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The purpose of this study was to examine the effects of lifestyle activity modification (LAM) and structured exercise on the non-traditional cardiovascular risk factors C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1), in sedentary, African American Women. Subjects were randomized to a control group, a structured exercise group, or LAM group for a twelve week intervention.

A repeated measures ANOVA showed that both the exercise and LAM group significantly improved their predicted VO_2max and daily physical activity level. The exercise group significantly decreased their percent body fat and the control group significantly increased their waist circumference, while there were no changes in the LAM group. There were no changes in insulin, glucose, CPR or PAI-1.

Results show that both LAM and structured exercise improved cardiovascular fitness and prevented an increase in waist circumference in this cohort, but neither improved levels of CRP or PAI-1.

THE EFFECTS OF LIFESTYLE ACTIVITY MODIFICATION (LAM) OR A STRUCTURED EXERICSE PROGRAM ON NON-TRADITIONAL CARDIOVASCULAR DISEASE (CVD) RISK FACTORS IN AFRICAN-AMERICAN WOMEN

by

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CHAPTER I

INTRODUCTION

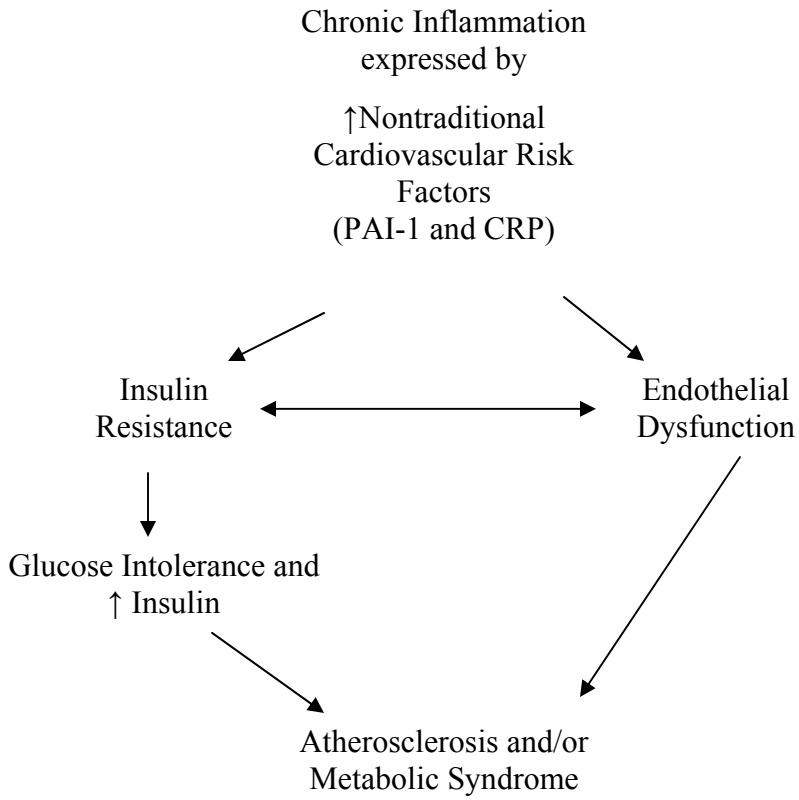
Background

Atherosclerosis is a disease of the arteries and is the primary cause of heart disease and stroke. It is a progressive disease in which lipids and fibrous debris accumulate in the arteries, often leading to thrombotic event(s) (Lusis, 2000). Metabolic syndrome, insulin resistance and type 2 diabetes are all metabolic disorders, often associated with atherosclerosis. Metabolic syndrome is a cluster of abnormalities including obesity, dyslipidemia, hyperglycemia, and high blood pressure (NCEP Panel III, 2001). When insulin resistance is present, insulin-mediated glucose uptake does not respond properly to changes in insulin (Vague and Raccah, 1992) which leads to hyperglycemia, a hallmark of dysfunction in glucose homeostasis. Obesity is a major component and contributor to both atherosclerosis and metabolic disorders and both are highly correlated with incidence of type 2 diabetes (Tkac, 2005, Vague and Raccah 1992). African-American women have higher rates of obesity and type 2 diabetes, making them a high risk group for the development of atherosclerosis and/or metabolic syndrome (Mokdad *et al*, 2003, Ogden *et al,* 2006).

Recently, it has been proposed that both atherosclerosis and metabolic disorders are related to inflammation (Manuel *et al*, 2003, Ross, 1999, Yeh, 2004). C-reactive

protein (CRP) and plasminogen activator inhibitor-1 (PAI-1) are two non-traditional cardiovascular risk markers that may be used to identify the possible risk of developing insulin resistance, type 2 diabetes, metabolic syndrome and/or atherosclerosis in the future. CRP is an acute phase reactant produced in response to infection and inflammation (Black *et al*, 2004, Du Clos, 2000, Munford, 2001). Elevated levels of CRP are associated with hypertension, obesity, diabetes, and dyslipidemia (Pearson *et al*, 2003). CRP is also directly associated with production of reactive oxygen species, platelet adhesion and aggregation, and production of adhesion molecules, all of which leads to endothelial dysfunction and an increased risk of developing atherosclerosis (Pasceri *et al*, 2000, Russo *et al*, 2002).

PAI-1 is the main regulator of plasminogen activation and the fibrinolytic system. The fibrinolytic system is responsible for disrupting blood coagulation, thus preventing the removal of intravascular thrombi. Elevated levels of PAI-1 disrupts this natural process, decreasing the degradation of extracellular matrix and increasing the risk of experiencing a cardiovascular event (Juhan-Vague *et al*, 1999, Mavri *et al*, 2004, Speiser *et al*, 1988).



It has been proposed that lifestyle modification of physical activity may prevent and/or reverse the development of atherosclerosis and metabolic disorders. The Centers for Disease Control and the American College of Sports Medicine now recommend that adults accumulate 30 minutes of moderate-intensity physical activity each day to improve cardiovascular health (Pate *et al*, 1995, US Department of Health and Human Services, 1996). It is important to investigate whether or not these new activity guidelines will alter levels of non-traditional cardiovascular risk factors in African-American women.

Statement of Problem

Regular structured physical activity has been shown to improve cardiovascular health and reduce the co-morbidities associated with obesity. Few research studies have been done to see if short exercise bouts done two-three times per day will yield the same benefits as long, continuous bouts, specifically for cardiovascular risk factors (Hardman, 2001). Recently, non-traditional cardiovascular risk factors have been examined in an attempt to identify new ways to track cardiovascular disease (CVD). The purpose of this investigation is to examine the effects of lifestyle activity modification (LAM) versus traditional structured exercise, compare to controls, on non-traditional cardiovascular risk factors in African-American (AA) women.

Specific Aim 1:

To determine the effects of 12 weeks of LAM and traditional exercise on non-traditional cardiovascular risk factors CRP and PAI-1 in previously sedentary, African-American women.

Hypothesis 1: Both LAM and traditional exercise will decrease fasting levels of CRP and PAI-1 in previously sedentary, African-American women in comparison to controls.

Rationale: The magnitude of the improvement in CRP and PAI-1 is often associated with the level of energy expenditure and/or amount of fat loss and decrease in BMI. Since both LAM and traditional exercise have been shown to increase energy expenditure, decrease percent body fat, and improve cardiovascular health in a similar fashion, CRP and PAI-1 are expected to decrease in both groups.

Specific Aim 2:

To compare the differences in the magnitude of the effect of LAM and traditional exercise on non-traditional cardiovascular risk factors CRP and PAI-1 in previously sedentary, African-American women.

Hypothesis 2: LAM and traditional exercise will be equally effective at lowering levels of CRP and PAI-1 in previously, sedentary, African-American women in comparison to controls.

Rationale: There has been no research comparing alterations in CRP and PAI-1 in LAM and traditional exercise groups. Both CRP and PAI-1 levels tend to decrease with a decrease in BMI and/or fat mass. Assuming energy expenditure and the resultant decreases in BMI and/or fat mass are similar in both groups, CRP and PAI-1 levels should decrease equally in both groups.

Specific Aim 3:

To compare the relationship between changes in body composition and changes in the non-traditional cardiovascular risk factors, CRP and PAI-1.

Hypothesis 3: Alterations in both CRP and PAI-1 will be positively correlated to changes in body composition.

Rationale: Since, both CRP and PAI-1 are closely related to BMI, percent body fat and fat mass, individuals with largest decrease in BMI or fat mass will show the largest decrease in CRP and PAI-1.

SUMMARY

This study will investigate the extent to which lifestyle activity modification and structured exercise effects PAI-1 and CRP, non-traditional risk-factors for cardiovascular disease. It will further investigate the difference in effect, if any, that the two different activity modes have on PAI-1 and CRP. More importantly, these risk factors will be examined in a high-risk population, African-American women, for which little research has been done.

CHAPTER II

REVIEW OF LITERATURE

This review of literature will discuss atherosclerosis and metabolic disorders as diseases of inflammation. Both C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1) are non-traditional cardiovascular risk factors that may be used to identify individuals at risk for future development of atherosclerosis and/or metabolic disorders. CRP and PAI-1 and their association with atherosclerosis, metabolic disorders, and obesity will be discussed. More specifically, CRP and PAI-1 in African-American women will be examined and the effects of exercise on CRP and PAI-1 will be explored.

Atherosclerosis

Atherosclerosis is a progressive disease in which lipids and fibrous debris accumulate in the arteries and it is the primary cause of heart disease and stroke (Lusis, 2000). Recently, research has suggested atherosclerosis may be a disease of inflammation, which develops in response to endothelial damage and/or injury. The inflammation hypothesis of atherosclerosis states that local inflammatory stimuli change the milieu of the arterial wall and prompt production of adhesion molecules and chemokines (Ross, 1999, Yeh, 2004). In a healthy, basal condition, the endothelium forms a barrier between elements in the blood and the vessel wall. An antithrombotic surface is maintained through secretion of vasoactive substances and the endothelium

modulates inflammatory responses, regulates vessel tone (via nitric oxide), and prohibits proliferation of the vascular smooth muscle cells (Russo *et al*, 2002). However, with chronic endothelial damage leading to inflammation, endothelial dysfunction may occur resulting in arterial lesions and ultimately the development of atherosclerosis (Libby and Ridker, 2004, Ross 1999, Yeh, 2004).

