AN ABSTRACT OF THE DISSERTATION OF

Patricia J. Manns for the degree of Doctor of Philosophy in Human Performance presented on October 12, 2001. Title: Physical Activity, Hormone Replacement Therapy, and Insulin Resistant Coronary Artery Disease Risk Factors in Postmenopausal Women.

Abstract Approved: Redacted for privacy
Daniel P. Williams

Low physical activity levels and high serum C-reactive protein (CRP) levels are risk factors for coronary artery disease (CAD) in both men and women. However, postmenopausal women who take hormone replacement therapy (HRT) may have increased risk of CAD because of HRT-related increases in serum CRP. There are two manuscripts in this dissertation. The purpose of the first manuscript was to determine whether higher physical activity energy expenditure was associated with lower serum CRP, independent of oral HRT status and body fatness, in 133 postmenopausal women. Higher physical activity energy expenditures were significantly associated with lower serum CRP levels ($r=-0.21$, $p=0.019$), independent of oral HRT use, age, smoking behavior, alcohol consumption, aspirin use, and statin use. However, in the complete multivariate model, which included body fat, the association between higher physical activity and lower serum CRP levels was abolished. The purpose of the second study was to quantify the biological variability of insulin resistant CAD risk factors in a sample of 8 postmenopausal women. Risk factor outcomes, including serum total cholesterol, serum triglycerides (TG), serum high-density lipoprotein cholesterol (HDL-C), serum glucose, plasma insulin, serum CRP, waist and hip circumferences, abdominal sagittal

diameter, body fat, systolic (SBP) and diastolic blood pressure, and self-reported physical activity energy expenditure, were measured on two occasions, 7-12 days apart. High *absolute* biological variability values (by standard error of measurement) were observed for serum TG (32.0 mg/dl), serum CRP (5.6 mg/l), SBP (4.0 mmHg), and physical activity (9.4 kcal/kg/week). High *relative* biological variability (by within-subjects coefficient of variation $\geq$ 27.3 %) was also observed for serum TG, serum CRP, and physical activity. Bland-Altman plots identified individual outliers for serum TG, serum CRP, plasma insulin, and SBP. Together, the results suggest that the correlations between lower levels of serum CRP and higher levels of physical activity, though significant, may have been attenuated by the high biological variability of both serum CRP and physical activity. Thus, the importance of higher levels of physical activity, in decreasing serum CRP and the concomitant risk of heart disease, may be underestimated in the absence of serial measurement of serum CRP and physical activity.

Physical Activity, Hormone Replacement Therapy, and Insulin Resistant Coronary Artery Disease Risk Factors in Postmenopausal Women

By Patricia J. Manns

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I understand that my dissertation will become part of the permanent collection of the Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Patricia J. Manns, Author

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CONTRIBUTION OF AUTHORS

Dr. Daniel P. Williams was involved in the design, analysis, writing and editing of both manuscripts. The assays were performed in the laboratory of Dr. Williams. Dr. Christine Snow conducted the dual energy x-ray absorptiometry scans for the first manuscript. Dr. Rosemary Wander and her staff performed the analysis of all the lipid outcomes for both studies.

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Physical Activity, Hormone Replacement Therapy, and Insulin Resistant Coronary Artery Disease Risk Factors in Postmenopausal Women

CHAPTER 1: INTRODUCTION

Heart disease is the leading cause of death in women in the United States. In 1998, there were 503,927 women who died from cardiovascular disease, as compared to lung cancer which claimed 64, 475 lives, and breast cancer which claimed 41,737 lives (American Heart Association, 2001). Thus, though there are understandable and widespread fears of cancer, particularly breast cancer, heart disease is a much larger public health problem. Furthermore, some statistics suggest that heart disease may be a more deadly disease for women than men. For instance, 38 % of women who suffer a heart attack die within a year. By comparison, only one quarter of the men who have heart attacks die within the year. Within 6 years of a first documented heart attack, 35 % of women will have a second heart attack, as compared to just 18 % of men having a second heart attack, in the same time period. Clearly, strategies designed to prevent heart disease, such as lifestyle physical activity interventions or improved diets, should command greater attention from postmenopausal women and their health care providers.

Additionally, hormone replacement therapy (HRT) had been recommended for its purported benefits to heart disease risk factors and endpoints (such as heart attack or death) in postmenopausal women. However, recent publications from the American Heart Association and the National Cholesterol Education Program no longer recommend HRT use for prevention of heart disease (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001; Mosca et al., 2001). The new recommendations now state that women who choose to initiate or continue HRT use

should do so for established non-coronary benefits such as increased bone density and decrease in menopausal symptoms (Mosca et al., 2001). Therefore, in spite of the change in recommendations regarding heart disease, it is likely that there will continue to be a large number of women taking HRT for its perceived health benefits to bone and its proven effects in reducing menopausal symptoms. Additionally, physician prescribing practices and patient health behaviors change slowly, and it is likely that any decline in HRT use because of the change in recommendations, will occur slowly.

Hormone Replacement Therapy, Heart Disease Risk, and C-reactive Protein

There are several reasons behind the recent change in the recommendations regarding HRT use and heart disease. The strongest evidence against HRT use for prevention of heart disease comes from the Heart and Estrogen Replacement Study (HERS), a randomized controlled trial that found that women with heart disease, who took HRT, did not decrease their risk of heart attack or death (Hulley et al., 1998). Other randomized controlled trials have strengthened the HERS evidence by showing that HRT use did not affect the progression of atherosclerosis in patients with coronary disease (Angerer, Stork, Kothny, Schmitt, & von Schacky, 2001; Herrington et al., 2000). In addition to the HERS trial, recent studies have determined that HRT use leads to consistently increased circulating levels of C-reactive protein (CRP) (Cushman et al., 1999; Ridker, Hennekens, Rifai, Buring, & Manson, 1999).

CRP is an acute phase protein, synthesized in the liver, whose circulating levels increase dramatically in response to acute bacterial infections, viral infections, or other inflammatory conditions (Tracy, 1998). Chronic yet modest elevations in CRP have

emerged as an independent risk factor for coronary artery disease (CAD) in both men (Koenig et al., 1999; Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker, Glynn, & Hennekens, 1998) and women (Ridker, Buring, Shih, Matias, & Hennekens, 1998). In addition, recent studies have reported a role of CRP in both the initiation of atherosclerosis and the precipitation of an acute CAD event (Pasceri, Willerson, & Yeh, 2000; Torzewski et al., 2000; Yudkin, Stehouwer, Emeis, & Coppack, 1999). Thus, the observation of experimental increases in serum CRP after oral HRT administration is cause for concern (Cushman et al., 1999).

C-Reactive Protein, Physical Activity and Exercise.

Because of the well-defined HRT-related increase in serum CRP, it is important to examine lifestyle changes, like increased physical activity, which may offset that increase. Serum CRP levels have been shown to decrease in response to endurance exercise training in young healthy men (Mattusch, Dufaux, Heine, Mertens, & Rost, 2000), and in middle-aged healthy men and women (Smith, Dykes, Douglas, Krishnaswamy, & Berk, 1999). Additionally, lower serum CRP levels have been reported in the more physically active elderly participants of the Macarthur study of successful aging (Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000). Similarly, investigators from the Cardiovascular Health Study have reported significantly lower serum CRP levels in the group of elderly men and women with the highest physical activity levels (Geffken et al., 2001). Taken together, the above findings (Geffken et al., 2001; Mattusch et al., 2000; Smith et al., 1999; Taaffe et al., 2000) provide evidence to hypothesize that regular physical activity may offset the HRT-related increases in serum

CRP (Cushman et al., 1999). However, previous exercise and physical activity studies (Geffken et al., 2001; Smith et al., 1999; Taaffe et al., 2000) have not examined the potential confounding influence of differences in oral HRT status among its postmenopausal female participants. Oral HRT use is an important factor to consider when exploring the relationship between serum CRP and physical activity, as higher CRP values have consistently been reported for women on HRT (Cushman et al., 1999; Ridker et al., 1999). Therefore, while physical activity and exercise have been shown to decrease markers of inflammation (Geffken et al., 2001; Smith et al., 1999), it is not known whether regular physical activity can independently offset the HRT-related increase in serum CRP in women with differing HRT status.

Statement of Purpose I

The aim of the first manuscript in this dissertation is to determine whether higher levels of physical activity are associated with decreased serum CRP levels in 133 postmenopausal women aged 50-78 years, independently of oral HRT use and other important confounding factors such as age, past smoking history, present alcohol consumption, statin use, aspirin use and body fat (both total and regional).

Insulin Resistant CAD Risk Factors and Biological Variability

The American Heart Association no longer recommends that HRT use be initiated for the sole purpose of primary CAD prevention in postmenopausal women (Mosca et al., 2001). Therefore, it may be especially important to determine the effectiveness of lifestyle physical activity interventions, for managing heart disease risk factors that

become more prevalent as women age. Insulin resistant CAD risk factors such as high fasting glucose, insulin, triglycerides, and serum CRP levels (Festa et al., 2000), low high-density lipoprotein cholesterol (HDL-C) levels, high blood pressure and abdominal obesity, may be particularly important to target in lifestyle interventions with women because diabetes is a more powerful risk factor for heart disease in women than men (Mosca et al., 1999). Yet, risk factors in the insulin resistant syndrome, particularly glucose, insulin, serum CRP levels (Festa et al., 2000), and abdominal obesity, have been understudied in lifestyle modification trials with women. Previous investigations with women have more narrowly focused on serum lipid and blood pressure outcomes. In addition, previous studies with postmenopausal women have reported ambiguous findings in that diet and physical activity interventions reduced some but not all of the CAD risk factors being studied (King, Haskell, Young, Oka, & Stefanick, 1995; Kuller, Simkin-Silverman, Wing, Meilahn, & Ives, 2001). These ambiguous findings may be explained by the biological actions of physical activity. Alternatively, the finding may be explained by unassessed differences in biological variability between outcomes. Thus, a better quantification of the biological variability of insulin resistant CAD risk factors in postmenopausal women is needed. Quantification of biological variability would improve the likelihood of detecting significant lifestyle treatment effects while maintaining feasible subject recruitment and retention estimates. By identifying those risk factors with the highest biological variability, specific CAD risk factors could be selectively targeted for serial assessment, prior to future intervention trials.

Statement of Purpose II

The purpose of the second study in this dissertation is to quantify the biological variability of insulin resistant CAD risk factors in 8 postmenopausal women, measured on two occasions, 7-12 days apart. By conducting a reliability study with different subjects, but subjects from the same population as the first manuscript, the findings about biological variability will be directly applicable to the first study.

CHAPTER 2

ASSOCIATION BETWEEN PHYSICAL ACTIVITY AND SERUM C-REACTIVE PROTEIN IN POSTMENOPAUSAL WOMEN WITH AND WITHOUT HORMONE REPLACEMENT

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Abstract

Objectives: To determine whether higher physical activity is associated with lower serum C-reactive protein (CRP), independent of oral hormone replacement therapy (HRT) status and body fatness, in 133 postmenopausal women.

Design: Cross-sectional exploratory design

Setting: University research laboratory setting

Subjects: 133 postmenopausal women, aged 50-73 years, with no evidence of coronary artery disease or diabetes.

Main Outcome Measures: Serum CRP, physical activity as measured by Stanford 7-day activity recall, body fat (both total and regional) as measured by dual energy x-ray absorptiometry (DXA) and anthropometry (waist and hip circumference). Secondary outcome measures were triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol.

Results: Higher physical activity energy expenditures were significantly associated with lower serum CRP levels ($r=-0.21$, $p=0.019$), independent of oral HRT use, age, smoking behaviour, alcohol consumption, aspirin use, and statin use. However, in the complete multivariate model, which included body fat, the association between higher physical activity and lower serum CRP levels was abolished and older ages ($p=0.029$), greater trunk fat masses ($p < 0.001$), and oral HRT use ($p < 0.001$) were independently associated with higher serum CRP levels. The complete multivariate model accounted for 48% of the variance in serum CRP.

Conclusions: This study is the first to report that greater amounts of physical activity are associated with lower serum CRP levels, independent of oral HRT use. Furthermore, the

present study suggests that physical activity-related reductions in body fat may be an effective way to diminish the pro-inflammatory effects of oral HRT in postmenopausal women.

Introduction

Recent studies have raised awareness of the importance of vascular inflammation in the biology, morbidity, and mortality of coronary artery disease (CAD) (26, 30, 31, 39). Elevated serum C-reactive protein (CRP) is an important marker of vascular inflammation (11), is recognized as a "conditional" risk factor for CAD (36), and becomes more prevalent with advancing age (17). Despite its effectiveness for preventing age-related changes in blood lipids (41), oral hormone replacement therapy (HRT) does not prevent CAD events (16) and increases serum CRP in postmenopausal women (5). However, there are unequivocal bone density benefits (25) (19) and probable cognitive benefits (14) of oral HRT use. Thus, it may be premature to avoid oral HRT use based solely on the findings of increased serum CRP. Furthermore, it is not known whether healthy lifestyle behaviours, such as regular physical activity, can partially offset the HRT-related increase in serum CRP.

Endurance exercise training has been reported to reduce serum CRP levels in healthy young men (21) and in healthy middle-aged men and women (35). Moreover, physically active elderly men and women have lower serum CRP levels than their less active counterparts (12, 38). However, to our knowledge, no study has examined whether the inverse association between physical activity and serum CRP is affected by oral HRT use in postmenopausal women.

Besides oral HRT, body fat is another important factor to consider because greater amounts of body fat are associated with higher serum CRP levels (6, 8). The production and secretion of the pro-inflammatory cytokine interleukin-6 (IL-6), from fat cells, provides an endocrine stimulus for hepatic CRP production (15), that links obesity to

chronic low-grade inflammation (42). One limitation of previous studies reporting an inverse association between physical activity and serum CRP levels is the use of body mass index (BMI) to assess body composition (12, 38). BMI may not accurately reflect body fatness in older women with declining stature and bone mass (13). Therefore, in the present study we used dual-energy x-ray absorptiometry (DXA) to assess total and regional fatness, a method that accurately reconstructs total body mass from its bone mineral, soft tissue lean and fat components (28).

Thus, while physical activity and exercise have been shown to reduce circulating markers of vascular inflammation (12, 35), it is not known whether higher physical activity is associated with lower serum CRP, independent of oral HRT use and body fatness, in postmenopausal women. We hypothesized that higher physical activity would be associated with lower serum CRP levels, regardless of oral HRT use. However, we also hypothesized that any association between physical activity and serum CRP would be dependent, in part, on the lower body fat of the more active women.

Methods

Participants:

A total of 423 women responded by telephone to newspaper advertisements and articles seeking participants in a study of physical activity and CAD risk factors in postmenopausal women. Two hundred eighty three women were excluded during telephone screening because of the use of non-oral forms of hormone replacement therapy or selective estrogen receptor modulators, or for the presence of thyroid disorders, cardiovascular disease, diabetes or gout. Women were also excluded if they

had experienced a menses in the past year, or if they had surgical menopause before the age of 40 years. Lastly, participants were excluded if they were taking corticosteroids, niacin or fibrate medications. Of the 140 eligible participants, three were excluded because of missing data, and four were excluded because of acute illnesses or infections at the time of the blood draw. Thus, the sample described herein consists of 133 postmenopausal women age 50-78 years with no evidence of CAD, diabetes, or infectious illness.

Procedures:

Metabolic outcomes, derived from morning fasting blood draws, included glucose, insulin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and C-reactive protein (CRP). Venous blood samples were collected in plasma EDTA tubes for glucose and insulin and in serum vacutainer tubes with gel clot activator for TC, TG, HDL-C, and CRP. Serum total cholesterol (2) and triglycerides (22) were determined with enzymatic techniques. After precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid and magnesium chloride (3), serum HDL-C was determined enzymatically (2). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedwald equation (9). A colorimetric glucose oxidase method (40) was used to assess plasma glucose, and a human insulin-specific radioimmunoassay (Linco Research, St. Charles, MO) was used to assess fasting plasma insulin levels. Our measurement errors, expressed as coefficients of variation (SD/Mean x 100) were, 2.4% for TC, 4.4% for HDL-C, 4.5% for TG, and 6.4% for glucose. For insulin, the coefficient of variation

ranged from 3.0% for the high plasma insulin control to 7.1% for the low plasma insulin control.

Serum CRP levels were measured in duplicate with a highly sensitive enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, TX). The serum dilution protocol recommended by the manufacturer is 1:500 and is primarily set to detect acute infection/inflammation in a clinical laboratory setting. As such it is not designed to record modest, chronic subclinical inflammation. Therefore, the dilution protocol was modified and serum samples were initially diluted 1:50 with assay buffer, which resulted in detectable serum CRP values of 0.5 to 25 mg/l. After initial analyses, samples that fell above the detectable range were reanalyzed with a serum dilution of 1:500 (detectable range 5-250 mg/l), and samples that fell below the detectable range were reanalyzed with a serum dilution of 1:5 (detectable range 0.05-2.5 mg/l). Our interassay coefficient of variation was 5.2 % for low-level serum CRP control that ranged from 1.2 to 1.4 mg/l, and 6.6% for high-level serum CRP control that ranged from 11.4 to 13.3 mg/l.

On the same day as the blood draws, body weight, height, anthropometric body composition measures (waist and hip circumference) and total and regional body composition were measured. Body weight was recorded with a Detecto balance beam scale (Metro Equipment Corp., Sunnydale, CA), and height was measured with a wall-mounted stadiometer. Waist and hip circumference were measured with a steel tape measure, according to the Anthropometric Standardization Manual (20). Total and regional body composition were measured using a Hologic QDR 1000/W DXA scanner, with analytical software version 5.71. Body composition is reported as percent body fat as well as trunk fat mass using the anatomical region of interest that excludes the head,

the arms, and the legs. Participants also completed a medical questionnaire that addressed medication use, smoking history (divided into 'never' or 'ever' smokers), alcohol consumption, and standard medical history questions.

Estimates of physical activity caloric expenditure were assessed by a trained interviewer using the Stanford 7-day physical activity recall (34). However, we deviated from the standard protocol for estimating physical activity energy expenditure in two important ways. First, to be consistent with current physical activity recommendations for accumulating moderate or higher intensity activities (27), we did not include a calculated estimate of unreported "low-intensity" physical activity. Second, to further improve accuracy, caloric expenditures were calculated with the recommended metabolic equivalents for each specific activity from the Ainsworth Compendium of Physical Activities, rather than rely on "blanket" metabolic equivalents that do not account for the specific *type* of activity reported (1). Physical activity is reported as kilocalories of energy expended per kilogram of body weight per week.

Data Analysis:

SPSS Version 9.0 was utilized for all data analysis. Descriptive statistics for three HRT groups (no HRT, estrogen plus progestin, unopposed estrogen) were used to determine the normality of metabolic and behavioral data. Because the distribution of the values for C-reactive protein was skewed, log-transformed CRP values were used for all parametric statistical hypothesis testing. However, the untransformed median CRP values are presented for clinical interpretation. Analysis of variance (ANOVA) was used to determine significant HRT-related differences in serum CRP, physical activity, body

composition, and other metabolic CAD risk factors. In addition, chi square analysis was used to determine whether there were HRT-related differences in the proportion of women who were former smokers, statin users, present alcohol consumers or aspirin users.

To determine the associations among physical activity, serum CRP, body fatness, and selected CAD risk factors, partial correlations were used with statistical adjustments for oral HRT use, age, smoking, alcohol consumption, statin use, and aspirin use. Older ages (17) and cigarette smoking (4) are associated with higher serum CRP levels, whereas aspirin use (29), statin use (32), and moderate alcohol consumption (18) are associated with lower serum CRP levels. Multiple linear regression analysis was then used to determine whether the association between physical activity and serum CRP was independent of body fat and all of the above potential confounding factors. Subsequently, an analysis of covariance (ANCOVA) was used to determine whether there were significant interactive differences in serum CRP in groups of women stratified by oral HRT use and physical activity tertile, with age, smoking status, alcohol consumption, statin use and aspirin use used as covariates. A similar ANCOVA was used to determine whether there were interactive differences in serum CRP in groups of women stratified by oral HRT use and abdominal obesity, defined by critical waist circumference measurements (>88 cm and ≤ 88cm) (24).

