The Relationship Between High Sensitivity C-Reactive Protein, Metabolic Syndrome and Exercise

Micheline A. Vargas

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LOMA LINDA UNIVERSITY

School of Public Health

THE RELATIONSHIP BETWEEN HIGH SENSITIVITY C-REACTIVE PROTEIN,
METABOLIC SYNDROME AND EXERCISE.

By

Micheline A. Vargas

A Dissertation Proposal in Partial Fulfillment of the Requirements for the
Degree of Doctor of Public Health in Preventive Care

June 2006
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ABSTRACT OF DISSERTATION

The Relationship Between High Sensitivity C-Reactive Protein, Metabolic Syndrome and Exercise.

by

Micheline A. Vargas

Doctor of Public Health Candidate in Preventive Care
Loma Linda University, Loma Linda University, 2005

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Background: It is well known that regular physical activity is associated with lower risk of chronic diseases such as diabetes and cardiovascular disease. Physical activity is also considered an important determinant of metabolic syndrome. All levels of metabolic syndrome, diabetes, and cardiovascular disease are thought to involve inflammation. Physical activity may reduce risk, at least in part, by modifying the inflammatory process. Recent studies have demonstrated an inverse relationship between inflammatory markers, such as high sensitivity C-reactive protein (hs-CRP), and physical activity. Elevated hs-CRP appears to be an independent predictor of both CVD and diabetes. Recent evidence also suggests that hs-CRP is positively associated with all metabolic syndrome characteristics: low high density lipoprotein (HDL), hypertriglyceridemia, hypertension, obesity, and abnormal glucose.

Purpose: The purpose of this study was to examine the relationship between hs-CRP, metabolic syndrome and physical activity. Understanding this relationship will
provide insight into the potential of physical activity as a therapeutic option to reduce cardiovascular disease risk.

**Methodology:** This study relied on a cross-sectional, retrospective analysis of an archival database from the Center for Health Promotion (CHP). Study participants were obtained from a pool of 1,072 men and women patients at the Center of Health Promotion (CHP) at Loma Linda University. Of the 1,072 pool, 173 individuals met the inclusion criteria and were the subjects of the study. Any missing data variables were imputed using the maximum likelihood method (EM) in SYSTAT version 10; SPSS©2000. The distribution of hs-CRP, the dependent variable of greatest interest, was positively skewed. Therefore, a log transformation was applied to hs-CRP values for all analyses. Simple regression/correlation analysis was used to evaluate the relationships between log (CRP) and each of the relevant variables: VO2max (ml•kg⁻¹•min⁻¹), metabolic syndrome, and the physical activity status. The following relationships were explored: (1) hs-CRP and metabolic syndrome characteristics, (2) hs-CRP and VO2max, (3) VO2max and metabolic syndrome characteristics, and (4) VO2max and physical activity status. Pearson Chi-square analysis was used to determine if a threshold level of physical activity was associated with hs-CRP changes.

**Results:** Hs-CRP increased linearly with the number of metabolic syndrome characteristics (p = 0.00022). Inverse associations were found between hs-CRP and VO2max (p = 0.00008) and between VO2max and the number of metabolic syndrome criteria (p = 0.00004). VO2max was positively associated with PA (p = 0.00000). Subjects engaging in 2-3 hours of PA per week had hs-CRP levels ≤ 2.5 mg/L (p = 0.01817).
**Conclusions:** Physical activity may reduce risk by modifying the inflammatory process. We found that hs-CRP and metabolic syndrome severity were reduced in those with a higher cardiorespiratory fitness level. These associations are suggested mechanisms by which cardiorespiratory fitness reduces the risk of CVD and diabetes. Our findings support physical activity recommendations set forth by both the Surgeon General and the American College of Sports Medicine. This study was also in support of recommendations set forth by the ATP-III report to use physical activity as first-line therapy for the management of metabolic syndrome.

**Significance to Preventive Care:** Preventive Care Specialists often prescribe physical activity as a way of reducing chronic diseases such as cardiovascular disease and diabetes. Reducing hs-CRP via regular exercise may be an intervention by which exercise reduces risk of chronic disease. This study strengthens the argument that regular physical activity reduces inflammation and metabolic syndrome risk factors thereby providing protection against the number one killer in the U.S., cardiovascular disease.
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ACKNOWLEDGEMENTS

I would like to express sincere appreciation for my dissertation committee. To Dr. Lee Berk and Dr. Edward Fujimoto, the co-chairmen of this project, thank you both for your kindness, your patience, and the hours you spent working with me. I will forever be grateful. To Dr. Dysinger and Dr. Peters, you were both priceless in providing input from a physician’s perspective as well as providing the data from the Center for Health Promotion. I appreciate the time you allotted me despite your busy schedules.

Jim Westengard played an invaluable role in this project. Without your statistical expertise and time this study would not have come to fruition. I truly can not thank you enough for your contribution. I would also like to thank the School of Public Health faculty, namely Dr. Brenda Rea and Dr. Jerry Lee for their support, attention to detail, and encouragement.

Lastly, I would like to thank my family for their patience and understanding while I undertook this long journey. I especially want to thank my husband, Doug Vargas. Thank you for your love, your encouragement, and your never-ending belief in me. I would not be where I am today without you. I am truly blessed to have you in my life!! I love you.

I am indebted to everyone who contributed to this project. I truly appreciated your effort and support. Thank you!
A. Statement of the Problem

It is well known that inflammation is involved in mediating all stages of the atherosclerotic disease process (Pearson et al., 2003; Libby, Ridker, & Maseri, 2002). Hence, markers of inflammation have been examined as potential tools in the prediction of cardiovascular events. Of these markers, the acute-phase reactant, high sensitivity C-reactive protein (hs-CRP), has generated significant interest (Szmitko et al., 2003). Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events (Szmitko et al., 2003; Yeh & Willerson, 2003). According to LaMonte et al. (2002), elevated hs-CRP is associated with a 2 to 5-fold increase in cardiovascular event risk.

Although hs-CRP has been considered only a biomarker, recent evidence suggests that it may directly promote atherosclerosis. According to Szmitko et al. (2003), hs-CRP provokes inflammation and endothelial dysfunction. It appears to reduce transcription of endothelial nitric oxide synthase (eNOS), which leads to a diminished release of nitric oxide (NO). This is turn reduces protection against vascular injury and inflammation. Hs-CRP causes further disruption to endothelial homeostasis by stimulating the release of endothelin-1 (ET-1) and interleukin (IL)-6, by upregulating adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule-1, selectins), and by stimulating the chemokine monocyte chemoattractant protein-1 (MCP-1). Hs-CRP also promotes foam cell formation by facilitating LDL uptake by macrophages (Szmitko et al., 2003; Yeh & Willerson, 2003). Preliminary observations from Szmitko et al. (2003) also
indicate that hs-CRP up-regulates nuclear factor *KappaB* (NF-κB), which facilitates transcription of several proatherosclerotic genes. Hs-CRP's proatherogenic effects extend beyond the endothelium to the vascular smooth muscle (VSM). In concentrations known to predict cardiovascular events, hs-CRP demonstrates up-regulation of angiotensin-type 1 receptors (AT₁-R) in vascular SMCs. This augments VSM proliferation, migration, reactive oxygen species (ROS) production, and restenosis (Szmitko et al., 2003).

Observational studies have provided evidence that physical inactivity and low cardiorespiratory fitness predict atherosclerotic cardiovascular disease (Blair et al., 1996; Ekelund et al., 1988; Lakka et al., 1994, Laukkanen et al., 2001; Lee, Blair, & Jackson, 1999; Sandvik et al., 1993), while regular physical activity is associated with lower risk of coronary heart disease, stroke, and cardiovascular mortality (Erikssen et al. 1998). Physical activity may reduce risk, at least in part, by modifying the inflammatory process and reducing endothelial dysfunction. Recent studies have demonstrated an inverse relationship between inflammatory markers, such as hs-CRP, and physical activity (Geffken et al., 2001; Rawson et al., 2003). Higher levels of hs-CRP have also been associated with obesity and insulin resistance, while physical activity has been associated with lesser degrees of obesity and insulin resistance. Based on these findings many researchers have speculated that physical activity may be associated with lower levels of inflammation via its inverse relationship with obesity and insulin resistance (LaMonte et al., 2002). Other researchers believe that physical activity reduces hs-CRP independently (Rawson et al., 2003).

Relatively few studies have examined the effects of physical activity on inflammatory markers and results are conflicting (Geffken et al., 2001; Smith et al., 1999;
Rawson et al., 2003). This may be due in part to research design. Several of the studies had an inadequate number of participants and others failed to adjust for body composition and other confounding variables. According to Pearson et al. (2003) many variables are associated with hs-CRP levels. Elevated body fat mass, weight loss, elevated blood pressure, cigarette smoking, metabolic syndrome, diabetes, low HDL cholesterol, high triglycerides, gender, age, and increased physical activity all appear to influence hs-CRP. More research is needed to assess the relationship between hs-CRP, metabolic syndrome and physical activity. Questions remain regarding: (1) the relationship between cardiorespiratory fitness level and hs-CRP, (2) the threshold level of physical activity that is associated with changes in hs-CRP, (3) the type of physical activity needed to reduce hs-CRP, (3) the association between hs-CRP, metabolic syndrome and cardiorespiratory fitness level, and (4) whether the associations seen between physical activity and lowered inflammatory markers is mediated by reduced body fat.

B. Purpose of the Study

The purpose of this study was to examine the relationship between hs-CRP, metabolic syndrome and physical activity. Understanding this relationship will provide insight into the potential of physical activity as a therapeutic option to reduce cardiovascular disease risk. This study relied on a cross-sectional, retrospective analysis of an archival database from the Center for Health Promotion (CHP). Study participants were obtained from a pool of 1,072 men and women patients at the Center of Health Promotion (CHP) at Loma Linda University. Of the 1,072 pool, 173 individuals met the inclusion criteria and were the subjects of the study. Any missing data variables were imputed using the maximum likelihood method (EM) in SYSTAT version 10;
SPSS©2000. Because the percent body fat variable required over 100 imputations to fill in all of the missing data it was not used in any analyses. The distribution of hs-CRP, the dependent variable of greatest interest, was positively skewed. Therefore, a log transformation was applied to hs-CRP values for all analyses. Simple regression/correlation analysis was used to evaluate the relationships between log(CRP) and each of the relevant variables: VO$_2$ max (ml•kg$^{-1}$•min$^{-1}$), metabolic syndrome, and the physical activity status. The following relationships were explored: (1) hs-CRP and metabolic syndrome characteristics, (2) hs-CRP and VO$_2$ max, (3) VO$_2$ max and metabolic syndrome characteristics, and (4) VO$_2$ max and physical activity status. Pearson Chi-square analysis was used to determine if a threshold level of physical activity was associated with hs-CRP changes. Understanding these relationships provides insight into the potential of physical activity as a therapeutic option to reduce cardiovascular disease risk (Church et al., 2002).

C. Research Questions

1. What is the relationship between fitness level and hs-CRP?

2. What is the relationship between hs-CRP, the metabolic syndrome and cardiorespiratory fitness?

3. Is there a threshold level of physical activity that is associated with changes in hs-CRP?

D. Mechanisms

Several theories exist as to how regular physical activity reduces hs-CRP and the inflammatory process. Mattusch et al. (2000) suggested that hs-CRP levels were reduced, at least in part, due to enhanced antioxidative defense mechanisms seen after regular exercise training. It has been reported that well trained athletes have increased...
activity in muscular and erythrocytic antioxidative enzymes. Animal studies have also shown improved antioxidant defense mechanisms after exercise training. It may be that enhanced antioxidative protection reduces the production of IL-6 and other cytokines in the exercising muscle.

Church et al. (2002) also proposed interleukins as a potential pathway. Evidence suggests that tumor necrosis factor-α (TNF-α) and IL-6 are involved in hs-CRP production. TNF-α stimulates the production of IL-6 and IL-6 stimulates the production of hs-CRP. Regular exercise training appears to lower basal plasma interleukin concentrations and TNF-α. The release of TNF-α and IL-6 is augmented by the downregulation of sympathetic stimulation via regular physical activity. Reductions in TNF-α and IL-6 have been found independently of weight loss, hence, TNF-α and IL-6 may be responsible for reduced hs-CRP in fit individuals (Church et al., 2002).

Smith et al. (1999) found that exercise reduced TNF-α. These researchers examined the possibility that long-term exercise could favorably alter the production of cytokines with atherogenic and atheroprotective properties. Cytokines with atherogenic properties include IL-1, TNF-α, and interferon-γ (IFN-γ). Cytokines with atheroprotective properties include IL-4, IL-10, and transforming growth factor beta (TGF-B). Smith et al. (1999) measured the effects of exercise on the production of these cytokines along with serum levels of hs-CRP. Following six months of moderate intensity training proatherosclerotic cytokine production was attenuated, while atheroprotective cytokine production was increased. These changes were proportionate to the time participants spent performing repetitive lower-body exercises, suggesting a dose-response relationship. A reduction in hs-CRP accompanied the changes in immune
cell function. This could indicate a systemic decrease in the production of both IL-1 and TNF-α. These alterations were seen independently of aspirin or other medication use, dieting, weight loss, and smoking cessation. In addition to reductions in IL-6 and TNF-α, Smith et al. (1999) found a significant attenuating effect on IFN-γ. IFN-γ and TNF-α have been identified in early and advanced atherosclerotic lesions. They appear to activate endothelial cells, monocytes, macrophages, and smooth muscle cells, hence they contribute to leukocyte recruitment, endothelial cell procoagulant activity, LDL oxidation, and foam cell formation. Therefore, reducing these and other proatherosclerotic cytokines via regular exercise is of value for those persons at risk of developing cardiovascular disease (Smith et al., 1999).