The initial insult to the arterial wall is often accompanied by the accumulation of oxidized low-density lipoproteins (LDL) and causes an alteration of normal homeostatic properties resulting in increased adhesiveness due to the up-regulation of adhesion molecules such as vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (Lusis, 2000, Ross, 1999). VCAM-1 and ICAM-1 receptors cause leukocytes in the blood to adhere to the vascular endothelium. Eventually these monocytes migrate into the intima and become intimial macrophages, a process that is promoted by monocyte chemoattractant protein-1 (MCP-1). The process of lesion formation, endothelial permeability, up-regulation of adhesion molecules, and migration of leukocytes into the artery wall are all part of endothelial dysfunction (Libby and Ridker, 2004, Ross, 1999).

Once inside the arterial wall, these intimial macrophages then express scavenger receptors which engulf lipoproteins and become foam cells. These pro-inflammatory foam cells become apoptotic, which leads to the production of a necrotic lipid core. Smooth muscle cells are then recruited from the media to help in the repair process and form a fibrous protective cap over the necrotic material. This remodeling process leads to an enlarged lesion, which leads to chronic dilation in an attempt to compensate for the

lesion. Eventually, the artery can no longer compensate and the lesion will invade the lumen, impeding blood flow and possibly resulting in a rupture which ultimately leads to a vascular event (Libby and Ridker, 2004, Munford, 2001, Ross 1999, Yeh, 2004).

Metabolic Disorders

Insulin resistance is defined as resistance to insulin-mediated glucose uptake, which is often accompanied by a compensatory increase in insulin levels (i.e. hyperinsulinemia). Insulin resistance is usually secondary to excessive body weight, especially visceral fat accumulation in the abdominal area. As visceral fat becomes less sensitive to insulin, lipolytic action is altered and visceral fat releases a large flux of free fatty acids into circulation. This increases hepatic gluconeogensis and lipoprotein synthesis and diminishes hepatic insulin clearance. This process leads to hepatic and muscle resistance to insulin-mediated glucose uptake and excess synthesis of triglycerides (Vague and Raccah, 1992). This insulin resistance is often present with metabolic syndrome. Metabolic syndrome is defined (according to the National Cholesterol Education Program-ATP III) as having three or more of the following criteria: abdominal obesity (waist >88 cm in females and >102 cm in males), low HDL (<50 mg/dl), elevated triglycerides (≥150 mg/dl), elevated glucose (≥110 mg/dl), and high blood pressure (≥130/85 mm Hg) (NCEP Panel III, 2001).

Insulin resistance and the metabolic syndrome tend to induce a prothrombotic state. Interleukin-6 (IL-6), tumor necrosis factor (TNF), PAI-1 and CRP are all elevated with the metabolic syndrome which suggests that inflammation may trigger insulin

resistance (Juhan-Vague *et al*, 2002). Metabolic syndrome and insulin resistance may further promote atherosclerosis by the formation of advanced glycosylation endpoints (AGEs) which interact with endothelial receptors. AGEs increase endothelial permeability, leukocyte adhesion and decrease nitric oxide production (Lusis, 2000). Given this, many metabolic conditions, such as insulin resistance, metabolic syndrome and type 2 diabetes, have recently been considered diseases of inflammation (Manuel *et al*, 2003).

Obesity is a major contributor to metabolic syndrome, more specifically visceral obesity (Juhan-Vague *et al*, 2002). From 1988-1994, the prevalence of metabolic syndrome in United States adults was 23.1%. This number increased to 26.7% in 1999-2000. Concomitantly, the percentage of adults with abdominal obesity went from 38.3% to 44%, which may contribute to the increase in metabolic syndrome (Ford *et al*, 2004). The 2001 Behavioral Risk Factor Surveillance System revealed that 58% of the population is overweight (BMI $\geq$ 25 kg/m^2) and 20.9% are obese (BMI $\geq$ 30 kg/m^2). Furthermore, 7.9% of the population has diabetes. When this is broken down by ethnic groups, 31.1% of the African-American population is obese and 11.2% have diabetes (Mokdad *et al*, 2003).

In 2003-2004, 66.3% of adults were defined as either overweight or obese and 32.2% were obese. The prevalence of obesity was higher in women (33.2%) than men (31.1%) and was significantly different across ethnic groups, with non-Hispanic Caucasian women at 30.2% and non-Hispanic black women at 53.9% (Ogden *et al*, 2006).

African-American Women

More specifically, the National Health and Nutrition Examination Survey for 2003-2004 reported that 81.6 % of African-American women are classified as overweight or obese and 53.9% are obese (Ogden *et al*, 2006). Additionally, African-American women have the highest percentage of diabetes (11.2%) compared to any other ethnic group (Mokdad *et al*, 2003). Generally, obesity is associated with increased risk of cardiovascular disease (CVD); however studies with African American women are controversial. Some studies show that in Africa-American women, body weight and/or body mass index (BMI) are not associated with higher rates of CVD (Calle *et al*, 1999, Schmidt *et al*, 1996, Stevens *et al*, 1998) while others have found a link between obesity and CVD morbidity (Adams-Campbell *et al,* 1995, Folsom *et al* 1998).

Data from the North Carolina Well Integrated Screening and Evaluation for Women Across the Nation (Nelson *et al,* 2002) showed that in a population of low income, middle-aged women, the prevalence of obesity was greater in African-Americans. In their sample, only 13% had a normal BMI, 28% were classified overweight, and 59% were considered obese. In comparison, the values for Caucasian women 31%, 34%, and 35%, respectively. Appel *et al* (2002) found that in a sample of 1110 women, African-American women had a significantly higher BMI (29.5 kg/m^2) and weight (78.5 kg) than Caucasian women (25.5 kg/m^2 and 69 kg, respectively). In addition, African-American women reported a higher incidence of CVD and diabetes. Appel *et al* (2002) argued that the differences observed between ethnicity and these

variables may be a function of socioeconomic status, as opposed to ethnicity itself. However, Winkleby *et al*, (1998) showed that the increased prevalence of obesity observed in African-American women compared to Caucasian women occurred at all socioeconomic levels and Sharma *et al* (2004) showed a higher rate of CVD risk factors in African-Americans compared to Caucasians regardless of education.

Mathews *et al* (2005) examined ethnic differences in cardiovascular risk factors in women. Results showed that 48.9% of African-American women were obese compared to 28.9% of Caucasian women. In addition, African-American women had higher CRP and PAI-1 levels. However, once adjusted for education, physical activity, total calories consumed and amount of calories from fat, PAI-1 levels were no longer significantly higher and CRP differences decreased, but still remained significant.

The National Heart, Lung and Blood Institute Growth and Health Study followed young girls over the course of 10 years to assess early childhood obesity and fat distribution as a predictor of the development of metabolic syndrome. At baseline (8-9 years of age), African-American girls had significantly higher BMI, waist circumference, insulin, glucose and HOMA-IR values compared to Caucasian girls. Ten years later, these differences remained significant. The average BMI for African-American girls was 27.5 kg/m^2 compared to 19.3 kg/m^2 for the Caucasian girls. With increasing age, the insulin and glucose levels decreased in both groups, but Caucasian girls had a greater decrease and the absolute value of HOMA-IR remained lower. The prevalence of metabolic syndrome at ages 18-19 was 3.6% in African-American and 3.0% in Caucasian girls (Morrison *et al*, 2006).

In a study comparing the differences in obesity and diabetes in different ethnic groups, African-American women had significantly higher BMI, waist girth, subcutaneous and truncal fat, [measured via dual-energy x-ray absorptiometry (DEXA)], compared to Caucasians. However, visceral adipose tissue, measured via computed tomography (CT), did not differ between Caucasian and African-American women. Age-adjusted prevalence of type 2 diabetes was 5.8% among Caucasians and 12.1% among African-Americans. Interestingly, type 2 diabetes prevalence increased with increasing levels of visceral adipose tissue in Caucasian women, but not in African-American women (Araneta and Barrett-Connor, 2005). These studies show that African-American women tend to have a higher prevalence of obesity, CVD, and diabetes than Caucasians.

C-Reactive Protein (CRP)

Proinflammatory risk factors that may contribute to the pathophysiology of atherosclerosis include elevated levels of oxidized LDL, proinflammatory cytokines such as interleukin-1 (IL-1) and TNFα, inflammatory stimuli such as IL-6, ICAM-1, VCAM-1 and selectins and acute-phase reactants such as CRP (Pearson *et al*, 2003).

Acute phase reactants or proteins bind to the surface of invading microbes and mark them for destruction by phagocytes and the complement system. They can also serve as anti-inflammatory agents, neutralizing proteases, oxidants, and pro-inflammatory cytokines. Additionally, acute phase reactants can promote the use of endogenous nutrients by activating lipolysis, gluconeogenesis, and glycogenolysis when necessary. Lastly, they can serve as procoagulants. Although these proteins provide an important

survival function, repeated activation of this system for prolonged periods can be harmful (Munford, 2001).

CRP is an acute phase reactant which is produced by the liver in response to infection, inflammation, and trauma. Clinically, CRP is used to monitor the inflammatory response. CRP can also function as an opsonin and can activate the complement system, which plays a role in host defense mechanisms and in the inflammatory response. CRP's influence on the complement system shows that CRP has an ability to protect the organism from infection. To date, no deficiencies in CRP have been found, suggesting that CRP is a crucial *in vivo* protein (Black *et al*, 2004, Du Clos, 2000, Munford, 2001).

Production of CRP by hepatocytes is regulated at the transcriptional level by IL-6 and can be enhanced by interleukin-1β (IL-1β) (Moshage *et al*, 1988). Specifically, C/enhancer binding protein family members are critical for induction of the CRP gene. CRP increases release of interleukin-10 (IL-10), represses synthesis of interferon-γ, stimulates interleukin-8 (IL-8) release, increases PAI-1 levels and activity, and increases the release of IL-1β, IL-6, and TNF-α (Black *et al*, 2004, Ballou and Lozanski, 1992).

Set levels for CRP, as a relative risk, in a clinical setting are: <1mg/L (low), 1-3 mg/L (moderate), >3mg/L (high). These risk cut-offs are based on samples from >15 different populations and > 40,000 samples (Pearson *et al*, 2003). It has been found that the high category has a 2-fold increase in relative risk for CVD compared to the low category. In general, elevated levels of CRP are seen with elevated blood pressure, high BMI, smoking, metabolic syndrome and/or diabetes, low HDL, high triglycerides,

hormone replacement therapy, chronic infection and chronic inflammation. Decreased levels of CRP are seen with moderate alcohol consumption, increased physical activity, weight loss, and certain medication such as statins, fibrates, and niacin (Pearson *et al*, 2003).

