated for major psychiatric disorders or depression. All subjects signed an approved informed consent form prior to data collection. The WOMAN study research protocol was approved by the University of Pittsburgh's Institutional Review Board (IRB# 000356). The secondary analysis of the WOMAN study for the purpose of this dissertation project was also approved (IRB# 0602160).

Table 1: Baseline Visit WOMAN Study Measurements

Completed Clinic Visit (demographic survey, medication inventory, anthropometric measures, blood pressure, and fasting blood draw)	n=508
Physical Activity Measures	
Past Year MAQ	n=504
Past Week MAQ	n=506
Pedometer	n=173
Cardiovascular Disease Risk Factors	
hs-CRP	n=201
Subclinical Measures of CHD	
Coronary Artery Calcification	n=507
Carotid Ultrasound	n=503

3.3 Collection of Data

Data was collected in the University of Pittsburgh's Department of Epidemiology Research Center located in Pittsburgh, PA by trained researchers. All clinical procedures used standardized protocols and calibrated equipment. The baseline data collection form can be located in Appendix A.

3.3.1 Phlebotomy

Blood samples were obtained after a 12 hour fast by venipuncture of an antecubital vein by a trained phlebotomist for analysis of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting glucose and insulin, and high sensitivity CRP. Analysis of glucose, insulin, and lipid profile was done in the Heinz Nutrition Laboratory in the Department of Epidemiology at the Graduate School of Public Health, University of Pittsburgh.

High sensitivity CRP was analyzed in the Lab for Clinical Chemical Research at the University of Vermont. C-reactive protein was measured using the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonepholometric assay. Polystyrene particles are coated with monoclonal antibodies to CRP, which, in the presence of antigen (CRP) agglutinate to cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of CRP in the sample. Suitable specimens for this assay are serum, heparin, or EDTA anticoagulated plasma. The assay range is 0.175 – 1100 mg/L. Expected values for CRP in normal, healthy individuals are < 3 mg/L. Intra-assay CVs range from 2.3 – 4.4% and inter-assay CVs range from 2.1 – 5.7%. The phlebotomy collection form used in the WOMAN study can be found in Appendix B.

3.3.2 Subject Characteristics

Subject characteristics including body weight, height, body mass index (BMI), and waist circumference were recorded. Body mass index was calculated from weight divided by height^2 ($kg*m^{-2}$). Waist circumference was measured at the level of the umbilicus and recorded in centimeters.

3.3.3 Subclinical Disease Measures

Electron Beam Computed Tomography (EBCT) was used to quantify calcification in the coronary arteries. An Imatron C-150 Ultrafast CT Scanner (Imatron, South San Francisco, CA) was used to perform two passes. The first is a scout pass that allows an evaluation of the subject's anatomy so that the landmarks for the coronary scan can be identified. The second pass is for evaluation of the coronary arteries. During this pass, 30 to 40 contiguous 3mm thick transverse images were obtained from the level of the aortic root to apex of the heart. Images were obtained during a maximal breath hold using ECG triggering so that each 100 millisecond exposure is obtained during the same phase of the cardiac cycle (60% of R-R interval). A DICOM work station and software by AcuImage was used to read the images. This software program implements the widely accepted Agatston scoring method. Coronary artery calcium will be considered to be present when 3 contiguous pixels greater than 130 HU are detected overlying the vessels of interest. A total calcium score as well as a total number of calcifications was obtained.

A carotid ultrasound was conducted to obtain average wall (intima-media) thickness measurements. A Toshiba SSA-270A scanner was used to take detailed B-mode images of the right and left common carotid artery, carotid bifurcation, and the first centimeter of the internal

carotid. Selected images were digitized for later measurement of intima-media thickness by central readers in Pittsburgh.

3.3.4 Physical Activity Assessment

To assess current physical activity behaviors, an interviewer administered the Modifiable Activity Questionnaire (MAQ), Appendix C, which assesses occupational and leisure activities done in the past year (45). The subject's average past year physical activity was calculated as the product of the duration and frequency of each activity (hours/week). Each activity then can be weighted by their estimated metabolic cost (METs) and ultimately expressed as MET-hours per week. A summation of each activity will give a total average past year physical activity score in either hours/week, or MET-hours. The MAQ has been shown to be both reliable and valid in adults through comparisons with activity monitors and doubly labeled water (46, 85).

A past week version of the MAQ was also used to assess participation in leisure activities performed over the past 7 days. Study participants were given the past week MAQ during the eligibility visit and were asked to record leisure activities for the 7 days prior to their clinic assessment. Past week leisure physical activity levels were calculated using the same methods as the past year MAQ.

Physical activity was also objectively measured in some subjects using the Yamax Digi-Walker pedometer. The pedometer was worn clipped to the waistband over the dominant hip for one week. At the end of each day, the subject was asked to record the total number of steps taken in a dairy which was then returned to the investigator (Appendix C). For each participant, a seven-day average of the number of the steps taken per day was calculated.

3.4 Treatment of Data

Data analysis was done using SAS Software (SAS Institute Inc., NC). All variables were tested for normality. Normally distributed variables were reported as mean $\pm$ standard deviation; not normally distributed variables were reported as median with 25^{th} and 75^{th} percentiles. Depending upon the characteristics of the variables, Pearson or Spearman correlation coefficients were utilized to examine associations between continuous variables. Chi Square models were used to examine relationships between categorical variables. Multiple linear multiple regression models with CRP value as the dependent variable and with physical activity, HRT status, waist circumference, age, smoking status, total cholesterol, HDL-c, triglycerides, glucose, CACS, and carotid artery intimal thickness as the independent variables were used to identify the factors that are related to CRP. A logistic stepwise regression model with detectable coronary artery calcium (0-10, $\geq$10) as the dependent variable and with CRP, physical activity, carotid artery intimal thickness, total cholesterol, HDL-c, triglycerides, waist circumference, HRT status, and age as the independent variables was used to determine the factors that are related to the CACS. Independent t-tests and Wilcoxon rank scores were used to determine if there were differences in CRP, CACS, intimal thickness, physical activity and other cardiovascular risk factors among those women with and without characteristics of metabolic syndrome. All statistical testing was conducted at the $p<0.05$ level of significance.

3.5 Summary

A cohort of 201 postmenopausal, 52-62 year old women who are enrolled in the Women On the Move through Activity and Nutrition (WOMAN) Study was examined at the baseline evaluation which occurred 30-42 months ago. Inclusion criteria for enrollment into the

WOMAN study included a waist circumference ≥80 centimeters, a LDL cholesterol level between 100-160 mg/dL, and the physical ability to participate in a walking intervention. Individuals were excluded if they had known heart disease, or diabetes mellitus, were currently on lipid lowering drug therapy, or were being treated for major psychiatric disorders or depression. All subjects signed an approved informed consent form prior to data collection. The WOMAN study research protocol was approved by the University of Pittsburgh's Institutional Review Board (IRB# 000356). The secondary analysis of the WOMAN study for the purpose of this dissertation project was also approved (IRB# 0602160).

Fasting blood samples were collected by venipuncture of an antecubital vein by a trained phlebotomist at the University of Pittsburgh's Department of Epidemiology Research Center located in Pittsburgh, PA for analysis of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting glucose and insulin, and high sensitivity CRP. Body weight and height were measured and BMI was calculated.

Subclinical disease measures were also obtained. Electron Beam Computed Tomography (EBCT) was used to quantify calcification in the coronary arteries. Coronary artery calcium was considered to be present when 3 contiguous pixels greater than 130 HU are detected overlying the vessels of interest. A total calcium score using the Agatston scoring method as well as a total number of calcifications was obtained. A carotid ultrasound was conducted to obtain average wall (intima-media) thickness measurements. Selected images were digitized for later measurement of intima-media thickness by central readers in Pittsburgh.

Lastly, the subjects completed the MAQ which assessed occupational and leisure activities done in the past year. The subject's average past year physical activity score can be expressed in either hours/week, or MET-hours. Data was analyzed using SAS. All statistical testing was conducted at the $p < 0.05$ level of significance.

4.0 RESULTS

4.1 Introduction

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD).

4.2 Subject Characteristics

A cohort of participants from the WOMAN study (n=508) was used for this investigation of baseline data. The 201 women included in this cohort had CRP blood analysis drawn at baseline thus meeting eligibility requirements. All of the women were postmenopausal with a mean age of 57.14 ($\pm$ 2.92) years with 160 (80%) taking HRT and 41 (20%) not on HRT. The majority of the sample was Caucasian (93%). Table 2 identifies further information regarding the characteristics of the sample. Baseline measurements taken from the cohort were tested for normality and are reported in Table 3 as mean $\pm$ standard deviation for normal variables and median (25^{th}, 75^{th} percentiles) for variables with skewed distribution. A comparison of the sample characteristics between the CRP cohort (n=201) and the rest of the WOMAN participants (n=307) revealed that the two groups were significantly different in the following variables: hormone therapy use and race (Tables 2).