Several theories exist as to how exercise reduces hs-CRP. These potential mechanisms along with a reduction in traditional risk factors, such as hypertension, elevated cholesterol, elevated triglycerides, impaired glucose tolerance, and diabetes mellitus likely lead to a reduction in hs-CRP and cardiovascular disease. Figure 1 shows the potential mechanisms by which exercise reduced hs-CRP and cardiovascular disease.
Figure 1. Potential mechanisms by which exercise reduces hs-CRP and Cardiovascular Disease (CVD). IL-6 = interleukin-6, TNF-α = tumor necrosis factor-α, CRP = c-reactive protein, HTN = hypertension, CH = cholesterol, TG = triglyceride, IGT = impaired glucose tolerance, DM = diabetes mellitus, NO = nitric oxide, ET-1 = endothelin-1, ICAM = intracellular adhesion molecule, VCAM = vascular cell adhesion molecule, AT1R = angiotensin type-1 receptor, MCP-1 = monocyte chemoattractant protein-1, NFκB = nuclear factor κB.

E. Significance to Preventive Care

Preventive Care Specialists often prescribe physical activity as a way of reducing chronic diseases such as cardiovascular disease and diabetes. Physical activity provides protection to the cardiovascular system via multiple mechanisms: (1) increased dimension of coronary arteries; (2) improved coagulation system; (3) increased stroke volume, and (4) increased maximal oxygen uptake (Geffken et al. 2000). Reducing inflammation (i.e. hs-CRP) via regular exercise may be yet another mechanism by which exercise decreases risk of chronic disease. This study investigates the impact of regular physical activity on the inflammatory process and cardiovascular disease risk to confirm and extend available data. Hence, it will strengthen the argument for regular physical
activity and support the Surgeon General’s recommendation to include a moderate amount of physical activity on most, if not all days of the week. This study will also likely support the theory that regular physical activity, regardless of body fat mass, reduces risk of chronic disease. This could send yet another public health message. Even low to moderate amounts of regular physical activity provide protection from the number one killer in the U.S., cardiovascular disease.
A. Overview

Atherosclerosis was formerly considered a mere lipid storage disease when in actuality it involves an ongoing inflammatory response with the endothelium being the primary site of dysfunction. Advances in basic and experimental science have established that inflammation is involved in mediating all stages of this disease from initiation through progression and, ultimately, to atherosclerotic plaque complications (Pearson et al., 2003; Libby, Ridker, & Maseri, 2002). Several markers of the inflammatory cascade have been examined as potential tools in the prediction of cardiovascular events. Among them are markers of systemic inflammation produced in the liver, such as high sensitivity C-reactive protein (hs-CRP) and serum amyloid A (SAA); cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α); and adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Of these markers, the acute-phase reactant, hs-CRP, has generated significant interest (Szmitko et al., 2003). Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events (Szmitko et al., 2003; Yeh & Willerson, 2003). Ridker, Hennekens, Burning, and Rifai (2000) found that hs-CRP was better than low-density lipoprotein (LDL) cholesterol at predicting cardiovascular events and according to LaMonte et al. (2002) elevated hs-CRP is associated with a 2 to 5-fold increase in cardiovascular event risk. Although, once thought to act only as a biomarker new evidence is showing that hs-CRP
plays a more functional role in the atherosclerotic disease process. This new information is profound and will likely change the way we screen and manage patients (Yeh & Willerson, 2003).

It is well known that regular physical activity reduces the risk of cardiovascular disease and recent studies have found an association between physical activity and hs-CRP. Greater understanding of this relationship may provide insight into the potential of exercise as a therapeutic option to reduce hs-CRP (Church et al., 2002). At this time there are no firm conclusions as to how exercise reduces the inflammatory process or hs-CRP. Only a handful of studies have examined the effects of exercise on inflammatory markers and of those many have presented conflicting results. This may be due in part to research design. Many lack either a reliable measure of regular physical activity, adjustments for body weight and other confounding variables, or an adequate number of participants. The cohorts used also differ significantly making it difficult to compare results.

This chapter will review epidemiological and experimental evidence regarding the role of hs-CRP in primary prevention, inflammation in atherosclerosis, the functional role of hs-CRP in the atherosclerosis process, and the effects of exercise on hs-CRP. Studies for this review were identified using computer searches of Medline, PubMed, and prominent peer reviewed journals.

B. Hs-CRP Use in Primary Prevention

Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events. It appears to be an even stronger predictor than LDL cholesterol (Yeh & Willerson, 2003) and it adds prognostic value to the Framingham risk
assessment score and all levels of the metabolic syndrome (Szmitko et al., 2003; Ridker, 2003). In the Framingham Heart Study, coronary heart disease occurred in approximately one third of the individuals with total cholesterol less than 200 mg/dL (Castelli, 1996).

According to Ridker (2003), over a dozen prospective epidemiological studies have found a single, non-fasting measure of hs-CRP to be a strong predictor of cardiovascular events in individuals with no prior history of cardiovascular disease. Initial data from the Multiple Risk Factor Interventional Trial (MRFIT) (Kuller, Tracey, Shaten, Meilahn, 1996), the Women’s Health Study (Ridker, Hennekens, Buring, and Rifai, 2000), the Rural Health Promotion Project (Tracy, Lemaitre, Psaty, Ives, Evans, Cushman, Meilahn, and Kuller, 1997), the NIH-funded Coronary Heart Study (Tracy et al., 1997), the Physicians’ Health Study (Ridker, Cushman, Stampfer, Tracy, and Hennekens, 1997; Ridker, Cushman, Stampfer, Tracy, and Hennekens, 1998a), the MONICA-Augsburg Cohort Study (Koenig, Sund, Frohlich, Fischer, Lowel, Doring, Hutchinson, Pepys, 1999), and the Helsinki Heart Study (Roivainen, Viik-Kajander, Fischer, Lowel, Doring, Hutchinson, et al., 2000) are just a few of the prospective studies conducted in the United States and Europe that have found an association between baseline hs-CRP levels and risk of first cardiovascular event. Hs-CRP’s strong predictive value may be partially explained by its long-term stability during storage. One study recently found that hs-CRP was a strong predictor of risk even 20 years after the initial blood samples were acquired (Ridker, 2003). Hs-CRP has a long half-life, it lacks diurnal variation, and it lacks age and sex dependence (Yeh & Willerson, 2003).
Simple clinical cut points have been established from event-free survival data allowing clinicians to interpret hs-CRP levels. An hs-CRP assay, such as Dade Behring, is needed to detect levels within the normal range (low grade levels). Hs-CRP predicts cardiovascular events within this normal range (Rifai, Tracy, and Ridker, 1999). Standard CRP tests determine levels that are increased several hundred-fold if not a thousand-fold during acute infection and tissue injury and are not able to detect low grade levels of CRP. Using the hs-CRP assays we can categorize patients as low (<1 mg/L), moderate (1-3 mg/L), or high risk (3-10 mg/L) for future cardiovascular events. Levels exceeding 10 mg/L may indicate a possible acute phase response. In this case, the test should be repeated within a few weeks (Ridker, 2003; Yeh & Willerson, 2003).

In addition to hs-CRP, other biomarkers have been shown to predict myocardial infarction and stroke. Sophisticated methods have been utilized to measure adhesion molecules, cytokine activity, and immunologic functions (i.e. ICAM-1, IL-6). Unfortunately, measures of these biomarkers are often inappropriate for clinical use: the assays used are often inappropriate for routine clinical use, the protein being measured has a short half-life, or the marker has not been standardized (Ridker, 2003). Hs-CRP on the other hand, has a standardized assay and is stable. It has a half life of about 18-20 hours, partly due to its pentraxin structure. This allows the measure to be made from either fresh or frozen plasma.

The Women's Health Study compared ten biomarkers for vascular disease (Ridker et al, 2000). The researchers compared lipoprotein(a), homocysteine, IL-6, soluble ICAM-1, serum amyloid A (SAA), total cholesterol (TC), LDL, Apolipoprotein B, TC to high density lipoprotein cholesterol (HDL) ratio, hs-CRP, hs-CRP plus TC:HDL.
Interestingly, they found that SAA and soluble ICAM-1 were better risk markers than TC or LDL. The best lipid marker was the TC:HDL ratio, which is consistent with many epidemiological studies. Hs-CRP was, however, the best predictor of risk. Hs-CRP screening, on its own, was associated with a 4.4-fold increase in vascular risk in the study's participants. But, the best overall risk estimate was found when hs-CRP was combined with TC:HDL ratio (Ridker et al., 2000). In an analysis from the Physicians' Health Study, Ridker, Glynn, and Hennekens, (1998b) found an interaction between hs-CRP and TC:HDL ratio. Hence, using both these parameters may do a better job of predicting risk than using either alone.

The Women's Health Study prospectively examined the predictive value of LDL and hs-CRP in 27,939 postmenopausal women for approximately eight years. The researchers found that hs-CRP was a better predictor of cardiovascular event risk than LDL (Ridker et al. 2002). Even after adjusting for all Framingham risk score components, hs-CRP appeared to be an independent risk factor for stroke, myocardial infarction, and cardiovascular mortality. Women with low LDL levels (<123.7 mg/dL), but high hs-CRP values were at higher risk for cardiovascular events than the women with higher LDL (>123.7) levels, but low hs-CRP values (Ridker et al., 2000). Hence, the addition of hs-CRP to traditional cholesterol testing may improve risk prediction, but might also improve compliance with preventive approaches. According to Ridker (2003), it is well established that absolute risk perceived by an individual is directly related to compliance with lifestyle recommendations. Hs-CRP evaluation may be useful for this reason alone.
In addition to its ability to add predictive value to traditional lipid screening, hs-CRP also adds prognostic value to all levels of the metabolic syndrome (Szmitko et al., 2003; Ridker, 2003). Inflammation, not elevated LDL, plays a major role in almost all processes associated with the metabolic syndrome. Ridker (2003) stated that hs-CRP levels are associated with triglycerides, obesity, blood pressure, and fasting glucose, all of which are components of the Adult Treatment Panel III (ATP III) metabolic syndrome definition. Ridker (2003) examined the possible interrelationships between the metabolic syndrome, hs-CRP, and cardiovascular events in participants in the Women's Health Study (n = 14,719). The women were aged 45 and older and apparently healthy with no prior history of cardiovascular disease or cancer and did not have diabetes at baseline. The participants had also complete data for all components of the metabolic syndrome. Ridker (2003) found a significant (p-trend <0.0001) linear relationship between the number of metabolic syndrome criteria and hs-CRP level (0.68 mg/L for women who met no criteria of the metabolic syndrome to 5.75 mg/L for women who met all 5 criteria).

Festa, D'Agostino, Howard, Mykkanen, Tracy, and Haffner (2000) also found an association between the metabolic syndrome and hs-CRP. In the Insulin Resistance Atherosclerosis Study (IRAS), Festa et al. (2000) found that hs-CRP levels in non-diabetic subjects increased in a monotonic fashion as the number of metabolic disorders increases. According to Ridker (2003), insulin sensitivity and impaired fibrinolysis, which are additional factors associated with the metabolic syndrome also appear to be associated with hs-CRP. Ridker (2003) also stated that several prospective studies have found hs-CRP to predict type 2 diabetes. Risk for developing type 2 diabetes appears to increase 4 to 6-fold in individuals with hs-CRP levels >3mg/L. These studies further
suggest inflammation, atherosclerosis, and diabetes as interconnected disorders of the innate immune system.

Studies conducted in both the U.S. and Europe have consistently found a relationship between an individual's baseline hs-CRP level and future vascular risk. In most cases this has been seen independent of cholesterol levels, blood pressure, age, smoking, and diabetes. Effects are seen in men and women, middle age and elderly, those with low absolute risk and those with high absolute risk for vascular events (Ridker, 2003). Although, hs-CRP appears to be associated with an increased risk for cardiovascular disease controversy surrounds the role hs-CRP actually plays in the atherosclerotic disease process. In order to further understand hs-CRPs role as an inflammatory biomarker or a possible risk factor, we must first understand the link between inflammation, endothelial dysfunction, and atherosclerosis.

C. Endothelial Dysfunction, Inflammation, and Atherosclerosis

The role of inflammation has become a well established theory in describing the atherosclerotic disease process (Pearson et al., 2003; Libby, Ridker, and Maseri et al., 2002). From a pathological standpoint all stages from initiation to plaque rupture are considered to be an inflammatory response to injury. The traditional triggers that promote atherogenesis include, cigarette smoking, atherogenic lipoproteins, hypertension, and hyperglycemia. These triggers encourage a number of noxious stimuli that elicit secretion of both leukocyte soluble adhesion molecules, which facilitate monocyte attachment to the endothelium, and chemotactic factors, which enable the monocytes to migrate into the subendothelial space (Pearson et al., 2003; Libby, Ridker, and Maseri et al., 2002).
Endothelial injury results in "dysfunction". According to Szmitko et al. (2003) dysfunction involves an imbalance in endothelium-derived relaxing factors, such as nitric oxide (NO) and contracting factors, such as endothelin-1 (ET-1), angiotensin, and oxidants. The imbalance between endothelial-derived vasodilating and vasoconstricting substances impairs regulation of vascular tone. As production or availability of NO is diminished, its ability to protect against vascular injury, inflammation, and thrombosis is diminished. Normally NO inhibits leukocyte adhesion, helps maintain non-proliferative vascular smooth muscle, and limits platelet aggregation (Szmitko et al., 2003).