CRP and atherosclerosis

There are several mechanisms by which CRP may be involved in atherosclerosis. When CRP is introduced into human aortic endothelial cells, there is a dose-dependent inhibition of endothelial nitric oxide synthase (eNOS) mRNA levels and a decrease in eNOS bioactivity (Venugopal *et al*, 2002). Lower eNOS activity reduces the synthesis of nitric oxide (NO). Decreased production of NO increases vascular oxidant stress via the production of reactive oxygen species (ROS) and promotes platelet adhesion and aggregation. In addition, decreased NO and the presence of ROS together stimulate vascular smooth muscle cell proliferation and migration into the intima leading to the production of extracellular matrix. These processes together exacerbate endothelial dysfunction which leads to atherosclerosis (Russo *et al*, 2002).

High levels of CRP can also induce the expression of adhesion molecules VCAM-1 and ICAM-1 by endothelial cells. Specifically, elevated levels of these adhesion molecules were seen at CRP levels of 5 mg/L and were maximal at 50 mg/L (Pasceri *et al*, 2000). Furthermore, CRP induces monocyte chemoattractant chemokine-1 synthesis which promotes leukocyte migration through arterial endothelium (Pasceri *et al*, 2001), and increases proliferation and migration of vascular smooth muscle cells and increases ROS production, all of which play a role in endothelial dysfunction and the development

of foam cells (Wang *et al*, 2003). These results indicate that CRP may not only be a marker of inflammation and atherosclerosis, but may actually promote the development of atherosclerosis.

Research indicates that CRP levels in the high-normal range (>3 mg/L) are a prognostic marker for future vascular events (Ridker *et al,* 1998, Ridker, 2001, Ridker *et al*, 2002). In male subjects followed over several years, those who were in the highest quartile range (≥ 2.11 mg/L) of baseline CRP had three times the risk of future myocardial infarction (MI) compared to those in the lowest quartile (Ridker *et al*, 1997). Ridker *et al* (1998) specifically looked at women and found that those who eventually developed cardiovascular events (over 3 years), had higher baseline CRP measures than their age and smoking matched controls.

In the Women's Health Study, investigators examined healthy postmenopausal women and compared CRP levels among those that had subsequent cardiovascular (CV) events to those who did not. In general, individuals with CV events had higher body weights at baseline and were more likely to have hypertension, diabetes, and/or parental history of early myocardial infarction. Baseline levels of CRP were significantly higher in women who subsequently had a CV event compared to those who did not. When compared to other inflammatory or lipid markers, CRP levels were the most powerful predictor of CV risk (Ridker *et al*, 2000).

Likewise, Ridker *et al* (2002) examined CRP and LDL cholesterol in women (45 years and older) over an eight year period, in order to establish the timing of the first CV event. A CV event was defined as nonfatal myocardial infarction, nonfatal ischemic

stroke, coronary revascularization procedures, and death from CV causes. Results showed that CRP was a stronger predictor of future CV events than LDL cholesterol. This prediction still held when analysis was adjusted for smoking, age, diabetes, blood pressure, and use of hormone-replacement therapy. Those with both high CRP and LDL cholesterol had the highest occurrence of a CV event.

CRP and metabolic disorders

In addition to playing a role in the development of CVD, inflammation may also play a role in the development of insulin resistance, metabolic syndrome and/or type 2 diabetes. In order to investigate this possibility, subjects from the Women's Health Study were screened to investigate whether or not an association existed between inflammation and later development of type 2 diabetes. At a four-year follow up, 188 confirmed cases of type 2 diabetes were identified (excluding those who developed type 2 diabetes within one year of baseline measures) and 362 matched controls were included. Anyone who developed type 2 diabetes within one year of baseline measures, had a HbA1c greater than 6.5%, or had a history of hypertension, hyperlipidemia, or used hormone replacement therapy were excluded (Pradhan *et al*, 2001).

At baseline, BMI, CRP, fasting insulin and HbA1c levels were significantly higher in those who subsequently developed diabetes compared to controls. In addition, those who later developed diabetes had a significantly lower frequency of exercise compared to controls at baseline. When baseline measures of CRP were broken into quartiles, the relative risk for increasing quartiles of CRP were, 1.0, 2.2, 8.7 and 15.7, respectively ($p < 0.001$) (Pradhan *et al*, 2001).

Since there was a significant correlation between BMI and CRP, BMI was divided and stratified into tertiles of low, medium and high and then the relative risk of incidence of diabetes was determined. Results indicated that higher levels of baseline CRP were associated with an increased risk of developing type 2 diabetes regardless of BMI. Even in women with a lower BMI (< 29 kg/m^2), there was an augmented stepwise elevation in risk with increased levels of CRP. Even after a fully adjusted model including BMI, family history, smoking, alcohol consumption, physical activity, and hormone replacement therapy, CRP remained a significant predictor of later development of type 2 diabetes (Pradhan *et al*, 2001). This association between CRP levels and type 2 diabetes is strengthened by Figaro *et al* (2006) who found that elderly patients with type 2 diabetes had significantly higher CRP values than those who did not. Additionally, Oberbach *et al* (2006), found that the mean CRP level in patients with type 2 diabetes, impaired glucose tolerance, and normal glucose tolerance was 7.5, 3.0, and 2.0 mg/L, respectively.

However, studies examining metabolic syndrome and CRP have not found similar results. Lamonte *et al* (2005) investigated the association between fitness, CRP and metabolic syndrome in a racially diverse sample of women. Metabolic syndrome was defined according to the National Cholesterol Education Program-ATP III criteria. For all women, the prevalence of metabolic syndrome, elevated CRP (> 2.0 mg/L), and combined metabolic syndrome and elevated CRP was 22.6%, 45.9%, and 17.8% respectively. Metabolic syndrome was significantly correlated with CRP levels. However, adjustment for fitness level (determined by maximal treadmill exercise test)

attenuated the association between metabolic syndrome and CRP. In addition, after adjusting for age and race, women with high CRP and metabolic syndrome were more likely to have an elevated Framingham score for coronary heart disease (CHD) risk than those without either risk factor. Again this association was eliminated when adjusting for maximal METs achieved on the treadmill test.

These results suggest that the association between CRP and metabolic syndrome is eliminated when fitness level is considered (Lamonte *et al*, 2005). However, it must be noted that BMI was controlled for in all analyses and BMI and fitness level are closely related. Controlling for BMI may explain why there was an association between parameters of metabolic syndrome and CRP and the lack of association between CRP and fitness level. Overall these data suggests that CRP may be an independent predictor for future development of diabetes or the metabolic syndrome and fitness level may attenuate this prediction.

CRP and obesity

Research indicates a significant correlation between obesity and CRP. Tchernof *et al* (2002) evaluated CRP levels in a group of obese Caucasian postmenopausal women. All women had a BMI > 27 kg/m^2 and did not participate in regular physical activity (<2 exercise periods per week). Body composition was measured via DEXA and CT scans. The average CRP for the group was 3.20 mg/L. CRP levels were positively and significantly associated with body weight, BMI, fat mass and fat free mass, intra-abdominal adipose tissue and triglycerides. There was a significant, negative correlation between CRP and insulin sensitivity (measured as glucose disposal via hyperinsulinemic-

euglycemic clamp). Multiple regression analysis revealed that body weight was the best predictor of CRP levels, explaining 18.1% of the variance.

The relationship between CRP, obesity, and the insulin resistant syndrome were investigated in 186 age-matched premenopausal and postmenopausal women. Regression coefficients showed that measures of obesity, i.e. BMI, waist and hip circumference, and waist-hip ratio, significantly correlated with levels of CRP. Association was the strongest for waist and hip circumference. After adjustment for BMI, waist circumference was the only measurement significantly associated with CRP. Additionally, waist circumference was the strongest predictor of CRP levels, accounting for 31.3% of the variance. This suggests that abdominal fat accumulation may be an important factor for the cardiovascular inflammatory response. Further analysis revealed that CRP levels were also associated with parameters of the insulin resistance syndrome: blood pressure, insulin, HDL-c, and triglycerides (Hak *et al*, 1999).

Furthermore, Rawson *et al* (2003) found that in a cross-sectional analysis of men and women, CRP was significantly related to BMI (measures were averaged over the course of the year). When subjects were distributed into tertiles based on BMI, mean CRP was significantly greater in obese (BMI >30 and mean CRP 3.2 mg/L) and overweight (BMI 25-29.9 and mean 2.1 mg/L) subjects. In this cohort, there was no significant association between level of physical activity (assessed by three 24 hour physical activity recalls where amount and intensity of activity was converted into MET levels) and CRP, thus the authors concluded that BMI, not physical activity, is an independent predictor of CRP levels. It must be noted that this lack of association can not

be extended to level of physical fitness since physical activity was measured based on activity recall and actual fitness level was not assessed. Previous studies finding a positive correlation between fitness and CRP often use maximal or sub-maximal testing. Overall, a clear positive relationship exists between CRP and body composition and/or adiposity.

CRP, gender and ethnicity

Several studies have shown that CRP levels are higher in females than males. Additionally, there seems to be a significant difference in CRP across different ethnic groups. Marcell *et al* (2005) demonstrated a gender difference in CRP levels in a group of overweight (mean BMI 33.7 ± 4.8 kg/m^2) middle-aged men and women. Results showed a significant positive correlation between CRP and % body fat (determined by DEXA) in women only. After comparing CRP between men and women, women's CRP was greater than 2-fold higher than men's, indicating gender may play a role in CRP.

Khera *et al* (2005) divided CRP values into low (< 1 mg/L), medium (1-3 mg/L) and high (>3 mg/L) in a group of men and women to investigate gender differences. Based on this division, women consisted of 40.7%, 41.9% and 61.9% of the groups, respectively. Based on the same division, African-Americans consisted of 25.8%, 24.3% and 32.3% of the groups, respectively. More specifically, 63.4% of African-American women had CRP levels > 3 mg/L compared to 50.4% of African-American men, 42.3% of Caucasian women and 29.1% of Caucasian men.

Lakoski *et al* (2006) found that a CRP cutoff of 3 mg/L was the 75^{th} percentile for men and 55^{th} percentile for women. The 75^{th} percentile cutoff for women was 5 mg/L

and 21% of all women had a CRP level > 10 mg/L. More specifically, the 75th percentile cutoff for African-American women was 7.5 mg/L. Furthermore, Heilbronn *et al* (2001) found in a population of obese women (BMI > 28 kg/m^2), that 74% of their CRP levels were > 3 mg/L.