Table 2 : Sample Characteristics

Variable	WOMAN CRP Cohort n=(201)	WOMAN n=(307)	p-value
Race			p=0.0062*
Caucasian	93%	88%	
Minorities	7%	12%	
Education			p=0.2621
Some High School	1.5%	1%	
High School Graduate	15.5%	18%	
Some College	30%	29%	
College Graduate	26%	24%	
Advanced Degree	27%	28%	
Current HRT Status			p<0.0001*
On HRT	80%	60%	
Off HRT	20%	40%	
Current Smoking Status			p=0.9125
Smoke	6%	6%	
Do Not Smoke	94%	94%	
Past Smoking Status			p=0.5811
Smoked	48%	50%	
Did Not Smoke	52%	50%	

*p<0.05

Table 3: Baseline Measurements

Variable	CRP Cohort n=(201)
Physical Activity Measures	**Value**
Past Year Leisure PA (MET-hr/week)	11.87 (5.37, 19.63)
Total PA (MET-hr/week)	16.08 (7.88, 30.63)
Past Week Leisure PA (MET-hr/week)	10.20 (4.67, 17.83)
7-Day Mean Pedometer Steps	6,345.30 (4801, 8918)
Bloodwork	
Total Cholesterol (mmol/L)	208 $\pm$ 23.95
HDL-C (mmol/L)	58.70 $\pm$ 13.92
LDL-C (mmol/L)	119.11 $\pm$ 19.70
Triglycerides	133 (99.0, 174.5)
Glucose	93.0 (88.0, 99.0)
Insulin	11.10 (8.9, 14.8)
C-Reactive Protein	2.5 (1.6, 7.0)
Physical Measurements	
Weight (pounds)	174.0 (158.0, 194.0)
Body Mass Index	29.87 (27.10, 32.67)
Waist Circumference	104.50 (98.8, 113.0)
Systolic Blood Pressure (mmHg)	125.05 $\pm$ 12.96
Diastolic Blood Pressure(mmHg)	76.35 $\pm$ 8.59
Subclinical Disease Measures	
Carotid IMT	0.7049 (0.6483, 0.7724)
Coronary Calcium Score	
0-10	86% of cohort
$\geq$10	14% of cohort

4.3 Specific Aim #1

The purpose of this aim was to examine the relation between CRP and physical activity in postmenopausal women. Three physical activity (PA) variables were obtained from the Modifiable Activity Questionnaire (MAQ) all expressed as MET-hr/week: 1) Past year leisure physical activity (LPA), 2) Total PA, and 3) Past week LPA. The fourth physical activity variable was objectively measured in 75 subjects using the Yamax Digi-Walker pedometer. A seven-day average of the number of the steps taken per day was calculated and expressed as steps/day. In addition to the four collected PA variables, the data obtained during the past week LPA assessment was transformed from MET-hr/week to Mins/Wk and is included as a separate variable. The physical activity measurements were strongly correlated to each other as illustrated in Table 4.

Table 4: Spearman Correlations of Physical Activity Measures

	Total PA	**Past Wk LPA**	**Mean Steps**	**Mins/Wk**
Past Yr LPA	0.6456 p<0.0001	0.3805 p<0.0001	0.3204 p=0.0057	0.3359 p<0.0001
Total PA		0.2983 p<0.0001	0.1932 p=0.1015	0.2529 p=0.0003
Past Wk LPA			0.4384 p<0.0001	0.8482 p<0.0001
Mean Steps				0.4876 p<0.0001

Spearman correlations revealed a significant inverse relationship between CRP and Mean pedometer steps (Table 5).

Table 5: Correlation between CRP and Physical Activity

	rho-value	p-value
Subjective		
Past Yr LPA	0.03082	0.6680
Total PA	0.01287	0.8575
Past Wk LPA	-0.01348	0.8497
Mins/Wk	0.0156	0.8265
Objective		
Mean Steps	-0.24413	0.0348*

*p<0.05

To examine the significant correlation between CRP and mean pedometer steps further, CRP was divided into two groups based upon clinically set standard values: <3.0 low/average risk (n=103) and ≥3.0 high risk (n=98). Mean pedometer steps were divided into two groups: <6,000 (n=35) and ≥6,000 steps (n=40). A chi square analysis revealed a significant relationship between the groupings of CRP and pedometer steps (p=0.0237). Figure 1 illustrates the trend of pedometer steps across CRP.

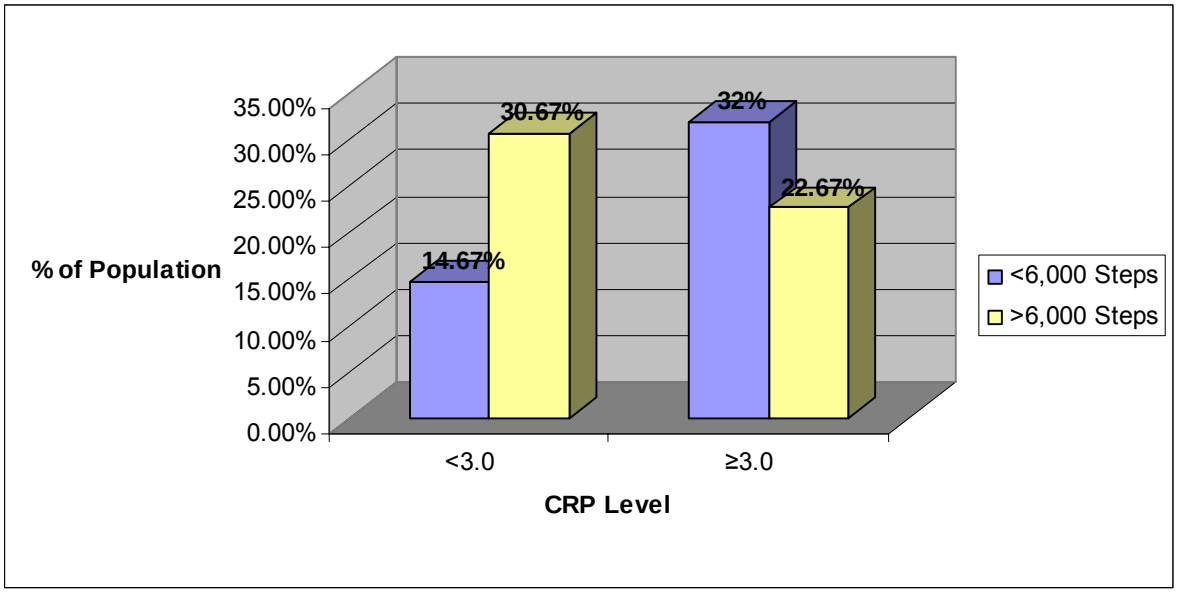

Figure 1: Trend of Pedometer Steps Across CRP Levels

The relationship between pedometer steps as a continuous variable across CRP groups was investigated with regard to HRT. For the women taking HRT, there was no significant difference (p=0.1447) in mean pedometer steps across CRP groups: CRP<3.0 (n=26), CRP≥3.0 (n=35). There also was no significant difference (p=0.0528) in the women not taking HRT in mean pedometer steps across CRP groups: CRP<3.0 (n=8), CRP≥3.0 (n=6). Pedometer steps as a continuous variable by HRT status and CRP group is illustrated in Figure 2.

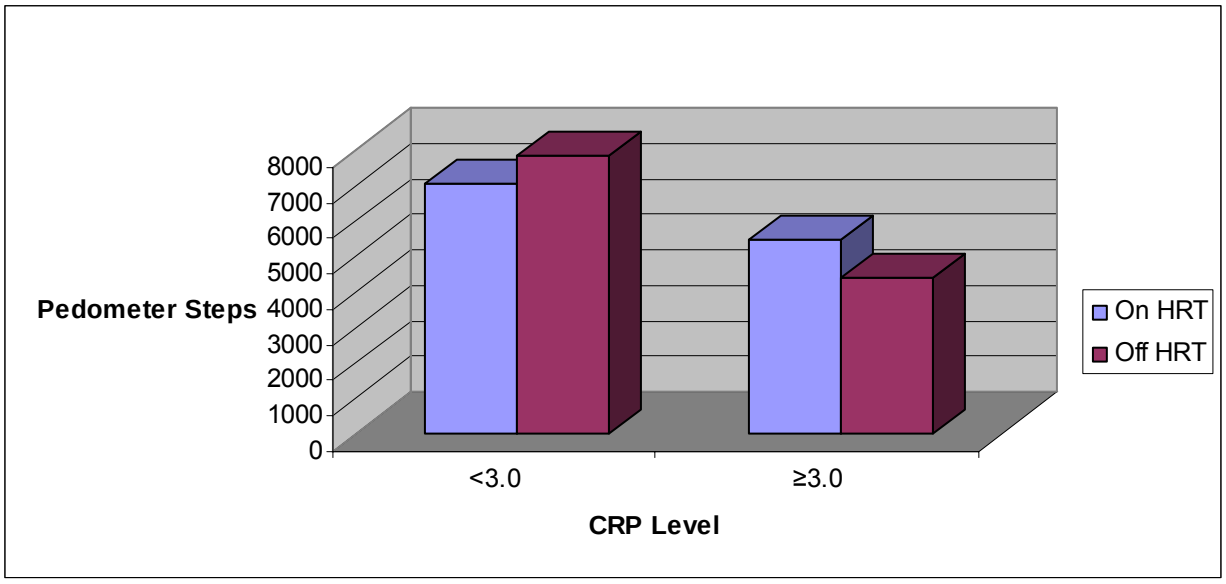

Figure 2: Pedometer Steps by HRT Across CRP Levels

4.4 Specific Aim #2

The purpose of the second specific aim was to determine the factors that are related to CRP in postmenopausal women. The correlations between CRP and the independent variables which were included in the multivariate linear regression analysis were adjusted for age and hormone therapy use (Table 6). Linear regression models were created with CRP as the dependent variable and the following independent variables: past year LPA, HRT status, age, smoking status, total cholesterol, waist circumference, body mass index, glucose, and insulin

(Table 7). The model most related to CRP was found to be: past year physical activity, hormone therapy status, age, smoking status, total cholesterol, body mass index, and fasting glucose (model R^2=0.1204).