Results

There were 50 women (38%) who were not taking any form of HRT. Fifty-one women (38%) were using oral conjugated estrogens plus progestin, and the remaining 32

women (24%) were taking oral unopposed conjugated estrogens. Table 2.1 presents participant characteristics by oral HRT use. Median (interquartile range) serum CRP values in mg/l were as follows: 1.6 (0.7-2.8) for the women not taking HRT, 5.8 (2.1-8.4) for the women taking combined estrogen and progestin, and 9.1 (5.2 –21.1) for the women taking unopposed estrogen. Thus, the median serum CRP levels for women taking oral HRT with or without oral progestins, were 3.5 to 5.5 times greater than the median serum CRP levels of the women not taking HRT. The serum CRP values in the

Table 2.1 Descriptive Characteristics of 133 Postmenopausal Women by Oral HRT use*

	No HRT n=50	Estrogen Plus Progestin n=51	Unopposed Estrogen n=32	P-value
Age (yrs)	62.4 (7.8)	61.4 (6.7)	62.9 (6.6)	0.593
Weight (kg)	66.1 (13.0)	67.2 (12.0)	71.3 (14.2)	0.194
Waist Circumference (cm)	80.5 (13.2)	79.9 (9.8)	83.3 (12.7)	0.324
Waist-to-Hip Ratio	0.78 (0.07)	0.78 (0.06)	0.78 (0.08)	0.835
Body mass index (kg/m^2)	25.1 (5.1)	25.3 (4.3)	27.0 (4.8)	0.171
DXA Total Body Fat (%) ‡	30.6 (6.6)	31.3 (6.5)	34.3 (6.3)	0.036
DXA Trunk Fat Mass (kg) ‡	8.6 (5.3)	9.0 (4.2)	11.2 (5.3)	0.048
Insulin (pmol/L)	80.7 (38.8)	78.0 (26.9)	82.0 (36.2)	0.856
Glucose (mmol/L)	4.9 (0.5)	4.8 (0.5)	4.9 (0.6)	0.348
Total Cholesterol (mmol/L)	5.5 (0.8)	5.4 (0.8)	5.6 (0.9)	0.401
Triglycerides (mmol/L) ‡	1.4 (0.9)	1.6 (0.8)	2.0 (1.1)	0.011
HDL-C (mmol/L) ‡ §	1.6 (0.3)	1.7 (0.4)	1.9 (0.5)	<0.001
LDL-C (mmol/L) † ‡	3.3 (0.8)	2.9 (0.8)	2.8 (0.8)	0.009
Physical Activity (kcal/kg/week)	51.5 (39.9)	47.1 (31.7)	41.3 (33.6)	0.449
LogCRP (mg/l) † ‡ §	0.16 (0.51)	0.62 (0.46)	0.97 (0.47)	<0.001
CRP > 10 mg/l (%) ‡ §	6	16	50	<0.001
Past Smoker (%)	32	27	34	0.781
Present Alcohol Consumption (%)	64	76	75	0.330
Statin Use (%)	4	2	9	0.278
Aspirin User (%)	22	26	16	0.570

*Values expressed as mean (SD) for continuous data, or percentages for categorical data.

† – Denotes significant difference between no HRT and Estrogen plus Progestin Groups
‡ – Denotes significant difference between no HRT and Unopposed Estrogen Groups
§ – Denotes significant difference between Estrogen Plus Progestin and Unopposed Estrogen Groups

women taking oral estrogen and progestins were significantly *higher* than the serum CRP of the women taking no oral HRT and significantly *lower* than the serum CRP of the women taking unopposed estrogen. Moderate or higher-intensity physical activity energy expenditure, expressed relative to body weight (kcal/kg/week), was not different among the HRT groups. As expected, lower serum LDL-C, higher serum HDL-C, and higher serum TG were associated with oral HRT use ($p \leq 0.011$). There were no HRT-related differences in fasting plasma glucose and insulin levels. However, body fat percentage and trunk fat mass, as measured by DXA, were significantly lower in the women not taking HRT, as compared to the women taking unopposed estrogen ($p \leq 0.048$). Lastly, there were no HRT-related differences in smoking status, alcohol consumption, aspirin use, and statin use.

Table 2.2 reports partial correlations among physical activity, serum CRP, and selected metabolic outcomes of the insulin resistance metabolic syndrome, after adjustment for potential confounding by oral HRT use, age, smoking behavior, alcohol consumption, statin use and aspirin use. Higher serum CRP levels were associated with higher fasting plasma glucose, higher fasting plasma insulin, higher fasting serum TG, higher BMI, larger waist circumferences, and greater trunk fat masses. In addition, higher weekly physical activity energy expenditures were associated with lower serum CRP, lower fasting plasma insulin, lower BMI, smaller waist circumferences, and lesser trunk fat masses.

Table 2.2 – Partial Correlations of Physical Activity and Serum CRP with Selected Metabolic Outcomes of the Insulin Resistance Metabolic Syndrome and Statistical Adjustments for Oral HRT Use, Age, Smoking, Alcohol Consumption, Statin Use and Aspirin Use.

	Physical Activity	Log CRP
Log CRP	-0.21*	-
TG	-0.15	0.37 ‡
HDL-C	0.14	0.01
Glucose	0.05	0.19*
Insulin	-0.18*	0.35 ‡
Body Mass Index	-0.29†	0.61 ‡
Waist Circumference	-0.25†	0.60 ‡
Waist-to Hip Ratio	-0.06	0.40 ‡
DXA Trunk Fat	-0.31‡	0.63 ‡

*p <0.050
†p <0.010
‡p <0.001

A multiple regression analysis showed that the independent association between higher physical activity and lower serum CRP did not persist after adjusting for individual differences in trunk fat mass (Table 2.3). In the complete multivariate model, older ages (p=0.029), greater trunk fat masses ($p < 0.001$), and oral HRT use ($p < 0.001$) were independently associated with higher serum CRP levels and accounted for 48% of the variance in serum CRP. Individually, DXA trunk fat mass explained the largest share of the variance in serum CRP (35%).

Table 2.3. Multiple Regression Analysis of Serum Log CRP from age, physical activity, DXA trunk fat, oral HRT use, and selected health behaviors in 133 postmenopausal women.

Variable	b	y-intercept	P-value	R^2	SEE
Age	0.01189	-1.037	0.029	0.482	0.426
Physical Activity	-0.00023		0.835		
DXA Trunk Fat Mass	0.00007		<0.001		
Oral HRT use	0.21800		<0.001		
Aspirin Use	-0.01848		0.837		
Alcohol Consumption	-0.02279		0.804		
Smoking	0.05315		0.527		
Statin Use	-0.06933		0.700		

SEE = standard error of the estimate

To illustrate the independent associations of oral HRT use and physical activity with serum CRP, we plotted median serum CRP levels by physical activity tertile and oral HRT use (Figure 2.1). There was no interaction (p=0.891) between oral HRT use and physical activity. However, lower serum CRP levels were associated with higher physical activity caloric expenditures at every type of oral HRT use (p=0.020). By contrast, higher serum CRP levels were associated with oral estrogen use at every tertile of physical activity ($p < 0.001$).

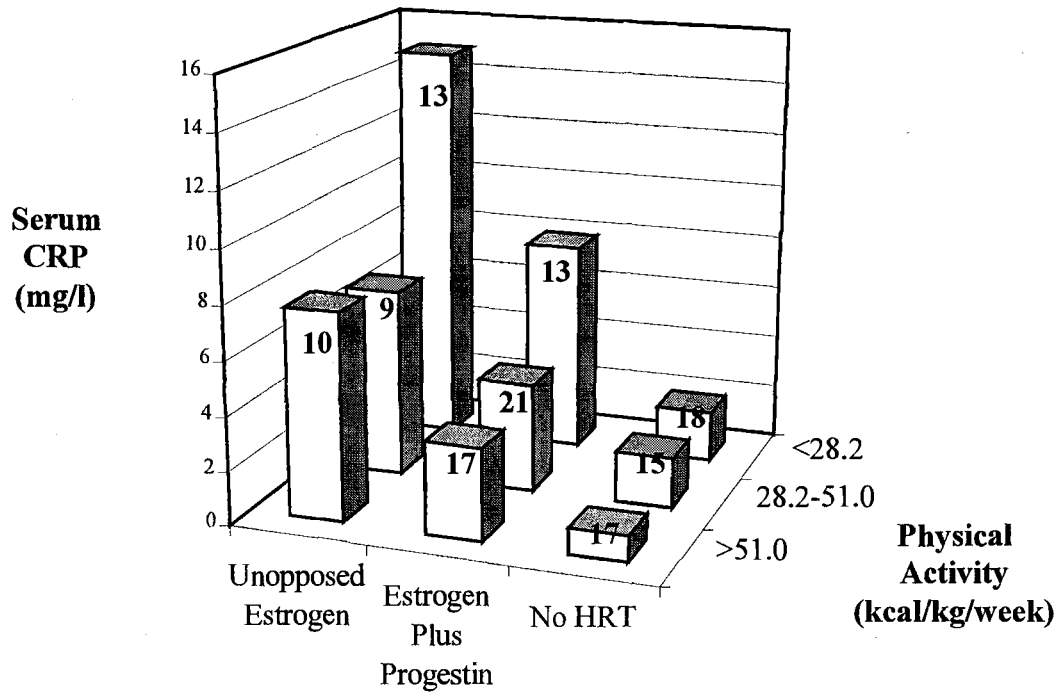

Figure 2.1. Stereogram of Serum CRP by oral HRT use and Physical Activity Tertile. Sample size of each group is shown on the bars. There were significant main effects of physical activity ($p=0.020$) and oral HRT use ($p<0.001$) on serum CRP.

Finally, to demonstrate the independent associations of oral HRT use and abdominal obesity with serum CRP, we plotted median serum CRP by oral HRT use and abdominal obesity (Figure 2.2). There was no interaction between oral HRT use and abdominal obesity ($p=0.485$). However, having a waist circumference above 88 cm was associated with higher serum CRP levels for every type of oral HRT use ($p < 0.001$). Moreover, higher serum CRP levels were associated with oral estrogen use for women with and without abdominal obesity ($p < 0.001$).

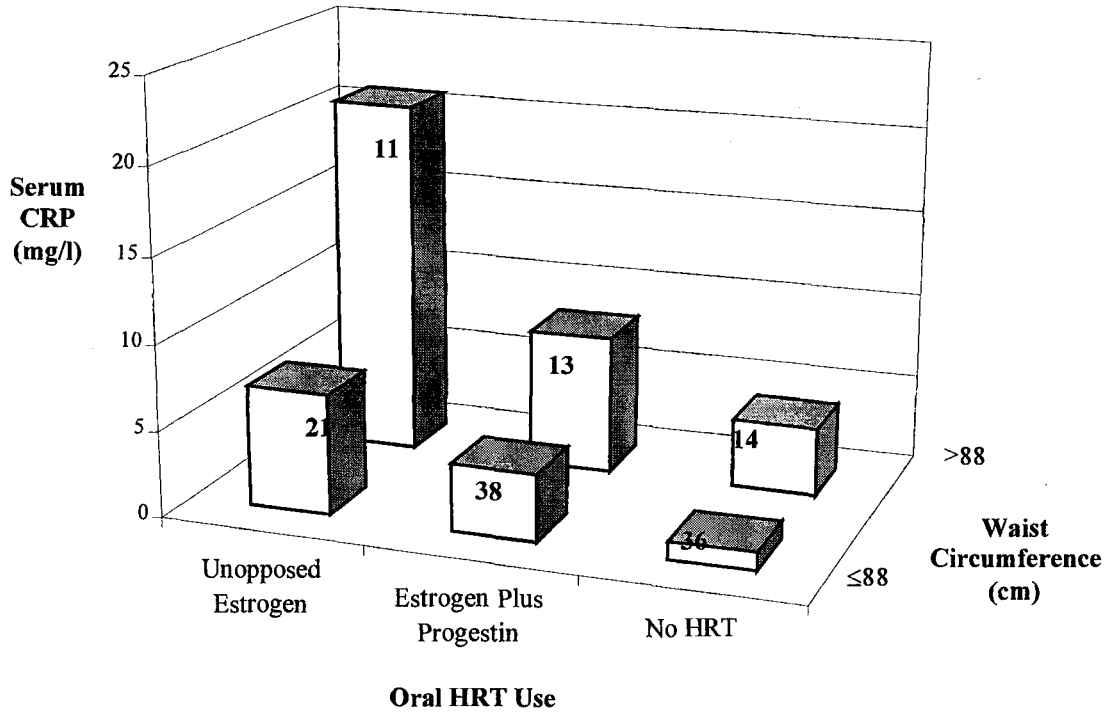

Figure 2.2. Serum CRP by oral HRT use and abdominal obesity. Sample size of each group is shown on the bars. There were significant main effects of abdominal obesity ($p<0.001$) and oral HRT use ($p<0.001$) on serum CRP.

Discussion

In this investigation of 133 postmenopausal women, aged 50-78, we determined that higher amounts of physical activity were associated with lower serum CRP levels independent of oral HRT use, age, smoking, alcohol consumption, statin use and aspirin use. Recently, investigators from the Cardiovascular Health Study (12) and the MacArthur studies of successful aging (38) have similarly reported an association between higher physical activity and lower serum CRP levels. However, the present investigation is the first to demonstrate a significant association between higher levels of physical activity and lower levels of serum CRP that is independent of oral HRT use.

The present findings also extend earlier observations (12, 38) to a younger (~10 years younger), more active, and likely, a healthier sample of postmenopausal women.

However, as hypothesized, we found that the association between higher physical activity and lower serum CRP and was *not* independent of lesser amounts of total and truncal body fat. It is plausible that exercise-related improvements in body fat control may be the most important mechanism whereby regular physical activity leads to reduced serum CRP levels. By contrast, our findings (Figure 2) and the findings of others (6) suggest that oral HRT use may increase serum CRP *independent* of the degree of body fatness or the magnitude of overweight.

Therefore, if oral HRT is capable of increasing serum CRP, independent of body fatness, and if regular physical activity can reduce serum CRP, dependent on the extent of body fat control, then it is likely that physical activity and oral HRT affect serum CRP levels through separate mechanisms. Interleukin-6 (IL-6) is an important pro-inflammatory cytokine that may link obesity to vascular inflammation. IL-6 is expressed and actively secreted by fat cells (23), and in turn, chronically high circulating IL-6 levels may stimulate the hepatic overexpression of CRP, thereby explaining the chronic, low-grade, vascular inflammation accompanying obesity. Furthermore, oral HRT *decreases* serum IL-6 levels (37), yet paradoxically *increases* serum CRP at every level of BMI (6). Thus, it is likely that the first pass hepatic effect of oral HRT directly increases CRP production in the liver through an exogenous pathway, bypassing the endogenous endocrine stimulus from fat cell-derived IL-6. Future prospective trials should determine whether exercise training is capable of reducing serum IL-6 and serum CRP in the presence or absence of weight loss, to elucidate the exact mechanisms whereby

regular physical activity may reduce vascular inflammation in postmenopausal women undergoing and not undergoing oral HRT.

In this study we expressed physical activity in terms relative to the participant's body weight. Thus, we purposely did not express physical activity in absolute terms (kcal). When relative energy expenditure (in kcal/kg) is simply multiplied by body weight (in kg) to determine absolute energy expenditure (in kcal), it assumes that the basal metabolic rate is invariant and equal to 1 kcal/kg of body weight/hour for every person (1). However, for individuals in whom the basal metabolic rate deviates from the assumed constant, the resulting estimates of absolute energy expenditure more closely reflect body weight than the reported physical activity behaviour (1). In addition, the present relative estimates of energy expenditure may be more useful because they emphasize that physical activity goals should account for individual differences in body weight.

We found strong associations between higher serum CRP levels and components of the insulin resistant metabolic syndrome, including higher fasting glucose, insulin, and triglyceride levels, and greater abdominal fat masses. These findings are in agreement with other reports (8, 10) and support the suggestion that chronic low-grade vascular inflammation, as measured by serum CRP, is part of the insulin resistant metabolic syndrome. However, the associations we found between serum CRP and two components of the insulin resistance metabolic syndrome, body fat and HDL-C, were somewhat unexpected. The correlations we found between serum CRP and body fatness are consistently greater in magnitude (i.e. $r \geq 0.60$ vs. $r \leq 0.40$) than those reported elsewhere (8, 10, 43). Although we used DXA to assess body fatness, the correlation of

DXA-derived body fat or BMI with serum CRP were almost identical in magnitude ($r \simeq 0.60$, $p < 0.001$). Thus, there are several possible explanations for the relatively high correlations observed herein. Previous investigations did not stratify their analysis by gender (8, 10, 43), possibly masking higher correlations between serum CRP and body fat in women than in men. Additionally, only one of the previous studies (10) measured serum CRP with a similar assay protocol as the present study, which carefully reanalyzed individual serum samples at the appropriate dilution to ensure that every sample fell within the detectable range of the assay. Lastly, the relatively high correlations between body fat and serum CRP in the present study may be due to the relative "healthiness" of our sample. In studies where a greater proportion of participants with clinical or subclinical CAD or diabetes are included, it becomes more likely that the serum CRP may be elevated independent of the degree of obesity (33). By contrast, in non-diseased individuals, obesity, rather than extensive vascular injury, may be the primary cause of chronic modest elevations in serum CRP.

Contrary to other published reports (8, 10, 43), we found a non-significant association between HDL-C and serum CRP. However, the simultaneous increases in HDL-C (41) and serum CRP (5) that accompany oral HRT use likely negated the expected inverse correlation between HDL-C and serum CRP. For instance, after adjusting for age, smoking behaviour, alcohol consumption, statin use, and aspirin use, we were able to detect a significant partial correlation between HDL-C and serum CRP ($r = -0.346$, $p = .02$) only in those women who were not taking oral HRT.

There are a number of study limitations to consider, including: 1) weakness of the cross-sectional study design; 2) limited sample size of the women taking unopposed oral

estrogen; and 3) lack of serum IL-6 measurement. Because of the cross-sectional design of this study, no causal inferences can be derived from these findings. Prospective exercise intervention trials are needed to determine the effectiveness of physical activity in reducing serum CRP in postmenopausal women taking oral HRT. The smaller sample size in the unopposed estrogen group is also a limitation. That group had significantly higher body fat than the group without any oral HRT, which broadens the potential explanations for the consistently higher serum CRP levels in the women using unopposed oral estrogen. Lastly, measurement of IL-6 would have strengthened the mechanistic insight underlying the present independent associations among physical activity, oral HRT use, and serum CRP in postmenopausal women.

In summary, this study is the first to report that higher physical activity levels are associated with lower serum CRP levels, independent of oral HRT use. Our finding that the physical activity-serum CRP association was not independent of body fat does not diminish the potential public health importance of regular physical activity. In addition, the newly revised National Cholesterol Education Program guidelines encourage the use of statins and discourage the use of oral HRT for lipid management and CAD risk reduction in postmenopausal women (7). Thus, the present study suggests that physical activity-related reductions in body fat may be an effective way to diminish the pro-inflammatory effects of the oral HRT that will likely continue to be prescribed to reduce bone loss and control menopausal symptoms. Therefore, with experimental confirmation, the present findings may broaden the preventive health benefits of diet and exercise programs aimed at reducing body fat or limiting age related body fat gains in postmenopausal women taking oral HRT.

References

1. Ainsworth, B. E., W. L. Haskell, A. S. Leon, D. R. Jacobs, Jr., H. J. Montoye, J. F. Sallis, and R. S. Paffenbarger, Jr. Compendium of physical activities: classification of energy costs of human physical activities [see comments]. *Med Sci Sports Exerc* 25: 71-80, 1993.

2. Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-5, 1974.

3. Assmann, G., H. Schriewer, G. Schmitz, and E. O. Hagele. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2. *Clin Chem* 29: 2026-30., 1983.

4. Chenillot, O., J. Henny, J. Steinmetz, B. Herbeth, C. Wagner, and G. Siest. High sensitivity C-reactive protein: biological variations and reference limits. *Clin Chem Lab Med* 38: 1003-11., 2000.

5. Cushman, M., C. Legault, E. Barrett-Connor, M. L. Stefanick, C. Kessler, H. L. Judd, P. A. Sakkinen, and R. P. Tracy. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation* 100: 717-22., 1999.

6. Cushman, M., E. N. Meilahn, B. M. Psaty, L. H. Kuller, A. S. Dobs, and R. P. Tracy. Hormone replacement therapy, inflammation, and hemostasis in elderly women. *Arterioscler Thromb Vasc Biol* 19: 893-9., 1999.

7. Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285: 2486-2497, 2001.

8. Festa, A., R. D'Agostino, Jr., G. Howard, L. Mykkanen, R. P. Tracy, and S. M. Haffner. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102: 42-7, 2000.

9. Freidwald, W. T., R. I. Levy, and D. S. Fredrickson. Estimation of the concentration of low density lipoprotein cholesterol in plasma, with the use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502, 1972.

10. Frohlich, M., A. Imhof, G. Berg, W. L. Hutchinson, M. B. Pepys, H. Boeing, R. Muche, H. Brenner, and W. Koenig. Association between C-reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care* 23: 1835-9., 2000.

11. Gabay, C., and I. Kushner. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448-54., 1999.

12. Geffken, D. F., M. Cushman, G. L. Burke, J. F. Polak, P. A. Sakkinen, and R. P. Tracy. Association between Physical Activity and Markers of Inflammation in a Healthy Elderly Population. *Am J Epidemiol* 153: 242-250., 2001.

13. Going, S., D. Williams, and T. Lohman. Aging and body composition: biological changes and methodological issues. *Exerc Sport Sci Rev* 23: 411-58, 1995.

14. Grodstein, F., J. Chen, D. A. Pollen, M. S. Albert, R. S. Wilson, M. F. Folstein, D. A. Evans, and M. J. Stampfer. Postmenopausal hormone therapy and cognitive function in healthy older women. *J Am Geriatr Soc* 48: 746-52., 2000.

15. Heinrich, P. C., J. V. Castell, and T. Andus. Interleukin-6 and the acute phase response. *Biochem J* 265: 621-36, 1990.

16. Hulley, S., D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs, and E. Vittinghoff. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 280: 605-13., 1998.

17. Hutchinson, W. L., W. Koenig, M. Frohlich, M. Sund, G. D. Lowe, and M. B. Pepys. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. *Clin Chem* 46: 934-8., 2000.

18. Imhof, A., M. Froehlich, H. Brenner, H. Boeing, M. B. Pepys, and W. Koenig. Effect of alcohol consumption on systemic markers of inflammation. *Lancet* 357: 763-7., 2001.

19. Kuller, L. H., K. A. Matthews, and E. N. Meilahn. Estrogens and women's health: interrelation of coronary heart disease, breast cancer and osteoporosis. *J Steroid Biochem Mol Biol* 74: 297-309., 2000.

20. Lohman, T. G., A. F. Roche, and R. Martorell. *Anthropometric Standardization Manual*. Champaign, IL: Human Kinetics, 1988.

21. Mattusch, F., B. Dufaux, O. Heine, I. Mertens, and R. Rost. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 21: 21-4, 2000.

22. McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. Zak. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-42, 1983.

23. Mohamed-Ali, V., S. Goodrick, A. Rawesh, D. R. Katz, J. M. Miles, J. S. Yudkin, S. Klein, and S. W. Coppack. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82: 4196-200., 1997.

24. National Institutes of Health/National Heart Lung and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. The evidence report. *Obesity Res* 6: 51S-209S, 1998.

25. Nawaz, H., and D. L. Katz. American College of Preventive Medicine Practice Policy Statement: perimenopausal and postmenopausal hormone replacement therapy. *Am J Prev Med* 17: 250-4., 1999.

26. Pasceri, V., J. T. Willerson, and E. T. Yeh. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 102: 2165-8., 2000.

27. Pate, R. R., M. Pratt, S. N. Blair, W. L. Haskell, C. A. Macera, C. Bouchard, D. Buchner, W. Ettinger, G. W. Heath, A. C. King, and et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine [see comments]. *JAMA* 273: 402-7, 1995.

28. Pietrobelli, A., C. Formica, Z. Wang, and S. B. Heymsfield. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *Am J Physiol* 271: E941-51., 1996.

29. Ridker, P. M., M. Cushman, M. J. Stampfer, R. P. Tracy, and C. H. Hennekens. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men [published erratum appears in N Engl J Med 1997 Jul 31;337(5):356] [see comments]. *N Engl J Med* 336: 973-9, 1997.

30. Ridker, P. M., R. J. Glynn, and C. H. Hennekens. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction [see comments]. *Circulation* 97: 2007-11, 1998.

31. Ridker, P. M., C. H. Hennekens, J. E. Buring, and N. Rifai. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342: 836-43, 2000.

32. Ridker, P. M., C. H. Hennekens, N. Rifai, J. E. Buring, and J. E. Manson. Hormone replacement therapy and increased plasma concentration of C- reactive protein. *Circulation* 100: 713-6, 1999.

33. Sakkinen, P. A., P. Wahl, M. Cushman, M. R. Lewis, and R. P. Tracy. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol* 152: 897-907., 2000.

34. Sallis, J. F., W. L. Haskell, P. D. Wood, S. P. Fortmann, T. Rogers, S. N. Blair, and R. S. Paffenbarger, Jr. Physical activity assessment methodology in the Five-City Project. *Am J Epidemiol* 121: 91-106, 1985.

35. Smith, J. K., R. Dykes, J. E. Douglas, G. Krishnaswamy, and S. Berk. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA* 281: 1722-7, 1999.

36. Smith, S. C., Jr., P. Greenland, and S. M. Grundy. AHA Conference Proceedings. Prevention conference V: Beyond secondary prevention: Identifying the high-risk patient for primary prevention: executive summary. American Heart Association. *Circulation* 101: 111-6., 2000.

37. Straub, R. H., H. W. Hense, T. Andus, J. Scholmerich, G. A. Riegger, and H. Schunkert. Hormone replacement therapy and interrelation between serum interleukin- 6 and body mass index in postmenopausal women: a population-based study. *J Clin Endocrinol Metab* 85: 1340-4., 2000.

38. Taaffe, D. R., T. B. Harris, L. Ferrucci, J. Rowe, and T. E. Seeman. Cross-sectional and prospective relationships of interleukin-6 and C- reactive protein with physical performance in elderly persons: MacArthur studies of successful aging. *J Gerontol A Biol Sci Med Sci* 55: M709-15., 2000.

39. Torzewski, M., C. Rist, R. F. Mortensen, T. P. Zwaka, M. Bienek, J. Waltenberger, W. Koenig, G. Schmitz, V. Hombach, and J. Torzewski. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol* 20: 2094-9, 2000.

40. Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 6: 24-27, 1969.

41. Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 273: 199-208., 1995.

42. Yudkin, J. S., M. Kumari, S. E. Humphries, and V. Mohamed-Ali. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 148: 209-14., 2000.

43. Yudkin, J. S., C. D. Stehouwer, J. J. Emeis, and S. W. Coppack. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19: 972-8, 1999.

CHAPTER 3

BIOLOGICAL VARIABILITY OF INSULIN RESISTANT CAD RISK FACTORS IN POSTMENOPAUSAL WOMEN

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Abstract

Purpose: To quantify the biological variability of insulin resistant coronary artery disease (CAD) risk factors in a sample of 8 postmenopausal women. **Methods:** Risk factor outcomes were measured on two occasions, 7-12 days apart. Outcome measures included serum total cholesterol, serum triglycerides (TG), serum high-density lipoprotein cholesterol (HDL-C), serum glucose, plasma insulin, serum C-reactive protein (CRP), waist and hip circumferences, abdominal sagittal diameter, systolic (SBP) and diastolic blood pressure, and self-reported physical activity energy expenditure. Biological variability was assessed using standard errors of measurement (SEM), intraclass correlation coefficients, within-subjects coefficients of variation (CV), and Bland-Altman plots. **Results:** With the exception of waist circumference, there were no mean differences between measurements 1 and 2 ($p \geq 0.160$). High *absolute* biological variability values (by SEM) were observed for serum TG (32.0 mg/dl), serum CRP (5.6 mg/l), SBP (4.0 mmHg), and physical activity (9.4 kcal/kg/week). High *relative* biological variability (by within-subjects CV's ≥ 27.3 %) was also observed for serum TG, serum CRP, and physical activity. The Bland-Altman plots identified individual outliers that accounted for much of the overall biological variability observed for serum TG, serum CRP, plasma insulin, and SBP. **Conclusions:** Serial measurement of serum CRP, serum TG, plasma insulin, SBP, and physical activity in a future lifestyle modification trials in postmenopausal women may help in detecting their true responses to the intervention.

Key Words: Serial assessment, serum C-reactive protein, serum lipids, blood pressure, body fat, and physical activity.

Introduction

The primary prevention of coronary artery disease (CAD) through lifestyle modifications such as improved diets and more regular physical activity may be a cost effective way to reduce the 650,000 first-time heart attacks occurring each year in the United States (3). However, a physical activity trial designed to increase primary prevention of CAD is not feasible due to the need for an unrealistically prolonged intervention requiring a prohibitively large number of subjects (32). Nonetheless, it is important to test the impact of physical activity interventions on modifiable CAD risk factors as more feasible surrogates for primary CAD events. Such interventions may be especially important for postmenopausal women. For instance, the most recent American Heart Association Advisory recommends that hormone replacement therapy (HRT) should not be initiated for the sole purpose of primary CAD prevention in postmenopausal women (22). Thus, it may be especially important to determine the effectiveness, efficacy and safety of non-pharmacological alternatives to HRT, like physical activity, for managing CAD risk factors in postmenopausal women.

One of the most important health benefits of regular physical activity is its ability to reduce insulin resistance (24). In fact, slowing or halting the progression of insulin resistance to type 2 diabetes may be particularly important for women because diabetes is a stronger risk factor for CAD in women than men (23). Today, diabetes (25) and CAD (26) are increasingly recognized as inflammatory diseases, and serum C-reactive protein levels (CRP) represent an important marker and amplifier of inflammation (33). Elevated serum CRP levels have also been advanced as a new component of the insulin resistant metabolic syndrome (10), which is a constellation of CAD risk factors that also includes

high fasting glucose, insulin, and triglyceride levels, low high-density lipoprotein cholesterol (HDL-C) levels, high blood pressure, and abdominal obesity. Thus, lifestyle modification trials for older women should include a full spectrum of insulin resistant CAD risk factors as biologically relevant outcomes that may be beneficially altered by regular physical activity.

However, most physical activity interventions, to date, have focused somewhat more narrowly on serum lipid and blood pressure outcomes (6, 15, 16). Moreover, the lack of consideration of differences in biological variability among CAD risk factor outcomes creates some ambiguity in interpreting the actual effects of physical activity. For instance, one exercise lifestyle intervention increased plasma HDL-C but failed to reduce plasma triglyceride or blood pressure levels in postmenopausal women (15). Thus, the inconsistent treatment effects may reflect the specific biological actions of physical activity, or alternatively, they may reflect unquantified differences in the biological variability of the specific CAD risk factors assessed. Thus, a better quantification of the biological variability of insulin resistant CAD risk factors in postmenopausal women is needed. By identifying those risk factors with the highest biological variability, specific CAD risk factors could be selectively targeted for serial assessment, prior to future intervention trials, which in turn, may improve the likelihood of detecting the true CAD risk factor response to lifestyle modification. Thus, the purpose of this study is to quantify the biological variability of insulin resistant CAD risk factors in 8 postmenopausal women, measured on two occasions, 7-12 days apart.

Methods

Participants:

Nine postmenopausal women volunteered to participate in the present study. Women were excluded during telephone screening if they used non-oral forms of hormone replacement therapy or selective estrogen receptor modulators, or had thyroid disorders, cardiovascular disease, diabetes or gout. Women were also excluded if they had experienced a menses in the past year, or if they had surgical menopause before the age of 40 years. Lastly, participants were excluded if they were taking corticosteroids, niacin or fibrate medications. One subject was unable to have blood drawn the second week. Therefore, the number of subjects with complete data for analysis was 8.

Procedures:

This study took place in the summer months and participants were measured on two occasions, 7-12 days apart. To minimize inter-observer measurement error, on both occasions, the same technicians were used to assess a given study outcome. Blood was drawn from an antecubital vein, between 8-9 in the morning, following a 12-hour fast. Venous blood samples were collected in plasma EDTA tubes for glucose and insulin and in serum vacutainer tubes with gel clot activator for serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and serum C-reactive protein (CRP). Serum and plasma cryovials were then stored at –70C until all blood collection was complete.

Every serum or plasma sample was assayed in duplicate, and the mean of each duplicate was utilized in the data analysis. To eliminate inter-assay variability, every

week 1 and week 2 serum or plasma analyte sample for every subject was analyzed within the same assay. Analytical variability, which is a component of biological variability, was determined by calculating the intra-assay coefficient of variation (CV=[standard deviation/mean] x 100) using control serum or plasma samples with known concentrations for all circulating CAD risk factors. Serum total cholesterol (2) and triglycerides (21) were determined with enzymatic techniques. After precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid and magnesium chloride (4), serum HDL-C was determined enzymatically (2). A colorimetric glucose oxidase method (31) was used to assess plasma glucose, and a human insulin-specific radioimmunoassay (Linco Research, St. Charles, MO) was used to assess fasting plasma insulin levels. Serum CRP levels were measured with a highly sensitive enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories, TX). The serum dilution protocol recommended by the manufacturer is 1:500 and is primarily set to detect acute infection/inflammation in a clinical laboratory setting. As such it is not designed to record modest, chronic subclinical inflammation. Therefore, the dilution protocol was modified and serum samples were initially diluted 1:50 with assay buffer, which resulted in detectable serum CRP values of 0.5 to 25 mg/l. After initial analyses, samples that fell above the detectable range were reanalyzed with a serum dilution of 1:500 (detectable range 5-250 mg/l), and samples that fell below the detectable range were reanalyzed with a serum dilution of 1:5 (detectable range 0.05-2.5 mg/l).

On the same day as the blood draws, body weight, height, anthropometric body composition measures (waist circumference, hip circumference, and abdominal sagittal diameter), and blood pressure were measured. Body weight was recorded with a Detecto

balance beam scale (Metro Equipment Corp., Sunnydale, CA), and height was measured with a wall-mounted stadiometer. Waist and hip circumference were measured with a steel tape measure, according to the Anthropometric Standardization Manual (18). Abdominal sagittal diameter (ASD) was measured with a commercially available abdominal caliper made by Holtain Ltd. (Dyfed, Wales, United Kingdom) (14). Body circumferences and ASD's were measured in rotational order, and the mean of three measurements that agreed within 1cm or less was used in the data analysis. Systolic and diastolic blood pressures were measured by auscultation with the subject seated and resting quietly for five minutes prior to cuff inflation. Blood pressure cuff size was determined by right arm circumference measurements, and the mean of three blood pressure measurements, one minute apart, was used in the data analysis.

Estimates of physical activity caloric expenditure were assessed, on both occasions, by the same trained interviewer using the Stanford 7-day physical activity recall (28). However, we deviated from the standard protocol for estimating physical activity energy expenditure in two important ways. First, to be consistent with national physical activity recommendations for accumulating moderate or higher intensity activities (24), we did not include a calculated estimate of unreported "low-intensity" physical activity. Second, in an attempt to improve accuracy, caloric expenditures were calculated with the recommended metabolic equivalents for each specific activity from the Ainsworth Compendium of Physical Activities, rather than rely on "blanket" metabolic equivalents that do not account for the specific type of activity reported (1). The same technician who interviewed the participants also converted the reported

physical activities to metabolic equivalents. Physical activity is reported as kilocalories per kilogram of body weight per week.

Statistical Analysis:

A paired t-test was used to determine if there was a significant difference between the measurements at week 1 and week 2. A repeated measures analysis of variance was used to quantify the within subjects variability, the between subjects variability, the intraclass correlation, the effect size, and the statistical power. The within and between subjects variabilities were expressed as a coefficient of variation (CV). Because the distribution of serum CRP values was positively skewed, the denominator of CVs calculated for serum CRP was the median rather than the mean value.

In addition to the within subjects CV, which was used as a *relative* expression of biological variability, the standard error of measurement (SEM) was calculated (18) and used as an *absolute* expression of biological variability. Thus, the percentage scale of the within subjects CV facilitates a *relative* comparison of biological variability *across* CAD risk factors, whereas the SEM quantifies the *absolute* biological variability *within* the measurement units of each specific CAD risk factor. To further interpret the present use of the SEM, 95% of the values for a specific CAD risk factor will fall within ± 2 SEM from the mean of the serial assessments. Lastly, to graphically illustrate the statistical agreement between serial measurements and to identify the presence of individual outliers, Bland-Altman plots (7) were drawn for the circulating CAD risk factors, systolic blood pressure, and for the self reported estimates of physical activity caloric expenditure.

Results

There were 8 postmenopausal women with complete data, who ranged in age from 61-77 years (mean 68.1 ± 5.3 years). Two of the women were taking oral HRT in the form of combined oral estrogen and progestin, and the remaining six women were not taking any form of HRT. All participants were non-smokers, and none were taking statin drugs for cholesterol management or antibiotics for an infectious illness. In addition, none of the women reported any acute infections, or any other acute or chronic inflammatory illness during the course of the study. One of the women who was taking combined HRT, also took aspirin regularly.

The descriptive statistics for both measurements, the test for differences between measurements, the effect size, the observed power, the intraclass correlation coefficient, and the standard error of measurement (SEM) are reported in Table 3.1. With the exception of waist circumference, there were no significant mean differences between the measurements at week 1 and week 2. Even though the same technician assessed waist circumferences on both occasions, there was, on average, a 2 cm increase in waist circumference at week 2 ($p=0.012$), which was not corroborated by any significant changes in mean body weight or ASDs ($p>0.222$). Despite the limited sample size of eight subjects with complete data, the small effect sizes suggest that a larger sample may not have altered the general trend towards non-significant week-to-week changes in CAD risk factors, body composition, and physical activity. For instance, with the exception of waist circumference and systolic blood pressure, the effect sizes are ≤ 0.18, which is below the conventional threshold of 0.20 for a small effect size (9).

Table 3.1. Descriptive statistics, power statistics, standard errors of measurement, and intraclass correlation coefficients for circulating CAD risk factors, body composition, blood pressure, and physical activity.