Participants in the women's health study (mean age of 54.6 ± 7.1) were stratified by race to investigate ethnic differences in CRP, metabolic and lipid parameters. Analysis included 24,455 Caucasian (CC) and 475 African American (AA) women. Results showed that AA women were more likely to have higher BMIs and more likely to have diabetes. CRP levels in AA women (median 2.96 mg/L) were significantly higher (44.6%) compared to CC women (median 2.02 mg/L). After adjusting for age, smoking, diabetes status, alcohol use, hypertension, exercise, family history of myocardial infarction, estrogen use, education, and lipid levels the differences between the two groups remained. When adjusted for BMI (this also included age and estrogen use), there was still a significant difference between the two groups, but the difference dropped from 44.6% (unadjusted) to 10.6%. It is clear that BMI has an effect on the differences in CRP among these two ethnic groups (Albert *et al*, 2004).

Lamonte *et al* (2005) investigated the association between fitness, CRP and metabolic syndrome in a racially diverse sample of women. Results indicated that for BMI, waist circumference, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), CRP, and percent abdominal obesity, CC women had significantly lower values than AA women. Additionally, in AA women compared to CC, the prevalence of metabolic syndrome was 29.5% vs. 8.9%, elevated CRP levels (> 2.0 mg/L) was 70.5%

vs. 28.2%, and both metabolic syndrome and elevated CRP levels was 22.7% vs. 4.3%. These results show that the prevalence of metabolic syndrome and/or elevated CRP is significantly higher in AA women versus CC in this cohort (Lamonte *et al*, 2005).

Forty-four AA and forty-six CC women were assessed during the Cross-Cultural Activity Participation Study. Overall, AA women had significantly higher BMI, waist circumference and insulin, but had significantly lower fitness levels compared to CC women. Within this study, CRP correlated significantly with fitness (defined as amount of time spent on treadmill during graded exercise test), BMI, waist circumference, insulin, and triglycerides across both ethnic groups. The mean CRP level was significantly higher in the AA women (LaMonte *et al*, 2002).

Effects of exercise on CRP

Research investigating the relationship between CRP and physical activity is inconclusive. It has been shown that females with higher CRP values have significantly lower estimated VO_2max (Kuo *et al*, 2006). Kondo *et al* (2005) investigated the relationship between CRP and estimated maximal oxygen uptake. Subjects included 50 healthy, middle-aged men and women. Body fat (measured via skinfold thickness), body weight, BMI, glucose, insulin, insulin resistance (HOMA-IR), lipids, and CRP were measured. Analyses showed that CRP was positively correlated with percent body fat, BMI, and HOMA-IR. CRP was negatively correlated with HDL-c and estimated maximal oxygen uptake. In multivariate analysis, estimated maximal oxygen uptake was an independent factor for CRP, suggesting that increased physical activity alone might decrease CRP levels, improving inflammation.

Women with Type 2 diabetes were assessed for cardiorespiratory fitness and divided into low (LCF) and average (ACF) cardiovascular fitness groups (determined via VO_2peak performance), (difference in group averages, $p<0.05$). In addition, eight healthy older women without Type 2 diabetes were used as controls. There were no differences in classic markers of CVD (i.e. total cholesterol, HDL, LDL, and triglycerides) between LCF and ACF groups, but the LCF group had a significantly higher CRP level (mean 6.3 mg/L) than the ACF group (mean 1.9 mg/L). There were no differences in CRP values between the ACF and control groups. Within the groups, there was a significant positive correlation between CRP and HOMA-IR This study shows that high CRP levels are associated with lower fitness levels and HOMA-IR values (McGavock *et al*, 2004).

In an elderly population (greater than 70 years), a cross-sectional study examined the association between physical activity and markers of inflammation. Traditional, structured exercise done by the participants was divided into none, low (>0 to < 180 min/wk), and high (≥ 180 min/wk). Results indicated that CRP levels were significantly lower in the high exercise group. These significant results were found after adjusting for age, sex, and race and were still significant after further adjustment for smoking, alcohol, use of anti-inflammatory drugs and antioxidants, hypertension, arthritis, cardiovascular, cerebrovascular and peripheral vascular disease, osteoporosis, diabetes, respiratory disease and acute upper respiratory tract infection. However, when adjustments for total and visceral body fat (measured via DEXA and CT) were added, significant differences were lost ($p = .096$) (Colbert *et al*, 2004).

To further investigate the relationship between fitness levels and body composition with CRP, models were used that adjusted for all other covariates noted above. There was a significant relationship between BMI and CRP levels, but no significant interaction between BMI and exercise for CRP. These results suggests that body fat and not amount of physical activity is most important in predicting CRP levels (Colbert *et al*, 2004).

In contrast, several studies have shown that an increase in activity leads to a decrease in CRP levels. In a recent adolescent study, subjects which included 21 male and female, age 15-16 years, were divided into lean and obese groups. The obese adolescents were matched and randomized in to a control or physical activity-behavioral-diet based lifestyle intervention group. The treatment group participated in 45 minutes of physical activity three times a week. In addition to the activity regimen, their diet and everyday activity was modified. For example, meal portion were reduced, sugar-based drinks were reduced, and television time was limited. During this 3 month study, both the obese and lean control groups did not make any other activity or lifestyle changes (Balagopal *et al*, 2005).

Baseline measurements were used to compare all obese subjects to the lean subjects. CRP, BMI and percent body fat were all significantly higher in the obese adolescents. Furthermore, the obese group had a significantly higher calculated HOMA-IR compared to the lean subjects. At baseline, CRP was significantly correlated with BMI, percent body fat, and calculated HOMA-IR (Balagopal *et al*, 2005).

Comparing the obese intervention group against the obese control group, the intervention group maintained their body weight while the control group had a significant gain in body weight and percent body fat. The intervention group had a significant decrease in percent body fat and increase in fat free mass (measured via DEXA). In the intervention group, there was a significant decrease in calculated HOMA-IR. CRP decreased by 20-62% in seven of the eight subjects and the overall mean decrease was ~30% ($p = .02$). Changes in fitness level were not reported. This study shows that the obesity-related inflammatory process can start early in adolescences and can be reversed through early lifestyle intervention (Balagopal *et al*, 2005).

Oberbach *et al* (2006) looked at the effects of 60 minutes of exercise, three times a week for four weeks on CRP in a group stratified by glucose tolerance. Subjects were classified as normal glucose tolerant, impaired glucose tolerant, or type 2 diabetic. After 4 weeks of training, all three groups decreased CRP levels, percent body fat, BMI, waist-to-hip ration and increased VO_2max. In this population, CRP was significantly, positively correlated with percent body fat, BMI, glucose and insulin and negatively correlated with VO_2max.

Jae *et al* (2006) investigated the effects of a lifestyle intervention, consisting of exercise training, on CRP, obesity measures and fitness levels. The intervention consisted of 50-60 minutes of home-based exercise, 60-80% maximum heart rate, 5 times per week for 3 months. In addition, the participants attended diet education sessions and met with a physiologist every 2 weeks to monitor their progress. The control group received no education or exercise intervention. After 3 months of treatment, there was a

significant decrease in body weight, BMI, and CRP levels and a significant increase in VO_2peak. Further analysis revealed that improved VO_2peak and weight loss were independent predictors of decreasing CRP levels.

A group of Japanese women (both pre- and postmenopausal) underwent a 2 month long aerobic exercise program to assess the effects of exercise on inflammatory markers. The subjects participated in 2 structured workouts per week that lasted 110 to 140 minutes. These sessions consisted of 80 minutes of aerobic dance followed by 30-60 minutes of bicycle or treadmill exercise. In addition, they were asked to perform home-based exercise one or more days per week. At baseline, CRP was significantly positively correlated to body weight, BMI, waist-hip ratio, total cholesterol, LDL cholesterol, triglycerides, fasting glucose and insulin, HOMA-IR, and HbA1c. CRP was significantly negatively associated with HDL-c and peak oxygen uptake (Okita *et al*, 2004).

After 2 months of training, BMI, waist-hip ratio, body weight, lipid and glycemic measures and CRP levels were significantly reduced and fitness levels were improved (significance not reported). Further analysis showed that percent weight reduction significantly correlated with changes in lipid (except HDL-c) and glycemic measures, but decreases in CRP were not proportional with changes in body weight. It must be noted that the average CRP at baseline in this group was much lower (0.60 mg/L with interquartile range of 0.26-1.16) than seen in previous studies of sedentary, overweight individuals. Since CRP levels greater than 3.0 mg/L are considered high, it may be that there was no association with changes in body weight in this study because the CRP levels in these women were not elevated enough. In addition, the quartile with the largest

weight reduction did not have significant decreases in CRP. Again, the authors suggested that adverse effects of strenuous exercise and high pace weight reduction may include increases in inflammation, canceling out any improvements in CRP (Okita *et al*, 2004).

Marcell *et al* (2005) set out to determine the effects of different exercise regimens on CRP levels and to assess whether or not exercise-induced changes in insulin sensitivity was partially explained by changes in inflammatory markers. Fifty-one Caucasian, middle aged, sedentary men and women were randomized based on sex and insulin sensitivity (measured via euglycemic hyperinsulinemic clamp) into 2 different exercise groups or a control group. The exercise groups participated in 30 minutes of activity 5 days per week for 16 weeks. The exercise intervention was divided into a moderate intensity group with no specific heart rate requirements and an intense group defined as 80-90% of age-predicted maximal heart rate.

After the intervention, both the moderate and intense groups had a significant decrease in body weight, percent body fat (measured via DEXA), and BMI compared to the control group. Only the intense exercise group had a significant increase in insulin sensitivity and predicted VO_2 max compared to the other two groups. This study revealed no changes in CRP after exercise in either group. To further investigate this lack of effect, the subjects were divided into tertiles based on absolute change in VO_2max. There was still no change in CRP associated with improved fitness. Finally, subjects were divided into tertiles based on changes in body fat. The group with the greatest body fat loss (average 4% decrease) had a significantly greater percent change in insulin sensitivity than the other two groups, but there were still no changes in CRP. The

lack of differences between groups may be partially attributed to the fact that the control group decreased CRP levels by 1.0 ± 0.5 mg/L, the same decrease seen in the moderate exercise group. However, it is still surprising that the intense exercise group only decreased CRP levels by 0.4 ± 0.5 mg/L (Marcell *et al*, 2005).

In general, the results from this study suggest that moderate to intense exercise associated with a decrease in body weight, was not associated with a reduction in CRP, even when subjects were stratified by changes in fitness level and body fat. The authors suggest that weight loss was not great enough to see a reduction in CRP as seen in other studies and that calorie restriction may be needed. Furthermore, the fact that exercise itself has an inflammatory component may mask the effects of exercise on CRP if weight loss is not significant enough (Marcell *et al*, 2005).