Table 6: Adjusted Correlations between CRP and Parameters

	rho-value	p-value
Past Yr LPA	0.0588	0.4332
Mean Pedometer Steps	-0.1666	0.1882
Waist Circumference	0.2212	0.0028*
Body Mass Index	0.2931	<0.0001*
Total Cholesterol	-0.0237	0.7522
HDL-Cholesterol	-0.0701	0.3495
Triglycerides	0.1472	0.0486*
Glucose	0.0336	0.6540
Insulin	0.0786	0.2945

*p<0.05

Table 7: Multivariate Linear Regression Models for Leisure Physical Activity and CRP

Model I	Estimate ± SE
Past Year Leisure PA	0.20 ± 0.21
HRT Status	-1.38 ± 0.84
Age	0.06 ± 0.12
Smoking Status	-1.87 ± 1.51
Total Cholesterol	-0.01 ± 0.01
Model R^2	0.034
Model II: Model I plus Waist Circumference	
Past Year Leisure PA	0.34 ± 0.21
HRT Status	-1.13 ± 0.83
Age	0.08 ± 0.12
Smoking Status	-2.23 ± 1.48
Total Cholesterol	-0.02 ± 0.01
Waist Circumference	0.10 ± 0.03**
Model R^2	0.081
Model III: Model I plus Body Mass Index	
Past Year Leisure PA	0.31 ± 0.20
HRT Status	-1.10 ± 0.81
Age	0.11 ± 0.12
Smoking Status	-2.65 ± 1.46
Total Cholesterol	-0.02 ± 0.01
Body Mass Index	0.38 ± 0.09***
Model R^2	0.114
Model IV: Model III plus Glucose	
Past Year Leisure PA	0.32 ± 0.20
HRT Status	-0.95 ± 0.82
Age	0.12 ± 0.12
Smoking Status	-2.78 ± 1.46*
Total Cholesterol	-0.02 ± 0.01
Body Mass Index	0.41 ± 0.10***
Glucose	-0.05 ± 0.04
Model R^2	0.1204

*p<0.05; **p<0.01; ***p<0.001. PA=Physical Activity. HRT=Hormone Therapy. CRP=C-reactive Protein.

Another set of Linear regression models were created replacing past year leisure PA with mean pedometer steps. The dependent variable was CRP and the independent variables

included: mean pedometer steps, HRT status, age, smoking status, total cholesterol, waist circumference, body mass index, glucose, and insulin (Table 8).

Table 8: Multivariate Linear Regression Models for Mean Pedometer Steps and CRP

Model I	Estimate ± SE
Mean Pedometer Steps	-0.0004 ± 0.0002
HRT Status	-1.90 ± 1.54
Age	0.03 ± 0.23
Smoking Status	-2.13 ± 2.32
Total Cholesterol	0.003 ± 0.03
Model R^2	0.0898
Model II: Model I plus Waist Circumference	
Mean Pedometer Steps	-0.0003 ± 0.0002
HRT Status	-1.70 ± 1.55
Age	0.07 ± 0.23
Smoking Status	-2.63 ± 2.36
Total Cholesterol	0.002 ± 0.03
Waist Circumference	0.06 ± 0.06
Model R^2	0.1052
Model III: Model I plus Body Mass Index	
Mean Pedometer Steps	-0.0003 ± 0.0002
HRT Status	-1.51 ± 1.53
Age	0.13 ± 0.23
Smoking Status	-3.61 ± 2.39
Total Cholesterol	0.00002 ± 0.03
Body Mass Index	0.38 ± 0.19
Model R^2	0.1391
Model IV: Model III plus Glucose	
Mean Pedometer Steps	-0.0002 ± 0.0002
HRT Status	-1.05 ± 1.53
Age	0.11 ± 0.23
Smoking Status	-3.36 ± 2.37
Total Cholesterol	0.02 ± 0.03
Body Mass Index	0.44 ± 0.19*
Glucose	-0.12 ± 0.08
Model R^2	0.1725

*p<0.05; **p<0.01; ***p<0.001. HRT=Hormone Therapy. CRP=C-reactive Protein.

4.5 Specific Aim #3

The purpose of this aim was to determine the association between CRP levels and coronary artery calcium score, carotid artery intimal thickness, and other cardiovascular risk factors in postmenopausal women. Table 9 illustrates the correlations between CRP and the other cardiovascular parameters. Significant positive correlations were identified between CRP and body mass index (rho=0.30810), waist circumference (rho=0.25707), and triglycerides (rho=0.19254).

Table 9: Correlation between CRP & Parameters

	rho-value	p-value	n
Glucose	0.01695	0.5266	200
Insulin	0.07371	0.3161	187
Lipid Profile			
Total Cholesterol	0.01294	0.8556	200
HDL-C	-0.07139	0.3151	200
LDL-C	-0.04504	0.5266	200
Triglycerides	0.19254	0.0063 *	200
Body Mass Index	0.30810	<.0001*	201
Waist Circumference	0.25707	0.0002*	201

*p<0.05

The interaction between CRP and BMI was analyzed. Body mass index was divided into quartiles: <27 (n=44), 27-29.99 (n=61), 30-32 (n= 36), and >32 (n=60). A significant linear relationship was observed between CRP continuously, and BMI quartiles (p=0.0008) as illustrated in Figure 3.

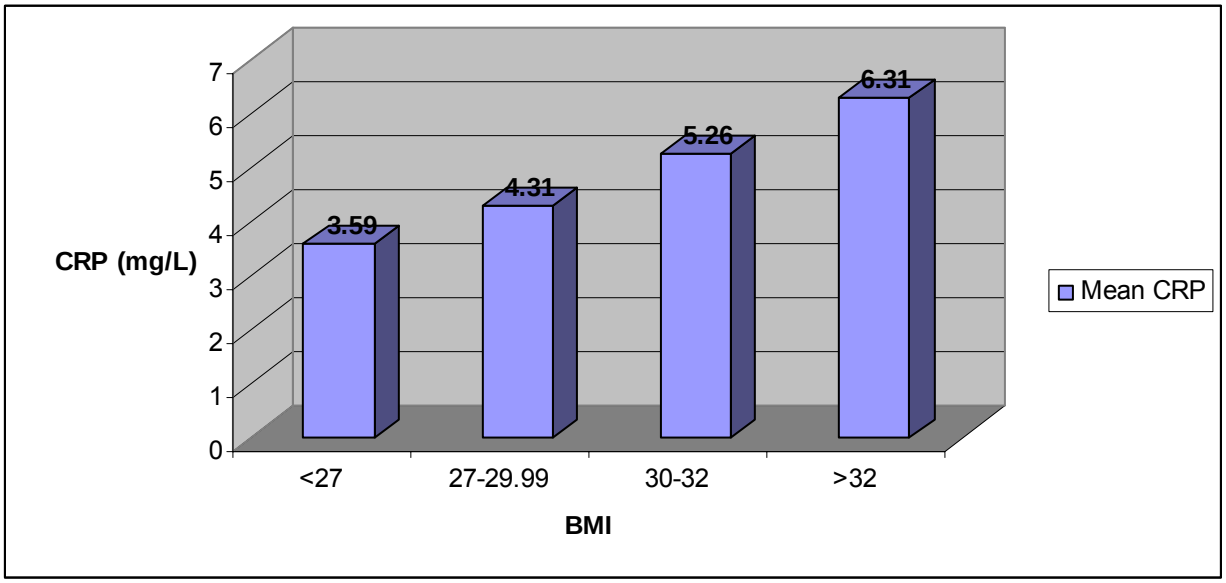

Figure 3: CRP Across BMI Quartiles

To further analyze the relationship between CRP and body mass index, a chi square test was conducted. CRP was divided into two groups based upon clinically set standard values: <3.0 low/average risk (n=103) and ≥3.0 high risk (n=98). Body mass index was divided into quartiles: <27 (n=44), 27-29.99 (n=61), 30-32 (n= 36), and >32 (n=60). The chi square analysis of CRP group * BMI group was statistically significant ($p<0.0003$; trend $p<0.0001$).

C-reactive protein and carotid intimal medial thickness was analyzed utilizing a Kruskal-Wallis statistic. CRP was divided into two groups based upon clinically significant values: <3.0 low/average risk (n=103) and ≥3.0 high risk (n=98). Carotid intimal medial thickness was entered as a continuous variable. There was not a significant relationship between CRP and intimal medial thickness ($p=0.6012$).

A Chi Square analysis was conducted to investigate the relationship between CRP and coronary artery calcium score. Again, CRP was divided into two groups based upon clinically significant values: <3.0 low/average risk (n=103) and ≥3.0 high risk (n=98). The coronary artery calcium score variable was divided into two groups: 0-10 (n=172) and ≥10 (n=29) based upon the University of Pittsburgh Lab protocol. There was not a significant relationship between CRP

and the coronary artery calcium score (p=0.4549). The median CRP values are reported by groups in Table 10.

Table 10: CRP Levels by CRP/CACS Group

Group	CRP Value
CRP <3 and CACS:1-10	1.60 (1.0, 2.2)
CRP <3 and CACS ≥ 10	1.70 (1.3, 2.2)
CRP ≥3 and CACS: 1-10	7.55 (5.3, 11.4)
CRP ≥3 and CACS ≥ 10	5.15 (4.4, 6.45)

To further explore the relationship between coronary artery calcium score and all of the cardiovascular parameters, the cohort was divided into two groups: coronary calcium score 0-10 (n=172), and coronary calcium score ≥10 (n=29). Between group differences were analyzed with either Wilcoxon rank-sum tests, or t-tests for normally distributed variables. Values in Table 11 are reported as mean ± standard deviation for normal variables and median (25^{th}, 75^{th} percentiles) for variables with skewed distribution. Overall, the women who had a coronary artery calcium score ≥10 had significantly higher glucose (p=0.0182), total cholesterol (p=0.0041), and LDL cholesterol (p=0.0119) levels.

All parameters were also analyzed by dividing the cohort by hormone therapy use: On HRT (n=160) and Off HRT (n=41). Women taking hormone therapy had significantly lower LDL-cholesterol (p=0.0363), glucose (p=0.0038), and diastolic blood pressure (p=0.0099) than those not taking hormone therapy. Also, women taking hormone therapy had significantly higher levels of CRP (p=0.0216) than those women not on hormone therapy. Between group differences were analyzed with either Wilcoxon rank-sum tests, or t-tests for normally distributed variables. Values in Table 12 are reported as mean ± standard deviation for normal variables and median (25^{th}, 75^{th} percentiles) for variables with skewed distribution.