Unfortunately, CVD risk factors, such as hypercholesterolemia, hypertension and diabetes, reduce endogenous defenses of the vascular endothelium and it begins to break down. Hypercholesterolemia enhances blood leukocyte attachment to the endothelium (Szmitko et al., 2003). This may lead to an increase in the amount of low-density lipoproteins (LDL) that becomes oxidized. Oxidized LDL (oxLDL) activate and change the biological characteristics of the endothelium reducing intracellular NO concentrations (Szmitko et al., 2003). Angiotensin II, a potent vasoconstrictor associated with hypertension, also has several negative effects. Angiotensin II, according to Wang et al. (2003), is a key bioactive factor involved in the atherosclerotic disease process. It inhibits NO action, elicits reactive oxygen species (ROS), increases IL-6 and monocyte-chemoattractant protein-1 (MCP-1), and up-regulated VCAM-1 on the endothelium (Szmitko et al., 2003). According to Szmitko et al. (2003) newer risk factors such as hs-CRP also appear to promote endothelial dysfunction. Hs-CRP quenches the production of NO and diminishes its bioactivity. All these endothelial alterations enhance inflammation (Szmitko et al., 2003).
Once endothelial cells undergo inflammatory activation, areas of the arterial endothelium start to express selective adhesion molecules on their surface (Charo, 1992; Libby, Ridker, and Maseri et al., 2002; Szmitko et al., 2003). These adhesion molecules bind to various classes of leukocytes (Libby, Ridker, and Maseri et al., 2002). Research has identified a number of vascular endothelial adhesion molecules that can mediate leukocyte adhesion to the endothelium in a selective manner. According to Luscinskas & Gimbrone (1996) the primary examples are from four gene families: 1) the selectins (E-, P-and L-selectin); 2) the immunoglobulin family [ICAM-1, -2, and -3; VCAM-1; platelet-endothelial cell adhesion molecule (PECAM-1)]; 3) the integrins (B1- and B2-integrins); and 4) mucin-like adhesion molecules [P-selectin glycoprotein ligand-1 (PSCG-1)]. Once activated, endothelial cells can also synthesize an array of leukocyte chemoattractants, such as members of the chemokine gene family [MCP-1 and interleukin-8 (IL-8)], platelet-activating factor (PAF), and leukotriene B4 (LTB4) (Luscinskas & Gimbrone, 1996; De Caterina et al., 2000). Chemoattractants such as LTB4 and PAF act broadly on neutrophils, eosinophils, basophils, and monocytes, whereas others such as MCP-1 and IL-8 are selective for leukocyte subsets (De Caterina et al., 2000). Multiple receptor-ligand pairs appear to function in a sequential manner (an adhesion cascade) to allow the initial interactions of leukocytes with the endothelial lining. This initial interaction is called phase I or the rolling phase. The initial rolling interactions are weak and reversible unless the leukocytes are activated to undergo stable adhesion. This stable adhesion is considered phase II or the arrest and spreading phase. During the rolling interval, chemoattractants derived from the endothelium, such as MCP-1 and IL-8, appear to critically affect adhesion of mononuclear leukocytes by
triggering the activation of $B1$- and $B2$-integrin (Devaraj, Kumaresan, and Jialal, 2003; Luscinskas & Gimbrone, 1996, Wang et al., 2004). Once the integrins are activated a dramatic increase in monocyte adhesiveness is seen, presumably via binding to ligands, such as ICAM-1 and -2 and VCAM-1. This results in stable arrest (phase II). The monocyte flattens and extends pseudopods toward the intercellular junctions between neighboring endothelial cells. The monocyte ultimately transmigrates through the endothelial junctions into the extravascular space (phase III, transmigration). PECAM-1 is involved in this last step, along with $B1$- and $B2$-integrins (Charo, 1992; Luscinskas & Gimbrone, 1996). This entire process takes place within minutes for monocytes, but significantly longer for T cells. Monocytes and lymphocytes can directly influence the endothelium and each other once inside the interstitial space by secreting growth factors, chemotactic substances, and cytokines such as interleukin-4 (IL-4), interferon-$\gamma$ (IFN-$\gamma$), interleukin-10 (IL-10), interleukin-2 (IL-2), and interleukin-12 (IL-12) (Luscinskas & Gimbrone, 1996).

Blood-derived inflammatory cells participate in and perpetuate a local inflammatory response once they have resided in the arterial wall (Libby, Ridker, and Maseri et al., 2002). Native LDL may be oxidized by resident vascular cells, such as endothelial cells, smooth-muscle cells, and macrophages, after it becomes trapped in the subendothelial space. The oxLDL can stimulate monocyte chemotaxis along with endothelial dysfunction and injury (Ross, 1999). Endothelial cells are activated by the oxLDL to express MCP-1, which attracts monocytes into the subendothelial space from the vessel lumen (Navab et al., 1991). Once inside the intima monocytes differentiate into macrophages. Macrophages begin to express scavenger receptors, such as SR-A,
CD36, and LOX-1, which allows them to ingest the oxLDL and become foam cells (Szmitko et al., 2003). Macrophage colony-stimulating factor (M-CSF) along with MCP-1 contributes to the monocyte differentiating into the macrophage foam cell. T-cells also produce inflammatory cytokines (INF-γ and TNF-β), which in turn stimulate macrophages, vascular endothelial cells, and smooth muscle cells (SMCs).

As the process continues, macrophages and foam cells release fibrogenic mediators (i.e. peptide growth factors). These mediators can promote SMC replication and contribute to proliferation and production of dense extracellular matrix, creating a fibrous cap (Libby, Ridker, and Maseri et al. 2002; Ross, 1999). IL-8, which is present in macrophage dense areas of the atheroma, can also induce proliferation and migration of SMC (Devaraj, Kumaresan, and Jialal, 2003). The vascular smooth muscle cells (VSMC) that accumulate in the intima play a key role in the development of the arterial lesion. These cells synthesize collagen that stabilize the fibrous cap. However, as the lesion progresses VSMC apoptosis occurs, contributing to plaque vulnerability.

Blaschke et al. (2004) found that hs-CRP induced VSMC apoptosis in cultured human VSMCs via up-regulation of growth arrest-and DNA damage- inducible gene 153 (GADD153) mRNA expression. According to Blaschke et al. (2004) GADD153 is involved in growth arrest and apoptosis in both vascular and non-vascular cells. Using immunohistochemistry, Blaschke et al. (2004) were able to identify hs-CRP deposition in the deeper parts of the intima adjacent to the media and in the plaque shoulder region in a fibrous cap atheroma. They were also able to identify VSMCs as hs-CRP containing cells with α-Smooth muscle actin stain.
As the atherosclerotic disease process continues the plaque becomes more unstable. Foam cells and macrophages produce metalloproteinases that contribute to matrix degradation. Hence, they contribute to both growth of the lesion and plaque instability. IL-8 also contributes to the breakdown of the fibrous cap as it begins to inhibit tissue inhibitor of metalloproteinase (TIMP) (Devaraj, Kumaresan, and Jialal, 2003). As the proteolytic enzymes degrade the collagen in the fibrous cap, it becomes thin, weak, and susceptible to rupture (Libby, Ridker, and Maseri et al., 2002). T-lymphocytes are also involved in the degradation of the fibrous cap. They produce INF-y, which can halt the synthesis of collagen by the SMC, thereby limiting the renewal of collagen.

The thinning fibrous cap is now more vulnerable to rupture (Ross, 1999). Once the plaque has been disrupted excessive apoptosis of the VSMCs can increase thrombogenicity. The VSMCs produce active thrombin, which activates platelet adherence (Devaraj, Kumaresan, and Jialal, 2003). Macrophages produce tissue factor, which is a major procoagulant and can trigger thrombosis. Thrombus formation can lead to vessel occlusion and trigger a cardiovascular event.

It has been thought for some time that inflammatory mediators are important regulators of the atherosclerotic disease process (Libby, Ridker, and Maseri et al., 2002), but only recent evidence has begun to demonstrate the functional role of hs-CRP. Hs-CRP is now emerging to be more than just a biomarker. It is being examined for its functional role in the atherosclerotic disease process.
D. Hs-CRP, Atherosclerotic Biomarker or Contributor

Recent evidence suggests that hs-CRP may directly promote atherosclerosis. Traditionally, it has been thought that hepatocytes, during the acute-phase response, produced hs-CRP exclusively. Recent evidence, however, suggests that hs-CRP is being produced by the atherosclerotic arteries themselves. Calabro, Willerson, and Yeh (2003) found that human coronary artery SMCs could produce hs-CRP when incubated with inflammatory cytokines: TNF-\(\alpha\), IL-6, interleukin-1B (IL-1B) or a combination of IL-1B and IL-6. Calabro, Willerson, and Yeh (2003) concluded that locally produced hs-CRP participated directly in atherogenesis and cardiovascular complications.

According to Szmitko et al. (2003), hs-CRP provokes inflammation and endothelial dysfunction via multiple mechanisms. It appears to reduce transcription of endothelial nitric oxide synthase (eNOS) and destabilizes eNOS mRNA, which leads to a diminished release of nitric oxide (NO). This in turn reduces protection against vascular injury and inflammation. Hs-CRP causes further disruption to endothelial homeostasis by stimulating the release of ET-1 and IL-6, by up-regulating adhesion molecules (ICAM-1, VCAM-1, selectins), and by stimulating MCP-1. Hs-CRP also appears to increase the expression of plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor. PAI-1 inhibits tissue plasminogen activator (tPA), thereby regulating fibrinolysis in atherothrombosis. (L. Li, Roumeliotis, Sawamura, and Renier, 2004; Wang et al. 2004). Nuclear factor-\(\kappa\)B (NF-\(\kappa\)B), a key transcription factor involved in controlling gene expression concerning the inflammatory response, also appears to be affected by hs-CRP (J. Li, Fang, Chen, Chen, and Lee, 2004). Preliminary observations from Szmitko et al. (2003) indicate that hs-CRP up-regulates NF-\(\kappa\)B. NF-\(\kappa\)B remains
inactive in the cytoplasm of monocytes, lymphocytes, smooth muscle cells, and endothelial cells, until it is stimulated (Li et al. 2004). Once stimulated NF-κB transcriptionally activates several proatherosclerotic genes (Szmitko et al., 2003): IL-1, IL-6, IL-8, interferon, TNF-α, endothelial cell adhesion molecule-1 (ECAM-1), ICAM-1, and VCAM-1 (Li et al., 2004). Activated NF-κB has been found in the coronary vasculature and in atherosclerotic lesions, even in early stages.

According to Ghanim et al. (2004), an increase in NF-κB in the nucleus and a decrease in its inhibitors IκB-α and/or IκB-B describe inflammation at the cellular level. Ghanim et al. (2004) examined the proinflammatory state of peripheral blood mononuclear cells (MNC) in obese subjects. They found that transcriptional activity was increased in IL-6, TNF-α, migration inhibitor factor (MIF), and matrix metalloproteinase-9 (MMP-9) genes of healthy non-diabetic obese female participants when compared to normal-weight controls. This increase in transcriptional activity was reflected by increased levels of mRNA, which is consistent with increased binding of intranuclear NF-κB. Obese participants had significantly higher (p<0.05) DNA binding of the transcriptionally active form of NF-κB (p65/p50) in MNCs compared with lean participants. Significant correlations between NF-κB binding and hs-CRP was also seen.

L. Li, Fang, et al. (2004) also found a positive correlation between serum levels of hs-CRP and NF-κB activity. These researchers investigated NF-κB activity in white blood cells from patients (n = 33) with coronary heart disease (CHD). Participants with unstable angina had an increase in NF-κB activity and elevated hs-CRP levels compared to those with stable angina. Li et al. (2004) concluded that hs-CRP and NF-κB are useful in detecting vulnerable plaque and unstable angina.
Wang et al. (2005), like other researchers (Ghanim et al. 2004; L. Li, Fang et al. 2004; Szmitko et al., 2003), concluded that hs-CRP can significantly influence vascular endothelial gene expression and therefore play a functional role in atherogenesis. In order to investigate the effects of hs-CRP on vascular endothelial cell gene expression, Wang et al. (2005) incubated human vascular endothelial cells with hs-CRP in concentrations ranging from 0-10 ug/ml. They found that eleven genes (IL-8, PAI-1, MCP-1, Fibronectin-1, Connexin-43, ZF9, Activin A, EXT1, Cited2, SORL-1, and Gravin) increased and six genes (PIN, AnnexinA1, MAT2A, WRB, RCN1, and TEB4) decreased on their mRNA levels. The IL-8 gene was the most significantly up-regulated gene, with a 13.6-fold increase. This up-regulation began as early as three hours after incubation with hs-CRP and continued for over 24 hours. This is a significant find because IL-8 is involved in many aspects of the atherosclerotic disease process. As stated by Devaraj, Kumaresan, and Jialal (2003), IL-8 mediates recruitment and transmigration of phagocytes across the endothelium and appears to be a powerful trigger for monocyte adhesion to the endothelium. Wang et al. (2005) found that monocyte adhesion to the endothelium was enhanced 2-fold (p<0.01) by hs-CRP. They also found that the response was partially blocked by an anti-IL-8 antibody. IL-8 is also important during later stages of atherogenesis. It is induced by lipid loading of macrophages and is found in macrophage-rich areas of human coronary atheroma. It stimulates proliferation and migration of VSMCs and inhibits tissue inhibitor of metalloproteinase (TIMP) seen during later stages of the disease (Devaraj, Kumaresan, and Jialal, 2003). Hence, IL-8 is involved in the building up and breaking down of the atheroma’s fibrous cap.
Devaraj, Kumaresan, and Jialal (2003) also found that hs-CRP effected IL-8 expression and synthesis. Like Wang et al., (2005), Devaraj, Kumaresan, and Jialal (2003) incubated human aortic endothelial cells (HAEC) with hs-CRP. Western blotting was used to assay IL-8 protein, and B-actin was used as a loading control. Devaraj, Kumaresan, and Jialal (2003) found that hs-CRP (5 to 50 μg/ml) increased IL-8 protein levels in a dose-dependent manner. Incubation with hs-CRP also resulted in a dose-dependent increase in IL-8 mRNA levels. Actinomycin D, a transcription inhibitor of IL-8, prevented an increase in IL-8 following incubation with hs-CRP. It would appear from these finding that hs-CRP effects IL-8 at the transcriptional level. Therefore, Devaraj, Kumaresan, and Jialal, 2003 further investigated the effects of hs-CRP on NF-κB. They found that hs-CRP did in fact augment NF-κB activity and that inhibitors of NF-κB reversed the up-regulation of IL-8 by hs-CRP significantly.