Effects of diet-induced body weight changes on CRP

Some research indicates that changes in CRP are mostly due to reductions in body weight and/or fat mass and not to physical activity in and of itself. Twenty-five women underwent a weight loss protocol that consisted of 1200 kcal/day with no change in physical activity. This portion of the study lasted approximately 14 months. These women lost an average of 14.5 ± 6.2 kg of body weight ($p < 0.0001$), with a 25% loss in fat mass (kg). Weight loss was accompanied by a decrease in triglyceride levels and an increase in HDL-c and glucose disposal. There was a 32.3% reduction in CRP levels, with the average values decreasing from 3.06 to 1.63 mg/L. Changes in body weight and fat mass were significantly correlated with a reduction in CRP even after adjustments for hormone replacement, aspirin, and statin use (Tchernof *et al*, 2002).

Heilbronn *et al* (2001) investigated CRP measures in an obese population of women (BMI > 28 kg/m^2) after 12 weeks of calorie restriction. Subjects ate a diet of 1350 kilocalories/day with no change in their physical activity. At baseline, CRP levels in 74% of the women were greater than 3.0 mg/L and CRP was associated with waist circumference and BMI. At baseline, there was no association between fasting glucose and lipid levels and CRP.

After treatment, there was a mean decrease in body weight by 7.9 kgs. Total cholesterol, LDL-c and triglyceride levels were significantly reduced. There was a significant reduction in CRP and this reduction was correlated with overall weight loss, percentage weight loss, and baseline CRP levels. After weight loss, CRP was significantly correlated with BMI, waist and hip circumference, and triglycerides and change in CRP was correlated with change in total cholesterol (Heilbronn *et al*, 2001). These results strengthen the idea that a decrease in CRP is more closely related to changes in body composition rather than increase physical activity and/or improvements in fitness.

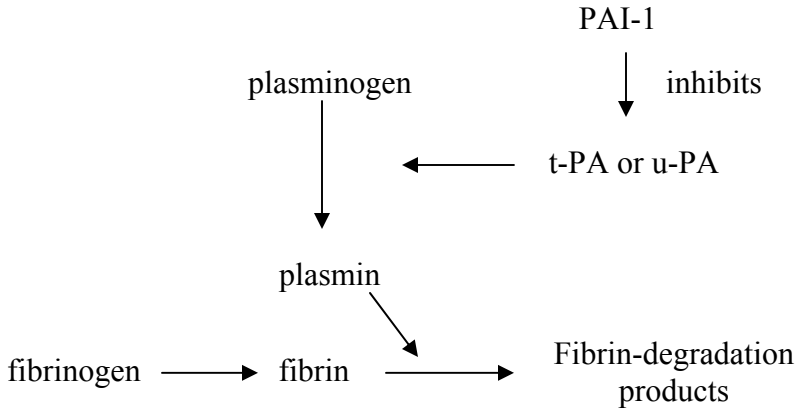
Plasminogen Activator Inhibitor-1 (PAI-1)

Type 1 PAI, or endothelial, is a single chain glycoprotein belonging to the serpin superfamily. It is synthesized in its active form and will remain this way if it binds to vitronectin, stabilizing the inhibitor. The synthesis of PAI-1 is increased by endotoxin, growth factors, hormones and cytokines (Juhan-Vague, 1999). PAI-1 is found in the blood in platelets (100-200 ng/ml) and in plasma (10-20 ng/ml). The levels of PAI-1 in

plasma can be measured as either antigen (ng/ml) or activity (units/ml). PAI-1 antigen levels are measured in plasma via ELISA with an antibody and activity is measured by a tPA coated ELISA, which assess PAI-1 binding. Both are highly correlated and either can be used for measurement purposes. Both PAI-1 activity and antigen fluctuates with circadian rhythm, with the highest measurements seen in the early morning (Kluft *et al*, 1988, Juhan-Vague *et al*, 1992).

The modulation of PAI-1 influences fibrin and extracellular matrix accumulation (Juhan-Vague *et al*, 1999). The fibrinolytic system dissolves fibrin deposits and disrupts blood coagulation, participating in lysis of intravascular thrombi. It is a proteolytic system in which plasminogen is converted to plasmin, often on the surface of thrombi, via tissue-type plasminogen activators (t-PA) or urokinase-type plasminogen activators (u-PA). Plasmin is a trypsin-like enzyme that degrades fibrin and extracellular matrix proteins. Degradation of fibrin is decreased with increased PAI-1 activity (Lindahl *et al*, 1996, Speiser *et al*, 1988).

t-PA is implicated in the degradation of intravascular fibrin and u-PA is produced mainly by macrophages and is implicated in tissue remodeling (Alessi *et al*, 2000). t-PA and u-PA are inhibited by PAI-1, and the modulation of PAI-1 influences fibrin and extracellular matrix accumulation thus making PAI-1 the main regulator of plasminogen activation and the fibrinolytic system. PAI-1 binds to t-PA or u-PA, forming an inactive complex, which is cleared from the system via the liver (Juhan-Vague *et al*, 1999, Mavri *et al*, 2004, Speiser *et al*, 1988).



In a normal system, there is an equilibrium between anticoagulant protease inhibitors (i.e. PAI-1) and activation of blood coagulation (often at the site of endothelial injury or inflammation). With elevated PAI-1 levels, there is excessive inhibition on t-PA and u-PA so that plasminogen does not convert to plasmin. Plasmin cannot degrade fibrin and the degradation of extracellular matrix is decreased. An excess of PAI-1 leads to hypofibrinolysis which decreases thrombolysis, diminishing the removal of thrombi, leading to a cardiovascular event (Lindahl *et al*, 1996, Mavri *et al*, 2004, Speiser *et al*, 1988).

PAI-1 and atherosclerosis

PAI-1 not only decreases fibrin degradation, but also influences cell adhesion and migration. This occurs because of the action of PAI-1 on uPA receptors (uPAR), specifically on smooth muscle cells (SMC). SMC migration requires cellular expression of integrins and vitronectin in the matrix. PAI-1 promotes u-PA clearance from its receptors which then decreases the interaction of uPAR with vitronectin. PAI-1 also directly interferes with uPARs interaction with vitronectin by acting as a competitive inhibitor for vitronectin. When PAI-1 is present, it inhibits the attachment of integrin to

vitronectin, which is necessary for SMC migration. These processes allow PAI-1 to influence the tissue remodeling processes, specifically cellular adhesion, migration and proliferation (Juhan-Vague *et al*, 1999, Stefansson and Lawrence, 1996).

The presence of atherosclerotic plaques are positively correlated with an increase in PAI-1 expression. Schneiderman *et al* (1992) studied gene expression of PAI-1 in sections of human atherosclerotic arteries. Results indicated that PAI-1 mRNA was significantly increased in atherosclerotic arteries as compared to normal or mildly affected vessels. *In situ* hybridization also revealed an intense signal for PAI-1 mRNA in cells surrounding plaque. Since it is known that PAI-1 is deposited into the extracellular matrix on smooth muscle and endothelial cells and preserves matrix structures from degradation (Mimuro *et al*, 1987), these authors suggest PAI-1 may play a significant role in the organization and incorporation of thrombi by limiting extracellular proteolysis in developing plaque lesions (Schneiderman *et al*, 1992).

Within SMCs, PAI-1 is significantly expressed in the fibrous cap. It is also detected in the area surrounding the necrotic core in macrophages. This evidence suggests that PAI-1 expression plays a role in thrombotic complications and extracellular matrix deposits (Lupu *et al*, 1993).

Some studies show that an increase in PAI-1 is predictive of cardiovascular events and mortality. Within a group of 109 men with a myocardial infarction before the age of 45, 16 men had at least one reinfarction and PAI-1 was independently related to reinfarction (Hamsten *et al*, 1987).

The ECAT (European Concerted Action on Thrombosis and Disabilites) Angina Pectoris Study followed patients for two years and compared subjects who had undergone coronary angiography who did or did not have a major coronary event (defined as sudden coronary death, and fatal and nonfatal myocardial infarctions). PAI-1 antigen and activity were significantly higher in the coronary event group. However, the significant differences between the means were washed out after adjusting for variables related to insulin resistance (triglycerides, HDL-c, BMI, systolic blood pressure, history of diabetes). In addition, correlational analysis showed a strong correlation between PAI-1 antigen and activity to BMI and triglyceride levels. In conclusion, the authors state that increased levels of PAI-1 antigen and/or activity are associated with an increase risk of subsequent coronary events (Juhan-Vague *et al*, 1996).

PAI-1 and metabolic disorders

Many studies have shown that PAI-1 plasma levels are strongly positively associated with insulin resistance syndrome and its parameters. Festa *et al* (2006) found that higher levels of PAI-1 over time are associated with a higher incidence of type 2 diabetes, independent of body weight and insulin resistance, and after adjusting for demographics, smoking, and baseline PAI-1 levels. Vague *et al* (1986) found that BMI and insulin were significantly, positively correlated with PAI-1 levels. Furthermore, stepwise analysis showed that these correlations were independent (Vague, *et al*, 1986). This is further supported by Eliasson *et al* (1994) who found that insulin levels are a strong predictor of PAI-1 activity. In addition, those with impaired glucose tolerance (measured via oral glucose tolerance test) tend to have increased PAI-1 activity.

Lindahl *et al* (1996) compared fibrinolytic activity to insulin resistance (via HOMA-IR), insulin sensitivity index (ISI), and fasting insulin levels in men and women. In both genders, PAI-1 activity was significantly positively correlated with fasting insulin and HOMA-IR and was significantly negatively correlated with ISI. When insulin resistance measures were divided into tertiles, there was a dose response. Men with the highest insulin resistance (upper tertile) had three times the PAI-1 activity compared to those in the lowest tertile, and in women PAI-1 activity was twice as high in the upper compared to lower tertile. Using multiple regression, HOMA-IR, triglycerides, DBP, and age significantly accounted for 42% of the variance for PAI-1 activity in men. In women, only HOMA-IR and BMI significantly accounted for 30% of the variance in PAI-1 activity (Lindahl *et al*, 1996).

Potter van Loon *et al* (1993) administered a hyperinsulinemic euglycemic clamp to obese nondiabetics and obese type 2 diabetics. Results showed that PAI-1 antigen was significantly related to level of insulin resistance. The combination of hepatic and peripheral insulin action, insulin and triglyceride levels, blood pressure, WHR, and BMI accounted for 76% of the variance of PAI antigen. The authors concluded that elevated levels of PAI-1 contribute to the increased risk of cardiovascular risk associated with insulin resistance.