Table 11: Coronary Calcium Score Group Differences

Variable	Calcium Score 0-10 n=172	Calcium Score ≥10 n=29	p-value
Physical Activity Measures			
Past Year Leisure PA (MET-hr/week)	11.69 (5.05, 19.26)	14.80 (8.38, 24.53)	0.1719
Total PA (MET-hr/week)	15.86 (7.13, 31.07)	17.99 (8.98, 28.58)	0.6877
Past Week Leisure PA (MET-hr/week)	10.31 (4.67, 18.50)	10.08 (4.67, 14.83)	0.9117
7-Day Mean Pedometer Steps	6345 (4951, 8690)	6218(4110, 9822)	0.8231
Bloodwork			
Total Cholesterol (mmol/L)	206.02 ± 22.91	219.66 ± 26.49	0.0041*
HDL-C (mmol/L)	58.90 ± 14.24	57.51 ± 11.99	0.5961
LDL-C (mmol/L)	117.77 ± 19.89	127.0 ± 16.75	0.0119*
Triglycerides	133.0 (101.0, 169.0)	139.0 (98.0, 202.0)	0.5513
Glucose	92.0 (87.0, 98.0)	96.0 (92.0, 103.0)	0.0182*
Insulin	11 (8.8, 14.4)	12.8 (10.0, 16.8)	0.1004
C-Reactive Protein	2.5 (1.5, 7.3)	3.8 (2.1, 5.4)	0.9204
Physical Measurements			
Weight (pounds)	173.5 (157.5, 192.75)	174.5 (162.5,200.0)	0.3998
Body Mass Index	29.73 (27.04, 32.52)	30.40 (27.83, 35.18)	0.1417
Waist Circumference	104.0 (98.35, 113.3)	106.8 (99.8, 110.7)	0.6906
Systolic Blood Pressure (mmHg)	124.31 ± 12.73	129.45 ± 13.63	0.0602
Diastolic Blood Pressure (mmHg)	76.15 ± 8.26	77.52 ± 10.40	0.4212
Subclinical Disease Measures			
Carotid IMT	0.6991 (.6473, .7694)	0.7356 (.6552, .8132)	0.1434

*$p<0.05$

Table 12: Group Differences by Hormone Therapy Use

Variable	On HRT n=160	Not On HRT n=41	p-value
Physical Activity Measures			
Past Year Leisure PA (MET-hr/week)	12.03 (5.54, 19.65)	11.30 (3.77, 18.92)	0.7832
Total PA (MET-hr/week)	16.52 (9.18, 31.23)	15.38 (4.62, 29.37)	0.3342
Past Week Leisure PA (MET-hr/week)	10.79 (5.50, 18.78)	8.46 (3.79, 13.79)	0.0644
7-Day Mean Pedometer Steps	6251 (4951, 8690)	6587(4110, 9799)	
Bloodwork			
Total Cholesterol (mmol/L)	207.42 $\pm$ 24.47	210.27 $\pm$ 21.94	0.4711
HDL-C (mmol/L)	58.91 $\pm$ 13.74	57.89 $\pm$ 14.74	0.6898
LDL-C (mmol/L)	117.72 $\pm$ 20.06	124.46 $\pm$ 17.47	0.0363*
Triglycerides	140.0 (102.0, 176.0)	113.0 (98.0, 150.0)	0.0684
Glucose	92.0 (87.0, 98.0)	98.0 (90.0, 103.0)	0.0038*
Insulin	11.0 (9.0, 15.20)	11.85 (8.35, 14.5)	0.9934
C-Reactive Protein	3.4 (1.85, 7.10)	1.70 (1.2, 4.7)	0.0216*
Physical Measurements			
Weight (pounds)	173.0 (157.8, 193.5)	175.0 (158.5,194.3)	0.8463
Body Mass Index	29.90 (27.11, 32.85)	29.46 (26.76, 31.89)	0.3963
Waist Circumference	105.30 (98.8, 113.3)	102.5 (97.0, 110.5)	0.2853
Systolic Blood Pressure (mmHg)	124.73 $\pm$ 13.09	126.29 $\pm$ 12.50	0.4824
Diastolic Blood Pressure (mmHg)	75.63 $\pm$ 8.76	79.17 $\pm$ 7.33	0.0099*
Subclinical Disease Measures			
Carotid IMT	0.7006 (.6473, .7694)	0.7060 (.6489, .7871)	0.7482
Coronary Calcium Score			
0-10	69% of cohort	17% of cohort	
$\geq$ 10	11% of cohort	3% of cohort	

*$p<0.05$

4.6 Specific Aim #4

The purpose of this aim was to determine the factors that are related to the coronary artery calcium score in postmenopausal women. A logistic stepwise regression analysis was completed with coronary artery calcium score as the dependent variable and the following

independent variables: CRP, past year LPA, carotid artery intimal medial thickness, total cholesterol, HDL-cholesterol, triglycerides, waist circumference, HRT status, and age. Past year LPA was forced into the model and all other variables were left in the model at the 0.05 significance level. The best subset of related factors for coronary artery calcium score (Table 13) was found to be: past year LPA, and average waist circumference (model p=0.0356).

Table 13: Logistic Stepwise Regression Model for Coronary Artery Calcium Score

Model[1]	Estimate $\pm$ SE	P value
Past Year LPA	0.0946 $\pm$ 0.0933	0.3109
Waist Circumference	0.0541 $\pm$ 0.0154	0.0004

[1]Variables in order of entry into multiple regression model

4.7 Specific Aim #5

The purpose of this aim was to identify subjects with characteristics of metabolic syndrome and to determine if there were differences in CRP, coronary artery calcium score, carotid artery intimal thickness, and physical activity among those women without metabolic syndrome. According to the ATP III guidelines, a woman would be classified as having metabolic syndrome if they exhibited any three or more of the following criteria: 1) waist circumference >88 cm, 2) serum triglycerides $\geq$ 1.7 mmol/L, 3) blood pressure $\geq$130/85 mmHg, 4) HDL cholesterol <1.3 mmol/L, and 5) serum glucose $\geq$6.1 mmol/L (32). In this cohort, 38% of the women (n=76) had characteristics of metabolic syndrome while 62% (n=125) did not. Table 14 outlines the distribution of the cohort with regards to the number of characteristics of metabolic syndrome.

Table 14: Frequency Table of Metabolic Syndrome Characteristics

# of Characteristics	≤ 1	2	3	≥4
n (%)	56 (28)	68 (34)	53 (26.5)	23 (11.5)

Between group differences were analyzed with either Wilcoxon rank-sum tests, or t-tests for normally distributed variables. Values in Table 15 are reported as mean ± standard deviation for normal variables and median (25^{th}, 75^{th} percentiles) for variables with skewed distribution.

Table 15: Group Differences by Metabolic Syndrome Classification

Variable	No Met. Syndrome n=125	Met. Syndrome n=76	p-value
Physical Activity Measures			
Past Year Leisure PA (MET-hr/week)	12.81 (5.18, 19.65)	11.17 (5.63, 18.80)	0.5040
Total PA (MET-hr/week)	17.01 (8.28, 35.49)	15.77 (7.25, 27.86)	0.6752
Past Week Leisure PA (MET-hr/week)	10.79 (5.38, 19.60)	9.54 (3.77, 15.12)	0.1278
7-Day Mean Pedometer Steps	6251 (5105, 10139)	6381(4526, 7887)	0.2902
Bloodwork			
Total Cholesterol (mmol/L)	204.46 ± 23.68	213.78 ± 23.39	0.0072*
HDL-C (mmol/L)	63.26 ± 13.13	51.26 ± 11.87	<0.0001*
LDL-C (mmol/L)	118.18 ± 20.51	120.62 ± 18.34	0.3839
Triglycerides	111.50 (89.0, 140.0)	179.0 (153.5, 240.0)	<0.0001*
Glucose	91.50 (87.0, 95.0)	98.0 (91.5, 103.0)	<0.0001*
Insulin	10.70 (8.3, 13.70)	12.95 (9.40, 16.85)	0.0183*
C-Reactive Protein	2.5 (1.5, 7.1)	3.15 (1.85, 6.30)	0.4509
Physical Measurements			
Weight (pounds)	170.5 (158.0, 191.0)	179.75 (158.0, 201.5)	0.1764
Body Mass Index	28.69 (27.07, 31.86)	31.12 (27.33, 34.15)	0.0210*
Waist Circumference	103.70 (98.3, 110.8)	106.95 (99.0, 116.9)	0.0942
Systolic Blood Pressure (mmHg)	122.25 ± 12.54	129.66 ± 12.37	<0.0001*
Diastolic Blood Pressure (mmHg)	75.39 ± 7.93	77.92 ± 9.42	0.0524
Subclinical Disease Measures			
Carotid IMT	0.6899 (.6483, .7583)	0.7287 (.6473, .7966)	0.0378*
Coronary Calcium Score			
0-10	56% of cohort	29% of cohort	
≥10	6% of cohort	9% of cohort	

*p<0.05

When compared to those women without characteristics of metabolic syndrome, women with metabolic syndrome had significantly lower HDL-cholesterol levels ($p<0.0001$) and higher total cholesterol ($p=0.0072$), triglycerides ($p<0.0001$), glucose ($p<0.0001$), and insulin ($p=0.0183$) levels. These women also had significantly higher body mass index ($p=0.0210$), systolic blood pressure ($p<0.0001$), and carotid intimal medial thickness ($p=0.0378$). Lastly, coronary artery calcium score and the prevalence of metabolic syndrome was investigated. A Chi Square analysis: metabolic syndrome * coronary calcium score was conducted. There was a significant relationship between the two parameters ($p=0.0125$).