	Measurement 1	Measurement 2	P	Effect Size	Power	SEM	ICC
Total Cholesterol (mg/dl)	232.5 (44.4)	235.3 (40.2)	0.680	0.03	6.6	12.1	0.95
Triglycerides (mg/dl)	130.9 (72.2)	119.9 (37.6)	0.529	0.06	8.9	32.0	0.90
HDL-C (mg/dl)	64.9 (11.1)	67.6 (11.8)	0.258	0.18	18.7	4.6	0.92
Glucose (mg/dl)	88.5 (9.3)	90.5 (11.0)	0.343	0.13	14.3	4.1	0.92
Insulin (μU/ml)	9.9 (2.7)	10.4 (3.6)	0.616	0.04	7.4	1.6	0.85
C-reactive Protein (mg/l)	3.5 (1.5-8.3)	2.5 (1.3-5.6)	0.338	0.13	14.5	5.6	0.34
Waist circumference (cm)	80.3 (8.8)	82.0 (9.2)	0.010	0.64	85.0	1.6	0.99
Hip Circumference (cm)	104.6 (6.1)	105.8 (5.7)	0.164	0.16	25.7	1.6	0.97
Abdominal Sagittal Diameter (cm)	19.8 (2.6)	19.8 (2.7)	0.693	0.02	6.5	0.3	0.99
Body Weight (kg)	66.4 (7.5)	66.0 (7.6)	0.222	0.21	21.4	0.5	0.99
Systolic Blood Pressure (mmHg)	128.3 (18.6)	131.1 (15.9)	0.160	0.26	27.6	4.0	0.98
Diastolic Blood Pressure (mmHg)	76.8 (11.9)	78.0 (9.2)	0.413	0.10	11.8	2.8	0.98
Physical Activity (kcal/kg/week)	34.4 (26.1)	37.6 (28.7)	0.536	0.06	8.7	9.4	0.93
Time* (minutes)	877.5 (285.3)	934.6 (324.2)	0.471	0.08	10.2	146.1	0.86

Power is expressed as a percentage.
ICC - intraclass correlation coefficient.
SEM – standard error of measurement, expressed in the units of each individual variable.
Time = time since last bout of moderate physical activity

The relatively large standard errors of measurement (SEM) for serum triglycerides (32.0 mg/dl), serum CRP (5.6 mg/l), systolic blood pressure (4.0 mmHg), and physical activity (9.4 kcal/kg/week) suggest that intervention-related changes in these CAD risk factors would be difficult to detect in the absence of serial measurement. The intraclass correlation coefficients (ICC) represent common variance between the two measurements. The ICCs, except for serum CRP, ranged from r=0.85 to r=0.99, indicating reasonably good reliability between week 1 and week 2 measurements. One

subject heavily influenced the low ICC of serum CRP. Thus, the ICC increased from R=0.34 to R=0.97 with the exclusion of the one subject with a large 22 mg/l drop in serum CRP from one week to the next.

In addition to the absolute measurement errors (in the form of SEMs), the relative errors (in the form of CVs) were partitioned into their intraassay, within subjects, and between subjects components (Table 3.2). The within subjects CVs reflect biological

Table 3.2. Intraassay, within subjects, and between subjects coefficients of variation for circulating CAD risk factors, body composition, blood pressure, and physical activity.

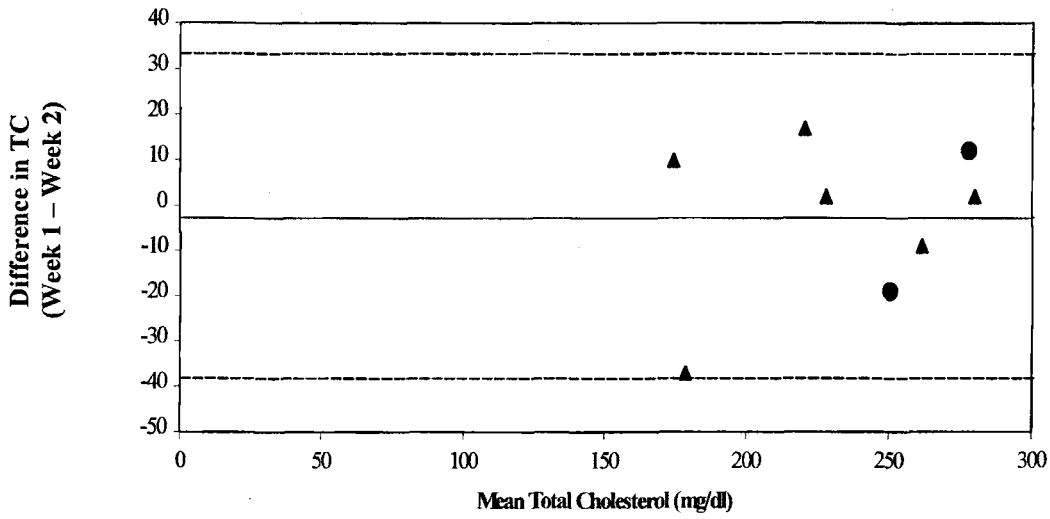
	CV (Intrassay)	CV (Within Subjects)	CV (Between Subjects)
Total Cholesterol (mg/dl)	2.4	5.5	25.0
Triglycerides (mg/dl)	4.5	27.3	59.2
HDL-C (mg/dl)	4.4	7.4	23.5
Glucose (mg/dl)	6.4	4.9	15.4
Insulin (μU/ml)	3.0-7.1	17.2	41.5
C-reactive Protein (mg/l)	6.4	239.8	254.8
Waist circumference (cm)		2.1	15.6
Hip Circumference (cm)		1.7	7.8
Abdominal Sagittal Diameter (cm)		1.9	18.9
Body Weight (kg)		0.9	16.1
Systolic Blood Pressure (mmHg)		3.3	18.7
Diastolic Blood Pressure (mmHg)		3.9	19.1
Physical Activity (kcal/kg/week)		27.9	104.3

CV – Coefficient of variation = (standard deviation/mean) x 100. Expressed as a percentage.
CV (Intraassay) = analytical variability
CV (Within Subjects) = biological variability

variability *relative* to the appropriate mean or median value. Thus, the large relative biological variability in serum triglycerides, serum CRP, and physical activity, which

were all ≥ 27.3%, further emphasizes the potential importance of serial measurement of these CAD risk factors. The between subjects CVs ranged from 7.8 % for hip circumference, to 254.8 % for serum CRP. For each variable, the between subjects variability was 2.2 to 9.9 times greater than the corresponding within subjects variability. Thus, despite the small sample of 8 postmenopausal women, there is a wide range of individual differences in CAD risk factors and body composition, which supports the generalizability of the findings.

The Bland-Altman plots illustrate the agreement between serial measurements for the circulating CAD risk factors, systolic blood pressure (SBP), and physical activity (Figures 3.1 and 3.2). Outliers are defined by Bland-Altman as difference scores that are greater than 2 standard deviations above or below the mean difference. Thus, the variables serum CRP, plasma insulin, serum triglycerides, and SBP all have one individual outlier. For physical activity, there were no individual outliers. However, the difference scores were widely dispersed around the mean difference, which suggests that serial measurement of self-reported physical activity may provide a more stable estimate of physical activity behavior. The Bland-Altman plots reinforce the earlier recommendation that serial measures may be important for serum CRP, serum triglycerides, plasma insulin, SBP, and physical activity, as they would help in identifying individual outliers or in averaging repeated measurements to achieve more stable baseline data prior to an intervention.



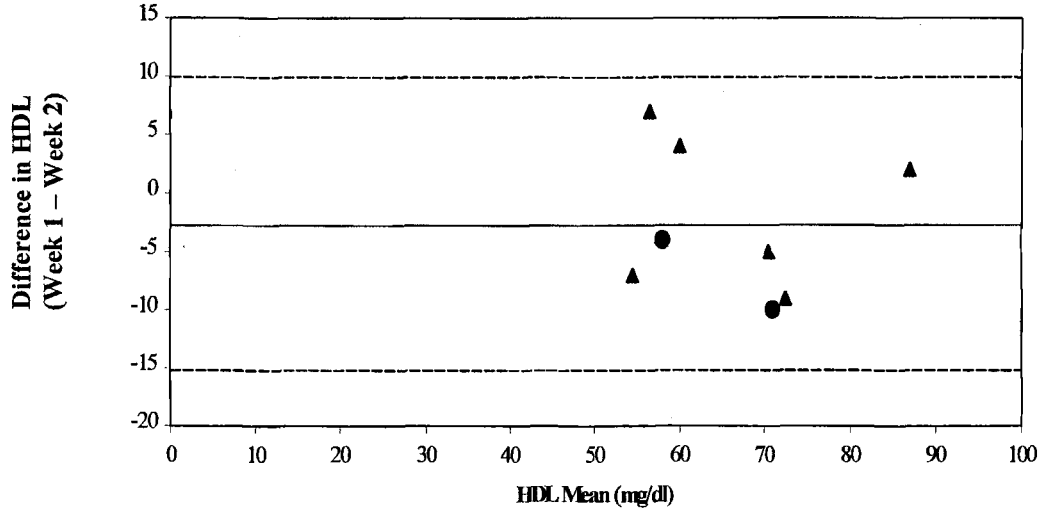
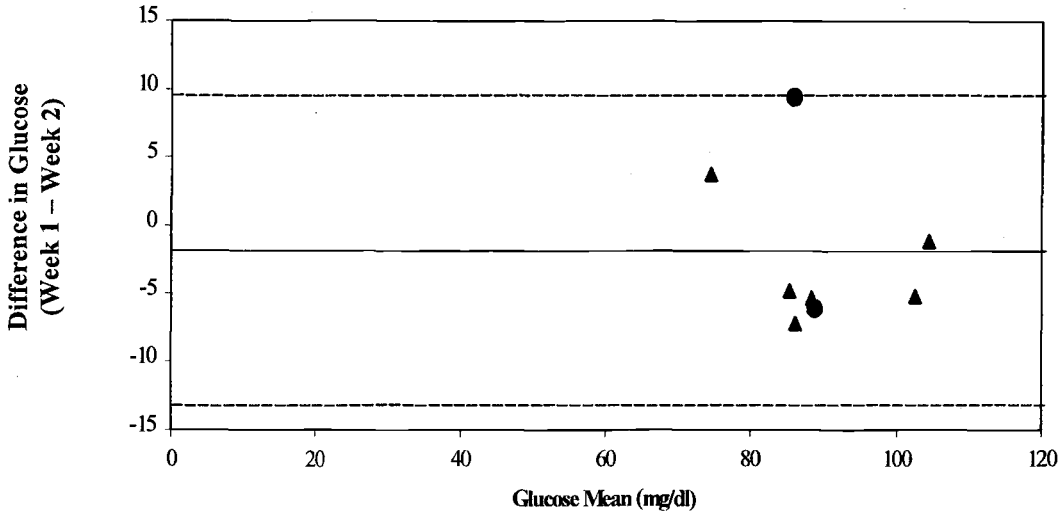


Figure 3.1 (Continued)



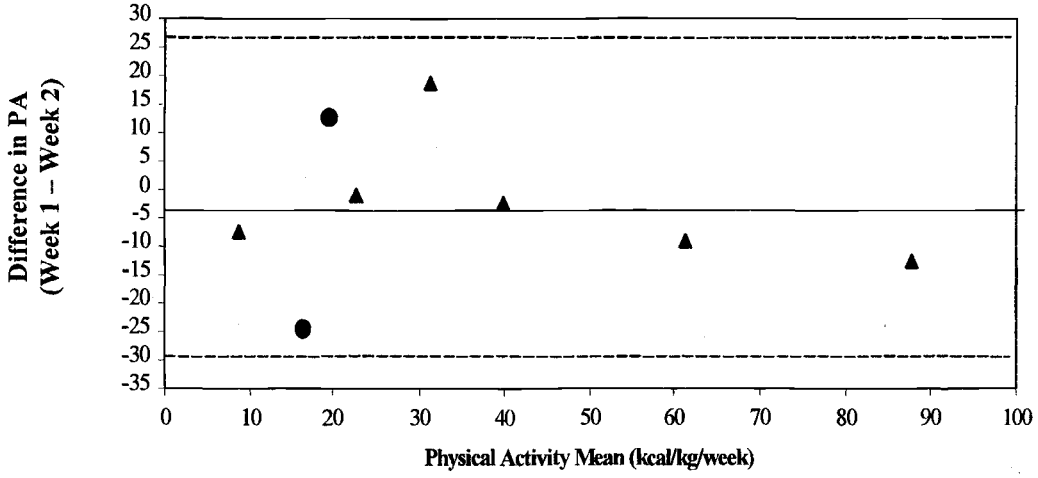
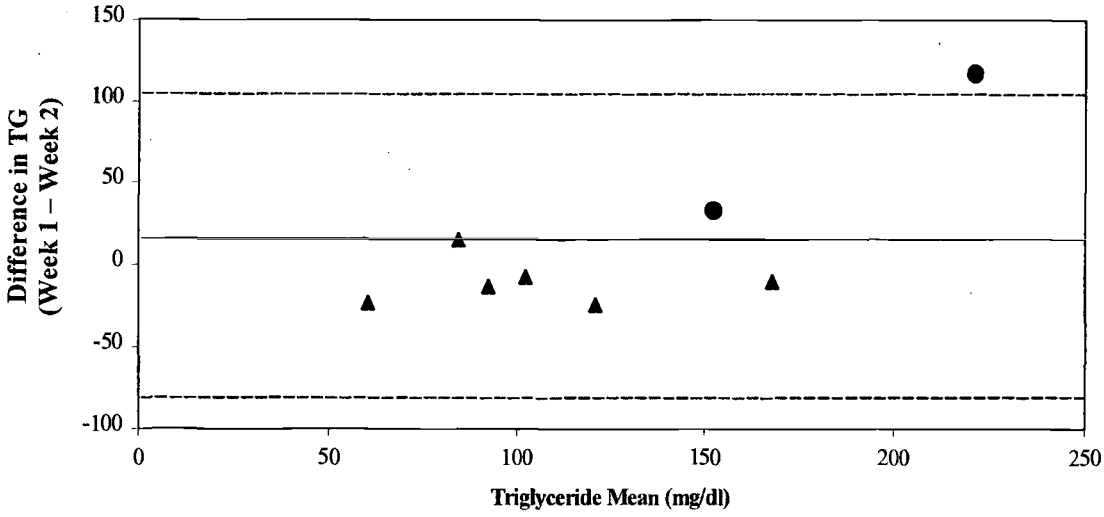


Figure 3.1. Bland –Altman Plots for Outcomes without Outliers including Total Cholesterol, HDL-C, Glucose and Physical Activity. The solid horizontal line indicates the mean of the difference scores. The dashed horizontal lines indicate ± 2 standard deviations from the mean difference score. The triangles represent women not on HRT and the circles represent women taking combined HRT.



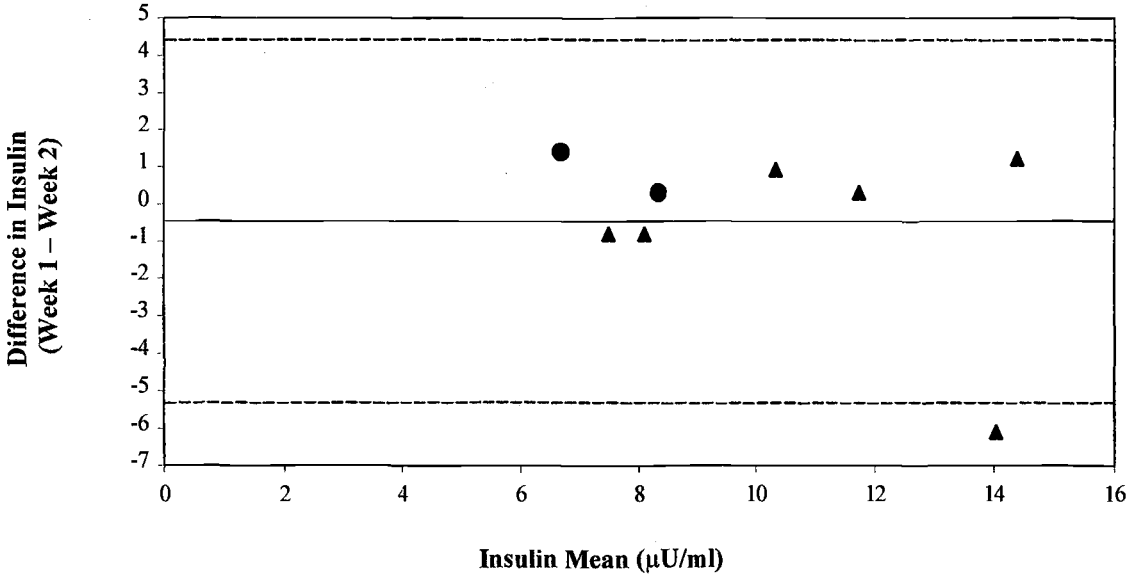
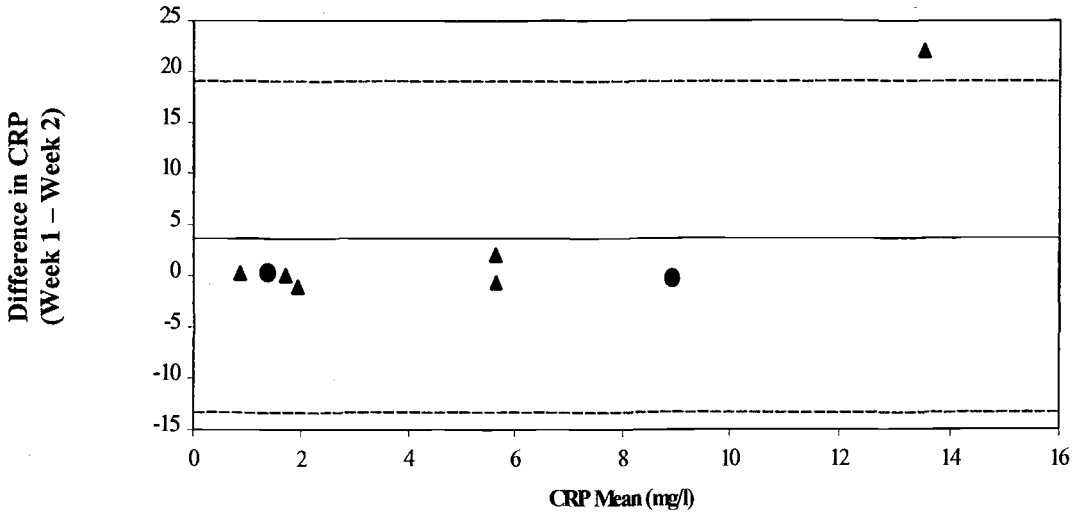


Figure 3.2 (Continued)



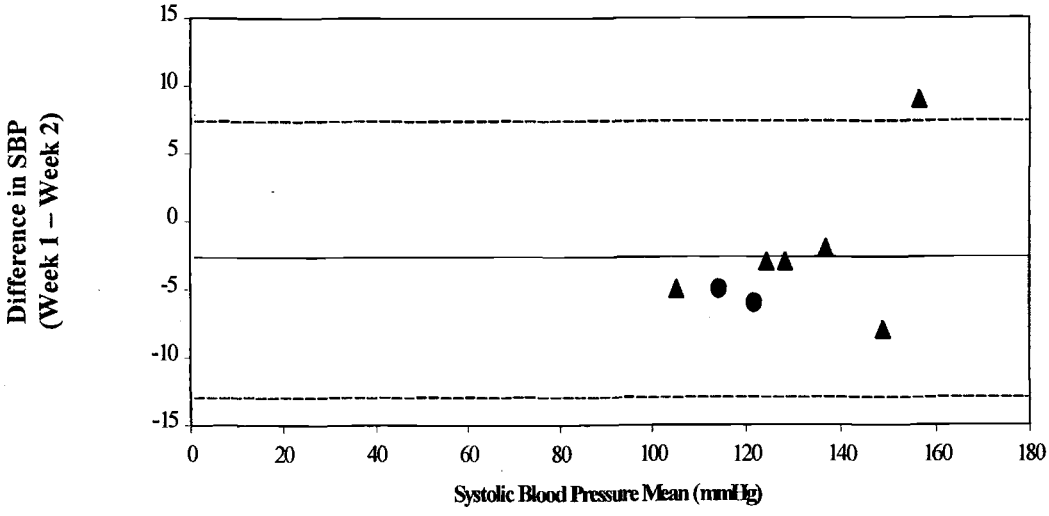


Figure 3.2. Bland –Altman Plots for Outcomes with Outliers including Triglycerides, Insulin, Serum CRP and Systolic Blood Pressure. The solid horizontal line indicates the mean of the difference scores. The dashed horizontal lines indicate ± 2 standard deviations from the mean difference score. The triangles represent women not on HRT and the circles represent women taking combined HRT.