In general, persons with type 2 diabetes have a higher BMI and higher levels of insulin, glucose, triglycerides, and total cholesterol and lower levels of HDL-c compared to those without type 2 diabetes. Additionally, PAI-1 activity has been found to be significantly higher (mean 21.8 units/ml) in the type 2 diabetics than in non-diabetics

(mean 7.7 units/ml). Correlation analysis showed that PAI-1 activity was significantly correlated with BMI and insulin and apolipoprotein B levels. However, partial correlation analysis showed that after adjustment for insulin, both BMI and apolipoprotein B levels no longer correlated with PAI-1 activity. Furthermore, the presence of coronary heart disease was associated with higher levels of PAI-1 activity (Juhan-Vague *et al*, 1989).

The Insulin Resistance Atherosclerosis Study (IRAS) examined subjects across different ethnic groups with varying states of glucose tolerance (measured via frequently sampled intravenous glucose tolerance test with minimal model analysis). Subjects were divided into one of three groups: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (DM). Mean PAI-1 antigen levels for the NGT, IGT, and DM were 19.8 ng/ml, 26.6 ng/ml, and 31.9 ng/ml respectively. PAI-1 antigen was significantly different between all three groups before and after adjustment for BMI, age, sex and ethnicity. However, after adjustment for insulin sensitivity, there were no longer significant differences between NGT and IGT groups. Differences between NGT and DM and IGT and DM remained significant (Festa *et al*, 1999).

PAI-1 antigen was significantly correlated with BMI, fasting insulin, proinsulin and insulin sensitivity within all groups. Even after adjustment for age, sex, ethnicity, BMI, and glucose tolerance, PAI-1 antigen was still significantly correlated with fasting insulin, proinsulin, and insulin sensitivity. This association of PAI-1 antigen and insulin and proinsulin remained after insulin sensitivity was factored into the model (Festa *et al*, 1999).

The multiple regression model suggested that BMI, insulin sensitivity, proinsulin, and fasting insulin were significant independent predictors of PAI-1 antigen and together accounted for 34.2% of the total variance. Furthermore, proinsulin levels were a stronger predictor than fasting insulin concentrations. This study demonstrates a positive relationship between insulin and it precursors and PAI-1 antigen levels. This association was found across varying states of glucose tolerance and was independent of insulin sensitivity. In addition, BMI and insulin sensitivity were found to have a significant relationship to PAI-1 antigen. Since BMI, glucose tolerance and fasting insulin levels are all variables associated with the insulin resistance, PAI-1 antigen may also be a contributing factor in insulin resistance (Festa *et al*, 1999).

A PAI-1 knockout mouse (-/-) model has been used to describe the relationship between PAI-1, obesity and insulin resistance markers. A euglycemic-hyperinsulinemic clamp was performed at baseline and at 12 weeks post-high fat diet. The control mice developed hyperglycemia, hyperinsulinemia, and hyperleptinemia in response to the high-fat diet, but the PAI -/- mice did not. In addition, glucose infusion rates to maintain euglycemia in the PAI -/- mice was significantly higher that in the controls, suggesting better insulin sensitivity. This suggests that a deficit in PAI-1 is helpful in protecting against diet-induced metabolic abnormalities (Ma *et al*, 2004).

PAI-1 and obesity

Obesity can be defined as the accumulation of excess body fat and PAI-1 seems to be linked to obesity. When women with different waist to hip circumference ratios (WHR) were matched with respect to age, body weight, lean body mass and body mass,

results indicated that women with elevated WHR also had elevated PAI-1 levels compared to those with low WHR (Landin *et al*, 1990).

In addition, it has been shown that weight loss, independent of triglyceride and insulin changes, is accompanied by a reduction in PAI-1 levels. When subjects underwent significant weight loss (7.4 kg in women and 9.4 kg in men), there was a significant reduction in mean PAI-1 antigen. There was a dose-dependent association between the degree of weigh-loss and decrease in PAI-1 antigen (Folsom *et al*, 1993).

It is generally accepted that PAI-1 is expressed mostly at the liver. However, expression at the adipose tissue may explain the correlation between changes in body weight and fatness and PAI-1. To investigate this hypothesis, Shimomura *et al* (1996) examined rat PAI-1 mRNA in fat tissue. PAI-1 mRNA expression was found in both visceral and subcutaneous fat. With the development of obesity over time (via ventromedial hypothalamus lesion), PAI-1 mRNA expression increased in visceral fat, but not in subcutaneous fat or the liver. Overall, PAI-1 mRNA was more abundant in visceral fat than in subcutaneous fat and liver. In support of these results, Bastelica *et al* (2002) found that *in vivo* visceral fat expressed almost 5-fold more PAI-1 mRNA than subcutaneous fat. These results suggest that visceral adipose tissue is an important factor in PAI-1 expression in obesity.

Furthermore, Shimomura *et al* (1996) looked at the expression of PAI-1 in different types of adipose tissue. In obese and non-obese subjects, the level of PAI-1 was significantly associated with the visceral fat area and not the subcutaneous fat area

(measured via computed tomography). When gender was factored in, these significant differences remained.

Histochemical studies have revealed interesting results concerning adipose tissue and PAI-1. When entire adipose tissue is analyzed for PAI-1 mRNA, little to no signal was detected. However, when tissue is broken down, the stromal cells elicited a much stronger signal than the adipocyte fractions. On the other hand, when adipocytes were incubated, they were the main source of PAI-1 synthesis. This suggests that adipocytes are the main source of PAI-1 synthesis under specific environmental conditions. Increased PAI-1 expression was found in both subcutaneous and omental fat, but omental tissue produced more PAI-1 than subcutaneous under incubation (Alessi *et al*, 1997). These findings are important since it is known that intra-abdominal visceral fat is a better indicator of heart disease than overall fat mass (Nakamura *et al*, 1994).

PAI-1, gender and ethnicity

Research examining gender and ethnic difference in PAI-1 are limited and inconclusive. Women tend to have higher fibrinogen levels than men and this elevation in fibrinogen is significantly associated with insulin resistance and hyperinsulinemia (Lindahl *et al*, 1996).

Toft *et al* (1997) investigated gender differences in a group of stable, untreated hypertensive patients. Age-adjusted correlations were run between several metabolic parameters and PAI-1 activity. In men, plasma glucose and insulin sensitivity index (ISI) were significantly associated with PAI-1 activity. In women, BMI, WHR, plasma glucose, insulin, proinsulin, c-peptide, ISI, and serum triglycerides and VLDL cholesterol

were significantly associated. Subjects who were glucose intolerant (measured via oral glucose tolerance test) had significantly higher PAI-1 activity, ($p=0.04$). When BMI and WHR ratio were correlated with metabolic parameters, only plasma glucose and insulin were significantly associated with PAI-1 activity in both genders. There were no difference in PAI-1 activity between males and females (Toft *et al*, 1997). In multiple stepwise regression analysis, plasma glucose, and age were significantly associated with PAI-1 and accounted for most of the variance in men. Neither WHR nor BMI was associated with PAI-1 activity in men. In women, insulin sensitivity accounted for most of the variability. BMI and WHR and to some degree ISI, were strong determinants of elevated PAI-1 activity in women and not men. Even though there were no gender differences, levels of PAI-1 activity in males and females are influenced by different factors (Toft *et al*, 1997).

Furthermore, van Harmelen *et al* (2000) found no difference in PAI-1 secretion in adipose tissue between men and women. They did find that plasma PAI-1 activity levels were higher in men than women, but this significance was lost when waist-to-hip ratio was factored in. In contrast, Mansfield *et al* (1996), found that PAI-1 activity was significantly higher in females than males in a population of type 2 diabetics. Even when BMI, glycosylated hemoglobin, and cholesterol were factored in, sex remained an independent predictor of PAI-1 activity level.

Festa *et al* (2003) investigated ethnic differences in plasma PAI-1 levels and genetic polymorphisms. African-Americans had a lower prevalence of the 4G/4G and 4G/5G genotypes that are often associated with higher PAI-1 levels. In addition, PAI-1

antigen levels were significantly lower in African-American (13.38 ng/ml) than non-Hispanic whites (18.19 ng/ml).

To further explore ethnic differences in PAI-1 and obesity, Solano *et al* (2003) compared obese and overweight African American (AA) and Caucasian (CC) premenopausal women. All subjects had BMI > 25 kg/m^2 and were in good health and both groups had comparable levels of physical activity and nutrient intake. There were no differences between AA and CC women in BMI, fat mass and % body fat (measured via BIA). However, AA women had a significantly lower visceral adipose tissue (VAT), area and volume (measured via MRI). In addition, AA women had significantly lower levels of triglycerides than CC women and there were no differences in other lipid measures, insulin, glucose, HOMA-IR, leptin, or PAI-1 antigen. Mean (SD) PAI-1 levels were 44.8 (± 29) ng/ml in Caucasian women and 48.1 (32) ng/ml in African-American women.

Correlational analysis showed that PAI-1 was significantly correlated with HOMA-IR and fasting insulin in all women. However, after adjusting for age and fat mass, this association was lost in the CC women but not in the AA women. After the age and fat mass adjustment, PAI-1 was significantly related to VAT volume, DBP, triglycerides, HDL, and glucose in the CC women only. No other significant partial correlations were found in the AA women other than insulin and HOMA-IR (Solano *et al*, 2003).

Multiple regression analysis that adjusted for age, HOMA-IR glucose and triglycerides revealed that VAT volume was the only independent predictor of PAI-1 in

CC women. In AA women, HOMA-IR was the only independent predictor of PAI-1 and explained 30.6% of the variance. This study showed a lack of association between PAI-1 and visceral adiposity in AA women. This may suggest that in African-American women, insulin resistance (measured via HOMA-IR) is associated with elevated PAI-1 and not obesity itself in AA women, whereas in CC women, VAT volume seems to play a major role in PAI-1 levels. This suggests that there may be an ethnic difference in the pathophysiologic impact of VAT (Solano *et al*, 2003).

Effects of exercise on PAI-1

It has been proposed that regular physical activity can deter age-related effects of the fibrinolytic system. DeSouza *et al* (1998) examined the differences in fibrinolytic variables in young and old sedentary and physically active women. Old sedentary women had significantly higher levels of PAI-1 antigen and PAI-1 activity than young sedentary women, suggesting an age factor in levels of PAI-1. There were no differences in PAI-1 antigen and PAI-1 activity between young active and old active women, however young and old active women had significantly lower levels of both compared to there age-matched counterparts. This suggests that physical activity may prevent the decline in fibrinolytic function associated with aging.