To better examine the characteristics of those women with metabolic syndrome, the metabolic syndrome characteristics were divided into four groups: $\leq$1 (n=56), 2 (n=68), 3 (n=53), and $\geq$4 (n=23). The group differences by metabolic syndrome characteristics are presented in Table 16. As the number of characteristics increased, a significant increase was also observed in total cholesterol ($p=0.0431$), triglycerides ($p<0.0001$), glucose ($p<0.0001$), insulin ($p= 0.0010$), body mass index ($p=0.0037$), and systolic blood pressure ($p=0.0007$). Also, HDL-cholesterol significantly decreased ($p<0.001$) as the number of metabolic syndrome characteristics increased.

The subclinical disease measures were also examined with regards to the number of characteristics of metabolic syndrome. Carotid artery intimal medial thickness was not statistically different among the number of metabolic syndrome characteristics ($p=0.1878$). The median intimal medial thickness of the carotid artery for each characteristic group is illustrated in Figure 5. There was also, no significant difference in coronary artery calcium score was observed between the number of metabolic syndrome characteristics ($p=0.0674$, trend $p=0.0452$).

Table 16: Group Differences by Metabolic Syndrome Characteristics

	≥1 n=56	2 n=68	3 n=53	≥4 n=23
Physical Activity Measures				
Past Year Leisure PA (MET-hr/week)	14.37 (5.35, 21.81)	11.64 (5.07, 19.0)	10.26 (4.62, 19.17)	11.74 (8.3, 17.62)
Total PA (MET-hr/week)	17.75 (9.76, 34.63)	15.82 (7.12, 49.34)	15.71 (6.46, 27.86)	15.77 (9.28, 28.99)
Past Week Leisure PA (MET-hr/week)	12.25 (5.25, 21.46)	10.23 (5.08, 18.14)	9.58 (3.79, 15.28)	9.50 (2.92, 14.96)
7-Day Mean Pedometer Steps	6251 (5111, 101461)	6128 (4732, 9822)	5687 (4308, 7060)	7032 (4526, 8690)
Bloodwork				
Total Cholesterol (mmol/L) *	201.63 ± 23.95	206.79 ± 23.38	212.79 ± 20.27	216.04 ± 29.78
HDL-C (mmol/L) ****	64.51 ± 10.60	62.23 ± 14.90	54.02 ± 12.88	44..90 ± 5.23
LDL-C (mmol/L)	116.80 ± 22.34	119.31 ± 18.96	120.75 ± 18.33	120.30 ± 18.77
Triglycerides ****	103.50 (80.0, 127.0)	123.0 (99.0, 146.5)	175.0 (140.0, 233.0)	196.0 (168, 276)
Glucose ****	90.0 (85.5, 93.0)	92.0 (88.0, 98.0)	97.0 (91.0, 103.0)	101.0 (92.0, 107.0)
Insulin ***	10.15 (7.4, 11.95)	11.80 (9.6, 15.90)	11.65 (9.30, 14.80)	13.65 (10.8, 20.40)
C-Reactive Protein	2.3 (1.2, 6.85)	3.8 (1.75, 7.40)	2.40 (2.1, 6.40)	4.8 (1.4, 6.2)
Physical Measurements				
Weight (pounds)	166.25 (152.3, 187.5)	178.25 (163.0, 194.2)	176.0 (157.5, 200.0)	188.0 (164, 213)
Body Mass Index **	28.08 (26.72, 31.43)	29.93 (27.54, 32.14)	30.38 (26.42, 33.40)	32.3 (29.7, 35.4)
Waist Circumference	103.60 (96.2, 109.7)	104.15 (99.95, 113.3)	104.80 (98.8, 116.8)	108.3 (99.8, 117.8)
Systolic Blood Pressure (mmHg)***	120.41 ± 11.08	123.71 ± 13.61	130.38 ± 12.46	128.0 ± 12.28
Diastolic Blood Pressure (mmHg)	74.79 ± 7.84	75.82 ± 8.07	78.11 ± 9.59	77.48 ± 9.21
Subclinical Disease Measures				
Carotid IMT	0.6866 (.6499, .7401)	0.7079 (.6440, .7695)	0.7325 (.6331, .8131)	.7249(.6676,.7694)
Coronary Calcium Score				
0-10	24% of cohort	31% of cohort	21% of cohort	9% of cohort
≥10	3.5% of cohort	2.5% of cohort	6% of cohort	3% of cohort

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

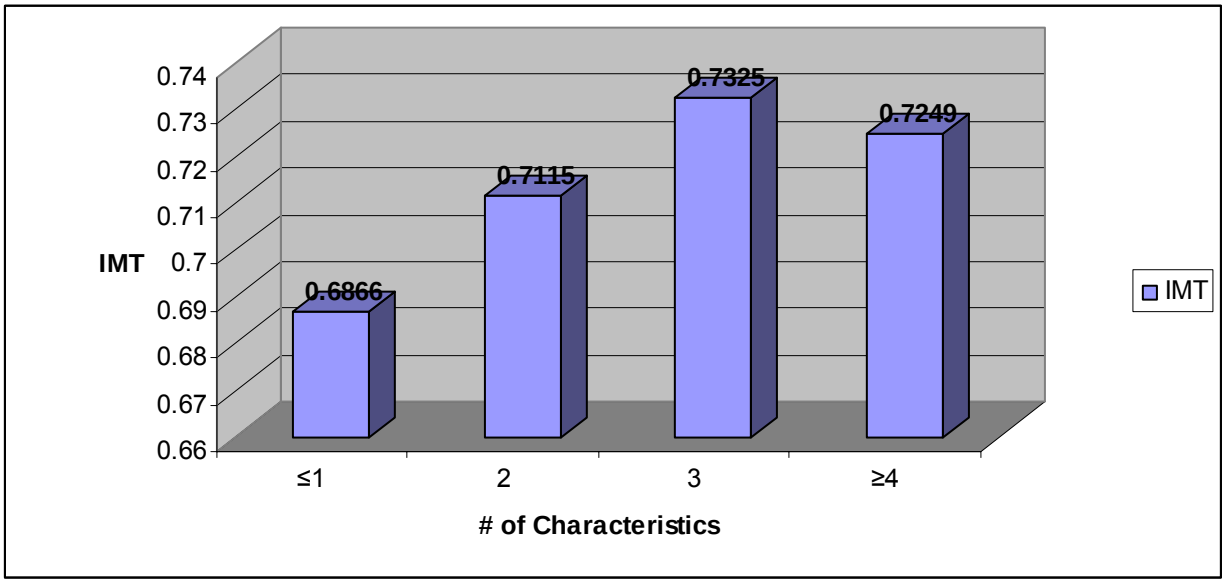

Figure 4: Median IMT Across Metabolic Syndrome Groups

5.0 DISCUSSION

5.1 Introduction

The primary purpose of this study was to examine the associations between C-reactive protein (CRP) and physical activity on the risk factor profile of postmenopausal women without known heart disease who were either taking or not taking hormone therapy. The secondary purpose of this investigation was to examine the relation of other cardiovascular risk factors on subclinical measures of coronary heart disease (CHD).

A cohort of 201 females who were participating in the WOMAN study was used for this investigation. All of the women were postmenopausal and most (80%) at the time of the collection of the baseline data were taking HRT. The majority of the sample was Caucasian (93%) and educated with 83% having at least some college education or completing college degrees. On average, the cohort had a lipid profile within normal range per ATP III Guidelines and an "average risk" CRP level. As a cohort, the women were overweight with a body mass index of 29.87 and a waist circumference of 104.5-cm which is not surprising due to the study inclusion criteria. To participate in the WOMAN study, their waist circumference had to be $\geq$80 centimeters.

The baseline data obtained on this cohort was analyzed for this investigation and the results will be discussed in the following sections: 1) Physical activity & CRP, 2) CRP & Cardiovascular Risk Markers, 3) HRT & Cardiovascular Risk Markers, 4) Coronary Artery Calcium Score, and 5) Metabolic Syndrome.

5.2 Physical Activity & CRP

As previously mentioned, coronary heart disease is increasingly being viewed as an inflammatory process and that high sensitivity C-reactive protein, a marker of inflammation, becomes elevated with atherogenesis and therefore can aid in prediction of CHD risk (54). It is widely recognized that physical activity can aid in the prevention of CHD and there is evidence that higher levels of physical activity are associated with lower levels of C-reactive protein (1,25). Although, the physiological mechanism responsible for this pattern is unclear, one could hypothesize that physical activity reduces the risk of CHD by reducing or preventing the inflammatory process.

In this cohort of postmenopausal women, there was not a significant relationship between self-reported subjective measures of physical activity and CRP levels. Three subjective physical activity variables were obtained from the Modifiable Activity Questionnaire: 1) Past year LPA, 2) Total PA, and 3) Past week LPA. Of the three, only past week LPA had a very weak inverse association (rho= -0.01348) with CRP. A fourth physical activity variable, pedometer steps, was objectively measured and did have a significant inverse relationship with CRP (rho= -0.24413).

The inconsistencies observed between the subjective and objective measures of physical activity may be due to many factors. First, the PA measures utilized in this study capture different intensities of exercise. The MAQ physical activity questionnaire captures moderate to high levels of physical activity as compared to the pedometer. The pedometer is an accurate tool for measuring activities of daily living (lower intensity), however, does not capture cycling, water activities, and arm movement. Thus, the inconsistent findings could be a product of methodological precision.

Second, unlike the three subjective PA measures which were obtained on 201 subjects, pedometer step counts were only collected on 75 women in this cohort. Another possible

explanation for these inconsistencies among PA measures could be due to the relatively small sample size; therefore, the strength of the relationship may have been weakened. If all of the physical activity measures had been collected on equal amounts of study participants, comparison among the measures would be more revealing.