Discussion

This study reports the biological variability of several insulin resistant CAD risk factors, which are biologically relevant outcomes to include in lifestyle intervention trials for women. Biological variability was primarily determined by the following criteria: a) relative variability (within subjects CV); b) absolute variability (SEM); or c) the presence of individual outliers in the Bland-Altman plots. The present study is unique and potentially significant because of its assessment of an extensive complement of insulin resistant CAD risk factors in a relatively understudied population of postmenopausal women. Furthermore, the present assessment of biological variability included important circulating markers of insulin resistance and vascular inflammation, which have been mechanistically implicated in the etiology of diabetes (25) and CAD (26) and may be reduced by more frequent participation in physical activity.

Serum CRP and serum triglycerides met all of the criteria for high biological variability by demonstrating high absolute and relative variability, and by the presence of individual outliers in the Bland-Altman plots. For serum CRP, the within subjects CV was 239.8%. The one outlying subject, with a 22 mg/l drop in serum CRP, reported a 250 mg/day increase in supplemental vitamin C intake from one week to the next. It is not entirely clear whether the subject's *reported* increase in supplemental vitamin C intake is the only explanation for her substantial drop in serum CRP. However, a recent study reports that higher serum vitamin C levels are strongly correlated with lower serum CRP levels ($r=-0.74$, $P < 0.0001$) (17). If the outlier for serum CRP is excluded from the analysis, the within subjects CV drops to 33.2 %. By comparison, previous studies have reported within subjects CVs for serum CRP ranging from 42% to 63% (8, 19, 27).

However, the previous reports were comprised of mixed study samples that included younger and male subjects (8, 27). Thus, the paucity of postmenopausal women in the earlier reports (8, 19, 27) makes it difficult to compare the present estimate of biological variability in serum CRP with the earlier reports.

Serum triglycerides have long been known to have high biological variability. In fact, our within subjects CV of 27.3 % for serum TG was similar to another study of women that reported a within subjects CV of 30% for serum TG that were also assessed on two occasions (13). Despite the similarity in the reported estimates of biological variability for serum TG, the prior study (13) included premenopausal women with lower TG levels than the postmenopausal women herein, and it used a longer sampling interval between blood draws (28 days vs. 7-12 days). The one outlying subject, with a 117 mg/dl drop in serum TG, was an oral HRT user. However, just prior to each of the two blood draws, she reported no change in premarin intake (0.625 mg/day), cycrin intake (2.5 mg/day), duration of fasting (14 to 14.5 hours), or length of time since the last acute bout of exercise (23-24 hours). Moreover, our within assay CV for serum TG was 4.5%, so excessive technical error is not a likely explanation for the 117 mg/dl drop in serum TG. Thus, there is no basis for removing this apparent "outlier" from our estimate of biological variability in serum TG. Furthermore, our within subjects CV for serum TG of 27.3% is comparable to a review of 26 reports of the biological variability for serum TG in humans (30). For instance, a mean within subjects CV of 21% was reported for serum TG across 26 reports with a wide range of subject numbers (N=10 to 7055), blood samples (N=2 to 19), and time intervals between first and last blood samples (N=2 to 141 days) (30).

Physical activity energy expenditure, as measured by a 7-day physical activity recall, displayed both high relative and absolute variability. Our within subjects CV of 27.9 % for total physical activity falls midway between the reported within subjects CVs of 7% for total activity and 52% for non-occupational (or recreational) activity in another study of older women (20). However, we used a 7-day physical activity recall, whereas the other study used a 24-hour physical activity recall (20). By comparison to the earlier report (20), our assessment tool required a relatively longer recall, which may have contributed to its greater variability. However, another report of the biological variability in self-reported physical activity, which used a 7-day physical activity recall (5) in much younger elementary school teachers reported that 14 days of assessment was required to achieve an estimate of physical activity that is 80% reliable in women. By comparison, we are reporting that the first of two consecutive 7-day recalls accounted for 86% of the common variance in physical activity reported in the second 7-day recall in older postmenopausal women. Our findings do not, in any way, imply that physical activity recall is more reliable in older as compared to younger women. Instead, the length of time between the repeated physical activity recalls was considerably shorter (1 week) in the present study as compared to the previous report of younger women (1 year). Moreover, the individual differences in self-reported physical activity between the two consecutively administered 7-day recalls herein ranged from –25 kcal/kg/week to +19 kcal/kg/week, which questions the usefulness of interpreting a squared reliability coefficient of > 80% as an acceptable standard of precision between repeated measurements. Therefore, correlational assessments of reliability between repeated

measurements may be misleading because they do not account for the magnitude of individual differences observed (7).

Fasting plasma insulin displayed low absolute variability (SEM=1.6 μU/ml) yet moderate relative variability (within subjects CV = 17.2 %). The one outlying subject, with a 6 μU/ml increase in plasma insulin, reported no medication or supplement use on either visit. Just prior to each of the two blood draws, she also reported little to no change in either the duration of fasting (13 to 14.5 hours) or the length of time since the last acute bout of exercise (11.5 to 13 hours). Moreover, our within assay CV for control plasma insulin in the range of our fasting values was only 3%, which is a relatively low technical error, that is also similar to an earlier report (29). Thus, there is no basis for removing this apparent "outlier" from our estimate of biological variability in fasting plasma insulin. Furthermore, our within subjects CV for fasting plasma insulin of 17.2% is slightly lower than an earlier report of a within subjects CV of 21% for fasting plasma insulin (29).

Systolic blood pressure (SBP) demonstrated somewhat high absolute variability (SEM = 4.0 mmHg), yet somewhat low relative variability (CV = 3.3%). We interpret the reported SEM for SBP (4 mmHg) as "somewhat high" in light of future attempts to detect relatively modest physical activity-related reductions in SBP in predominantly normotensive women. The one outlying subject, with a 9 mm Hg decrease in SBP from week 1 to week 2, reported taking a non-prescription antihistamine the night before the second morning visit. She also reported a greater length of time since the last acute bout of exercise at the second laboratory visit (23 vs. 16 hours). Neither the medication change nor the greater time since the last acute exercise bout, is a plausible explanation

for the 9 mmHg decrease in systolic blood pressure. Besides, our SEM of 4.0 mmHg for SBP is similar to an earlier study that reported a SEM of 4.5 mmHg for SBP in older adults with peripheral arterial disease (11).

Similar to earlier reports (18), our anthropometric estimates of regional body fat demonstrated low biological variability (within subjects CV < 2.1%). Because of their low biological variability, a single anthropometric estimate of body fat may suffice, as long as the same technician is used at every measurement session. However, the systematic difference in waist circumference, observed from week 1 to week 2, is troubling. The difference is likely explained by the lack of easy-to-identify soft tissue landmarks for waist circumference. By contrast, we observed no systematic difference between the week 1 and week 2 abdominal sagittal diameter measurements, which use easier-to-identify bony landmarks. Thus, researchers may want to consider measuring the abdominal sagittal diameter as a useful alternative or additional anthropometric estimate of abdominal fat.

The findings from the present study may help to explain some of the inconsistent effects previously attributed to lifestyle modification. One study, of group vs. home-based exercise reported a significant 24-month increase in plasma HDL-C but no change in either plasma TG or blood pressure in older men and postmenopausal women (15). The comparatively higher biological variability of serum TG and SBP, relative to serum HDL-C, reported herein, suggests that the unassessed and unaccounted for biological variability of plasma TG and blood pressure may have contributed to the null effects of exercise reported for these CAD risk factor outcomes (15). Another study of group-based exercise reported a significant 11-month decrease in serum total cholesterol but no

change in serum TG, serum HDL-C, or SBP in postmenopausal women (6). The comparatively higher biological variability of serum TG, relative to serum total cholesterol, reported herein, suggests that the unassessed and unaccounted for biological variability of plasma TG and SBP may have again contributed to the null effects of exercise reported for serum TG and SBP (6). However, it is not entirely clear why exercise-related increases in serum HDL-C, which had a low biological variability herein, were detected in one study (15) but not the other (6). We suspect that the ability to detect exercise-related increases in serum HDL-C in only one of the studies (15) may be explained by its inclusion of older men in the data analysis or by its longer study duration (24 vs. 11 months).

The main limitations of the present study include the small sample size and the use of only two rather than multiple measurements for study outcomes. A larger sample size may have improved the quality of the estimates of biological variability. However, an earlier review suggests that larger samples sizes are not imperative for obtaining similar estimates of biological variability, in well-defined samples of subjects (12). However, our assessment of insulin resistant CAD risk factors on only two occasions is a limitation because we can make no recommendation regarding the optimal number of serial assessments needed to obtain a truly stable estimate of a given risk factor. Moreover, three or more serial measurements would improve the confidence in isolating an outlying value for a given subject. However, due to the need to contain both study costs and subject burdens, researchers conducting future lifestyle interventions who choose to use serial measurements may not be able to measure a given study outcome more than twice either before or after delivering the intervention. Two measurements are

certainly better than one, as the average of two serial measurements may provide a more stable estimate of an individual's true value at a given point in time. Further, a comparison of serial measurements may help to identify aberrant values caused by non-compliance with the measurement protocol, unavoidable changes in health behaviors, unexpected cases of acute illness, and inconsistent uses of medications and nutritional supplements.

In conclusion, we report that serum triglycerides, plasma insulin, serum CRP, SBP, and self-reported physical activity all exhibit higher biological variability than serum total and HDL cholesterol, serum glucose, diastolic blood pressure, and body fat in postmenopausal women. Thus, the serial assessment of serum triglycerides, plasma insulin, serum CRP, SBP, and physical activity in a lifestyle modification trial may help in detecting their true responses to an intervention.

References

1. Ainsworth, B. E., W. L. Haskell, A. S. Leon, D. R. Jacobs, Jr., H. J. Montoye, J. F. Sallis, and R. S. Paffenbarger, Jr. Compendium of physical activities: classification of energy costs of human physical activities [see comments]. *Med Sci Sports Exerc* 25: 71-80, 1993.

2. Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-5, 1974.

3. American Heart Association. 2001 Heart and Stroke Statistical Update. *Retrieved August 16, 2001 from http://www.americanheart.org/statistics/cvd.html:*, 2001.

4. Assmann, G., H. Schriewer, G. Schmitz, and E. O. Hagele. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2. *Clin Chem* 29: 2026-30., 1983.

5. Baranowski, T., M. Smith, W. O. Thompson, J. Baranowski, D. Hebert, and C. de Moor. Intraindividual variability and reliability in a 7-day exercise record. *Med Sci Sports Exerc* 31: 1619-22., 1999.

6. Binder, E. F., S. J. Birge, and W. M. Kohrt. Effects of endurance exercise and hormone replacement therapy on serum lipids in older women. *J Am Geriatr Soc* 44: 231-6., 1996.

7. Bland, J. M., and D. G. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-10., 1986.

8. Clark, G. H., and C. G. Fraser. Biological variation of acute phase proteins. *Ann Clin Biochem* 30: 373-6., 1993.

9. Cohen, J. *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: Laurence Erlbaum Inc., Publishers, 1988.

10. Festa, A., R. D'Agostino, Jr., G. Howard, L. Mykkanen, R. P. Tracy, and S. M. Haffner. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102: 42-7, 2000.

11. Fowkes, F. G., E. Housley, C. C. Macintyre, R. J. Prescott, and C. V. Ruckley. Variability of ankle and brachial systolic pressures in the measurement of atherosclerotic peripheral arterial disease. *J Epidemiol Community Health* 42: 128-33., 1988.

12. Fraser, C. G., and E. K. Harris. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 27: 409-37, 1989.

13. Friedlander, Y., J. D. Kark, and Y. Stein. Variability of plasma lipids and lipoproteins: the Jerusalem Lipid Research Clinic Study. *Clin Chem* 31: 1121-6., 1985.

14. Kahn, H. S., H. Austin, D. F. Williamson, and D. Arensberg. Simple anthropometric indices associated with ischemic heart disease. *J Clin Epidemiol* 49: 1017-24., 1996.

15. King, A. C., W. L. Haskell, D. R. Young, R. K. Oka, and M. L. Stefanick. Long-term effects of varying intensities and formats of physical activity on participation rates, fitness, and lipoproteins in men and women aged 50 to 65 years. *Circulation* 91: 2596-604., 1995.

16. Kuller, L. H., L. R. Simkin-Silverman, R. R. Wing, E. N. Meilahn, and D. G. Ives. Women's Healthy Lifestyle Project: A randomized clinical trial: results at 54 months. *Circulation* 103: 32-7., 2001.

17. Langlois, M., D. Duprez, J. Delanghe, M. De Buyzere, and D. L. Clement. Serum vitamin C concentration is low in peripheral arterial disease and is associated with inflammation and severity of atherosclerosis. *Circulation* 103: 1863-8., 2001.

18. Lohman, T. G., A. F. Roche, and R. Martorell. *Anthropometric Standardization Manual.* Champaign, IL: Human Kinetics, 1988.

19. Macy, E. M., T. E. Hayes, and R. P. Tracy. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clin Chem* 43: 52-8., 1997.

20. Matthews, C. E., J. R. Hebert, P. S. Freedson, E. J. Stanek, 3rd, P. A. Merriam, C. B. Ebbeling, and I. S. Ockene. Sources of variance in daily physical activity levels in the seasonal variation of blood cholesterol study. *Am J Epidemiol* 153: 987-95., 2001.

21. McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. Zak. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-42, 1983.

22. Mosca, L., P. Collins, D. M. Herrington, M. E. Mendelsohn, R. C. Pasternak, R. M. Robertson, K. Schenck-Gustafsson, S. C. Smith, Jr., K. A. Taubert, and N. K. Wenger. Hormone replacement therapy and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. *Circulation* 104: 499-503., 2001.

23. Mosca, L., S. M. Grundy, D. Judelson, K. King, M. Limacher, S. Oparil, R. Pasternak, T. A. Pearson, R. F. Redberg, S. C. Smith, Jr., M. Winston, and S. Zinberg. Guide to Preventive Cardiology for Women.AHA/ACC Scientific Statement Consensus panel statement. *Circulation* 99: 2480-4., 1999.

24. Pate, R. R., M. Pratt, S. N. Blair, W. L. Haskell, C. A. Macera, C. Bouchard, D. Buchner, W. Ettinger, G. W. Heath, A. C. King, and et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine [see comments]. *JAMA* 273: 402-7, 1995.

25. Pickup, J. C., and M. A. Crook. Is type II diabetes mellitus a disease of the innate immune system? [see comments]. *Diabetologia* 41: 1241-8, 1998.

26. Ross, R. Atherosclerosis--an inflammatory disease [see comments]. *N Engl J Med* 340: 115-26, 1999.

27. Sakkinen, P. A., E. M. Macy, P. W. Callas, E. S. Cornell, T. E. Hayes, L. H. Kuller, and R. P. Tracy. Analytical and biologic variability in measures of hemostasis, fibrinolysis, and inflammation: assessment and implications for epidemiology. *Am J Epidemiol* 149: 261-7., 1999.

28. Sallis, J. F., W. L. Haskell, P. D. Wood, S. P. Fortmann, T. Rogers, S. N. Blair, and R. S. Paffenbarger, Jr. Physical activity assessment methodology in the Five-City Project. *Am J Epidemiol* 121: 91-106, 1985.

29. Sebastian-Gambaro, M. A., F. J. Liron-Hernandez, and X. Fuentes-Arderiu. Intra- and inter-individual biological variability data bank. *Eur J Clin Chem Clin Biochem* 35: 845-52., 1997.

30. Smith, S. J., G. R. Cooper, G. L. Myers, and E. J. Sampson. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. *Clin Chem* 39: 1012-22., 1993.

31. Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 6: 24-27, 1969.

32. United States Department of Health and Human Services. *Physical activity and health: A report of the Surgeon General.* Atlanta, GA: United States Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion., 1996.

33. Yeh, E. T., H. V. Anderson, V. Pasceri, and J. T. Willerson. C-reactive protein: linking inflammation to cardiovascular complications. *Circulation* 104: 974-5., 2001.

CHAPTER 4: CONCLUSION

Heart disease is the leading cause of death for men and women in the United States, with almost 40% of all deaths each year, attributable to cardiovascular disease (American Heart Association, 2001). This rate amounted to almost 1 million deaths in 1998. Up until the last 3-5 years, hormone replacement therapy (HRT) for post-menopausal women was recommended, in part, for its purported improvements in heart disease risk factors and the decrease in the incidence of heart disease events and/or deaths. Recently, however, the American Heart Association has published a statement saying that they do not recommend HRT use for primary or secondary prevention of cardiovascular disease in postmenopausal women (Mosca et al., 2001). The American Heart Association further recommends that the decision to take, or initiate HRT, should be based on established benefits of HRT, including improvements in bone density and menopausal symptoms (Mosca et al., 2001). Thus, there are millions of women and doctors who will continue to use and prescribe HRT for its benefits to bone density and for the control of menopausal symptoms.

Nevertheless, HRT use consistently leads to elevated serum C-reactive protein (CRP) levels, which are a marker of inflammation, and a strong predictor of heart disease risk in men and women. Additionally, more and more evidence is suggesting that increased levels of serum CRP have a significant role in the initiation and progression of atherosclerotic plaque formation. Thus, the increased levels of serum CRP that accompany HRT use are troubling. Therefore, the aim of the first study was to determine whether higher physical activity levels could offset adverse HRT-related

increases in serum CRP, independent of HRT use. The study, using a cross-sectional design with 133 postmenopausal women, found a significant inverse association ($r= -0.21$, $p=0.018$) between increased physical activity energy expenditure and decreased circulating levels of serum CRP. That association was independent of oral HRT use, but dependent on body fat. This suggests that the most important mechanism by which increased levels of physical activity, lead to decreased levels of serum CRP, may be through a decrease in body fat.

The second study used a repeated measures design to examine the biological variability of physical activity energy expenditure, and the insulin resistant CAD risk factors measured in study one. Eight postmenopausal women were measured on two occasions, 7-12 days apart. Using several statistical procedures, including standard errors of measurement (SEM), intraclass correlation coefficients (ICC), coefficients of variation, and Bland-Altman plots, the results showed that serum triglycerides, serum CRP, plasma insulin, systolic blood pressure, and physical activity energy expenditure had higher biological variability than total cholesterol, high density lipoprotein cholesterol, glucose, and all body composition measures.

The biological variability findings may enhance our interpretation of the results of study one, and provide recommendations for future exercise intervention trials with postmenopausal women. For instance, study one reported a significant but relatively low inverse correlation between higher levels of physical activity energy expenditure and lower levels of serum CRP. We know, from the second study, that the biological variability of both physical activity and serum CRP is large. Thus, it is likely that the strength of the association between serum CRP and physical activity is being

underrepresented in the absence of serial measurement techniques. There are several future lines of research that would help to determine more clearly the role of increased physical activity levels in the reduction of serum CRP.

First, longitudinal physical activity intervention studies with postmenopausal women are necessary to determine the mechanism by which physical activity may decrease serum CRP. Longitudinal studies would allow testing of hypothesized mechanisms for the decrease in serum CRP reported with increased physical activity, including a decrease in body fat or decreased insulin resistance. Secondly, measurement of interleukin-6 (IL-6), the primary mediator of the acute phase inflammatory response, would improve our understanding of the complicated relationships between IL-6, CRP and HRT in postmenopausal women. Intervention studies, measuring IL-6, in addition to CRP, may be particularly important in the determination of the mechanism by which increased physical activity decreases serum CRP. Lastly, future studies with postmenopausal women measuring insulin resistant CAD risk factors, particularly, serum triglycerides, serum CRP, plasma insulin, systolic blood pressure, and physical activity, would benefit from the use of serial measurement techniques. Serial measurement of those outcomes, in particular, may improve the likelihood of detecting the true CAD risk factor response to lifestyle modification.