Several studies have investigated the effect of physical activity on PAI-1. Fifty-two obese (BMI $\geq$ 25 kg/m^2) and nineteen lean premenopausal women were recruited to participate in a body weight reduction program. At baseline, the obese women had significantly higher BMI, estimated % body fat ([0.439 x waist circumference in cm] + [0.221 x age in years] – 9.4), and insulin, triglyceride, leptin and PAI-1 antigen. In

addition there was a significant correlation with PAI-1 activity and antigen and BMI, wait-to-hip ratio, estimated % body fat, triglycerides, glucose, insulin, and leptin levels (Marvi *et al*, 1999).

The weight reduction program consisted of a low-calorie diet and physical activity prescribed as two ten minute aerobic bouts a day and 1 hour of supervised activity twice a week. After one week of the program, there was a 31% decrease in PAI-1 activity and 26% reduction in PAI-1 antigen ($p<0.01$). At the end of the program (10-12 weeks), there was a significant decrease in BMI, WHR, % body fat, glucose, leptin, and PAI-1 activity and antigen. PAI-1 activity and antigen was reduced to the levels seen in the lean controls and PAI-1 activity actually became lower than observed in the lean controls. Overall, PAI-1 activity decreased by 74% and antigen decreased by 54%. At a 5 month follow-up, those who regained greater than 25% of their weight had a significant increase in PAI-1 and activity, which paralleled the increase in BMI (Marvi *et al*, 1999).

Correlation coefficients revealed that change in PAI-1 activity and antigen were significantly related to change in BMI, % body fat, insulin and leptin at the end of the program. At the 5 month follow-up, change in PAI-1 activity was significantly related to change in BMI and % body fat, whereas PAI-1 antigen was only related to change in % body fat. Multiple regression analysis revealed that only changes in BMI were significantly and independently associated with changes in PAI-1 and accounted for 34% of the variance. This study showed that a reduction in body weight resulted in a reduction in PAI-1 levels (Marvi *et al*, 1999).

In a group of obese subjects with impaired glucose tolerance, Lindahl *et al* (1999) administered an intense lifestyle intervention to assess changes in PAI-1 activity. Treatment subjects were admitted to a wellness center for one month and participated in an intense activity schedule of 2.5 hours of daily low-moderate activity. In addition, subjects were subjected to a low-fat, high-fiber diet and counseled on lifestyle changes. The control group was only counseled and given written and oral instructions on lifestyle change. Twelve months from initial evaluation, subjects were called back in for a follow-up. At follow-up, the intense treatment group had significant changes in (group x time effect) in BMI, body weight, WHR, VO_2 max, and PAI-1 activity.

In a 10 week training study, post- and pre-menopausal women participated in aqua exercise and resistance training 3 days per week. The training program resulted in a decrease in percent body fat and total fat mass (TFM, measured via BodPod), BMI, and PAI-1 antigen. VO_2max trended towards an increase, but was not significant. The relationship of PAI-1 to fat mass was examined and found that the ratio of PAI-1/TFM significantly decreased. However, further analysis revealed that there was no correlation between TFM and PAI-1 antigen or changes in PAI-1 antigen before or after training (Hayase *et al*, 2002).

Although there is an obvious association between PAI-1 and exercise, limited research has attempted to explain the mechanisms behind it. Specifically, can exercise decrease PAI-1 levels independent of body weight changes? In an attempt to investigate this, Bodary *et al* (2003) examined the effects of short-term exercise on PAI-1 activity and antigen and other metabolic parameters, independent of body weight changes.

Thirty-two sedentary men and women between the age of 50 and 70 years participated in either an eleven day exercise protocol or were assigned to a control group. The exercise protocol was 50 minutes per day at 65% heart rate reserve. There were no differences between the groups on any of the measures before or after exercise. After 21 days of training, neither resting levels of PAI-1 activity or antigen had changed. This study design found no significant reductions in body weight or PAI-1 activity, suggesting that decreases in PAI-1 with exercise may be due to body weight changes and/or a more long-term training effect (Bodary *et al*, 2003).

Relationship between CRP and PAI-1

Devaraj *et al* (2003) has shown a significant relationship between levels of CRP and PAI-1. CRP increases the expression of PAI-1 in human aortic endothelial cells (HACEs). Incubation of HAECs with CRP resulted in a significant increase in PAI-1 and activity. In addition, increased CRP caused a dose-dependent increase in intracellular PAI-1 protein and mRNA levels. All of these effects were maximal at 12 hours of incubation. These results suggest that CRP and PAI-1 may be closely linked in the progression of atherosclerosis.

Lifestyle Activity Modification versus Structured Physical Activity

Past traditional exercise guidelines consisted of structured exercise of at least 60% of maximal oxygen uptake for up to 60 minutes, three times a week. More recent guidelines introduced by the Centers for Disease Control and the American College of

Sports Medicine recommend that adults perform 30 minutes of continuous or accumulated, moderate-intensity physical activity most, if not all, days of the week (Pate *et al*, 1995, US Department of Health and Human Services, 1996). There have been few randomized controlled studies investigating the differences between several short bouts of exercise (i.e. intermittent exercise) and long, continuous bouts of exercise (i.e. structured exercise) and their effects on cardiovascular fitness.

Dunn *et al* (1999) investigated the hypothesis that lifestyle activity modification (LAM) results in higher levels of overall physical activity and improved cardiovascular risk factors at 24 months compared to baseline. Healthy sedentary men and women were divided into either a structured exercise protocol or a lifestyle physical activity program. Both groups underwent a psychological behavior change program. For the first 6 months, both groups took part in an intensive intervention and for the following 18 months, a maintenance program was established. The structured exercise group participated in exercise at 50-85% maximal aerobic power for 20-60 minutes on 3-5 days per week. The LAM group was instructed to participate in 30 minutes of moderate-intensity physical activity most, preferably all days of the week in a manner that was adapted to their individual lifestyle.

At baseline both groups were similar with a mean BMI of 28.4 and 28.0 kg/m^2 and a mean percent body fat of 31.5 and 30.9 for the LAM and structured exercise groups, respectively. At 24 months, both groups significantly increased their VO_2peak and energy expenditure, and decreased their percent body fat (measured via 7-site skinfold thickness) and overall blood pressure with no significant differences between the

groups. Only the structured exercise group significantly decreased their total cholesterol and LDL-C and increased their HDL-C levels. The structured exercise program decreased their body weight (kg) more than the LAM group, but not significantly. The LAM group increased their moderate-intensity physical activities nearly 3 times that of the structured exercise group (Dunn *et al*, 1999).

This study illustrated that the LAM group improved physical activity and overall fitness similar to those in the structured group and supports the hypothesis that the new LAM recommendation will lead to favorable fitness outcomes in the same manner as the old recommendation. In addition, the LAM treatment was beneficial for decreasing percent body fat and blood pressure, variables known to be associated with CVD. Additionally, the structured exercise group significantly decreased their fitness level during the maintenance period (6 months to 24 months) compared to the LAM group . These results indicate LAM may be more effective for long-term behavior change (Dunn *et al*, 1999).

Using a similar study design, Schmidt *et al* (2001) recruited thirty-eight, sedentary, overweight (BMI >27 kg/m^2) females to participate in a 12 week exercise study. Subjects were divided into one of four groups: control, or 30 minutes of exercise that was either continuous, 2 bouts of 15 minutes, or 3 bouts of 10 minutes. The three intervention groups were asked to participate in their exercise protocol 3-5 days per week at 75% heart rate reserve. All subjects were instructed to follow a calorie-restricted diet of 80% of their resting energy expenditure (measured via metabolic cart).

Results showed that for all groups, there was a significant decrease in calorie consumption by 6 weeks that remained constant for the rest of the study. There were no differences in calorie consumption between any of the four groups. For the three exercise groups, there was a significant decrease in body weight, BMI, sum of skinfolds, and waist circumference at 12 weeks compared to baseline. In addition, there was a significant increase in VO_2max in all three exercise groups. There was no difference between groups in any of these measures and there were no significant changes in the control group. This study illustrates that all three exercise protocols resulted in similar improvements in health. While the reduction in calories probably contributed to these improvements, all groups reduced calories to the same extent. Therefore, the diet effects should have been similar across groups and any differences due to exercise regimen should have been teased out (Schmidt *et al*, 2001).

Donnelly and colleagues (2000) investigated the difference between an 18 month traditional continuous exercise program and an intermittent exercise program. Twenty-two overweight (BMI > 25), sedentary women were divided into either a continuous (CON) or intermittent (INT) exercise group. The CON group performed 30 minutes of continuous exercise at 60-75% of maximal aerobic capacity, three times per week. The INT group performed two 15 minute bouts of walking at 50-65% HRR, five days per week.

All measurements were assessed at baseline, 9 months, and 18 months. Total kilocalorie intake did not significantly change during the 18 month period as reported by 3-day recall. However, the INT group did significantly decrease the amount of calories

from fat over the course of the study. Body weight and percent body fat (measure via hydrostatic weighing) decreased significantly across the 18 months of the study in the CON group. The INT group had a significant decrease in these measures at 9 months, but returned to baseline by 18 months. Both groups had a significant increase in maximal oxygen consumption after 18 months. Additionally, both groups had a significant increase in HDL cholesterol. The results of the oral glucose tolerance tests revealed a decrease in the area under the curve for insulin in both groups, but only the INT group had a decrease in fasting insulin (Donnelly *et al*, 2000). This suggests that both groups improved their insulin response and/or glucose uptake during a glucose challenge, but only the INT group had an improvement in baseline insulin measures. This could indicate an improvement in either β-cell function or an improvement in basal insulin sensitivity.

Overall there was a 2.1% decrease in body weight in the CON group by 18 months and a 1% decrease in the INT group at 9 months only. The INT group returned to baseline body weight by 18 months. The authors acknowledge that the changes in body weight for both groups were small and that these small changes are not usually associated with improvements in cardiovascular risk factors such as lipids and insulin. However, they found an increase in HDL cholesterol and an improved insulin response to glucose load in the INT group irrespective of body weight changes. Therefore, they conclude that these improvements may be contributed to their change in physical activity alone. In addition, improvements in HDL and metabolic parameters were similar between groups

suggesting that intermittent exercise is as effective as continuous exercise in improving these variables (Donnelly *et al*, 2000).