The induction of IL-8 expression and synthesis via up-regulation of NF-κB activity in HAEC found by Devaraj, Kumaresan, and Jialal (2003) provides additional evidence that hs-CRP has atherogenic properties. This information is valuable because it provides a possible pathway for risk reduction. Because IL-8 is a key player in atherosclerosis, its reduction could potentially reduce risk. Therefore, a reduction in hs-CRP could possibly decrease risk, at least in part, by reducing IL-8.

As mentioned previously Wang et al. (2005) found several genes to be down-regulated following incubation with hs-CRP. One of those genes was PIN, a protein inhibitor of neuronal nitric oxide synthase (nNOS). According to Wang et al. (2005), the nitric oxide (NO) derived from nNOS is neurotoxic during the initial phase of ischemia. Inhibition of nNOS might decrease ischemic injury of neurons after a cerebral vascular
accident (CVA). According to Wang et al. (2005), clinical observations support this theory. Several studies have found that higher concentrations of hs-CRP predict worse outcomes in CVA patients (Di Napoli, Papa, and Bocola, 2001; Winbeck, Poppert, Etgen, Conrad, and Sander, 2002). By preventing the down-regulation of PIN, via hs-CRP reduction, we may thereby reduce ischemic injury and improve CVA patient outcomes.

Another mechanism by which hs-CRP provokes endothelial dysfunction is via its promotion of foam cell formation. Hs-CRP facilitates oxLDL uptake by macrophages (Szmitko et al., 2003; Yeh & Willerson, 2003). It is well known that a crucial step in atherogenesis is the unregulated uptake of oxLDL by vascular cells (L. Li, Roumeliotis, Sawamura, and Renier, 2004). This uptake causes further activation of the endothelium and changes the biological characteristics. A newly identified receptor of oxLDL is endothelial lectin-like oxidized LDL receptor-1 (LOX-1). Accumulating evidence suggests uptake via LOX-1 may also induce endothelial dysfunction. The binding of oxLDL to endothelial LOX-1 decreases NO production, generates superoxide anions, and activates NF-κB, all of which contribute to endothelial dysfunction (L. Li et al., 2004).

L. Li et al. (2004) investigated the effects of hs-CRP on LOX-1 expression, monocyte adhesion to the endothelium, and endothelial uptake of oxLDL. They incubated human aortic endothelial cells (HAECs) with hs-CRP. Hs-CRP, in concentration of just 5 μg/mL, enhanced LOX-1 mRNA levels. Concentrations between 10-25 μg/mL produced maximal effects. L. Li, Roumeliotis et al. (2004) also found that, through LOX-1, hs-CRP increased monocyte adhesion and uptake of oxLDL by the endothelial cells. They concluded that increased LOX-1 expression via hs-CRP was another mechanism by which hs-CRP promotes endothelial dysfunction.
Hs-CRPs proatherogenic effects extend beyond the endothelium to the VSMC. Hs-CRP appears to stimulate VSMC, at least in part, by activating NF-κB (J. Li, Wang, Huang, Xue, and Li, 2005) and by up-regulating angiotensin-type 1 receptors (AT$_1$-R) (Szmitko et al. 2003; Wang et al. 2003). In concentrations known to predict cardiovascular events, hs-CRP demonstrates up-regulation of AT$_1$-R in VSMC. AT$_1$-R mediates the majority of angiotensin II effects. AT$_1$-R up-regulation augments ROS production, restenosis, and VSMC proliferation and migration (Szmitko et al. 2003; Wang et al. 2003). Wang et al. (2003) examined the effects of human VSMCs treated with human recombinant CRP (0 to 100 ug/mL). They found that AT$_1$-R mRNA and protein expression were up-regulated. AT$_1$-R number was also increased on VSMCs. VSMC migration and proliferation was promoted by hs-CRP and hs-CRP increased production of ROS.

Current research is beginning to support the theory that hs-CRP is more than just a biomarker for CVD. Hence, therapies directed at its reduction appear to be valuable. Therapies, such as aspirin or statin-class medications, have been shown to reduce hs-CRP. Obisesan et al. (2004) stated that statin therapy has been shown to decrease hs-CRP by 13-17%. Ridker et al. (2005) found that individuals with low hs-CRP concentrations following statin therapy had better clinical outcomes compared to those with higher hs-CRP concentrations. They found that event-free survival was significantly improved in acute coronary syndrome patients that reduced hs-CRP levels to less than 2 mg/L following statin therapy. This effect was seen at all levels of LDL. Ridker et al. (2005) stated, “Our data also provide support for ongoing efforts to find agents capable of lowering CRP as a potential method of reducing vascular risk”. Controversy,
unfortunately, exists as to whether statin therapy is appropriate in primary prevention in individuals with high hs-CRP, but normal lipid levels. Therefore, lifestyle therapy may be more appropriate in these individuals. Physical activity, for example, may actually reduce hs-CRP to a greater extent than pharmacological therapies.

E. Hs-CRP and Physical Activity

It is well known that regular physical activity reduces the risk of cardiovascular disease (Eriksson et al., 1998), and recent studies have found an association between physical activity and hs-CRP. Regular exercise appears to diminish the acute phase reaction, which suggests suppression of the inflammatory response (Mattusch, Cufaux, Heine, Mertens, and Rost, 2000). Intervention studies have shown reductions in hs-CRP in exercise groups compared to controls. Geffken et al. (2001) found an inverse relationship between hs-CRP and regular physical activity among healthy elderly (≥ 65 years) participants (n = 5,201 participants, 2,961 females, 2,239 males). Mattusch et al. (2000) found that nine months of endurance training reduced hs-CRP levels by 31% when compared to non-training controls. Following six months of supervised exercise training Smith, Dykes, Douglas, Krishnaswamy, and Berk (1999) observed a 35% reduction in hs-CRP in individuals (n = 43) at high risk of ischemic heart disease.

Although these studies supply evidence that hs-CRP levels are reduced by regular physical activity, they lack either generalizability, fail to adjust for body weight and other confounding variables, or fail to account for drop outs. For example, Mattusch et al. (2000) failed to control for known confounding variables such as the presence of cardiovascular disease, race, smoking, age, diabetes, hypertension, and body mass index, which improved the quality of the study.
Mattusch et al. (2000) measured hs-CRP before and after nine months of marathon training in fourteen participants. During the training, distance run increased significantly as did the subjects aerobic capacity. Following training hs-CRP levels were reduced (1.19 mg/L to 0.82 mg/L). This was an unexpected result since intense exercise has been associated with inflammation of muscles and tendons. Mattusch et al. (2000) concluded that a decrease in hs-CRP following intense endurance training suggests that intense regular exercise has a systemic anti-inflammatory effect.

Mattusch et al. (2000) provided preliminary intervention data showing reduced hs-CRP levels following training. Results of this study are limited to males training for a marathon, therefore we can not generalize the results. Data from several participants was thrown out and not used in the statistical analyses, which could have created bias. Mattusch et al. (2000) failed to control for diet. Several dietary factors influence hs-CRP levels. Omega-3 fatty acid consumption for example, may reduce hs-CRP level (McCarty, 1999). Mattusch et al. (2000) also failed to adjust for body weight. Reductions in body weight can reduce hs-CRP (Pearson et al., 2003). Hence, we don’t know if the reduction in hs-CRP were due to exercise independently.

In many individuals inflammation is a result of obesity, particularly central obesity (Ridker, 2003). Many researchers have found strong positive associations between hs-CRP and waist girth, body fat mass, and visceral adiposity. LaMonte et al. (2002) found significant correlation between hs-CRP and waist girth, BMI, insulin, fitness level, and triglycerides. They examined the relationship between plasma hs-CRP and fitness level in 45 Native American (NA), 44 African American (AA), and 46 Caucasian (CA) women. Hs- CRP was measured following a 12-hour fast and 24-hour
abstinence from exercise and smoking. Duration of a maximal treadmill test was used to quantify fitness. A general linear model was used to analyze differences in hs-CRP concentrations across categories of race, fitness, and BMI. Treadmill exercise times were adjusted for age with linear regression and used to quantify fitness level. LaMonte et al. (2002) found hs-CRP was decreased in those with higher fitness levels and was increased in those with higher BMI and waist girth. They also found that hs-CRP levels varied by race. Lower hs-CRP levels were observed across tertiles of race-specific treadmill times in CA and NA, but not in AA after adjusting for BMI, smoking, diabetes, and estrogen status. The mechanisms for racial differences were unknown.

Rawson et al. (2003) found that BMI, not physical activity was associated with a reduction in hs-CRP. They measured hs-CRP five times over one year and examined the effects of BMI and both current and previous year physical activity in healthy men and women (n = 109). Current physical activity was measured using a telephone interview with a standardized interview script and a Baecke questionnaire was used to assess the previous year's physical activity. Over the course of the study physical activity increased, while BMI and hs-CRP remained unchanged. Only BMI was significantly related to hs-CRP when current physical activity, BMI, age, gender, and smoking were included in the statistical model. Current physical activity was similar when grouped by BMI (obese, overweight, normal) and was unrelated to hs-CRP. It may be that BMI was measured with greater reliability than physical activity, hence the association between BMI and hs-CRP was stronger than the association between physical activity and hs-CRP. Rawson et al. (2003) also found that as BMI increased, levels of hs-CRP increased. The lack of an association between physical activity and hs-CRP was surprising, as other
studies have found reductions in hs-CRP with increased physical activity. Discrepancies between this study and others could have resulted from cohort differences, subject screening and study design. For instance, the participants in Geffken et al. were older (79 years vs. 49 years). Hs-CRP is affected by age (Pearson et al., 2003). The study conducted by Smith et al. (1999) assessed hs-CRP in participants at risk for cardiovascular disease taking lipid-lowering medications. Some lipid lowering medications are known to reduce hs-CRP levels (Pearson et al., 2003). The cohort in the Rawsen et al. study was largely sedentary throughout the study while the participants in the Smith et al. and Geffken et al. studies were more physically active. In order to reduce hs-CRP, higher levels of physical activity may be needed when BMI remains stable. Respondent bias could also have lead to discrepancies between this study and others. It is well known that retrospective judgments can be biased. A number of factors influence retrospective data, such as the length of time since the event being recalled and specificity versus generality of information needed. Recalling physical activity over the past year may have been difficult, resulting in greater respondent error. If the survey used asked the participants specific events, the chance of inaccurate recall would have increased. Interviewer technique could also have introduced bias.

Physical activity has also been associated with reduced insulin resistance and reduced visceral obesity (Tracey, 2001). According to an editorial written by Tracey (2001) the Insulin Resistance and Atherosclerosis Study (IRSA) reported a strong association between insulin resistance and visceral adiposity. According to Tracey the study concluded that central obesity was the critical correlate of hs-CRP. Other researchers have found cardiorespiratory fitness levels to be inversely associated with hs-
CRP independent of body mass index (BMI). Wannamethee et al. (2002) examined the association between regular leisure-time activity and hemostatic and inflammatory variables. They found that physical activity was significantly and inversely associated with several hemostatic and inflammatory variables, including hs-CRP. This association persisted after adjustment for age, BMI, smoking, alcohol, and preexisting CVD.

Church et al. (2002) also found an association between cardiorespiratory fitness and hs-CRP independent of BMI. Cardiorespiratory fitness was assessed by a maximal treadmill test using a modified Balke protocol. They adjusted for weight and within weight categories. Participants (n = 722) were part of the Aerobic Center Longitudinal Study (ACLS), an epidemiological study of patients who received a preventive medical examination at the Cooper Clinic in Dallas, Texas. Church et al. calculated median and adjusted geometric mean hs-CRP levels, odds ratios of elevated hs-CRP across five levels of cardiorespiratory fitness, and percentages of individuals with hs-CRP ≥ 2 mg/L. They adjusted for age, BMI, smoking habit, vitamin use, statin medication, use of aspirin, and known inflammatory disease, CVD, and diabetes. An inverse association was found across fitness levels. Hs-CRP was highest in the lowest fitness quintile and lowest in the most fit quintile. Results were similar when the hs-CRP-fitness relationship was examined within categories of body fatness (normal weight, overweight, and obese).