In summary, the present studies suggest that increased physical activity levels play an important, possibly underrepresented role in decreasing serum CRP in postmenopausal women. For future intervention studies, the present findings provide a strong basis for the hypothesis that increased physical activity levels may decrease or maintain more normal serum CRP levels in postmenopausal women who take HRT.

BIBLIOGRAPHY

1. Ainsworth, B. E., W. L. Haskell, A. S. Leon, D. R. Jacobs, Jr., H. J. Montoye, J. F. Sallis, and R. S. Paffenbarger, Jr. Compendium of physical activities: classification of energy costs of human physical activities [see comments]. *Med Sci Sports Exerc* 25: 71-80, 1993.

2. Allain, C. C., L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470-5, 1974.

3. American Heart Association. 2001 Heart and Stroke Statistical Update. *Retrieved August 16, 2001 from http://www.americanheart.org/statistics/cvd.html:*, 2001.

4. Angerer, P., S. Stork, W. Kothny, P. Schmitt, and C. von Schacky. Effect of oral postmenopausal hormone replacement on progression of atherosclerosis : a randomized, controlled trial. *Arterioscler Thromb Vasc Biol* 21: 262-8., 2001.

5. Assmann, G., H. Schriewer, G. Schmitz, and E. O. Hagele. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2. *Clin Chem* 29: 2026-30., 1983.

6. Baranowski, T., M. Smith, W. O. Thompson, J. Baranowski, D. Hebert, and C. de Moor. Intraindividual variability and reliability in a 7-day exercise record. *Med Sci Sports Exerc* 31: 1619-22., 1999.

7. Binder, E. F., S. J. Birge, and W. M. Kohrt. Effects of endurance exercise and hormone replacement therapy on serum lipids in older women. *J Am Geriatr Soc* 44: 231-6., 1996.

8. Bland, J. M., and D. G. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307-10., 1986.

9. Brett, K., and Y. Chong. Hormone Replacement Therapy: Knowledge and Use in the United States. Hyattsville, Maryland: National Center for Health Statistics, 2001.

10. Bush, T. L. Preserving cardiovascular benefits of hormone replacement therapy. *J Reprod Med* 45: 259-73., 2000.

11. Cauley, J. A., D. M. Black, E. Barrett-Connor, F. Harris, K. Shields, W. Applegate, and S. R. Cummings. Effects of hormone replacement therapy on clinical fractures and height loss: The Heart and Estrogen/Progestin Replacement Study (HERS). *Am J Med* 110: 442-50., 2001.

12. Cauley, J. A., D. G. Seeley, K. Ensrud, B. Ettinger, D. Black, and S. R. Cummings. Estrogen replacement therapy and fractures in older women. Study of Osteoporotic Fractures Research Group. *Ann Intern Med* 122: 9-16., 1995.

13. Chenillot, O., J. Henny, J. Steinmetz, B. Herbeth, C. Wagner, and G. Siest. High sensitivity C-reactive protein: biological variations and reference limits. *Clin Chem Lab Med* 38: 1003-11., 2000.

14. Clark, G. H., and C. G. Fraser. Biological variation of acute phase proteins. *Ann Clin Biochem* 30: 373-6., 1993.

15. Cohen, J. *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: Laurence Erlbaum Inc., Publishers, 1988.

16. Cooper, G. R., G. L. Myers, S. J. Smith, and R. C. Schlant. Blood lipid measurements. Variations and practical utility. *JAMA* 267: 1652-60., 1992.

17. Cushman, M., C. Legault, E. Barrett-Connor, M. L. Stefanick, C. Kessler, H. L. Judd, P. A. Sakkinen, and R. P. Tracy. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. *Circulation* 100: 717-22., 1999.

18. Cushman, M., E. N. Meilahn, B. M. Psaty, L. H. Kuller, A. S. Dobs, and R. P. Tracy. Hormone replacement therapy, inflammation, and hemostasis in elderly women. *Arterioscler Thromb Vasc Biol* 19: 893-9., 1999.

19. de Maat, M. P., and C. Kluft. Determinants of C-reactive protein concentration in blood. *Ital Heart J* 2: 189-95., 2001.

20. Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285: 2486-2497, 2001.

21. Festa, A., R. D'Agostino, Jr., G. Howard, L. Mykkanen, R. P. Tracy, and S. M. Haffner. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102: 42-7, 2000.

22. Folsom, A. R., J. S. Pankow, R. P. Tracy, D. K. Arnett, J. M. Peacock, Y. Hong, L. Djousse, and J. H. Eckfeldt. Association of C-reactive protein with markers of prevalent atherosclerotic disease. *Am J Cardiol* 88: 112-7., 2001.

23. Fowkes, F. G., E. Housley, C. C. Macintyre, R. J. Prescott, and C. V. Ruckley. Variability of ankle and brachial systolic pressures in the measurement of atherosclerotic peripheral arterial disease. *J Epidemiol Community Health* 42: 128-33., 1988.

24. Fraser, C. G., and E. K. Harris. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 27: 409-37, 1989.

25. Freidwald, W. T., R. I. Levy, and D. S. Fredrickson. Estimation of the concentration of low density lipoprotein cholesterol in plasma, with the use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502, 1972.

26. Friedlander, Y., J. D. Kark, and Y. Stein. Variability of plasma lipids and lipoproteins: the Jerusalem Lipid Research Clinic Study. *Clin Chem* 31: 1121-6., 1985.

27. Frohlich, M., A. Imhof, G. Berg, W. L. Hutchinson, M. B. Pepys, H. Boeing, R. Muche, H. Brenner, and W. Koenig. Association between C-reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care* 23: 1835-9., 2000.

28. Gabay, C., and I. Kushner. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448-54., 1999.

29. Geffken, D. F., M. Cushman, G. L. Burke, J. F. Polak, P. A. Sakkinen, and R. P. Tracy. Association between Physical Activity and Markers of Inflammation in a Healthy Elderly Population. *Am J Epidemiol* 153: 242-250., 2001.

30. Going, S., D. Williams, and T. Lohman. Aging and body composition: biological changes and methodological issues. *Exerc Sport Sci Rev* 23: 411-58, 1995.

31. Grady, D., and S. R. Cummings. Postmenopausal hormone therapy for prevention of fractures: how good is the evidence? *JAMA* 285: 2909-10., 2001.

32. Greenblatt, R. B., and L. D. Stoddard. The estrogen--cancer controversy. *J Am Geriatr Soc* 26: 1-8., 1978.

33. Grodstein, F., J. Chen, D. A. Pollen, M. S. Albert, R. S. Wilson, M. F. Folstein, D. A. Evans, and M. J. Stampfer. Postmenopausal hormone therapy and cognitive function in healthy older women. *J Am Geriatr Soc* 48: 746-52., 2000.

34. Grodstein, F., M. J. Stampfer, G. A. Colditz, W. C. Willett, J. E. Manson, M. Joffe, B. Rosner, C. Fuchs, S. E. Hankinson, D. J. Hunter, C. H. Hennekens, and F. E. Speizer. Postmenopausal hormone therapy and mortality. *N Engl J Med* 336: 1769-75., 1997.

35. Hak, A. E., C. D. Stehouwer, M. L. Bots, K. H. Polderman, C. G. Schalkwijk, I. C. Westendorp, A. Hofman, and J. C. Witteman. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. *Arterioscler Thromb Vasc Biol* 19: 1986-91., 1999.

36. Heinrich, P. C., J. V. Castell, and T. Andus. Interleukin-6 and the acute phase response. *Biochem J* 265: 621-36, 1990.

37. Herrington, D. M., D. M. Reboussin, K. B. Brosnihan, P. C. Sharp, S. A. Shumaker, T. E. Snyder, C. D. Furberg, G. J. Kowalchuk, T. D. Stuckey, W. J. Rogers, D. H. Givens, and D. Waters. Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. *N Engl J Med* 343: 522-9., 2000.

38. Hulley, S., D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs, and E. Vittinghoff. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 280: 605-13., 1998.

39. Hutchinson, W. L., W. Koenig, M. Frohlich, M. Sund, G. D. Lowe, and M. B. Pepys. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. *Clin Chem* 46: 934-8., 2000.

40. Imhof, A., M. Froehlich, H. Brenner, H. Boeing, M. B. Pepys, and W. Koenig. Effect of alcohol consumption on systemic markers of inflammation. *Lancet* 357: 763-7., 2001.

41. Kahn, H. S., H. Austin, D. F. Williamson, and D. Arensberg. Simple anthropometric indices associated with ischemic heart disease. *J Clin Epidemiol* 49: 1017-24., 1996.

42. King, A. C., W. L. Haskell, D. R. Young, R. K. Oka, and M. L. Stefanick. Long-term effects of varying intensities and formats of physical activity on participation rates, fitness, and lipoproteins in men and women aged 50 to 65 years. *Circulation* 91: 2596-604., 1995.

43. Koenig, W., M. Sund, M. Frohlich, H. G. Fischer, H. Lowel, A. Doring, W. L. Hutchinson, and M. B. Pepys. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 99: 237-42., 1999.

44. Kuller, L. H., K. A. Matthews, and E. N. Meilahn. Estrogens and women's health: interrelation of coronary heart disease, breast cancer and osteoporosis. *J Steroid Biochem Mol Biol* 74: 297-309., 2000.

45. Kuller, L. H., L. R. Simkin-Silverman, R. R. Wing, E. N. Meilahn, and D. G. Ives. Women's Healthy Lifestyle Project: A randomized clinical trial: results at 54 months. *Circulation* 103: 32-7., 2001.

46. Langlois, M., D. Duprez, J. Delanghe, M. De Buyzere, and D. L. Clement. Serum vitamin C concentration is low in peripheral arterial disease and is associated with inflammation and severity of atherosclerosis. *Circulation* 103: 1863-8., 2001.

47. Lohman, T. G., A. F. Roche, and R. Martorell. *Anthropometric Standardization Manual*. Champaign, IL: Human Kinetics, 1988.

48. Lorrain, J., L. Plouffe, V. Ravnikar, L. Speroff, and N. Watts. Comprehensive Management of Menopause. New York: Springer-Verlag, 1994.

49. Macy, E. M., T. E. Hayes, and R. P. Tracy. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clin Chem* 43: 52-8., 1997.

50. Marcovina, S. M., V. P. Gaur, and J. J. Albers. Biological variability of cholesterol, triglyceride, low- and high- density lipoprotein cholesterol, lipoprotein(a), and apolipoproteins A- I and B. *Clin Chem* 40: 574-8., 1994.

51. Matthews, C. E., J. R. Hebert, P. S. Freedson, E. J. Stanek, 3rd, P. A. Merriam, C. B. Ebbeling, and I. S. Ockene. Sources of variance in daily physical activity levels in the seasonal variation of blood cholesterol study. *Am J Epidemiol* 153: 987-95., 2001.

52. Mattusch, F., B. Dufaux, O. Heine, I. Mertens, and R. Rost. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med* 21: 21-4, 2000.

53. Mayer-Davis, E. J., R. D'Agostino, Jr., A. J. Karter, S. M. Haffner, M. J. Rewers, M. Saad, and R. N. Bergman. Intensity and amount of physical activity in relation to insulin sensitivity: the Insulin Resistance Atherosclerosis Study. *JAMA* 279: 669-74., 1998.

54. McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. Zak. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29: 538-42, 1983.

55. Mohamed-Ali, V., S. Goodrick, A. Rawesh, D. R. Katz, J. M. Miles, J. S. Yudkin, S. Klein, and S. W. Coppack. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab* 82: 4196-200., 1997.

56. Mosca, L., P. Collins, D. M. Herrington, M. E. Mendelsohn, R. C. Pasternak, R. M. Robertson, K. Schenck-Gustafsson, S. C. Smith, Jr., K. A. Taubert, and N. K. Wenger. Hormone replacement therapy and cardiovascular disease: a statement for healthcare professionals from the american heart association. *Circulation* 104: 499-503., 2001.

57. Mosca, L., S. M. Grundy, D. Judelson, K. King, M. Limacher, S. Oparil, R. Pasternak, T. A. Pearson, R. F. Redberg, S. C. Smith, Jr., M. Winston, and S. Zinberg. Guide to Preventive Cardiology for Women.AHA/ACC Scientific Statement Consensus panel statement. *Circulation* 99: 2480-4., 1999.

58. Naessen, T., I. Persson, H. O. Adami, R. Bergstrom, and L. Bergkvist. Hormone replacement therapy and the risk for first hip fracture. A prospective, population-based cohort study. *Ann Intern Med* 113: 95-103., 1990.

59. National Institutes of Health/National Heart Lung and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. The evidence report. *Obesity Res* 6: 51S-209S, 1998.

60. Nawaz, H., and D. L. Katz. American College of Preventive Medicine Practice Policy Statement: perimenopausal and postmenopausal hormone replacement therapy. *Am J Prev Med* 17: 250-4., 1999.

61. Pasceri, V., J. T. Willerson, and E. T. Yeh. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 102: 2165-8., 2000.

62. Pate, R. R., M. Pratt, S. N. Blair, W. L. Haskell, C. A. Macera, C. Bouchard, D. Buchner, W. Ettinger, G. W. Heath, A. C. King, and et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine [see comments]. *JAMA* 273: 402-7, 1995.

63. Pickup, J. C., and M. A. Crook. Is type II diabetes mellitus a disease of the innate immune system? [see comments]. *Diabetologia* 41: 1241-8, 1998.

64. Pietrobelli, A., C. Formica, Z. Wang, and S. B. Heymsfield. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *Am J Physiol* 271: E941-51., 1996.

65. Ridker, P. M., J. E. Buring, J. Shih, M. Matias, and C. H. Hennekens. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women [see comments]. *Circulation* 98: 731-3, 1998.

66. Ridker, P. M., M. Cushman, M. J. Stampfer, R. P. Tracy, and C. H. Hennekens. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men [published erratum appears in N Engl J Med 1997 Jul 31;337(5):356] [see comments]. *N Engl J Med* 336: 973-9, 1997.

67. Ridker, P. M., R. J. Glynn, and C. H. Hennekens. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction [see comments]. *Circulation* 97: 2007-11, 1998.

68. Ridker, P. M., C. H. Hennekens, J. E. Buring, and N. Rifai. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 342: 836-43, 2000.

69. Ridker, P. M., C. H. Hennekens, N. Rifai, J. E. Buring, and J. E. Manson. Hormone replacement therapy and increased plasma concentration of C- reactive protein. *Circulation* 100: 713-6, 1999.

70. Ridker, P. M., N. Rifai, M. A. Pfeffer, F. Sacks, and E. Braunwald. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* 100: 230-5., 1999.

71. Ross, R. Atherosclerosis--an inflammatory disease [see comments]. *N Engl J Med* 340: 115-26, 1999.

72. Rousseau, M. E. Hormone replacement therapy. *Nurse Pract Forum* 9: 147-53., 1998.

73. Sakkinen, P. A., E. M. Macy, P. W. Callas, E. S. Cornell, T. E. Hayes, L. H. Kuller, and R. P. Tracy. Analytical and biologic variability in measures of hemostasis, fibrinolysis, and inflammation: assessment and implications for epidemiology. *Am J Epidemiol* 149: 261-7., 1999.

74. Sakkinen, P. A., P. Wahl, M. Cushman, M. R. Lewis, and R. P. Tracy. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol* 152: 897-907., 2000.

75. Sallis, J. F., W. L. Haskell, P. D. Wood, S. P. Fortmann, T. Rogers, S. N. Blair, and R. S. Paffenbarger, Jr. Physical activity assessment methodology in the Five-City Project. *Am J Epidemiol* 121: 91-106, 1985.

76. Samaras, K., C. S. Hayward, D. Sullivan, R. P. Kelly, and L. V. Campbell. Effects of postmenopausal hormone replacement therapy on central abdominal fat, glycemic control, lipid metabolism, and vascular factors in type 2 diabetes: a prospective study. *Diabetes Care* 22: 1401-7., 1999.

77. Sebastian-Gambaro, M. A., F. J. Liron-Hernandez, and X. Fuentes-Arderiu. Intra- and inter-individual biological variability data bank. *Eur J Clin Chem Clin Biochem* 35: 845-52., 1997.

78. Shah, S., T. J. Harris, and D. G. Cook. Differences in hormone replacement therapy use by social class, region and psychological symptoms. *BJOG* 108: 269-75., 2001.

79. Sidney, S., D. B. Petitti, and C. P. Quesenberry. Myocardial infarction and the use of estrogen and estrogen-progestogen in postmenopausal women. *Ann Intern Med* 127: 501-8., 1997.

80. Smith, J. K., R. Dykes, J. E. Douglas, G. Krishnaswamy, and S. Berk. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *JAMA* 281: 1722-7, 1999.

81. Smith, S. C., Jr., P. Greenland, and S. M. Grundy. AHA Conference Proceedings. Prevention conference V: Beyond secondary prevention: Identifying the high-risk patient for primary prevention: executive summary. American Heart Association. *Circulation* 101: 111-6., 2000.

82. Smith, S. J., G. R. Cooper, G. L. Myers, and E. J. Sampson. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. *Clin Chem* 39: 1012-22., 1993.

83. Straub, R. H., H. W. Hense, T. Andus, J. Scholmerich, G. A. Riegger, and H. Schunkert. Hormone replacement therapy and interrelation between serum interleukin- 6 and body mass index in postmenopausal women: a population-based study. *J Clin Endocrinol Metab* 85: 1340-4., 2000.

84. Taaffe, D. R., T. B. Harris, L. Ferrucci, J. Rowe, and T. E. Seeman. Cross-sectional and prospective relationships of interleukin-6 and C- reactive protein with physical performance in elderly persons: MacArthur studies of successful aging. *J Gerontol A Biol Sci Med Sci* 55: M709-15., 2000.

85. Torgerson, D. J., and S. E. Bell-Syer. Hormone replacement therapy and prevention of nonvertebral fractures: a meta-analysis of randomized trials. *JAMA* 285: 2891-7., 2001.

86. Torzewski, M., C. Rist, R. F. Mortensen, T. P. Zwaka, M. Bienek, J. Waltenberger, W. Koenig, G. Schmitz, V. Hombach, and J. Torzewski. C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. *Arterioscler Thromb Vasc Biol* 20: 2094-9, 2000.

87. Tracy, R. P. Inflammation in cardiovascular disease: cart, horse, or both? [editorial; comment] [see comments]. *Circulation* 97: 2000-2, 1998.

88. Trinder, P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 6: 24-27, 1969.

89. United States Department of Health and Human Services. *Physical activity and health: A report of the Surgeon General.* Atlanta, GA: United States Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion., 1996.

90. van Baal, W. M., P. Kenemans, M. J. van der Mooren, H. Kessel, J. J. Emeis, and C. D. Stehouwer. Increased C-reactive protein levels during short-term hormone replacement therapy in healthy postmenopausal women. *Thromb Haemost* 81: 925-8., 1999.

91. Women's Health Initiative Study Group. Design of the Women's Health Initiative clinical trial and observational study. *Control Clin Trials* 19: 61-109, 1998.

92. Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 273: 199-208., 1995.

93. Writing Group for the PEPI Trial. Effects of hormone therapy on bone mineral density: results from the postmenopausal estrogen/progestin interventions (PEPI) trial. *JAMA* 276: 1389-96., 1996.

94. Yaffe, K., G. Sawaya, I. Lieberburg, and D. Grady. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. *Jama* 279: 688-95., 1998.

95. Yeh, E. T., H. V. Anderson, V. Pasceri, and J. T. Willerson. C-reactive protein: linking inflammation to cardiovascular complications. *Circulation* 104: 974-5., 2001.

96. Yudkin, J. S., M. Kumari, S. E. Humphries, and V. Mohamed-Ali. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 148: 209-14., 2000.

97. Yudkin, J. S., C. D. Stehouwer, J. J. Emeis, and S. W. Coppack. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19: 972-8, 1999.