Third, there might not have been a strong association between CRP levels and physical activity observed in this study because of the activity level of the cohort. As a whole, they completed a median of 16.08 (7.88, 30.63) MET-hrs per week of physical activity. To get a better look at the volume of exercise completed, the data obtained during the past week LPA assessment was transformed from MET-hr/week to Mins/Wk which reveled that the women completed a median of 140 (60, 220) mins/wk of moderate physical activity. Also, as recorded by the pedometer they walked a median of 6,345.30 (4,801, 8,918) steps per week. Current recommendations for physical activity as per the United States Surgeon General suggest that individuals accumulate at least 30 minutes of moderate physical activity on most days of the week (approximately 150 mins/wk) (86). Pedometer indices for public health have been created and can be used to classify adults into the following activity levels: 1) <5,000 steps/day – Sedentary, 2) 5,000-7,499 steps/day – Low active, 3) 7,500-9,999 steps/day –Somewhat active, and 4) $\geq$ 10,000 steps/day –Active (97). Thus, in comparison to public health standards, this cohort of women was low-active. Perhaps, if there was a larger range of activity levels within the cohort, a stronger association would have been observed between CRP and PA.

Overall, in the research investigating CRP and physical activity, two distinct and conflicting conclusions have been supported. The first is that there is an inverse relationship between CRP and physical activity supporting the hypothesis that physical activity may reduce inflammation (5, 25). This relationship remained after adjusting for confounding variables including BMI and waist-hip ratio (5, 25). The second conclusion from previous research

suggests that the relationship between CRP and physical activity is not as strong as the impact of body mass index and adiposity on CRP levels (71, 97).

The results of the present investigation show that physical activity may play a role in CRP levels as objectively measured by pedometer steps but also suggests that anthropometic measures have a significant impact on CRP levels. Overall, physical activity was significantly related to the anthropometric measures. Past year physical activity was inversely related to waist circumference (rho= -.019014). Also, there was a significant inverse relationship between mean pedometer steps and body mass index (rho= -0.33990) as well as to waist circumference (rho= -0.26009). Due to these varied results within previous research and within this current investigation, more research investigating CRP and its relationship with physical activity and adiposity is warranted.

5.3 CRP & Cardiovascular Risk Markers

The relationship between CRP and other cardiovascular risk markers was investigated. In this cohort, significant positive correlations were identified between CRP and body mass index (rho=0.30810), waist circumference (rho=0.25707), and triglycerides (rho=0.19254). In the group of women who had a CRP level >3.0 (high relative risk), a significantly larger proportion of them had a BMI $\geq$ 29.9. In this investigation, the best subset of predictors for CRP was found to be average waist circumference, smoking status, past year physical activity, and total cholesterol levels. These results also support the theory that adiposity is strongly linked to CRP levels.

As mentioned earlier, obesity has multiple roles in triggering the inflammation associated with atherogenesis. Not only does obesity increase the risk of insulin resistance and diabetes, but it also contributes to dyslipidemia; all which may cause inflammation (54). Obesity directly

increases inflammation due to adipose tissue synthesizing proinflammatory cytokines such as interleukin-6, a primary hepatic stimulant for CRP production (107).

5.4 HRT & Cardiovascular Risk Markers

The present cohort was divided into two groups consisting of those who were taking HRT (n=113), and those who were not (n=88). The women taking HRT had significantly lower LDL-cholesterol (117.72 $\pm$ 20.06 mmol/L), glucose [92.0 (87.0, 98.0)], and diastolic blood pressure (75.63 $\pm$ 8.76 mmHg) and significantly higher levels of CRP [3.4 (1.85, 7.10)] than the women who were not taking HRT.

Previous research has supported the results of this current investigation. Generally, estrogen replacement has been shown to decrease total cholesterol and LDL cholesterol while increasing HDL cholesterol and triglycerides. Also, women taking HRT have been found to have elevated CRP levels. The study conducted by Manns et al. (2003) found that the median CRP levels for women on hormone therapy (with or without progestin) was 3.5 – 5.5 times higher than those not taking hormone therapy.

In a different study, investigators examined the hypothesis that hormone replacement therapy related increases in CRP would either be blunted or absent in those postmenopausal women who regularly exercised (95). Plasma CRP levels were ~75% higher in the sedentary group who were hormone therapy users when compared to sedentary non-users. There was no significant difference in CRP levels between hormone therapy groups for the physically active women. The researchers concluded that since the elevation in CRP due to hormone replacement therapy observed in sedentary women is absent in women who engage in regular physical activity, physical activity may prevent the increase in CRP secondary to hormone replacement therapy (95).

In the cohort from the WOMAN study, there were no differences in physical activity between those women taking HRT and those not on HRT. It should be noted however that 80% of the cohort was on HRT thus possibly minimizing the between group differences.

5.5 Coronary Artery Calcium Score

Coronary calcification as measured by EBCT has been shown to be associated with traditional risk factors of coronary artery disease in women (24, 47). In both of these investigations, most of the traditional risk factors were associated with the coronary calcium score after adjustment for age and gender. Coronary calcification was associated with LDL-cholesterol, HDL cholesterol, systolic blood pressure, glucose, trigylcerides, body mass index, and cigarette smoking (24, 47).

In this current research project, to investigate the relationship between coronary calcification and other cardiovascular risk markers, the cohort was divided into two groups based upon the coronary calcium score: 0-10, or $\geq$10. According to the University of Pittsburgh EBCT lab, coronary calcium scores ranging from 0-10 have limited clinical value. Therefore it was recommended that this dichotomous grouping be used. The women who had coronary calcification scores greater than 10 had significantly higher glucose, total cholesterol, and LDL cholesterol levels than those women with coronary calcium levels <10. There was no significant association between coronary calcification and CRP. An analysis was completed to find the best subset of related factors for coronary artery calcium score. The model reveled that past year LPA, and average waist circumference were the best predictors of the coronary artery calcium score.

The association of nontraditional cardiovascular risk factors with coronary artery calcification was examined in a cohort of the Atherosclerosis Risk in Communities (ARIC)

Study (24). Interestingly, the researchers did not find significant associations between the coronary calcium score and sports physical activity, insulin, nor with the inflammation marker CRP. Similarly, in the present investigation, only the traditional cardiovascular risk factors were associated with coronary calcification and no evidence was found for the inclusion of the "non-traditional" risk factors in the overall CHD risk assessment model.

5.6 Metabolic Syndrome

Since most instances of premature CHD occur in women who have a clustering of risk factors as seen in the "metabolic syndrome", these individuals should be targeted for more intensive cardiovascular risk reduction. According to the ATP III guidelines, a woman would be classified as having metabolic syndrome if they exhibited any three or more of the following criteria: 1) waist circumference >88 cm, 2) serum triglycerides $\geq$ 1.7 mmol/L, 3) blood pressure $\geq$130/85 mmHg, 4) HDL cholesterol <1.3 mmol/L, and 5) serum glucose $\geq$6.1 mmol/L (32).

Based upon these guidelines, the cohort in this investigation was divided into two groups: those with characteristics of metabolic syndrome (n=76), and those without (n=125). Between group differences were analyzed. Expectedly, those women with metabolic syndrome had significantly lower HDL-cholesterol, higher triglycerides and glucose levels. Also, those with metabolic syndrome were found to have higher total cholesterol levels, insulin levels, body mass index, systolic blood pressure, and carotid intimal medial thickness. There was not a statistically significant difference in CRP levels between groups; however those women in the metabolic syndrome group had a median CRP level of 3.15 which is considered high relative risk as compared to those without (CRP level of 2.5, normal risk).

5.7 Limitations and Recommendations for Future Research

This study is not without limitations and these may minimize the application of these findings. Future studies should address these factors to improve the generalizability and impact of these findings.

1) This was a cross-sectional investigation of a cohort of women participating in the WOMAN study. A longitudinal analysis may better show the associations between CRP and physical activity on the risk factor profile of postmenopausal women. Also, a longitudinal design would be better to look at the predictive value of cardiovascular risk factors on subclinical measures of coronary heart disease.

2) Although physical activity was measured both subjectively and objectively in this investigation, a much smaller sample of the cohort (37%) completed the objective pedometer step measurement. Future research should equally collect physical activity variables both subjectively through questionnaires and objectively using a tool such as a pedometer. Also, future research could include other objective measures of physical activity such as an accelerometer, the Body Media armband, and/or doubly-labeled water. A physical activity index utilizing the Ratings of Perceived Exertion (RPE) Scale and pedometer step count could be utilized to quantify the total aerobic exercise training load.

3) In this investigation, the women may not have been a true representation of all post-menopausal women due to the inclusion criteria of the WOMAN study. For inclusion, the waist circumference was >80cm, thus this population could have had more waist adiposity than the general population. This could have limited the effect of physical activity on CRP. Future research should investigate the relationship between physical activity and CRP without any body measurement inclusion criteria, therefore increasing generalizability of the results.

4) This cohort of women was 93% Caucasian and educated. Therefore the generalizability of the findings is limited to women with similar demographics to the subjects who participated in this investigation. In future research, ethnic differences could be investigated with regard to CRP, and cardiovascular risk factors in general.

APPENDIX A

PARTICIPANT INFORMATION

WOMAN Study

Participant Information

Please only complete lines where there are changes from last visit.

ID:_____

Date:_____/_____/_____(MM/DD/YY)

Staff:_____

Name:_____

Address:_____

_____

Phone #: **(Home)**_(____)_____

(Work)_(____)_____

(Other)_(____)_____

Email: _____

Alternative Contact:_____**Phone (Home):** _(____)_____

(Preferably somebody who does not live with you)

Relation to Participant:_____ **(Work):** _(____)_____

Emergency Contact:_____**Phone (Home):** _(____)_____

Relation to Participant:_____ **(Work):** _(____)_____

Primary Physician's Name:_____

Address:_____

_____

PCP_Phone#:_(____)_____

ID _____

Date_____

The WOMAN on the Move Study

HEIGHT, WEIGHT, AND WAIST MEASUREMENTS:

1. **Standing Height**- Record to nearest half-inch
   (round up) without shoes: ____. ____**inches**
   ____.____cm

2. **Weight -** record to nearest half-pound, (round up)
   clothed, without shoes: _____. ____**lbs.**
   _____.____**kg.**

WAIST MEASUREMENTS (cm) - Record to nearest (.1) cm

1st reading_____ 2nd reading_____ 3rdreading_____

If first 2 readings are within 2 cm, average the 2. If not, do a third measurement and average the 2 measures within range (2 cm). If even after 3 measures there are no matches, take the average of the 2 closest measurements.