Church et al. (2002) had a number of strengths in their study. They used metabolic equivalents (METs) as an objective measure of cardiorespiratory fitness, they had a large sample size, a thorough medical history, and in depth information on smoking, medication and vitamin use. Another important strength was the examination of fitness and hs-CRP with different levels of body composition and fat distribution.
Although this study had numerous strengths it also had limitations. Diet was not
evaluated. Diet may influence hs-CRP and those that exercise regularly often have
healthier diets than non-exercisers. The population studied was predominantly white,
middle-to-upper class, which limits the ability to generalize the results. Although this
homogenous group reduces external validity it improved internal validity by reducing the
likelihood of confounding variables.

F. Possible Mechanisms for Reduced Hs-CRP Following Training

Several theories exist as to how regular exercise reduces hs-CRP and the
inflammatory process. Mattusch et al. (2000) suggested that hs-CRP levels were
reduced, at least in part, due to enhanced antioxidative defense mechanisms seen after
regular exercise training. It has been reported that well trained athletes have increased
activity in muscular and erythrocytic antioxidative enzymes. Animal studies have also
shown improved antioxidant defense mechanisms after exercise training. It may be that
enhanced antioxidative protection reduces the production of IL-6 and other cytokines in
the exercising muscle (Mattusch, 2000).

Church et al. (2002) also proposed interleukins as a potential pathway. Evidence
suggests that TNF-α and IL-6 are involved in hs-CRP production. TNF-α and IL-6 are
released from adipose tissue, particularly visceral sites. Their release is augmented by
the down-regulation of sympathetic stimulation via regular physical activity. This may
lead to a reduction in hs-CRP. TNF-α stimulates the production of IL-6 and IL-6
stimulates the production of hs-CRP. Unlike a single bout of exercise which may
increase plasma levels of IL-6, IL-1B, and other associated inflammatory markers,
repeated exercise training appears to lower basal plasma interleukin concentrations.
TNF-α also appears to be reduced following regular exercise training. Reduction in TNF-α and IL-6 have been found independently of weight loss, hence, TNF-α and IL-6 may be responsible for reduced hs-CRP in fit individuals (Church et al., 2002).

Smith et al. (1999) found that exercise reduced TNF-α. These researchers examined the possibility that long-term exercise could favorably alter the production of cytokines with atherogenic and atheroprotective properties. Cytokines with atherogenic properties include IL-1, TNF-α, and IFN-γ. Cytokines with atheroprotective properties include IL-4, IL-10, and transforming growth factor beta (TGF-B). Smith et al. (1999) measured the effects of exercise on the production of these cytokines by blood mononuclear cells. They also examined the effects of exercise on serum levels of hs-CRP. Following six months of moderate intensity training, blood mononuclear cell production of cytokines was attenuated, while the production of cytokines with atheroprotective properties was increased. These changes were proportionate to the time participants spent performing repetitive lower-body exercises, which would suggest a dose-response relationship. A reduction in hs-CRP accompanied the changes in immune cell function. This could indicate a systemic reflection of decreased IL-1α and TNF-α production. These alterations were independent of aspirin or other medication use, dieting, weight loss, or smoking cessation. Smith et al. (1999) also found a significant attenuating effect on IFN-γ and TNF-α following training. Because both of these cytokines have been identified in early and advanced atherosclerotic lesions this is a favorable sign in persons at risk of developing cardiovascular disease. Endothelial cells, monocytes, macrophages, and smooth muscle cells are activated by these cytokines,
hence they contribute to leukocyte recruitment, endothelial cell procoagulant activity, LDL oxidation, and foam cell formation in atherosclerotic lesions.

Few studies have examined the effects of exercise on hs-CRP therefore the mechanisms remain largely unknown at this time. Further studies are clearly warranted.

G. Conclusions

There is increasing evidence that atherosclerosis is a dynamic and progressive disease resulting from the combination of inflammation and endothelial dysfunction. Advances in basic and experimental science have established that inflammation is involved in mediating all stages of this disease from initiation through progression and, ultimately, to atherosclerotic plaque complications (Pearson et al., 2003; Libby, Ridker, & Maseri, 2002). These new findings not only provide a link between risk factors and the mechanisms of atherogenesis, but the connection between inflammation and atherosclerosis also provides predictive and prognostic information (Libby, Ridker, & Maseri, 2002). Several markers of the inflammatory cascade have been examined as potential tools in the prediction of cardiovascular events. Hs-CRP is one of those markers and has generated much interest (Szmitko et al., 2003). Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events and may play a functional role in the atherosclerotic disease process (Szmitko et al., 2003; Yeh & Willerson, 2003). It appears to be even better than low-density lipoprotein (LDL) cholesterol at predicting cardiovascular events. This new information is profound and is beginning to change the way we screen and manage patients (Yeh & Willerson, 2003).
It is well known that regular physical activity is associated with lower risk of coronary heart disease, stroke, and cardiovascular mortality. Although the mechanisms are unclear, evidence suggests that physical activity modifies the inflammatory process. Recent studies have demonstrated an inverse relationship between inflammatory markers such as hs-CRP and physical activity. At this time only a handful of studies have examined the effects of exercise on hs-CRP and results are conflicting. More research is clearly needed to assess the relationship between hs-CRP and physical activity.

Questions remain regarding: (1) the type and intensity of exercise needed to reduce hs-CRP, (2) whether the association seen between physical activity and lowered hs-CRP is mediated by reduced body fat, and (3) whether fitness level modifies hs-CRP in those with the metabolic syndrome.
CHAPTER 3

METHODS

A. Overview

The purpose of this study was to examine the relationship between hs-CRP, metabolic syndrome and physical activity. Understanding this relationship will provide insight into the potential of physical activity as a therapeutic option to reduce cardiovascular disease risk. This study relied on a cross-sectional, retrospective analysis of an archival database. Study participants were obtained from a pool of 1,072 men and women patients at the Center of Health Promotion (CHP) at Loma Linda University. Of the 1,072 pool, 173 individuals met the inclusion criteria and were the subjects of the study. Any missing data variables were imputed using the maximum likelihood method (EM) in SYSTAT version 10; SPSS©2000. Simple regression/correlation analysis was used to evaluate the relationships between log (CRP) and each of the relevant variables: VO$_2$max (ml·kg$^{-1}$·min$^{-1}$), metabolic syndrome, and the physical activity status. The following relationships were explored: (1) hs-CRP and metabolic syndrome characteristics, (2) hs-CRP and VO$_2$max, (3) VO$_2$max and metabolic syndrome characteristics, and (4) VO$_2$max and physical activity status. Pearson Chi-square analysis was used to determine if a threshold level of physical activity was associated with hs-CRP changes.

B. Subjects

Study participants were selected from a pool of 1,072 men and women that underwent a medical exam at the Center for Health Promotion (CHP). The CHP is a
preventive health clinic that provides preventive medicine services to Southern California communities. Participants were, at the time of measurement, residents of Southern California’s Inland Empire residing in San Bernardino and Riverside counties.

Participants were included in this study if they: (1) underwent a medical exam at the CHP at Loma Linda University, which included blood pressure screening and blood analysis (hs-CRP, lipid profile and fasting blood glucose), (3) completed a Comprehensive Personal Wellness Profile (PWP) questionnaire regarding their medical history and lifestyle health practices (Wellsource Inc., Clachamus, OR), (4) attained 85% of estimated maximal heart rate on a Bruce protocol treadmill stress test at the CHP, and 5) were able to give informed consent. Failure to meet all of these criteria resulted in exclusion from the study. A total of 173 patients fulfilled the criteria and were included as the subjects of this study.

C. Measurement Variables

Cardiorespiratory fitness level was assessed via maximal oxygen uptake (VO2max, expressed in ml•kg^-1•min^-1) estimated from a Bruce protocol treadmill stress test. The PWP questionnaire was used to collect information regarding the participant’s medical history and lifestyle behaviors such as: smoking habits, alcohol intake patterns, eating habits, stress and coping patterns, social health, and physical activity status.

Several questions were asked regarding the participant’s physical activity status. Participants were asked about their current physical activity status under the headings of: (1) no regular exercise program, (2) occasionally walk for pleasure, (3) regular exercise in work or recreation requiring modest physical activity such as golf, yard work, calisthenics, up to one hour per week, (4) regular exercise in work or recreation requiring
modest physical activity such as golf, yard work, calisthenics, more than one hour per week, or (5) more active physical exercise (brisk walking, jogging, swimming). If the participant answered yes to the last question they indicated how much time was spent engaging in that activity each week.

Quest diagnostics performed the blood chemistry analyses. They annually perform over 100 million personal health tests, over 250 million diagnostic laboratory tests, and more than 6.5 million gene-based tests. They are CLIA Certified and their main laboratories are College of American Pathologists accredited. Plasma CRP concentrations were measured with the Dade Bering BN II high-sensitivity immunoassay (Dade Behring, Inc. IL). Fasting glucose were performed on the Olympus AU5400 Analyzer (Olympus America Inc., NY). Total cholesterol and triglyceride analyses were performed on the Olympus AU5400 Analyzer, while HDL-cholesterol was performed on a Roche Analyzer (Roche Molecular Systems Inc., CA).

Participants had their resting blood pressure measured by auscultation. Standard procedures outlined by the American Medical Association were followed (Reeves, 1995). Weight was measured on a standardized physician’s balance beam scale and stadiometer. BMI (weight (kg)/height (m)²) was also calculated for each.

Although the definition of Metabolic Syndrome has not been agreed upon internationally, a working definition continues to be used (Ford, Giles, and Mokdad, 2004; Kahn, Buse, Ferrannini, and Stern, 2005). Our definition utilizes criteria set forth by both the WHO and the U.S. National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines (The Expert Panel, 2001; Kahn, Buse, Ferrannini, and Stern, 2005). Participants had to meet at least three of the following variables in order to
be labeled as having metabolic syndrome: (1) blood pressure \( \geq 130/85 \text{ mm Hg} \), (2) HDL-C \( \leq 40 \text{ mg/dl} \) for males, \( \leq 50 \text{ mg/dl} \) for females, (3) fasting blood glucose \( \geq 110 \text{mg/dl} \), (4) elevated TG \( \geq 150 \text{ mg/dl} \), and (5) BMI \( \geq 30 \text{kg/m}^2 \). Due to the retrospective design of this study and the lack of waist circumference measurements we chose to use BMI \( \geq 30 \text{kg/m}^2 \) as our measure of obesity rather than waist circumference. The WHO definition utilizes \( \geq 30 \text{kg/m}^2 \) as criteria for obesity.\textsuperscript{12}

D. Data Analysis

A total of 173 patients fulfilled the inclusion criteria and were included as the subjects of this study. Any missing data variables were imputed using the maximum likelihood method (EM) in SYSTAT version 10; SPSS©2000. Because the percent body fat variable required over 100 imputations to fill in all of the missing data it was not used in any analyses. The distribution of hs-CRP, the dependent variable of greatest interest, was positively skewed. Therefore, a log transformation was applied to hs-CRP values for all analyses. Simple regression/correlation analysis was used to evaluate the relationships between log (CRP) and each of the relevant variables: VO\textsubscript{2}max (ml•kg\textsuperscript{-1}•min\textsuperscript{-1}), metabolic syndrome, and the physical activity status. The following relationships were explored: (1) hs-CRP and metabolic syndrome characteristics, (2) hs-CRP and VO\textsubscript{2}max, (3) VO\textsubscript{2}max and metabolic syndrome characteristics, and (4) VO\textsubscript{2}max and physical activity status. Pearson Chi-square analysis was used to determine if a threshold level of physical activity was associated with hs-CRP changes.
E. Power Analysis

When designing the study a medium effect size was assumed. Power analysis was conducted using the statistical software GPOWER (Faul & Erdfelder, 1992). Multiple regression analysis was the statistical test used to determine power along with a medium effect size (0.15), an alpha of 0.05, and power of 0.80. The total sample size needed according to the GPOWER analysis was 131 participants.

F. Limitations

The proposed study has several limitations: (1) reporting bias, (2) health habits of regular exercisers, (3) failure to control physical activity prior to blood draw, and (4) lack of detailed medication history prior to blood draw. Participants may have embellished when filling out the physical activity portion of the PWP. This respondent bias could threaten the studies validity.

People that exercise regularly may also engage in other positive health behaviors, thereby reducing hs-CRP levels. Regular exercisers, for example, may have healthier diets than those that do not exercise regularly (Church 2002). This could influence their hs-CRP levels. In addition to healthier eating habits, regular exercisers may have reduced stress, anxiety, and depression (Petruzzello, Landers, Hatfield, Kubitz, and Salazar, 1991; Plante, Unger, Hutchinson, and Faigenbaum, 1990) compared to non-exercisers. Mental state may influence markers of inflammation (Appels, Bar, Bar, Bruggeman, and DeBaets, 2000). According to Black (2003), an acute phase reaction may be induced by stress alone. IL-6 is the central mediator of the acute phase reaction and it facilitates the production of several acute phase proteins, including hs-CRP. Hence, if a person
exercises regularly, thereby reducing stress, he or she may in turn decrease hs-CRP levels independently of exercise.

Intense exercise leads to increased inflammation, hence hs-CRP levels will be elevated (Mattusch et al. 2000). The participants were not instructed to refrain from exercising prior to the blood draw. If a participant engaged in some form of exercise prior to the blood draw hs-CRP levels could have been transiently elevated, thereby affecting the study's validity.

Research is finding that certain medication may also influence hs-CRP values. Medications such as statins, fibrates, and niacin appear to reduce hs-CRP, while hormone replacement therapy appears to elevate hs-CRP levels (Pearson et al., 2003). The PWP does not provide detailed medication history. Lack of this knowledge could affect validity.