APPENDICES

APPENDIX A –LITERATURE REVIEW

Heart Disease

Heart disease is currently the number one killer of American men and women, as it has been for all but one of the past 102 years (American Heart Association, 2001). The National Health and Nutrition Examination Survey III (1988-94) (NHANES) reports that one in five people in America have some form of cardiovascular disease, which may include high blood pressure, coronary artery disease (CAD), stroke and congestive heart failure (American Heart Association, 2001). In 1998, cardiovascular disease resulted in the death of almost a million people in the United States, with over half of those deaths occurring in women. Though there are slightly more overall cardiovascular disease deaths in women than men (53% vs. 47%), women as a group continue to live longer lives than men. Researchers have long believed that greater longevity in women was associated with the cardio protective effect of estrogen, as prior to the age of menopause, women have a lower prevalence of heart disease than men (American Heart Association, 2001). At the age range of 55-64, for the first time, the prevalence of heart disease is almost equal in men and women. By age 75, cardiovascular disease is more prevalent in women, with almost 80% of women having some form of cardiovascular disease, as compared to 71% of males. The mortality statistics for cardiovascular disease are staggering, yet risk of cardiovascular disease could be significantly decreased with lifestyle changes like smoking cessation, improved diet, and increased physical activity. In addition to lifestyle changes, cardiovascular disease risk in postmenopausal women

has historically been reduced by hormone replacement therapy (HRT) following menopause.

Hormone Replacement Therapy

Menopause is a natural hormonal process in women, occurring between the ages of 45 and 55 years, and is characterized by a decline in ovarian estrogen production and the cessation of menses (Rousseau, 1998). There are a number of adverse symptoms associated with menopause, including hot flashes, sleep disturbances, and mood swings. Additionally, there is a marked increase in the rate of bone density loss (Lorrain, Plouffe, Ravnikar, Speroff, & Watts, 1994), and an increased risk of cardiovascular disease (Bush, 2000). Because of the adverse changes associated with decreased estrogen levels, hormone replacement therapy (HRT) was introduced for postmenopausal women, starting in the 1940's.

HRT with oral estrogens has a 60-year history, with the first oral estrogen, premarin, approved by the Federal Drug Administration in 1942 (Rousseau, 1998). Conjugated equine estrogens (CEE), primarily premarin, hold the greatest market share in hormone replacement therapy (Rousseau, 1998). In the United States, about 25 % of postmenopausal women who experience a natural menopause, take or have taken in the past, oral estrogen and progestin therapy. Of those 25 % of women who have taken HRT, 10 % are current HRT users (Brett & Chong, 2001).

The large number of women who take and then stop HRT is likely explained in part by the increased risk of endometrial hyperplasia, a transitory stage between healthy and cancerous cells, and endometrial cancer with an unopposed estrogen regime

(Greenblatt & Stoddard, 1978). For women with an intact uterus, the standard postmenopausal treatment has become CEE combined with progestins (Rousseau, 1998). The addition of progestin to unopposed estrogen regime decreases the incidence of endometrial hyperplasia from 15-30% to 4% (Lorrain et al., 1994) and protects against the risk of endometrial cancer (Rousseau, 1998). Furthermore, oral HRT use leads to an increased risk of breast cancer, and women who are identified as having a higher risk for breast cancer should not be prescribed oral HRT (Rousseau, 1998).

The primary benefits of traditional hormone replacement therapy include: 1) relief from menopausal symptoms; 2) prevention or control of the loss of bone density associated with menopause; and 3) improvements in cardiovascular disease risk factors (Rousseau, 1998). It has also been suggested that oral HRT use improves cognitive function but findings are inconsistent (Grodstein et al., 2000; Yaffe, Sawaya, Lieberburg, & Grady, 1998).

A Closer Look at the Benefits of Oral HRT Use

One of the most important and least controversial benefits of oral HRT use has been the benefit to the maintenance of bone density and decrease in fracture rate. Several observational studies and reviews have reported that oral HRT reduces the fracture rate in postmenopausal women (Cauley et al., 1995; Naessen, Persson, Adami, Bergstrom, & Bergkvist, 1990). However, because HRT users tend to be white, more educated, and more affluent than those who do not use HRT (Brett & Chong, 2001; Shah, Harris, & Cook, 2001), findings from observational studies have been weakened by the potential for selection bias. For example, greater bone density in women who take HRT may be

partly explained by the higher levels of education and affluence of women who take HRT. HRT users, as a group are more likely to have been exposed to education and intervention programs or mineral supplementation, than those with less education or financial means. However, randomized controlled trials, a design that theoretically minimizes or abolishes selection bias, report conflicting results with respect to bone density.

For instance, the PEPI trial, a randomized controlled trial that examined the effect of three different HRT regimes on a number of health outcomes, found that postmenopausal bone loss was prevented by oral HRT use (Writing Group for the PEPI Trial, 1996). Yet, a recent meta-analysis found that the evidence is weak about the efficacy of oral HRT use in the prevention of fractures (Torgerson & Bell-Syer, 2001). Moreover, evidence from the Heart and Estrogen/Progestin Replacement Study (HERS) found no reduction in the incidence of fractures or in the rate of height loss in non-osteoporotic women taking oral HRT for 4 years (Cauley et al., 2001). Subsequently, an editorial from the investigators of the HERS trial questions how good the evidence is about HRT use and fractures and does not recommend oral HRT use for the prevention of bone loss and fractures (Grady & Cummings, 2001). Nonetheless, findings about the effect of HRT on bone density are not consistent in randomized controlled trials and questions remain to be answered. Additionally, there are few randomized trials examining bone density and HRT use in women with and without osteoporosis (Cauley et al., 2001).

By contrast to the bone benefits of oral HRT use, which have only recently been questioned, the cardiovascular benefits have been challenged since the results of the

HERS trial were published in 1998. The evidence about the positive effects of oral HRT use on cardiovascular disease mortality and morbidity has primarily been derived from observational studies (Grodstein et al., 1997; Sidney, Petitti, & Quesenberry, 1997). The Heart and Estrogen/Progestin Replacement Study (HERS) trial of 2763 women with known heart disease, determined that there was no difference in the number of cardiac events and deaths between women treated with HRT and those treated with placebo (Hulley et al., 1998). In subsequent analyses, the HERS trial actually found that the number of CAD events was significantly increased in the first year of oral HRT treatment, as compared to the placebo group. The HERS study findings were disturbing because they refuted the commonly held belief that oral HRT use reduced the risk for CAD (Grodstein et al., 1997; Sidney et al., 1997). However, other studies have corroborated the findings from the HERS trial. For example, the recently completed Estrogen Replacement and Atherosclerosis (ERA) study found that HRT had no benefit on slowing the progression of coronary atherosclerosis (Herrington et al., 2000). Another recent randomized controlled trial confirms the ERA findings (Angerer, Stork, Kothny, Schmitt, & von Schacky, 2001). The findings from these secondary prevention studies (Angerer et al., 2001; Herrington et al., 2000; Hulley et al., 1998) have prompted the National Cholesterol Education Program (NCEP) and the American Heart Association to recently recommend that oral HRT not be used for the purpose of CAD risk reduction in postmenopausal women (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001; Mosca et al., 2001).

Even though the NCEP and the American Heart Association no longer recommend the use of oral HRT for CAD risk reduction, there is no evidence to support

or refute the benefit of oral HRT in the primary prevention of CAD. The Women's Health Initiative (Women's Health Initiative Study Group, 1998) is a randomized clinical trial designed to determine the effect of HRT on primary CAD events (i.e. in women with no history of CAD). The WHI is slated to be completed by 2005 and will provide much needed information about HRT and the primary prevention of CAD. In the interim, there are reasons to believe that oral HRT use may have an important positive role in the primary prevention of CAD. For instance, the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial (Writing Group for the PEPI Trial, 1995), a randomized controlled trial that examined differences in CAD risk factors in women randomly assigned to 3 different HRT regimes and placebo, has reported improvements in serum lipids (i.e. increased high-density lipoprotein cholesterol and decreased low-density lipoprotein cholesterol) and fibrinogen in women who take HRT. Higher HDL-C and lower LDL-C have been associated with decreased risk of CAD. Alternatively, researchers may hypothesize that oral HRT use has a similar null or detrimental effect in primary prevention, as in secondary prevention (Hulley et al., 1998) because of HRT-related increases in serum C-reactive protein (CRP) (Cushman, Legault et al., 1999; Cushman, Meilahn et al., 1999). However, until the WHI and other primary prevention studies are complete, the effect of the HRT-related increase in serum CRP is not known for women without heart disease. Some have speculated that high levels of serum CRP are most detrimental to women with known CAD because of the destabilizing effect that increased inflammation may have on unstable arterial plaques.

HRT and CRP

The PEPI trial (Writing Group for the PEPI Trial, 1995) reported that women treated with oral HRT demonstrated an 85% increase in serum CRP levels over three years (Cushman, Legault et al., 1999), as compared to the placebo group. Similarly, the Women's Health Study, a trial of aspirin and Vitamin E in the primary prevention of cancer and cardiovascular disease among apparently health women, reported that CRP levels in women taking oral HRT were two times higher than women not taking HRT (Ridker, Hennekens, Rifai, Buring, & Manson, 1999). An extension of the Women's Health Study observations showed that cases (i.e. women who subsequently had a myocardial infarction, stroke, coronary artery bypass graft or cardiovascular death) had median CRP values that were twice that of controls (Ridker, Buring, Shih, Matias, & Hennekens, 1998). Thus, it is well established that serum CRP levels are elevated in women treated with oral HRT.

CRP is an acute phase protein, synthesized in the liver, whose circulating levels increase dramatically in response to acute bacterial infections, viral infections, or other inflammatory conditions (Tracy, 1998). Historically, the clinical focus has been on detecting large elevations in CRP as a marker of acute infection and/or inflammation. More recently, and with improvements in assay sensitivity, chronic yet modest elevations in CRP have emerged as an independent risk factor for CAD in both men (Koenig et al., 1999; Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker, Glynn, & Hennekens, 1998) and women (Ridker, Buring et al., 1998). In addition, recent studies have reported a role of CRP in both the initiation of atherosclerosis and the precipitation of an acute CAD event. For example, CRP may play an important role in the

recruitment of monocytes to the arterial wall, an early event in atherogenesis (Torzewski et al., 2000). Moreover, CRP has been found to induce adhesion molecule expression in human endothelial cells, an action that could potentially advance an atherosclerotic lesion (Pasceri, Willerson, & Yeh, 2000). Inflammation may also facilitate the transition of stable athersclerotic plaques to unstable plaques that are more susceptible to rupture, thereby precipitating acute coronary events (Yudkin, Stehouwer, Emeis, & Coppack, 1999). Thus, due to the important roles of elevated serum CRP and vascular inflammation in atherogenesis, atherosclerotic plaque progression, and plaque destabilization, the observation of experimental increases in serum CRP after oral HRT administration is cause for concern (Cushman, Legault et al., 1999). However, the mechanism by which HRT use leads to increased serum CRP is not clear (Folsom et al., 2001).

The primary regulator and mediator of CRP synthesis in the liver is a cytokine called interleukin-6 (IL-6). Thus, the first obvious mechanism of the HRT-mediated increase in serum CRP is an up regulation of production of IL-6. However, circulating IL-6, which is released from leukocytes, fibroblast, endothelial cells and adipose tissue in response to disturbances in homeostasis (Heinrich, Castell, & Andus, 1990), is decreased in women who take HRT (Straub et al., 2000). Thus, the HRT-related increase in serum CRP does not appear to be mediated by IL-6. It is more likely that increased hepatic CRP synthesis with HRT use is the result of a direct hormonal effect on gene regulation (van Baal et al., 1999). Nevertheless, the mechanism of the HRT-related increase in CRP is unclear. However, it is clear that serum CRP levels are increased by HRT use (Cushman, Legault et al., 1999; Cushman, Meilahn et al., 1999; Ridker, Hennekens et al., 1999) and

therefore it is important to explore lifestyle behaviors that may help to offset that increase in women who take HRT.

Lifestyle Behaviors to Offset HRT-related Increases in CRP

With research about the benefits and risks of oral HRT use ongoing, it is important to determine whether lifestyle behaviors, such as regular physical activity, can offset the HRT-related increase in serum CRP. Serum CRP levels have been shown to decrease in response to endurance exercise training in young healthy men (Mattusch, Dufaux, Heine, Mertens, & Rost, 2000), and in middle-aged healthy men and women (Smith, Dykes, Douglas, Krishnaswamy, & Berk, 1999). Additionally, lower serum CRP levels have been reported in the more physically active elderly participants of the Macarthur study of successful aging (Taaffe, Harris, Ferrucci, Rowe, & Seeman, 2000). Similarly, investigators from the Cardiovascular Health Study have reported significantly lower serum CRP levels in the group of elderly men and women with the highest physical activity levels (Geffken et al., 2001).

The mechanism by which physical activity may decrease serum CRP levels has not been fully elucidated (Folsom et al., 2001). However, there is a strong association between serum CRP and abdominal obesity (Hak et al., 1999; Yudkin et al., 1999) and it has been hypothesized that the lower body fat associated with increased physical activity levels may explain the lower serum CRP levels in those who exercise (Geffken et al., 2001). In addition, insulin sensitivity and variables in the insulin resistance metabolic syndrome, particularly triglycerides, fasting glucose and fasting insulin, and abdominal obesity, have strong associations with serum CRP (Festa et al., 2000). Studies have

shown that increased participation in both vigorous and non-vigorous activity, leads to improved insulin sensitivity (Mayer-Davis et al., 1998). Thus, the lower levels of serum CRP reported with increased physical activity may be mediated through improved insulin sensitivity. Taken together, the above findings (Geffken et al., 2001; Mattusch et al., 2000; Smith et al., 1999; Taaffe et al., 2000) and the biological plausibility of the proposed mechanisms of physical activity related reductions in serum CRP, provide evidence to hypothesize that regular physical activity may offset the HRT-related increases in serum CRP (Cushman, Legault et al., 1999). However, previous exercise and physical activity studies (Geffken et al., 2001; Smith et al., 1999; Taaffe et al., 2000) have not examined the potential confounding influence of differences in oral HRT status among its postmenopausal female participants. Oral HRT use is an important factor to consider when exploring the relationship between serum CRP and physical activity, as higher CRP values have consistently been reported for women on HRT (Cushman, Legault et al., 1999; Ridker, Hennekens et al., 1999).

Other Confounding Influences to Consider When Examining the Association Between Serum CRP and Physical Activity

It is important to control for the potential confounding influence of body fat when examining the independent associations among HRT use, physical activity and serum CRP. Greater amounts of body fat are strongly associated with higher serum CRP levels in both men and women (Festa et al., 2000; Hak et al., 1999). Physical activity has been shown to decrease total and regional body fat (Going, Williams, & Lohman, 1995). Moreover, oral HRT administration may reduce abdominal fatness in postmenopausal

women (Samaras, Hayward, Sullivan, Kelly, & Campbell, 1999). Thus, physical activity and HRT use may independently prevent some of the age-related gain in body fatness, thereby confounding an association among HRT use, physical activity and serum CRP. To account for potential confounding, previous investigations examining the physical activity – serum CRP relationship in postmenopausal women have used body mass index (BMI) to assess body composition (Geffken et al., 2001; Taaffe et al., 2000). Unfortunately, BMI is more inaccurate for women than men and is especially inaccurate for older adults with declining stature (Going et al., 1995). Therefore, we have used dual x-ray absorbtiometry (DXA), which accurately reconstructs total body mass from its bone mineral, soft tissue lean, and fat components. BMI cannot differentiate between fat and fat-free tissues. In addition to oral HRT use and body fat, other potential confounding factors include statin use (Ridker, Rifai, Pfeffer, Sacks, & Braunwald, 1999), aspirin use (Ridker et al., 1997) and moderate alcohol consumption (Imhof et al., 2001), all of which have been shown to decrease serum CRP. Increasing age (Hutchinson et al., 2000) and current smoking (de Maat & Kluft, 2001), are also confounding factors as both have been reported to increase serum CRP.

Therefore, while physical activity and exercise have been shown to decrease markers of inflammation (Geffken et al., 2001; Smith et al., 1999), it is not known whether regular physical activity can independently offset the HRT-related increase in serum CRP in women with differing HRT status. *Thus, the specific aims of this investigation are: 1) to determine whether physical activity is associated with serum CRP in 133 postmenopausal women aged 50-78 years; and 2) to determine whether a significant physical activity –serum CRP association, if found, is independent from HRT*

status, age, past smoking history, present alcohol consumption, statin use, aspirin use and body fat (both total and regional). We hypothesize that higher physical activity will be associated with lower serum CRP levels, regardless of oral HRT use. However, we also hypothesize that any physical activity serum CRP association will be dependent, in part, on the lower body fat of the more active women.

Biological Variability of Insulin Resistant CAD Risk Factors

Lifestyle physical activity intervention trials with postmenopausal women have, in the past, focused primarily on lipid and blood pressure outcomes. However, diabetes is a more powerful risk factor for CAD in women than men. Thus lifestyle intervention trials should increase the range of outcomes they examine, to include the full spectrum of insulin resistant CAD risk factors. The insulin resistant CAD risk factors that link insulin resistance to type II diabetes, include high fasting glucose, insulin, and triglyceride levels, high serum CRP, low high-density lipoprotein cholesterol (HDL-C) levels, high blood pressure, and abdominal obesity. However, to fully understand and interpret changes in outcomes pre and post physical activity intervention or to interpret associations between insulin resistant CAD outcomes and physical activity, it is essential to quantify the analytical and biological variability of those outcomes, postmenopausal women.

Analytical variability is the variation due to measurement error and is often expressed as a coefficient of variation (standard deviation/mean) as calculated from laboratory measurements of control samples. For metabolic outcomes, analytical variability may be reported as both intraassay and interassay coefficients of variation. Researchers attempt to minimize analytical variation by using only experienced

assessors, having the same assessors on all occasions, and having the same person analyzing all the metabolic assays. With sound procedures and appropriate assays available, analytical variability can be effectively minimized.

By contrast, the magnitude of biological variability, or the variation within subjects, is more dependent on pre-analytical variables, individual factors, and the characteristics of the analytes measured (Marcovina, Gaur, & Albers, 1994). Pre-analytical factors that influence biological variability include diet, smoking, exercise, and alcohol consumption (Cooper, Myers, Smith, & Schlant, 1992). With standardized testing conditions, the effect of pre-analytical factors on biological variability is minimized. Thus, individual factors and characteristics of the analyte determine the biological variability of an outcome measure under standardized testing conditions.

In order to determine biological variability, more than one measurement of an outcome is required. Measurement of an outcome, on two or more separate days pre and post physical activity trial is uncommon. Nonetheless, if biological variability was measured in physical activity trials, it could be separated from treatment effects, thereby increasing the likelihood that a significant treatment effect will be found. Similarly, in a cross-sectional study, measurement of outcomes on more than one day is rare. Thus, if an outcome, such as CRP or physical activity, has high biological variability, correlations between variables will be attenuated (Baranowski et al., 1999).

The primary outcomes of interest for this dissertation are serum CRP and physical activity. The biological variability of serum CRP has been calculated previously for healthy subjects and variability was large, ranging from 42% to 63% (Clark & Fraser, 1993; Macy, Hayes, & Tracy, 1997; Sakkinen et al., 1999). Measurement of physical

activity has also been shown to have large biological variability and two recent studies reported that 14-21 days of physical activity assessment are required in order to achieve a measure that is 80% reliable (Baranowski et al., 1999; Matthews et al., 2001). The biological variability of serum CRP and physical activity, and other important insulin resistant CAD risk factors have not been characterized for postmenopausal women. *Therefore the aim of the second study in this dissertation is to determine the biological variability of insulin-resistant CAD risk factors in postmenopausal women.* The findings about biological variability will be directly applicable to the first study in this dissertation, which examined the associations between serum CRP and physical activity.

APPENDIX B: INFORMED CONSENT FORM

OREGON STATE UNIVERSITY ENDOCRINE AND METABOLISM LABORATORY

A. Title of Research Project

Hormones, Physical Activity, and Coronary Heart Disease Risk Factors in Postmenopausal Women.