3. Average waist circumference _____. ___cm
   (≥ 80 cm–eligible)

HEART RATE

4. Beats in 30 seconds: _____X2 = _____

BLOOD PRESSURE MEASUREMENTS:

Cuff Size: ____ Regular ____Large ____Pediatric _____Thigh

Arm: _____Left _____Right

5. First Reading _____/_____
   Systolic Diastolic

6. Second Reading _____/_____
   Systolic Diastolic

7. Average of blood pressure Readings_____/_____
   Systolic Diastolic

ID _____

BLOOD DRAW (12 HOUR FAST)

8. When did you last have anything other than water to eat or drink? _____ a.m./p.m.

Note:If less than 10 hours from clinic appointment, participant should be rescheduled for the blood draw.

Time Blood Drawn: _____ a.m./p.m.

Difficult blood draw? 1. ☐Yes 2. ☐No

Was blood sample collected? 1. ☐Yes 2. ☐ No, specify reason

Tubes collected: (T if completed)

EDTA 10mL: _____
Serum 15 mL: _____
Serum 10 mL: _____
Na Nitrate 5mL:_____

MEDICATION(S) LIST

Office Use Only
 # meds _____

9. Over the past 2 weeks have you taken any prescribed medications?

1. ☐Yes 2. ☐ No

Medication 1:	Medication 2:	Medication 3:
Type _____	Type _____	Type_____
Date began_____mo/yr	Date began_____mo/yr	Date began_____mo/yr
Dose_____mg	Dose_____mg	Dose_____mg
Freq._____x /day	Freq._____x /day	Freq._____x /day
Reason_____	Reason_____	Reason_____

Medication 4:	Medication 5:	Medication 6:
Type _____	Type _____	Type_____
Date began_____mo/yr	Date began_____mo/yr	Date began_____mo/yr
Dose_____mg	Dose_____mg	Dose_____mg
Freq._____x /day	Freq._____x /day	Freq._____x /day
Reason_____	Reason_____	Reason_____

Medication 7: Medication 8: Medication 9:

Type _____ Type _____ Type_____

Date began_____mo/yr Date began_____mo/yr Date began_____mo/yr

Dose_____mg Dose_____mg Dose_____mg

Freq._____x /day Freq._____x /day Freq._____x /day

Reason_____ Reason_____ Reason_____

9a. Do you take aspirin (not Tylenol): (0) ☐ daily
(1) ☐ 1-6 times/week
(2) ☐ less than once/week
(3) ☐ rarely
(4) ☐ never (go to blood results)

9b. When you take aspirin, how many on the average do you take per day?_____per day

9c. How many aspirin have you taken in the last 48 hours _____aspirin
(00 if none)

HORMONE REPLACEMENT USE

10. Are you currently taking hormone therapy? 1. ☐ Yes (if yes, proceed to #11) 2. ☐ No

10a. If no, what was the date that you stopped taking HRT? _____(MM/YY) (proceed to #13)

11. What are the hormones that you are taking?

Hormone 1: (Please circle one)
Oral Patch Injection Other

Name_____
Date Began_____(MM/YY)
Dose_____mg
Days/Month_____
Months Taken_____

Hormone 2: (Please circle one)
Oral Patch Injection Other

Name_____
Date Began_____(MM/YY)
Dose_____mg
Days/Month_____
Months Taken_____

Hormone 3: (Please circle one)
Oral Patch Injection Other

Name_____
Date Began_____(MM/YY)
Dose_____mg
Days/Month_____
Months Taken_____

Hormone 4: (Please circle one)
Oral Patch Injection Other

Name_____
Date Began_____(MM/YY)
Dose_____mg
Days/Month_____
Months Taken_____

12. Have you stopped taking your hormones in the past year for more than 1 week?

1. □ Yes 2. □ No | **Go to #14**

a. If yes, how many occasions did you stop taking your hormones? _____ (Proceed to #14)

13. What were the primary reasons you stopped taking hormone replacement therapy? (Check all that apply)

□ Directed by physician

□ Needed second opinion from another doctor

□ Felt like I didn't need it

□ Thought the medication was harmful

□ The cost of the medication

□ Developed a new health problem

□ Heard about the Women's Health Initiative HRT results and decided to stop because of health risks

□ Had undesirable side effects (check all that apply below):

___ Weight gain ___ Depression/mood swings

___ Bleeding ___ Flu-like symptoms

___ Breast lumps ___ Headaches

___ Breast tenderness ___ Other_____

☐ Other (please specify)_____

14. Have you experienced any side effects while on HRT?

1. □ Yes 2. □ No

(if yes, □ check all that apply below)

____Weight gain ____Depression/mood swings

____Bleeding ____Flu-like symptoms

____Breast lumps ____Headaches

____Breast tenderness ____Other_____

15. Do you currently smoke?

1. □ Yes 2. □ No

16. Have you had a mammogram in the last year?

1. □ Yes 2. □ No 1a. **If yes, Date: _____/_____mo/yr**

If no, explain importance of mammography and where to have them done

17. Have you ever been diagnosed by a physician with cancer?

1. ☐ Yes 2. ☐ No (If no, proceed to #18)

17a. If yes, Date: _____/_____mo/yr

17b. If yes, which type?

☐ Breast

☐ Uterine (Endometrium)

☐ Ovary

☐ Other, describe _____

18. Have you had a cardiovascular event since the start of your participation in this study?

1. ☐ Yes 2. ☐ No (If no, questionnaire, go to #19)

18a. If yes, which type of event?

☐ Myocardial infarction; Date:_____/_____mo/yr

☐ Stroke; Date:_____/_____mo/yr

☐ Venous thrombosis: Date:_____/_____mo/yr

☐ Aneurysm, in what location (e.g.,aortic)?_____; Date:_____/____mo/yr

☐ Other?, describe_____; Date:_____/_____mo/yr

19. Has your doctor ever told you that you have:

Arthritis	1. ☐ Yes	2. ☐ No
Diabetes (Sugar)	1. ☐ Yes	2. ☐ No
Lung Disease	1. ☐ Yes	2. ☐ No
Kidney Disease	1. ☐ Yes	2. ☐ No
Pancreatitis	1. ☐ Yes	2. ☐ No
Gallstone	1. ☐ Yes	2. ☐ No
Thyroid Disease	1. ☐ Yes	2. ☐ No

20. Have you ever had gastric bypass surgery?

1. ☐ Yes 2. ☐ No

If yes; Date:_____/_____mo/yr

ID_____

Corridor Walk

Exclusion Questions

Script: "*The following tests will assess your physical fitness by having you walk 1/4 mile at a steady pace. First I need to ask you a few questions to see if you should try the test."*

a. Does the participant use a walking aid, such as a cane?	□ Yes	□ No
b. Within the past 3 months, have you had a heart attack?	□ Yes	□ No
c. Within the past 3 months, have you had angioplasty?	□ Yes	□ No
d. Within the past 3 months, have you had heart surgery?	□ Yes	□ No
e. Within the past 3 months, have you had any new or worsening heart pain or angina?	□Yes	□ No

Demonstration

a. Attachment for heart rate monitor:

Script: "This device measures your pulse, or how often your heart beats."

400-Meter Walk Test Performance

Script: "You will be walking 10 complete laps around the course, about 1/4 mile. We would like you to walk at a normal pace you can maintain over the 10 laps. After you complete the 10 laps I will tell you to stop, and measure your blood pressure and heart rate. "STOP*" symptoms Script: "Please tell me if you fell any chest pain, tightness or pressure in your chest, if you become short of breath or if you feel faint, lightheaded or dizzy, or if you feel knee, hip, calf, or back pain. If you feel any of these symptoms, you may slow down or stop. Do you have any questions?" (Start walking when I say 'GO' and try to complete 10 laps at a pace you can maintain. Ready, go.")*

a. Baseline heart rate: _____bpm

b. Cross off as each lap is completed: **1 2 3 4 5 6 7 8 9 10**

c. Number of laps completed: _____laps

d. Did participant complete all 10 laps 1. □Yes 2. □ No

e. Record time at 400m or at stop: ____:_____.____

(Min:sec.hundreths/sec)

ID_____

f. Did the heart rate exceed 135 bpm at any time during the 400-meter walk? 1. □ Yes 2. □ No

g. Heart rate at 400m or at stop: _____bpm

h. Blood pressure at 400m or at stop: _____/_____mmHg

i. Heart rate **2 minutes** after completion of 400m walk: _____bpm

j. Did the participant complete the 400-meter walk? 1. □ Yes 2. □ No

Reason walk was incomplete or not done:

□ Excluded based on criteria

□ Refused

□ Heart rate > 135 bpm during 2-minute walk

□ Heart rate < 40 bpm

□ Symptoms reported during walk:

*Specify reason*_____

□ Stopped by technician:

*Specify reason*_____

□ Other:

*Specify reason*_____

While you were walking, did you have any of the following symptoms?