G. Research Ethics

Risk to participants is minimal. In order to protect participant confidentiality individual data will be coded and sorted by identification numbers. The list matching names and identification numbers will be stored separately from the data and both will be kept in locked filing cabinets. The identity of the participants will be known to the principal investigator, co-investigators, and statistical consultant. Signed consent forms will be kept in a locked storage facility at the CHP at Loma Linda University. Access will be restricted to the principal investigator, co-investigator, and designated representatives of the Human Subjects Committee.
CHAPTER 4

PUBLISHABLE PAPER

The Relationship Between C-Reactive Protein, Metabolic Syndrome and Exercise.

Micheline Vargas, Lee Berk, Edward Fujimoto, Wayne Dysinger, Warren Peters, Jim Westengard

To be submitted to the journal Circulation June 2006

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Total manuscript word count is 3,902.
The Relationship Between High Sensitivity C-Reactive Protein, Metabolic Syndrome, and Exercise

**Background**—Metabolic syndrome (MetS) is characterized by three or more of the following criteria: low high density lipoprotein (HDL), hypertriglyceridemia, hypertension, obesity, and abnormal glucose. High sensitivity C-reactive protein (hs-CRP) increases linearly with the number of MetS criteria. Both MetS and hs-CRP are associated with increased risk of cardiovascular disease (CVD) and diabetes. Cardiorespiratory fitness (CRF) is negatively associated with both the MetS and hs-CRP. CRF is associated with reduced risk of diabetes and CVD.

**Methods and Results**—We examined the relationship between hs-CRP, MetS, and CRF in 173 men and women. CRF was assessed using a Bruce protocol treadmill test and expressed as maximal oxygen uptake (VO₂max). Physical activity status was also assessed via self report. Plasma hs-CRP levels were determined using standardized immunoassay. Hs-CRP increased linearly with the number of MetS characteristics (p = 0.00022). An inverse association was found between hs-CRP and VO₂max (p = 0.00008) and between VO₂max and the number of MetS criteria (p = 0.00004). VO₂max was positively associated with physical activity pattern (p = 0.00000). Subjects engaging in 2-3 hours of exercise per week had hs-CRP levels ≤ 2.5 mg/L (p = 0.01817).

**Conclusion**—Higher hs-CRP levels are associated with lowered CRF and higher number of MetS criteria. These associations are suggested mechanisms by which CRF reduces the risk of CVD and diabetes.

**Key Words:** C-reactive protein □ Exercise □ Metabolic Syndrome □ Atherosclerosis □ Inflammation
The leading cause of death in the U.S. is cardiovascular disease (CVD).\textsuperscript{1} Today, it is well known that insulin resistance plays an integral role in CVD etiology.\textsuperscript{2-3} Certain CVD risk factors appear to be exacerbated by insulin resistance. According to the Adult Treatment Panel-III (ATP-III) Guidelines\textsuperscript{4} metabolic syndrome is characterized by three or more CVD risk factors which include the following: abdominal obesity, low high density lipoprotein (HDL) cholesterol, elevated serum triglycerides (TG), elevated resting blood pressure, and impaired fasting glucose.\textsuperscript{1-5} The prevalence of metabolic syndrome in adults aged \( \geq 20 \) years has increased from 41 million in 1990 to approximately 55 million in 2000.\textsuperscript{6} Individuals with metabolic syndrome are at increased risk for both diabetes and cardiovascular disease.\textsuperscript{6} Coronary artery disease, stroke, and myocardial infarction appear to be increased nearly threefold.\textsuperscript{7} Hence, the ATP-III report stresses the significance of preventive therapies in these individuals.

It is well known that regular physical activity reduces the risk of chronic diseases such as diabetes and cardiovascular disease.\textsuperscript{8} Physical activity is also considered an important determinant of metabolic syndrome.\textsuperscript{6} All levels of metabolic syndrome, diabetes, and cardiovascular disease are thought to involve inflammation. Physical activity may reduce risk, at least in part, by modifying the inflammatory process. Recent studies have demonstrated an inverse relationship between inflammatory markers, such as high sensitivity C-reactive protein (hs-CRP), and physical activity.\textsuperscript{9,10} Elevated hs-CRP appears to be an independent predictor of both cardiovascular disease and diabetes. Recent evidence also suggests that hs-CRP is positively associated with all metabolic syndrome characteristics.\textsuperscript{5} Ridker et al,\textsuperscript{5} stated that hs-CRP adds prognostic value to all levels of metabolic syndrome. Based on these findings we sought to evaluate the
relationship between hs-CRP, metabolic syndrome, and cardiorespiratory fitness (CRF), expressed as maximal oxygen uptake (ml·kg⁻¹·min⁻¹).

Methods

Study sample

Study participants were selected from a pool of 1,072 men and women from the Center of Health Promotion (CHP) at Loma Linda University. Participants were included in this study if they: (1) underwent a preventive medicine health exam at the CHP, which included blood pressure screening and blood analysis (hs-CRP, lipid profile, and fasting blood glucose), (3) completed Comprehensive Personal Wellness Profile (PWP) questionnaire regarding their medical history and lifestyle health practices (Wellsource Inc., Clachamus, OR), (4) reached ≥ 85% of age predicted maximal heart rate on a Bruce protocol treadmill stress test, and 5) were able to give informed consent. 173 subjects met the criteria and were included in the study.

Clinical Measurements

Cardiorespiratory fitness level was assessed via maximal oxygen uptake (VO₂max, expressed in ml·kg⁻¹·min⁻¹) estimated from a Bruce protocol treadmill stress test. The PWP questionnaire was used to collect information regarding the participant’s medical history, and lifestyle behaviors such as: smoking habits, alcohol intake patterns, eating habits, stress and coping patterns, social health, and physical activity status. Several questions were asked regarding the participant’s physical activity status. Participants were asked about their current physical activity status under the headings of: (1) no regular exercise program, (2) occasionally walk for pleasure, (3) regular exercise in work or recreation requiring modest physical activity such as golf, yard work, calisthenics, up
to one hour per week, (4) regular exercise in work or recreation requiring modest physical activity such as golf, yard work, calisthenics, more than one hour per week, or (5) more active physical exercise (brisk walking, jogging, swimming). If the participant answered yes to the last question they indicated how much time was spent engaging in that activity each week.

Quest diagnostics performed the blood chemistry analyses. Plasma hs-CRP concentrations were measured with the Dade Bering BN II high-sensitivity immunoassay (Dade Behring, Inc. IL). Fasting glucose was performed on the Olympus AU5400 Analyzer (Olympus America Inc., NY). Total cholesterol and triglyceride analyses were performed on the Olympus AU5400 Analyzer, while HDL-cholesterol was performed on a Roche Analyzer (Roche Molecular Systems Inc., CA).

Participants had their resting blood pressure measured by auscultation. Standard procedures outlined by the American Medical Association were followed. Weight was measured on a standardized physician’s balance beam scale and stadiometer. BMI (weight (kg)/height (m)²) was also calculated for each participant.

Definitions

Although the definition of Metabolic Syndrome has not been agreed upon internationally, a working definition continues to be used. Our definition utilizes criteria set forth by both the WHO (Table 1) and the U.S. National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines (Table 2). Participants had to meet at least three of the following variables in order to be labeled as having metabolic syndrome: (1) blood pressure \( \geq 130/85 \text{ mm Hg} \), (2) HDL-C \( \leq 40 \text{ mg/dl} \) for males, \( \leq 50 \text{ mg/dl} \) for females, (3) fasting blood glucose \( \geq 110 \text{ mg/dl} \), (4) elevated TG \( \geq 150 \text{ mg/dl} \), and (5) BMI
≥30kg/m². Due to the retrospective design of this study and the lack of waist
circumference measurements we chose to use BMI (≥30kg/m²) as our measure of obesity
rather than waist circumference. The WHO definition utilizes ≥30kg/m² as criteria for
obesity.12

Statistical Methods

Any missing data variables were imputed using the maximum likelihood method (EM) in
SYSTAT version 10; SPSS©2000. Because the percent body fat variable required over
100 imputations to fill in all of the missing data it was not used in any analyses. The
distribution of hs-CRP, the dependent variable of greatest interest, was positively skewed.
Therefore, a log transformation was applied to hs-CRP values for all analyses. Simple
regression/correlation analysis was used to evaluate the relationships between log (CRP)
and each of the relevant variables: VO₂max (ml·kg⁻¹·min⁻¹), metabolic syndrome, and the
physical activity status. The following relationships were explored: (1) hs-CRP and
metabolic syndrome characteristics, (2) hs-CRP and VO₂max, (3) VO₂max and metabolic
syndrome characteristics, and (4) VO₂max and physical activity status. Pearson Chi-
square analysis was used to determine if a threshold level of physical activity was
associated with hs-CRP changes.

Results

The mean age of the 173 subjects evaluated in the study was 49.5± (9.8) years. Clinical
characteristics of the study participant are shown in Table 3. 8.7% of our subjects met
the metabolic syndrome criteria. As shown in Figure 1, hs-CRP increased linearly with
the number of metabolic syndrome criteria (p = 0.00022). Figure 2 describes the
relationship between hs-CRP and VO_{2}\max. An inverse association was found between
hs-CRP and VO_{2}\max (p = 0.00008). An inverse relationship was also found between
VO_{2}\max and the number of metabolic syndrome criteria (p = 0.00004). VO_{2}\max was
positively related with physical activity status (p = 0.00000). Hence, fitness level was
increased, at least in part, by the amount of physical activity the participants engaged in.

The Surgeon General advises individuals of all ages to engage in a minimum of
30 minutes of moderate physical activity on most, if not all, days of the week to improve
health and reduce risk of chronic disease.\textsuperscript{14} The American College of Sports Medicine
(ACSM) recommends a minimum of 150 minutes per week of moderate-intensity
exercise for health benefits.\textsuperscript{15} Our findings supports the Surgeon General's physical
activity recommendations and recommendations set by the ACSM.\textsuperscript{14,15} Subjects in our
study engaging in 2-3 hours of exercise per week had hs-CRP levels < 2.5 mg/L (p =
0.01817), which is considered low to moderate risk.

Discussion
An apparent common theme that appears to connect most of the metabolic syndrome risk
factors is inflammation (Figure 3).\textsuperscript{19,21,22} Inflammation plays a role in elevated blood
pressure, dyslipidemia, impaired glucose tolerance, and obesity.\textsuperscript{19,21,22} The present study,
in agreement with others,\textsuperscript{5,7} found a positive relationship between the acute phase protein
(APP), hs-CRP, and metabolic syndrome characteristics. We found inverse associations
between: (1) hs-CRP and CRF level and (2) CRF level and metabolic syndrome severity.
It may be postulated that CRF reduces each of the risk factors seen in the metabolic
syndrome via a reduction in the inflammatory process.
Several studies have found an inverse relationship between inflammatory markers and physical activity. The proinflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α) are involved in hs-CRP production. Regular exercise training appears to lower basal plasma interleukin concentrations, such as IL-6, and TNF-α, which in turn downregulates hs-CRP production. According to Ghanim et al., as transcriptional activity of TNF-α and IL-6 is increased, intranuclear binding of the proinflammatory transcription factor nuclear factor-kappa B (NF-κB) is increased. It is suggested that exercise down modulates activation of NF-κB, thereby reducing TNF-α, IL-6, and CRP. This reduction in inflammation may in turn reduce risk factors seen in the metabolic syndrome.

It is well known that exercise improves blood pressure. This improvement is likely due to several mechanisms, including reduced cytokines and APP. Hypertension appears to stimulate production of cytokines such as IL-6 and other inflammatory mediators from the endothelium. Elevated cytokines along with APPs may impair endothelial dependent vasodilation. In addition to impaired vasodilation, chronic cytokinemia may contribute to insulin resistance, which leads to hyperinsulinemia. Hyperinsulinemia in turn stimulates the autonomic centers of the brain, which leads to an increase in catecholamine secretion, hence, the strong correlation between hypertension and insulin resistance syndrome. Catecholamines, such as norepinephrine (NE), may induce an acute phase reaction (APR). Thus, atherosclerotic plaque buildup can ensue due to the endothelial damage resulting from these events.

Numerous studies have found exercise training reduces proinflammatory cytokines, such as IL-6, and APPs such as CRP. These reductions may in turn...
improve insulin resistance, hyperinsulinemia, and reduce NE secretion, thereby reducing high blood pressure. Exercise may also reduce blood pressure via improved vasodilation. Studies have found exercise enhances the action of vasodilators, such as nitric oxide (NO). This may be due to the reduction in cytokines and APPs.

Another important metabolic syndrome characteristic, impaired glucose tolerance, is also improved with regular exercise. This improvement is likely due to several mechanisms, including reduced inflammation. Inflammation may activate NF-κB signaling pathways via up-regulation of TNF-α and IL-6, which stimulate hepatic CRP production. TNF-α may then contribute to insulin resistance by increasing oxidation of free fatty acid (FFA), stimulating additional cytokines (i.e. IL-6), inhibiting GLUT-4 transporters, harming endothelial function, and/or impairing glucose-stimulated release of insulin by B-cells. Hyperglycemia induces IL-6 from macrophages and the endothelium. IL-6 appears to reduce insulin signaling at the insulin receptor substrate-1 level. Hyperglycemia also decreases insulin sensitivity, which appears to enhance CRP production. The ensuing hyperinsulinemia stimulates IL-6 and TNF-α. Endothelial nitric oxide synthase-mediated vasodilation becomes impaired via the inflammatory cytokines and associated abnormal FFA oxidation. Reducing inflammation may be an important mechanism by which exercise improves glycemic control. Regular exercise has been found to increase the expression of GLUT 4 transporters and the expression of insulin receptor substrate-1 (IRS-1). These alterations may be due in part to a reductions in TNF-α and IL-6, respectively. Exercise has been found to improve glucose tolerance and both peripheral and hepatic insulin sensitivity.
improvements will likely lead to reduced cytokine and APP release, further improving glycemic control.