B. Investigators

Daniel P. Williams, Ph.D. (principal investigator), Rosemary C. Wander, PhD. (co-investigator), Christine M. Snow, Ph.D. (co-investigator), and Janet M. Shaw, PhD. (co-investigator).

C. Purpose of Research Project

You are invited to participate in an investigation of hormones and physical activity as possible determinants of abdominal fat and other risk factors for coronary heart disease (CHD) in postmenopausal women. It is known that higher amounts of abdominal fat are associated with an increased risk for diabetes and CHD and that physical activity reduces the risk for diabetes and CHD, in part, by minimizing the amount of abdominal fat that accumulates in women after menopause. However, we do not understand all of the hormonal factors that contribute to abdominal fat accumulation or to the possible reductions in abdominal fat and other CHD risk factors that may be achieved through physical activity.

Some preliminary studies in premenopausal women suggest that higher levels of male sex hormones (androgens) and a stress hormone (cortisol) may contribute to abdominal fat accumulation in women. Thus, the purpose of the present study is two-fold. First, the study will determine whether greater amounts of abdominal fat, higher blood pressure levels, and unfavorable blood cholesterol levels are associated with elevated blood levels of insulin (a risk factor for diabetes and CHD), elevated blood levels of androgen and with an elevated urine level of cortisol in postmenopausal women. *Second, the study will determine the amount or "dose" of physical activity associated with a lowering of known risk factors for CHD, so that we may better advise older women about how much physical activity they should do to reduce their risk for CHD.*

We hope to better understand the hormonal factors which explain why women tend to accumulate more body fat in the abdominal region, tend to have higher blood pressures, and tend to have more unfavorable blood cholesterol levels after menopause. We also hope to better understand the biological explanations for why physically active women are at lower risk for CHD, so that we may better understand how to prevent the increased risk for diabetes and CHD and postmenopausal women.

D. Procedures

I have received and oral and a written explanation of this study, and I understand that I was selected to participate in this study because I am a healthy postmenopausal woman with no prior or existing diagnoses of cardiovascular disease, diabetes, or gout. In addition, I was selected because I do not take a corticosteroid (i.e. prednisone), thyroid, or gout medications. Furthermore, I understand that a participant in this study the following things will happen over the course of a 3-4 hour laboratory visit and a second trip to the laboratory to return a 24-hour urine sample:

BLOOD AND URINE COLLECTIONS. On the day of the laboratory measurement session, I will arrive at the testing site in the morning, without having consumed anything but water for the previous 12 hours. No more than 25 ml (4-5 teaspoons) of blood will be drawn from a forearm vein. After the blood draw, I will be given a labeled 2.5 liter container with 10 g of boric acid (as a preservative) and instructed to collect a 24-hour urine sample. I will be asked to collect the 24-hour urine sample during an ordinary weekday from 8:00 AM to 8:00AM. I will also avoid smoking and alcohol intake during the 24-hr collection period, and I will promptly return the capped urine container to the laboratory personnel within the first few hours after completing the 24-hr urine collection.

BODY MEASUREMENTS. I will wear light shorts and a short sleeve shirt that can be easily moved for the accurate measurement of body weight, height, skinfold thicknesses, and girth measurements. My body weight will be measured on a beam scale, and my height will be measured with a wall mounted measuring stick. My total body and abdominal body fat will be assessed by skinfold thickness, body girths, and by the dual energy x-ray absorptiometry scan (DXA), which is also used to measure bone mineral (see below). My skinfold thicknesses will be measured with a caliper on the upper arm, on the upper back, on the side of the torso, on the abdomen, and on the thigh. My body girths will be measured with a measuring tape at the upper arm, waist, abdomen, hip, and thigh.

BLOOD PRESSURE AND HEART RHYTHMS. My blood pressure and heart rhythm at rest will be assessed by a device that automatically measures and records blood pressures and heart rhythms. After cleaning the skin with alcohol swabs, adhesive surface electrodes will be applied to the chest. A blood pressure cuff will then be placed on the upper arm, and electrical wires will be connected to a Quinton Model 410 automated blood pressure and heart rhythm monitor (Quinton Instrument Company, Seattle, WA). The heart rhythms will be used to determine heart rates and to determine whether there are any heart rhythm changes that frequently accompany high blood pressure.

DXA SCAN. My DXA scan will be obtained using a Hoologic QR 1000/W absorptiometer. The scans require that I lie quietly on a table for 15-20 minutes. The technique gives an accurate measure of total body and abdominal fat with a very low

exposure to radiation. The radiation dose is considered safe to administer on several occasions to postmenopausal women who may no longer become pregnant. The calculated radiation exposure is much less than a standard 2-position chest x-ray. The dose is approximately 1.5 millirads per scan, as compared to and average dose of 0.5 millirads per day from normal environmental exposure. Therefore, risk from participation in the present study is negligible. I further understand that I will experience no physical discomfort from the DXA scan.

MEDICAL HISTORY AND PHYSICAL ACTIVITY. I will complete a medical history questionnaire, and I will respond to questions posed during and interview with a member of the laboratory staff about my physical activity over the past 7 days. I understand that the medical history questionnaire is detailed and includes many questions about the health history of myself and my family that may even seem burdensome and intrusive. Previous studies have shown that women on hormone replacement therapy and physically active women tend to be healthier, have fewer medical problems and complaints, see their physicians more regularly, and report a family history of chronic diseases and conditions less frequently than women who are not on hormone replacement therapy and women who are not physically active. Thus, the purpose of the extensive medical history questionnaire is to determine whether the association of known risk factors for CHD with hormone replacement therapy and physical activity are independent of other health behaviors, medical conditions, and family histories for disease that may also be associated with the known risk factors for CHD, hormone replacement therapy and physical activity. Despite the scientific relevance of the extensive medical history questionnaire, I understand that is I find any part or parts of the questionnaire to be overly burdensome or intrusive, I am under no obligation to complete it, and that my decision will in no way affect my participation in other parts of the study.

FORSEEABLE RISKS OR DISCOMFORTS. FORESEEABLE RISKS OR DISCOMFORTS. I understand that I may experience some discomfort while my blood is being drawn and that this procedure could result in a small amount of bleeding, bruising and slight soreness at the site of the needle insertion. I have been informed that my blood will be drawn by trained personnel who will apply a bandage to my arm to prevent bleeding or bruising as a result of the blood draw.

I understand that the urine collection procedure may result in some inconvenience relate to carrying the urine container and collecting all the urine produced over and entire 24-hour period. I understand that I must keep that I must keep the urine container dry and capped when not in use. I have also been informed to keep the urine container in a cool, dry location that is relatively safe from disturbance by family members and pets.

I understand that the skinfold measurement thickness measurements may produce some mild discomfort associated with pinching of the skin. I have been informed that skinfold thicknesses will be measured by trained personnel who will minimize the risk of discomfort by using a standardized measurement protocol which involves a minimal amount of time that the skin is pinched.

I understand that the blood pressure measurements may also result in some mild arm discomfort associated with the inflation of the blood pressure cuff. I have been informed that the blood pressure measurements will be made by trained personnel who will minimize the risk for discomfort by using standardized measurement protocol which requires a measurement of upper arm girth to ensure that a properly sized blood pressure cuff is used.

BENEFITS FROM RESEARCH. I understand that the measurements of body fat, physical activity, insulin, blood pressure, and blood cholesterol, which will be sent to me, may be important to me or my physician form assessing my own personal risk for diabetes and CHD. Furthermore, I have been made aware that the information obtained during this study may help Dr. Williams and his associates, learn how to better prevent the increased risk for diabetes and CHD in postmenopausal women.

E. Confidentiality.

Any information obtained in connection with this study that can be identified with me will be kept confidential to the extent permitted by law. A code number will be used to identify any test results or other information I provide. Neither my name nor any information from which I might be identified will be used in any data summaries or publications.

F. Compensation for Injury

I understand that Oregon State University does not provide research subjects with compensation or medical treatment in the event that I become injured as a result of participation in this research project. However, if I sustain any injury during the data collection, trained personnel will provide first aid.

G. Voluntary Participation Statement.

I understand that my participation in this study is completely voluntary and that I may either refuse to participate or withdraw from the study at any time without penalty or loss of benefits to which I am otherwise entitled.

H. Questions Regarding the Study

I understand that any questions I have about the research study or any specific procedures should be directed to Daniel P. Williams, PhD at 737-5922..

I. Understanding and Consent.

My signature below indicates that I have read and that I understand the procedures described above. I give my informed consent to participate in this study. I understand that will receive a copy of this consent form.

Signature of subject _____

Name of Subject _____

Date Signed _____

Subject's Present Address _____

Subject's Phone Number _____

Signature of Principal Investigator _____

Date Signed _____

APPENDIX C

INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS

OREGON STATE UNIVERSITY

Report of Review

TO: Daniel Williams, ExSS

RE: Hormones, physical activity, and coronary heart disease risk factors in postmenopausal women

The referenced project was reviewed under the guidelines of Oregon State University's Committee for the Protection of Human Subjects and the U.S. Department of Health and Human Services. The committee has **approved** your application. The informed consent form obtained from each subject should be retained in program/project's files for three years beyond the end date of the project.

Any proposed change to the protocol or informed consent form that is not included in the approved application must be submitted to the IRB for review and must be approved by the committee before it can be implemented. The approval of this application expires upon the completion of the project or one year from the approval date, whichever is sooner.

Date: 4/4/97

Warren N. Suzuki, Chair
Committee for the Protection of Human Subjects
(Education, 7-6393, suzukiw@ccmail.orst.edu)

APPENDIX D

ID #_____
DATE_____
INITIALS _____

Medical Questionniare

Medication Allergies- state reactions:

Current Medications - give dosage and include non-prescription drugs:

Previous Surgeries/Hospitalizations:

Medical Problems:

Accidents - specify type of injury incurred:

Current Medical Concerns:

Primary Care Physician
Address
Phone

Medical Questionnnaire

RESPIRATORY	No	Yes (month, year)
Have you ever had any of the following?	(If so, indicate when)	
Asthma (wheezing)		
Pneumonia		
Severe bronchitis		
Emphysema		
Other lung trouble		
Trouble breathing		
Do you have chest pain?		
Pleurisy		
Tuberculosis skin test (state Pos or Neg)		
Tuberculosis (infection or contact)		
Chronic Bronchitis		
Exposure to dangerous dust or fumes		
Excessive snoring		
Abnormal chest x-ray		
Have you ever coughed up blood?		
Do you often or regularly:		
Cough?		
Raise sputum?		
Do you often get chest colds?		
When was your last chest x-ray		

CIRCULATORY		
Have you ever had any of the following?	(If so, indicate when)	
Angina pectoris		
Heart trouble		
Heart attack (coronary)		
Chest pain		
High blood pressure		
Blackouts		
Racing of heart		
Rheumatic fever		
Heart failure		
Abnormal electrocardiogram		
Swelling of your ankles		
Have you ever taken heart or water pills?		
Have you ever had a exercise stress test?		
Have you ever had a coronary angiogram?		

ENDOCRINOLOGY	No	Yes (month, year)
Have you ever had any of the following?	(If so, indicate when)	
Hormone problems		
Thyroid disease		
Diabetes		

DIGESTIVE		
Do you often or regularly have:		
Poor appetite		
Trouble swallowing		
"Heartburn"		
Regurgitation of food or bile		
Abdominal pain		
Any history of anorexia/ bulimia		
Constipation		
Diarrhea		
Has there been any change in your bowel movements in the last 6 months?		
Have you ever had any of the following?	(If so, indicate when)	
Hiatal or esophageal hernia		
Duodenal or gastric ulcer		
Vomiting of blood		
Black or tarry stool		
Blood in your stool		
Yellow jaundice		
Liver trouble or hepatitis		
Gallbladder trouble or stones		
Persistent diarrhea or colitis		
Inflammatory bowel disease		
Diverticulitis		
Parasite infection		
Hernia		
Rectal growth		
Rectal bleeding		
Other digestive disease		
When was your last:		
Upper G.I. X-ray		
Barium enema x-ray		
Hemoccult cards		
UGI endoscopy (gastroscopy)		
Sigmoidoscopy		
Rectal Exam		
Have you had recent weight loss?		

Medical Questionnnaire

JOINTS

Have you, or do you have:	No	Yes (month, year)
Back pain/ injuries		
Joint pain / injuries/ swelling		
Sciatica		
Muscle injuries / pain		
Gout		
Osteoarthritis		
Rheumatoid arthritis, lupus, or other connective tissue disorders		
Fractures		
Orthopedic surgeries		

CUTANOUS

Have you ever had:		
Skin rashes		
Skin cancer		
Changes in a mole		

URINARY

Have you ever had or been told you had any of the following?	(If so, indicate when)	
Kidney disease or nephritis		
Protein or albumin in urine		
Blood or pus in urine		
Kidney stones		
Urinary infection		
Sexually transmitted disease (STD)		
How many times do you urinate:		
at night		
during the day		
Do you have discomfort passing urine?		
Have you ever had a kidney x-ray (I.V.P.)?		

OBSTETRICAL & GYNNECOLOGICAL

Have you ever had tumor(s), cyst(s), or other breast disease?		
Are you now taking hormones or birth control pills?		
brand name and dose		
Have you had a partial or total hysterectomy?		
Were your ovaries removed also		
right / left / both / don't know		
When was your last Pap smear		
Do you examine your breasts for lumps?		
Are your menstrual periods normal?		

At what age did your menstrual periods begin?		
At what age did menopause begin?		
Are you sexually active?		

HEMATOLGY & ONCOLOGY

Have you ever had		
Anemia		
Bleeding or bruising tendency		
Cancer or tumor		
X-ray or radiation treatment		
Chemotherapy		

NEUROLOGICAL

Have you ever had any of the following?		(If so, indicate when)
Neurological disease		
Frequent or recurrent headaches		
Loss of consciousness		
Convulsions or seizures		
Severe head injury		
Stroke		
Paralysis or muscular weakness		
Tremor or abnormal movements		
Difficulty with coordination		
Difficulty in walking		
Difficulty in speaking		
Double vision or loss of vision		
Numbness		
Difficulty with memory		
Dizziness		

SPECIAL SENSES

Have you ever had:		
Glaucoma		
Other major eye disease		
Deafness		
Abnormal noises in the ear		

IMMUNIZATIONS

Date last immunized for :		
Tetanus- diphtheria (every 5-10 years)		
Pneumococcal Pneumonia (Pneeumovax)		
Influenza (annual- Fall)		

Medical Questionnnaire

PERSONAL HISTORY

What is your occupation? _____

Highest level of formal education you have attained? _____

MOOD

In the last year, have you:		
Experienced severe panic, anxiety, phobias		
Found it hard to concentrate?		
Felt unable to enjoy your usual activities?		
Had a weight change or eating disorder?		
Had insomnia or excessive daytime sleepiness?		
Felt hopeless?		
Felt excessively fatigued?		
Felt depressed?		
Would you like a consultation with a mental health professional?		

HABITS

☐ Never smoked

☐ Quit smoking in _____ (fill out below)
year

☐ Smoke _____ packs a day for _____ years
# #

☐ Never drink alcohol

☐ Quit drinking alcohol in _____
year

☐ _____ alcoholic drinks per ☐ day ☐ week ☐ month
#

☐ Never drink caffinated beverages

☐ Drink _____ cups of _____ a day
caffinated beverage

Type of Exercise and Frequency:

FAMILY HEALTH

Please give the following information about the health of your immediate family:

RELATION	Age	Age at death	State of health or cause of death
Mother			
Father			
Brothers and sisters			
Spouse			
Children			

Have any of your immediate family or grandparents ever had any of the following? (If so, indicate relationship)

"Heart attack" _____
Breast cancer _____
Colon cancer _____
Lung cancer _____
Other cancer (specify type) _____
High blood pressure _____
Blood disease _____
Abnormal bleeding _____
Diabetes _____
Kidney disease _____
Osteoporosis _____
Seizures or epilepsy _____
Parkinson's _____
Alzheimer's _____
Migraines _____
Allergies _____
Alcoholism _____
Psychiatric disease or suicide _____
A disease which "runs in the family" _____

Endocrine and Metabolism Laboratory
Oregon State University
Department of Health and Human Performance

7-Day Physical Activity Recall

Acrostic ☐☐☐☐☐☐-☐☐
Name _____
Birthdate _____
Weight _____
Date _____

Day of the week form completed: _____

1. Were you employed in the last 7 days (work & volunteering)? ☐ Yes ☐ No
2. How many total days of the last 7 did you work outside the home? ☐ no. of days (round to nearest day)
3. How many total hours did you work in the last seven days? ☐☐ at work ☐☐ at home ☐☐ total hours
4. What days of the week do you consider to be your weekend or non-work days? For most people this would be Saturday and and Sunday but it may be different for you.
   ☐ Sunday ☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday
5. If you did not work your usual week, why did you work less than usual?
   _____
   _____
6. For the past seven days, and thinking only about activities that are at least of **moderate** intensity how many days did you do activity or exercise that added up to at least 30 minutes each day? ☐ number of days (0 to 7)

7-day recall — explain segments of day
am - wake-lunch
pm - lunch-dinner
eve - dinner-sleep

One Week Ago

Yesterday

Morning	Sleep							
	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly.
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

One Week Ago

Yesterday

	Sleep							
Afternoon	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly,
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

One Week Ago

Yesterday

Evening	Sleep							
	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly,
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

7. Was this a typical week in terms of your usual pattern of activity or exercise?

   1 ☐ Yes

   2 ☐ No Were you more or less active in the past week than you usually are? 1 ☐ More 2 ☐ Less

3-Month Physical Activity Recall

8. During your **work week**, on average how many hours per day do you spend sitting quietly? ☐☐ average hours per day
   (e.g., **watching TV, working at a desk or computer, eating, or reading**) (consider all waking time - before work and after)

   During your **weekend**, on average how many hours per day do you spend sitting quietly? ☐☐ average hours per day
   (e.g., **watching TV, working at a desk or computer, eating, or reading**) (consider all waking time - before work and after)

9. How many flights of stairs do you climb up each day? (1 flight = 10 steps) ☐☐ number of flights

10. If you had to add together the **total minutes you spend walking** during the day, how many minutes would that be? Remember, add up your **actual walking time** and don't add in the time spent just standing. Include your to and from walking and any fitness walking. Don't try to remember every step, just give a general idea of the time spent walking. ☐☐☐ total minutes per day

11. What is your usual pace of walking? Mark **one** only.

    1 ☐ Casual or strolling (less than 2 miles per hour)

    2 ☐ Average or normal (2 to 3 miles per hour)

    3 ☐ Fairly brisk (3 to 4 miles per hour)

    4 ☐ Brisk or striding (4 miles per hour or faster)

12. Do you regularly do strength and flexibility exercises like sit-ups, push-ups, yoga, or stretching?

    1 ☐ Yes How many days per week do you do these exercises? ☐ number of days (0-7) ☐☐☐ total minutes ea session

    2 ☐ No

12 Months Physical Activity Recall

1. Thinking about the things you usually did **at work** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy _____ Not applicable

2. Thinking about the things you usually did **in your home** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy

3. Thinking about the things you usually did **in your leisure time** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy

Inactive	Light	Moderate	Heavy
sitting	teaching	mail carrier	regular vigorous activity
standing quietly	light gardening	construction	
	light regular household		carrying heavy boxes
deskwork		brisk walking	strenuous farm work
	walking >10min	recreational tennis	strenuous gardening
reading	leisure bicycle ride	swimming	
watching TV	fishing	moderate housework	jogging
quiet pursuits	bowling	moderate gardening	singles tennis
	golf		high-intensity aerobics

Interviewer Evaluation

1. Were there any problems with this survey?

Yes No Explain

2. Do you think this was a valid interview?

Yes No Explain

3. List any activities reported by participant which you don't know how to classify

FORM completed by _____ (initials) @ baseline