Chest pain?	□Yes	□ No	□ Don't Know
Shortness of breath?	□Yes	□ No	□ Don't Know
Knee pain?	□Yes	□ No	□ Don't Know
Hip pain?	□Yes	□ No	□ Don't Know
Calf pain?	□Yes	□ No	□ Don't Know
Foot pain?	□Yes	□ No	□ Don't Know
Numbness or tingling in your legs or feet?	□Yes	□ No	□ Don't Know
Leg cramps?	□Yes	□ No	□ Don't Know
Back pain?	□Yes	□ No	□ Don't Know
Other symptoms*?	□Yes	□ No	□ Don't Know

**Please specify*: _____

_____

APPENDIX B

PHLEBOTOMY FORM

WOMAN Phlebotomy/Processing Form

DATE_____ Phlebotomist ID: _____ ID: _____

PHLEBOTOMY

Participant Questions:

1. Do you bleed or bruise easily?
   No: _____ Yes: _____ Don't Know: _____
2. Have you ever been told you have a disorder related to blood clotting or coagulation?
   No: _____ Yes: _____ Don't Know: _____
3. Have you ever experienced fainting spells while having blood drawn?
   No: _____ Yes: _____ Don't Know: _____
4. Do you have diabetes?
   No: _____ Yes: _____ Don't Know: _____
5. Are you fasting?
   No: _____ Yes: _____ **If yes**, how long? _____

Was any blood drawn: (circle one) Yes No Time Drawn: _____

If no, reason not drawn: _____

Venipuncture:

Venipuncture time elapsed: _____minutes

Time elapsed until tourniquet released: _____(2 min optimum)

Quality of venipuncture: C (Clean) T (Traumatic - please specify)

A. Vein collapse B. Hematoma C. Vein Hard to get D. Multiple sticks
E. Excessive duration of draw F. Leakage at venipuncture site

Site of venipuncture: _____

Position of participant during blood drawing: supine sitting

Blood drawing:

Blood Volume per Tube:	Fill (✓)	Other (specify volume)
1. EDTA 10mL	_____	_____
2. Serum 15mL	_____	_____
3. Serum 10mL	_____	_____
4. Na citrate 5mL	_____	_____
5. Li Heparin 4mL	_____	_____

****

PROCESSING Processor ID: _____

Centrifuge after 30 minutes:

Indicate after centrifugation complete whether plasma is Lipemic (L), Icteric (I), Hemolyzed (H), or clotted (C).

Tube	(✓) Done	* Lab	Number vol(ml)	Suggested Tube	Aliquot dupl. (✓)	Blind Comment
#1 NMR EDTA	( )	L	_____	2ml	Cryovial( )	_____
#2 DNA EDTA	( )	S	_____	1ml	Cryovial( )	_____
#3 Extra EDTA	( )	S	_____	1ml	Cryovial( )	_____
#4 Extra EDTA	( )	S	_____	1ml	Cryovial( )	_____
#5 Na citrate	( )	V	_____	1ml	Cryovial( )	_____
#6 Na citrate	( )	V	_____	1ml	Cryovial( )	_____
#7 Serum	( )	H	_____	3ml	Cryovial( )	_____
#8 Serum L(a)	( )	H	_____	1ml	Cryovial( )	_____
#9 Serum CRP	( )	V	_____	1ml	Cryovial( )	_____
#10 Serum IL-6	( )	V	_____	1ml	Cryovial( )	_____
#11 Serum Est. Met.	( )	I	_____	1ml	Cryovial( )	_____
#12 Serum TSH	( )	I	_____	1ml	Cryovial( )	_____
#13 Extra Serum	( )	S	_____	1ml	Cryovial( )	_____
#14 Extra Serum	( )	S	_____	1ml	Cryovial( )	_____

***L=Lipomed V=University of Vermont H=Heinz Lab S=Storage**

CBAL Use Only

Received Date: _____ Time: _____ Frozen: _____

Comment: _____

APPENDIX C

PHYSICAL ACTIVITY MEASURES

THE MODIFIABLE ACTIVITY QUESTIONNAIRE (MAQ)

1. Please circle all activities listed below that you have done more than 10 times in the past year from _____ to _____.

1 Jogging (outdoor, treadmill)	14 Football/Socer	27 Stair Master
2 Swimming (laps, snorkeling)	15 Raquetball/Squash	28 Fencing
3 Bicycling (indoor, outdoor)	16 Horseback Riding	29 Hiking
4 Softball/Baseball	17 Hunting	30 Tennis
5 Volleyball	18 Fishing	31 Golf
6 Bowling	19 Aerobic Dance/Step Aerobic	32 Canoeing/Rowing Kayaking
7 Basketball	20 Water Aerobics	33 Water Skiing
8 Skating (roller, ice, blading)	21 Dancing (square, line, ballroom)	34 Jumping Rope
9 Martial Arts (karate, judo, kendo)	22 Gardening or Yardwork	35 Snow Skiing (x-country)
10 Tai Chi	23 Badminton	36 Snow Skiing (downhill)
11 Calisthenics/Toning Exercises	24 Strength/Weight Training	37 Snow Shoeing
12 Wood Chopping	25 Rock Climbing	38 Yoga
13 Elliptical Trainer	26 Scuba Diving	39 Other

40 Walking for Exercise (outdoor, indoor at a mall or fitness center, treadmill)

List each activity that you circled in the "activity" box below, check the months that you did each activity over the past year and then estimate the average amount of time spent in that activity. If participant did not participate in any activities, please check the box below marked none.

None ☐

ACTIVITY	Activity #	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC	Average # of Times per Month	Average # of Months Each Time

2. Excluding time at work, in general how many HOURS per DAY do you usually spend watching television or walking on the computer? _____ hours

3. Over this past year, have you spent more than one week confined to a bed or chair as a result of an injury, illness, or surgery? Yes _____ No _____

If yes, how many weeks over the past year were you confined to a bed or chair? _____ weeks

4. Do you have difficulty doing any of the following activities?

a. getting in or out of a bed or chair? Yes _____ No _____
b. walking across a small room without resting? Yes _____ No _____
c. walking for 10 minutes without resting? Yes _____ No _____

5. Did you ever compete in an individual or team sport (not including any time spent in sports performed during school physical education classes)? Yes _____ No _____

If yes, how many total years did you participate in competitive sports? _____ years

6. Have you had a job for more than one month over the past year, from last _____ to this_____?

List all JOBS that the individual held over the past 12 months for more than one month. Account for all 12 months of the past year. IF unemployed/disabled/homemaker/student during all or part of the past 12 months, list as such and probe for job activities of a normal 8 hour day, 5 day week.

Job Name	Job Code	Walk or bicycle to/from work	Average Job Schedule			Hrs. spent sitting at work	Out of the total # of "hrs/day" the individual reported working at this "job", how much of this time was usually spent sitting? Enter this # in "hrs sitting" column, then place a check in the category that best describes their job activities when not sitting. Check the category that best describes job activities when not sitting		
		Min/Day	Mos/Yr	Days/Wk	Hrs/Day	Hrs Sitting	A	B	C

Category A (Includes all sitting activities)	**Category B** (Includes most indoor activities)	**Category C** (heavy industrial work, outdoor construction, farming)
Sitting	Carrying light loads	Carrying moderate to heavy loads
Standing still w/o heavy lifting	Continuous walking	Heavy construction
Light cleaning	Heavy cleaning	Farming
Driving a bus, taxi, tractor	Gardening	Digging ditches, shoveling
Jewelry making/weaving	Painting/Plastering	Chopping (ax), sawing wood
General Office Work	Plumbing/Welding	Tree/pole climbing
Occasional/short distance walking	Sheep Herding	Water/Coal/Wood Hauling

JOB CODES	
Not employed outside of the home:	Employed (or volunteer):
1. Student	6. Armed Services
2. Home Maker	7. Office Worker
3. Retired	8. Non-Office Worker
4. Disabled	
5. Unemployed	

Seven Day
Physical Activity Diary

ID_____

Dates Recorded

_____ / _____ / _____ to

_____ / _____ / _____

Phone (____)_____

Reviewer Initials _____

Directions

Please complete your log the <u>week following</u> your clinic visit. This log consists of two parts 1). the pedometer and 2). activity diary. We are asking you to wear the pedometer and record your activities every day for a full seven-day period. For each of the seven days, please check the day of the week (ex. Monday) and write in the date. Each of the seven days is to be recorded on a separate sheet.

<u>Pedometer Instructions</u>: The monitor is to be clipped snuggly to your clothes or on a belt at the waist. First thing every morning, just before you put on the monitor, push the reset button and make sure the monitor reads "zero". **Record the time** you put on the monitor in your activity log and circle am or pm. Keep the monitor on all day, except when swimming or bathing. Remove the monitor at night just before bedtime and record **the number of steps per day** along with the **time that it was removed** in the activity log and circle am or pm. Please repeat these steps for the next seven days.

<u>Activity Diary Instructions</u>: **Only include activities or exercises you do for 10 minutes or longer*.** Please include ***only*** the time that you are actually active. Examples of physical activity includes brisk walking, gardening or yardwork, aerobics, swimming, stairclimbing, dancing, golf, weight training, etc. List each activity that you did during the day as well as the amount of time you spent doing that particular activity.

Day 1: ☐ Sunday ☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday

Date: __ __ /__ __ / __ __
(month) (day) (year)

Time you put the monitor on in the morning: __ __ : __ __ am or pm

Time you took the monitor off in the evening: __ __ : __ __ am or pm

The number of steps taken today from the pedometer: _____

Did you remove the monitor for longer than **30 minutes** for any reason today such as bathing, swimming, or water aerobics? If YES, please fill in the time that the monitor was taken off and the time it was put back on.

Activity	YES	NO	Time Monitor Removed	Time Monitor Put Back On
Bathing	☐	☐	__ __ : __ __ am or pm	__ __ : __ __ am or pm
Swimming/Water Aerobics	☐	☐	__ __ : __ __ am or pm	__ __ : __ __ am or pm

Leisure Physical Activity Done Today for 10 minutes or more

Activity/Exercise	Total Minutes/ Day	Activity Code
WALKING	_____	_____
2. _____	_____	_____
3. _____	_____	_____
4. _____	_____	_____
5. _____	_____	_____
6. _____	_____	_____

*NOTE: This form is replicated for an additional 6 days to obtain 7 days of pedometer step counts and reported leisure physical activity levels.

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