Dyslipidemia, another risk factor seen in metabolic syndrome is also improved by exercise. Exercise, particularly aerobic exercise, appears to increase HDL and reduces TG. 25,28 Sympathetically induced cytokines depress lipoprotein lipase (LPL). 21 In mice, TNF-α and IL-6 have been found to reduce LPL activity. 21 Exercise appears to enhance activity of several enzymes involved in lipid metabolism, including LPL. 28 Hence, exercise may enhance LPL activity via cytokine reduction thereby improving dyslipidemia.

The last metabolic syndrome characteristic is obesity. It is well known that exercise has a positive effect on this risk factor as measured by BMI or body fat percent. 21 It may be that exercise reduces inflammation via a reduction in adiposity. 16,29,30,31 Exercise appears to reduce visceral adipose tissue. 32 This may be an important mechanism by which exercise reduces inflammation. It is now recognized that adipose tissue functions in part as an immune organ. 33 It secretes many immunomodulatory factors and sends inflammatory signals known to cause insulin resistance. 32 Ghanim et al 19 found increased intranuclear NF-κB binding and increased concentrations of TNF-α and IL-6 in obese subjects. This proinflammatory state may contribute to insulin resistance in obese individuals as well as those with the metabolic syndrome.

In adipose tissue, TNF-α reduces the expression of the insulin receptor and causes a reduction in tyrosine phosphorylation of the insulin receptor thereby interfering with insulin action 19,33 Although the intracellular pathways activated by TNF-α remain to be
established they are thought to involve NF-κB. 33 Once activated, TNF-α receptors activate inhibitor kappa B kinase (IKK) complex, which appears to be a mediator of insulin resistance. 33 The key catalytic subunit of the IKK complex, IKK2, phosphorylates inhibitor kappa B. 33 This leads to NF-κB translocation into the nucleus, which activates inflammatory target genes. 33 IKK also inactivates the insulin receptor and IRS-1, which leads to decreased activation of phosphoinositol-3 kinase, a second messenger involved in the metabolic effects of insulin. 33 IL-6 also affects insulin’s metabolic effects via a reduction in insulin signaling at the insulin receptor substrate-1 level.19

As previously mentioned, exercise increases the expression of IRS-1.27 It may be that exercise enhances expression of IRS-1 via a reduction in TNF-α and IL-6. Enhanced expression of IRS-1 may reduce interference in insulin’s action. It may be that exercise reduces proinflammatory cytokines and the inflammatory response via a reduction in body fat, particularly a reduction in visceral adiposity. In this context, the present study has a couple important limitations. Inflammatory markers, such as cytokines, were not measured and waist circumference was not measured.

Conclusions

Elevated inflammation and the presence of the metabolic syndrome significantly increase the risk of CVD, diabetes, and premature mortality.34 We postulate that exercise reduces the risk of these conditions by reducing inflammation. We found that hs-CRP and the metabolic syndrome severity were reduced in those with a higher cardiopulmonary fitness level. We also found that those engaging in 2-3 hours of physical activity per week had lower hs-CRP levels (≤ 2.5 mg/L). Further clarification is needed to show
whether exercise reduces risk factors, thereby reducing inflammation or whether exercise reduces inflammation, thereby reducing risk factors. Regardless of which comes first, it appears that exercise is an important means of reducing risk factors seen in those individuals with metabolic syndrome. Aggressive therapeutic interventions of these individuals are imperative. The ATP-III report strongly supports the use of exercise as first-line therapy for the management of the metabolic syndrome. It would appear that our research findings add support to this recommendation.
Table 1. ATP III Clinical Identification of the Metabolic Syndrome*

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Defining Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity, (waist circumference)</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>&gt;102 cm (&gt;40 in)</td>
</tr>
<tr>
<td>Women</td>
<td>&gt;88 cm (&gt;35 in)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥150 mg/dL</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>&lt;40 mg/dL</td>
</tr>
<tr>
<td>Women</td>
<td>&lt;50 mg/dL</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>≥130/≥85 mm Hg</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>≥110 mg/dL</td>
</tr>
</tbody>
</table>

*Derived from Kahn et al. 12
**Table 2. WHO Clinical Identification of the Metabolic Syndrome**

Insulin Resistance: (1 of the following)
- 1) Type 2 diabetes
- 2) Impaired fasting glucose
- 3) Impaired glucose tolerance

Plus any 2 of the following:
- 1) Elevated BP (≥140/90) or Antihypertensive medication
- 2) Plasma TG ≥150mg/dl
- 3) HDL <35 in men, <40 in women
- 4) BMI >30 &/or waist:hip ratio >0.9 in men, >0.85 in women
- 5) Urinary albumin >20mg/min, or Albumin:Creatinine ratio ≥ 30mg/g

*Derived from Kahn et al.¹²*
### Table 3. Characteristics of Study Participants (Mean±SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n = 173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.5 ± 9.8</td>
</tr>
<tr>
<td>Weight, lbs</td>
<td>187.7 ± 44.9</td>
</tr>
<tr>
<td>Height, in.</td>
<td>68.3 ± 4.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.14 ± 5.8</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>110.0 ± 12.7</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>69.1 ± 8.6</td>
</tr>
<tr>
<td>Fasting Glucose, mg/dL</td>
<td>93.6 ± 16.4</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>129.5 ± 83.3</td>
</tr>
<tr>
<td>Total-C, mg/dL</td>
<td>192.5 ± 39.9</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>53.4 ± 15.6</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>113.2 ± 36.5</td>
</tr>
<tr>
<td>Metabolic Syndrome Criteria</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.0 ± 2.0</td>
</tr>
<tr>
<td>VO₂ max, ml·kg⁻¹·min⁻¹</td>
<td>45.0 ± 9.2</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Total-C, total cholesterol; CRP, C-reactive protein; VO₂ max, maximal oxygen uptake.
Figure 1. Relationship Between Log CRP and Metabolic Syndrome Criteria. Distribution of log CRP levels among 173 participants according to the presence of 0, 1, 2, or \( \geq 3 \) metabolic syndrome criteria. Box plots demonstrate median, 25\(^{th}\), and 75\(^{th}\) percentile values of log CRP.
Figure 2. Inverse Relationship Between Log CRP and VO₂max.
Figure 3. A Model Describing the Relationship Between Inflammation and Metabolic Syndrome Characteristics. IL-6 = interleukin-6, TNF-α = tumor necrosis factor-α, CRP = c-reactive protein, BP = blood pressure, TG = triglyceride, IGT = impaired glucose tolerance, DM = diabetes mellitus, NO = nitric oxide, ET-1 = endothelin-1, ICAM = intracellular adhesion molecule, VCAM = vascular cell adhesion molecule, AT1R = angiotensin type-1 receptor, MCP-1 = monocyte chemoattractant protein-1, NFKB = nuclear factor κB, Ang. II = angiotensin type II, IRS-1 = insulin receptor substrate 1, LPL = lipoprotein lipase, SNS = sympathetic nervous system.
**Figure 1.** Distribution of log CRP levels among 173 participants according to the presence of 0, 1, 2, or ≥ 3 metabolic syndrome criteria. Box plots demonstrate median, 25th, and 75th percentile values of log CRP.

**Figure 2.** Inverse relationship between log CRP and VO₂ max.

**Figure 3.** A model describing the relationship between inflammation and metabolic syndrome characteristics. IL-6 = interleukin-6, TNF-α = tumor necrosis factor-α, CRP = c-reactive protein, BP = blood pressure, TG = triglyceride, IGT = impaired glucose tolerance, DM = diabetes mellitus, NO = nitric oxide, ET-1 = endothelin-1, ICAM = intracellular adhesion molecule, VCAM = vascular cell adhesion molecule, AT₁R = angiotensin type-1 receptor, MCP-1 = monocyte chemoattractant protein-1, NFκB = nuclear factor κB, Ang. II = angiotension type II, IRS-1 = insulin receptor substrate 1, LPL = lipoprotein lipase, SNS = sympathetic nervous system.
References


A. Discussion

Markers of inflammation have been examined as potential tools in the prediction of cardiovascular events. Of these markers, the acute-phase reactant, high sensitivity C-reactive protein (hs-CRP), has generated significant interest (Szmitko et al., 2003). Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events (Szmitko et al., 2003; Yeh & Willerson, 2003). According to LaMonte et al. (2002), elevated hs-CRP is associated with a 2 to 5-fold increase in cardiovascular event risk.

Although hs-CRP has been considered only a biomarker, recent evidence suggests that it may directly promote atherosclerosis. According to Szmitko et al. (2003), hs-CRP provokes inflammation and endothelial dysfunction. It appears to reduce transcription of endothelial nitric oxide synthase (eNOS), which leads to a diminished release of nitric oxide (NO). This in turn reduces protection against vascular injury and inflammation. Hs-CRP causes further disruption to endothelial homeostasis by stimulating the release of endothelin-1 (ET-1) and interleukin (IL)-6, by upregulating adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule-1, selectins), and by stimulating the chemokine monocyte chemoattractant protein-1 (MCP-1). Hs-CRP also promotes foam cell formation by facilitating LDL uptake by macrophages (Szmitko et al., 2003; Yeh & Willerson, 2003). Preliminary observations from Szmitko et al. (2003) also
indicate that hs-CRP up-regulates nuclear factor \textit{Kappa} B (NF-\kappa B), which facilitates transcription of several proatherosclerotic genes. Hs-CRP's proatherogenic effects extend beyond the endothelium to the vascular smooth muscle (VSM). In concentrations known to predict cardiovascular events, hs-CRP demonstrates up-regulation of angiotensin-type 1 receptors (AT$_1$-R) in vascular SMCs. This augments VSM proliferation, migration, reactive oxygen species (ROS) production, and restenosis (Szmitko et al., 2003).

Observational studies have provided evidence that physical inactivity and low cardiorespiratory fitness predict atherosclerotic cardiovascular disease (Blair et al., 1996; Ekelund et al., 1988; Lakka et al., 1994, Laukkana et al., 2001; Lee, Blair, & Jackson, 1999; Sandvik et al., 1993), while regular physical activity is associated with lower risk of coronary heart disease, stroke, and cardiovascular mortality (Erikssen et al. 1998; U.S. Department of Health and Human Services). Physical activity may reduce risk, at least in part, by modifying the inflammatory process and reducing endothelial dysfunction.

Recent studies have demonstrated an inverse relationship between inflammatory markers, such as hs-CRP, and physical activity (Geffken et al., 2001; Rawsen et al., 2003). Higher levels of hs-CRP have also been associated with obesity and insulin resistance, while physical activity has been associated with lesser degrees of obesity and insulin resistance. Based on these findings many researchers have speculated that physical activity may be associated with lower levels of inflammation via its inverse relationship with obesity and insulin resistance (LaMonte et al., 2002).

Obesity and insulin resistance, along with elevated triglycerides, low HDL cholesterol, and elevated blood pressure, are characteristics seen in metabolic syndrome (Szmitko et al., 2003; Ridker, 2003). Inflammation plays a major role in almost all
processes associated with metabolic syndrome. Hs-CRP levels are associated with triglycerides, obesity, blood pressure, and fasting glucose (Ridker, 2003; Ridker, Buring, Cook, & Rifai, 2003). Ridker (2003) examined the possible interrelationships between metabolic syndrome, hs-CRP, and cardiovascular events in participants in the Women's Health Study (n = 14,719). The women were aged 45 and older and apparently healthy with no prior history of cardiovascular disease or cancer and did not have diabetes at baseline. The participants completed data for all components of metabolic syndrome. Ridker (2003) found a significant (p-trend < 0.0001) linear relationship between the number of metabolic syndrome criteria and hs-CRP level (0.68 mg/L for women who met no criteria of the metabolic syndrome to 5.75 mg/L for women who met all 5 criteria). Our findings are in support of Ridker's findings. We too found a positive linear relationship between hs-CRP and the number of metabolic syndrome characteristics.

According to Ford, Giles, & Mokdad (2004), physical activity is considered an important determinant of the metabolic syndrome. Our results are in support of this conclusion. The present study found an inverse relationship between the number of metabolic syndrome characteristics and VO2max. In addition, we found an inverse association between hs-CRP and VO2max, which was in concordance with previous research (Church et al., 2002; Mattusch et al., 2000; Smith et al., 1999). It may be that high cardiorespiratory fitness (CRF) reduces metabolic syndrome risk factors via a reduction in the inflammatory process.

Regular exercise training appears to lower basal plasma interleukin concentrations, such as IL-6, and TNF-α, (Church et al., 2002; Mattusch et al., 2000; Smith et al., 1999) which in turn reduces hs-CRP production. According to Ghanim et al.
(2004), as transcriptional activity of TNF-α and IL-6 is increased, intranuclear binding of
the proinflammatory transcription factor NF-κB is increased. It may be that exercise
modulates activation of NF-κB, (Aronson, 2004) thereby reducing TNF-α, IL-6, and CRP.
This reduction in inflammation may in turn reduce cardiovascular disease risk factors
seen in metabolic syndrome.

Inflammation is a common theme connecting all the metabolic syndrome risk
blood pressure, dyslipidemia, impaired glucose tolerance, and obesity (Black, 2003;
Ghanim, et al. 2004; Wexler, et al. 2005). Exercise may reduce these risk factors by
reducing levels of proinflammatory cytokines and APPs. Reductions in these
inflammatory modulators may, for example, reduce blood pressure by improving insulin
resistance and hyperinsulinemia, by reducing norepinephrine (NE) secretion, and by

Exercise may also improve dyslipidemia, via reductions in inflammatory
modulators. Exercise, particularly aerobic exercise, leads to a significant increase in
HDL cholesterol concentrations and a significant decrease in TG concentrations
(Rattigan, 2001; LeMura & von Duvillard, 2004). HDL elevation ranges from 2 mg/dl to
8 mg/dl or 4-22%, while TG concentrations are reduced by 5 mg/dl to 38 mg/dl or 4-
37%. It is thought that the metabolic demands of exercise produce changes in lipid
regulatory enzyme activity (LeMura & von Duvillard, 2004). A link between
lipoprotein lipase (LPL) and increased HDL, particularly HDL₂, and decreased TG
concentrations following exercise has been firmly established (LeMura & von Duvillard,
2004). LPL activity appears to increase transiently after a single bout of exercise. It
reaches a peak approximately 24 hours following exercise (Greiwe, JS, Holloszy J, Semenkovich, 2000; LeMura & von Duvillard, 2004). It may be that exercise enhances LPL activity via reductions in proinflammatory cytokines. Sympathetically induced cytokines depress lipoprotein lipase (LPL) (Black, 2003). In mice, TNF-α and IL-6 have been found to reduce LPL activity (Black, 2003). Hence, exercise may reduce dyslipidemia, at least in part, via a reduction in inflammation.

Regular exercise has also been found to improve glycemic control. This too may be due to a reduced inflammatory response. Regular exercise has been found to increase the expression of GLUT 4 transporters (Greiwe, Holloszy, & Semenkovich, 2000; Henriksen, 2002; Rattigan, 2001; Zheng, 2001) and the expression of insulin receptor substrate-1 (IRS-1) (Henriksen, 2002). These alterations may be due in part to a reduction in TNF-α and IL-6, respectively. Exercise has been found to improve glucose tolerance (Rattigan, 2001) and both peripheral and hepatic insulin sensitivity (LeMura & von Duvillard, 2004). Improvements in glucose tolerance and insulin sensitivity will likely lead to reduced cytokine and APP release, further improving glycemic control.

It may be that exercise reduces the inflammatory response via a reduction in body mass, particularly a reduction in visceral adiposity (Riechman, et al., 2002; Tracy, 2001). Numerous researchers have found strong positive associations between hs-CRP and waist girth, body fat mass, and visceral adiposity (LaMonte et al., 2002; Rawson et al., 2003; Wannamethee et al., 2002). It is now recognized that adipose tissue functions, in part, as an immune organ (Wisse, 2004). It secretes many immunomodulatory factors and sends inflammatory signals known to cause insulin resistance (Wisse, 2004). Ghanim et al. (2004) found increased intranuclear NF-κB binding and increased concentrations of TNF-
α and IL-6 in obese subjects. This proinflammatory state may contribute to insulin resistance in obese individuals as well as those with metabolic syndrome. Hence, exercise may reduce the inflammatory response via a reduction in body mass.

At this time there are no specific guidelines suggesting the amount of physical activity required to reduce inflammation. We found, through Pearson Chi-square analysis, that participants engaging in 2-3 hours of exercise per week had hs-CRP levels \( \leq 2.5 \text{ mg/L} \) (\( p = 0.01817 \)), which is considered low to moderate risk. This finding supports the Surgeon General's physical activity recommendations and recommendations set by the American College of Sports Medicine (U.S. Department of Health and Human Services, 1996; Jakicic, 2001).

**B. Strengths and Limitations**

The present study was based on sound theoretical measurements. Validated measures of blood pressure, body mass index, body fat percent, cardiorespiratory fitness, and hs-CRP were used. These tools, which have been used for years, increase the probability that variables were measured correctly.

When designing the study a medium effect size was assumed. Power analysis was conducted using the statistical software GPOWER (Faul & Erdfelder, 1992). Multiple regression analysis was the statistical test used to determine power along with a medium effect size (0.15), an alpha of 0.05, and power of 0.80. The total sample size needed according to the GPOWER analysis was 131 participants.

Due to the retrospective nature of this study we had a few limitations. Although we had a sufficient number of participants (\( n = 173 \)), some data was lacking. The variable, percent body fat, required over 100 imputations to fill in missing data, hence,
we chose not to include it in any analyses. We were, therefore, unable to determine if exercise reduced hs-CRP independent of body fat percent.

The proposed study had a few other possible limitations: (1) reporting bias, (2) health habits of regular exercisers, (3) failure to control physical activity prior to blood draw, and (4) lack of detailed medication history prior to blood draw. Participants may have embellished when filling out the physical activity portion of the PWP. This respondent bias could threaten the studies validity. To compensate for this possible bias we utilized a second measure of cardiorespiratory fitness level, a Bruce Protocol treadmill test. Treadmill test time is highly correlated with measured maximal oxygen uptake (Pollock, et al., 1976). Therefore, the present study used an objective laboratory measurement of cardiorespiratory fitness that reduces the misclassification bias often seen from physical activity self-reports.

People that exercise regularly may also engage in other positive health behaviors, thereby reducing hs-CRP levels. Regular exercisers, for example, may have healthier diets than those that do not exercise regularly (Church 2002). This could influence their hs-CRP levels. In addition to healthier eating habits, regular exercisers may have reduced stress, anxiety, and depression (Petruzzello, Landers, Hatfield, Kubitz, and Salazar, 1991; Plante, Unger, Hutchinson, and Faigenbaum, 1990) compared to non-exercisers. Mental state may influence markers of inflammation (Appels, Bar, Bar, Bruggeman, and DeBaets, 2000). According to Black (2003), an acute phase reaction may be induced by stress alone. IL-6 is the central mediator of the acute phase reaction and it facilitates the production of several acute phase proteins, including hs-CRP.
Hence, if a person exercises regularly, thereby reducing stress, he or she may in turn decrease hs-CRP levels independently of exercise.

Intense exercise leads to increased inflammation, hence hs-CRP levels will be elevated (Mattusch et al. 2000). The participants were not instructed to refrain from exercising prior to the blood draw. If a participant engaged in some form of exercise prior to the blood draw hs-CRP levels could have been transiently elevated, thereby affecting the study's validity.

Research has found certain medication influence hs-CRP values. Medications such as statins, fibrates, and niacin appear to reduce hs-CRP, while hormone replacement therapy appears to elevate hs-CRP levels (Pearson et al., 2003). The PWP does not provide detailed medication history and lack of this knowledge could have affected validity.
A. Conclusions

There is increasing evidence that atherosclerosis is a dynamic and progressive disease resulting from the combination of inflammation and endothelial dysfunction. Advances in basic and experimental science have established that inflammation is involved in mediating all stages of this disease from initiation through progression and, ultimately, to atherosclerotic plaque complications (Pearson et al., 2003; Libby, Ridker, & Maseri, 2002). These new findings not only provide a link between risk factors and the mechanisms of atherogenesis, but the connection between inflammation and atherosclerosis also provides predictive and prognostic information (Libby, Ridker, & Maseri, 2002). Several markers of the inflammatory cascade have been examined as potential tools in the prediction of cardiovascular events. Hs-CRP is one of those markers and has generated much interest (Szmitko et al., 2003). Emerging evidence suggests that hs-CRP is one of the strongest independent predictors of cardiovascular events and may play a functional role in the atherosclerotic disease process (Szmitko et al., 2003; Yeh & Willerson, 2003). Hs-CRP appears to be even better than low-density lipoprotein (LDL) cholesterol at predicting cardiovascular events. This new information is profound and is beginning to change the way we screen and manage patients (Yeh & Willerson, 2003).
In addition to hs-CRP's ability to predict cardiovascular events, it also appears to add prognostic value to all levels of the metabolic syndrome (Szmitko et al., 2003; Ridker, 2003; Festa, D'Agostino, Howard, Mykkanen, Tracy, and Haffner, 2000). In the Insulin Resistance Atherosclerosis Study (IRAS), Festa et al. (2000) found that hs-CRP levels in non-diabetic subjects increased in a monotonic fashion as the number of metabolic disorders increased. According to Ridker (2003), insulin sensitivity and impaired fibrinolysis, which are additional factors associated with the metabolic syndrome, also appear to be associated with hs-CRP. Hs-CRP also appears to predict type 2 diabetes (Ridker, 2003). Risk for developing type 2 diabetes appears to increase 4 to 6-fold in individuals with hs-CRP levels >3mg/L. These studies support the interconnection between atherosclerosis, metabolic disorders, and inflammation.

Although the mechanisms are unclear, evidence suggests that physical activity modifies the inflammatory process. This reduction in inflammation may in turn reduce risk factors seen in the metabolic syndrome. At this time only a few studies have examined the effects of exercise, hs-CRP, and the metabolic syndrome. Hence, the purpose of this study was to confirm and extend existing data regarding the relationship between hs-CRP, the metabolic syndrome, and cardiorespiratory fitness (CRF). The following is a list of study conclusions:

1. Hs-CRP increases linearly with the number of metabolic syndrome characteristics.
2. Hs-CRP is inversely associated with CRF level.
3. CRF level is inversely associated with the number of metabolic syndrome characteristics.
(4) A positive relationship was seen between CRF level and exercise pattern as measured by self report.

(5) Participants engaging in 2-3 hours of physical activity per week had hs-CRP levels \( \leq 2.5 \text{ mg/L} \) placing them in low to moderate risk categories.

B. Research Recommendations

More research is clearly needed to assess the relationship between hs-CRP, physical activity, metabolic syndrome, and atherosclerosis. A number of questions still remain.

(1) Does the type of physical activity affect hs-CRP level? For example, do anaerobic activities such as weight lifting or soccer reduce hs-CRP to the same extent as activities that primarily stress the aerobic energy system.

(2) Does one's enjoyment of the physical activity affect the inflammatory response? Psychological states have been associated with increased biomarkers of inflammation (Appels, Bar, Bar, Bruggeman, and DeBaets, 2000). Hence, if an individual perceives the type of physical activity as unpleasant the inflammatory response may be activated rather than reduced.

(3) Although the present study and others have demonstrated an inverse relationship between inflammatory markers, such as hs-CRP, and physical activity, mechanisms remain unclear. Several theories exist, however, more research is required to determine possible mechanisms.

(4) It is unclear whether exercise reduces risk factors seen in the metabolic syndrome, thereby reducing inflammation or whether exercise reduces inflammation, thereby reducing the metabolic syndrome risk factors. More research is required to
address the inflammatory response with regard to exercise, hs-CRP, and the metabolic syndrome criteria.

(5) Is the association seen between physical activity and lowered hs-CRP mediated by reduced body fat? At this time study results are conflicting regarding the role body fat plays in this relationship. It was our intent to examine this controversial question, however, we did not have an adequate number of participants with percent body fat measured to answer it confidently.

C. Practice Recommendations

Current research is beginning to support the theory that hs-CRP is more than just a biomarker for CVD. Hence, therapies directed at its reduction appear to be valuable. Therapies, such as aspirin or statin-class medications, have been shown to reduce hs-CRP. Obisesan et al. (2004) stated that statin therapy has been shown to decrease hs-CRP by 13-17%. Controversy, however, exists as to whether statin therapy is appropriate in primary prevention in individuals with high hs-CRP, but normal lipid levels. Therefore, lifestyle therapy may be more appropriate in these individuals.

Physical activity may even reduce hs-CRP to a greater extent than some pharmacological therapies. Mattusch et al. (2000) found that nine months of endurance training reduced hs-CRP levels by 31% when compared to non-training controls and Smith, Dykes, Douglas, Krishnaswamy, and Berk (1999) observed a 35% reduction in hs-CRP in individuals (n = 43) at high risk of ischemic heart disease following six months of supervised exercise training.

Our findings strengthen the argument that regular physical activity is a valuable form of lifestyle therapy. Regular exercise and improved cardiorespiratory fitness level
are important means of reducing risk factors associated with atherosclerosis, diabetes, and the metabolic syndrome. Our findings support the ATP-III report, which stresses the importance of using exercise as first-line therapy for the management of the metabolic syndrome (Festa et al., 2000, Tracey, 1997).

Identifying individuals with elevated hs-CRP and the metabolic syndrome is imperative. Once a patient has been identified as having the metabolic syndrome and/or elevated hs-CRP, the Preventive Care Specialist should prescribe exercise therapy via health education classes and/or individual counseling. Unfortunately, guidelines have not been established for the reduction of hs-CRP.

The present study found that participants engaging in 2-3 hours of exercise per week had hs-CRP levels ≤ 2.5 mg/L, which is considered low to moderate risk. This finding supports the Surgeon General’s physical activity recommendations and recommendations set by the American College of Sports Medicine (ACSM). The Surgeon General’s report on physical activity advices people of all ages engage in a minimum of 30 minutes of moderate physical activity, such as brisk walking, on most, if not all, days of the week to improve health and reduce risk of chronic disease (U.S. Department of Health and Human Services, 1996). The ACSM recommends individuals attain a minimum of 150 minutes per week of moderate-intensity exercise for health benefits (Jakicic, 2001). This moderate amount of physical activity may be ideal for reducing inflammation and its associated risk factors. Participating in moderate amounts of physical activity along with additional lifestyle therapies, such as stress reduction and proper nutrition, will likely reduce inflammation, the metabolic syndrome, and the number one killer in the U.S., cardiovascular disease.
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Appendix A

Center for Health Promotion Informed Consent
Appendix B

Personal Wellness Profile (PWP)