Further studies have reported similar results. Jakicic *et al* (1995), prescribed a twenty week intervention, for obese, sedentary females, in which there was a short bout group and a long bout group. Both groups exercised for 20-40 minutes, the short group did 10 minute bouts while the long group did one continuous bout, five days per week. In addition, both groups participated in a dietary program that reduced total caloric intake and fat intake. Results indicated that even though both groups significantly improved their predicted VO_2max, the short bout group may produce better overall health gains since this group's adherence was better and they reported exercising more days per week and the trend for weight loss was greater in this group.

DeBusk *et al* (1990), compared the difference between 30 minutes of continuous exercise and three, 10 minute bouts in healthy middle-aged men for 8 weeks. For both groups, VO_2max increased significantly. Murphy and Hardman (1998) compared long and short bouts of walking in sedentary women. Both groups walked for 30 minutes, 5 days a week, at 70-80% of their maximum heart rate for 10 weeks. The long bout consisted to one 30 minute walk and the short bout consisted of three, ten minute bouts. Again, both groups significantly increased their VO_2max. In addition, Murphy and Hardman (1998) found a decrease in the sum of skinfolds in both groups, but only the short bout groups saw an improvement in body weight and waist circumference.

Summary

Past research is still controversial as to what extent LAM improves cardiovascular fitness, increases physical activity and improve body composition and decreases body weight. Additionally, more research is needed to determine if LAM, in comparison to structured exercise, will also improve non-traditional cardiovascular risk factors that are linked to cardiovascular disease, possibly decreasing the risk of atherosclerosis and the development of insulin resistance syndrome and/or type 2 diabetes. It is clear that PAI-1 and CRP are nontraditional risk factors that may be useful in examining this. Specifically, these studies need to be investigated in populations at risk for cardiovascular disease and for which little information has been accumulated, such as African-American women.

CHAPTER III

OUTLINE OF PROCEDURES

Subjects

Twenty-four apparently healthy, sedentary African-American female volunteers, 22-53 years of age, with a BMI ≥ 23 kg/m^2, were recruited and randomly assigned to one of three groups; control, lifestyle-activity modification, or traditional exercise. Sedentary individuals were defined as those not participating in any regular physical activity for at least the past 6 months. Recruitment included placement of flyers on the University of North Carolina at Greensboro, North Carolina Agricultural and Technical State University, and Bennett College campuses, around the Triad area, and announcements made through local women's organizations. Exclusion criteria included:

1. History of myocardial infarction, stroke, or diagnosed diabetes.
2. Currently engaging in physical activity at least 3 days per week for ≥ 20 minutes each time, for at least the past 6 months.
3. Currently engaging in an organized weight loss program.
4. Planning to move from the local area during the length of the study.
5. Pregnant or planning to become pregnant.
6. Taking medication that could alter exercise performance, appetite or metabolism (excluding oral contraceptives).

Assessment

Subjects underwent an orientation session at which time the details and protocol for the study were explained and eligibility criteria were assessed. Eligible subjects completed a medical history (see Appendix A) and physical activity readiness (PAR-Q) questionnaire (see Appendix B), which were used to determine eligibility. Informed consent forms were signed (see Appendix C). All assessments were completed at pre- and post-intervention.

Metabolic and Inflammatory Markers

Approximately 60 milliliters of blood was collected into plain and EDTA filled vacutainer tubes after an overnight fast of at least 10 hours. Standard venipuncture technique was used to collect blood from the antecubical vein by a certified phlebotomist. After collection, the EDTA tube was placed on ice and the other tube was left at room temperature for at least 30 minutes. Both tubes were then centrifuged and serum and plasma were drawn off and stored at -80^{o}C until analysis.

C-reactive protein levels were determined via an Ultra-sensitive ELISA from Diagnostic Systems Laboratories, Inc. PAI-1 was measured by immuno-assay from HYPHEN BioMed. Insulin was determined by ELISA from Mercodia and glucose was determined by colorimetric assay from Roche Diagnostics Systems (see Appendix D). Serum was used for CRP and EDTA plasma was used for PAI-1, insulin and glucose. Intra-assay coefficients for all measurements were less than 10%.

Insulin resistance was assessed using the Homeostasis Model Assessment (HOMA-IR), which was calculated as insulin (uIU/ml) * glucose (mmol/L) / 22.5 (Matthews, 1985).

Anthropometric Measurements

Height was measured using a wall-mounted stadiometer and weight using a standard physician and hospital balance beam scale. Body weight and height was measured barefoot, to the nearest 0.1 kg for weight and the nearest 0.1 cm for height. BMI was calculated using body weigh and height as follows: weight (kg)/height (m^2). Waist circumference (cm) was measured three times for reliability and the average was taken. Circumference was measured at the narrowest part of the torso, above the umbilicus, using a tension-scale gauged measurement tape (ACSM, 2005).

Body Composition

Body composition was assessed via 3-site skinfold (tricep, suprailiac, and thigh) using Harpendon skin fold calipers. Body density was calculated from three-site skinfold using the Jackson and Pollock equation and percent body fat was calculated using the Siri equation (ACSM, 2005). Although there are ethnic specific calculations for percent body fat, the Jackson and Pollock equation is widely used and has been extensively validated.

Physical Activity

Energy expenditure was measured via Omron pedometer (Model HJ105). The total number of steps was averaged from seven days at both baseline and at 12-weeks post-intervention.

Cardiorespiratory Fitness

Subjects completed a sub-maximal cycle ergometer test following the YMCA protocol set by the American College of Sports Medicine (ACSM, 2005). After habituation to testing procedures and equipment, subjects were equipped with a heart rate monitor and testing began. The following YMCA sub-maximal procedure was used:

1. A 2-3 minute warm-up on the cycle ergometer at the workload that was used for stage one.
2. The workload for the first stage of the test was 150 kgm/min (0.5 kg at 50 rpm).
3. Once steady-state was reached, the heart-rate in the third minute of the stage dictated the workload for the next stage in accordance with the ACSM's Guidelines for Exercise Testing and Prescription (2005).
4. The third and fourth (if required) stages were set according to the same criteria used in stage 2.
5. Upon completion of two consecutive stages that met the criteria for cessation, the workload was dropped to the level of the warm up or less for at least a 2-minute cool-down stage.
6. The subject's heart rate was monitored following exercise and the subject was asked to remain in the lab until heart rate reached the individual's pre-exercise level.

7. The heart rate measured during the last stage of the exercise protocol was plotted against work rate as outlined by the ACSM's Guidelines for Exercise Testing and Prescription (2005).

Exercise Protocols

Subjects were randomly assigned to one of three intervention groups for 12 weeks: control group, lifestyle activity modification (LAM) group, or traditional exercise (EXE) group.

Control Group

The control group met for risk factor education seminars during weekly one-hour meetings for the first six weeks and bi-weekly meetings for the second six weeks. They were instructed not to change their physical activity, which was verified via pedometer data. Subjects in this group were offered free participation in an exercise program upon completion of this study.

Lifestyle Activity Modification

The participants were instructed to increase levels of low to moderate intensity activity in order to accumulate approximately 150 minutes of activity weekly, or approximately 30 minutes of accumulated activity on most days of the week. This group also participated in a lifestyle activity curriculum during weekly one-hour meetings for the first six weeks and bi-weekly meetings for the second six weeks. They were educated about the incorporation of exercise into activities of daily living. Information was

disseminated according to the curriculum recommended by the CDC's Division of Nutrition and Physical Activity in its Guide for Community Action (http:/www.cdc.gov/nccdphp/dnpa/physical/index.htm).

Traditional exercise group

The participants were prescribed traditional exercise using exercise equipment of the subjects' choice. Each subject participated in four low to moderate level (50-65% predicted maximum heart rate) group exercise sessions per week (40-60 minutes per session) to accommodate the recommendation that individuals accumulate at least 150 minutes of low-moderate intensity exercise each week. Each participant was equipped with a heart rate monitor to ensure accuracy of exercise level.

Adherence

Adherence the control and LAM groups were based on attendance to group meetings. Adherence for the traditional exercise group was based on attendance to exercise sessions. In order to be included in analysis, subjects attended at least 75% of their prescribed intervention, similar to methods for assessing adherence and attrition used in Project Active (Dunn,1997).

Data Analysis

All analysis was performed using STATISTCA 6 software package. Statistical significance was set at $p = 0.05$. All measures were checked for normality using Shapiro-Wilks test. Transformations were performed on measures with a significant difference in normality ($p < 0.05$). Transformations were done in the following order until the p-value was greater than 0.05: logarithm, inverse logarithm, square root. Given the small number of subjects in the study, normality was checked and transformations were made in order to meet the assumption for parametric statistics. Descriptive statistics and baseline group differences were checked using a one-way ANOVA. Repeated measures ANOVA was performed to check for a group x time interaction effect. Tukey HSD was used for all post-hoc analysis. Correlational statistics were run on all data as pre- and post-values combined. Spearman correlations were run on all the delta change data.

CHAPTER IV

RESULTS

Twenty-four subjects finished the study. One subject was eliminated because a baseline blood sample was not available. Two subjects were eliminated because baseline subject characteristics could not be clarified, leaving twenty-one subjects for data analysis. Descriptive statistics are shown in Tables 1 and 2, stratified by group. Predicted VO_2max, steps, CRP, PAI-1, insulin and HOMA-IR were significant for normality check and transformations were necessary to normalize the data. Steps, PAI-1, insulin, and HOMA-IR required log transformations, predicted VO_2max required inverse log and CRP required square root transformations. All tables show original, non-modified data, but statistical analyses were performed on the appropriately transformed data. There were no significant differences between groups on any baseline measurements.

Table 1: Baseline subject characteristics for each group. Values are mean (SD).

	Ctrl N=4	LAM N=9	Exe N=8	p-value
Age (years)	40.8 (10.7)	40.1 (9.5)	38.9 (10.7)	0.95
Weight (kg)	94.1 (15.6)	80.2 (18.5)	80.2 (18.5)	0.58
Waist (cm)	93.5 (12.4)	87.4 (18.7)	85.4 (14.8)	0.72
BMI (kg/m^2)	34.7 (4.8)	31.5 (8.0)	30.1 (6.4)	0.57
Predicted VO_2max (ml/kg/min)	23.43 (10.74)	20.73 (5.8)	22.94 (6.98)	0.76
% Body Fat	37.2 (6.3)	33.8 (8.3)	35.2 (8.5)	0.79
Steps	5457 (4120)	6934 (4651)	5424 (1433)	0.72
CRP (mg/L)	33.0 (12.7)	22.0 (22.2)	21.6 (21.7)	0.46
PAI-1 (ng/ml)	24.9 (17.9)	20.1 (24.0)	24.9 (17.4)	0.42
Insulin (uU/ml)	9.0 (3.8)	9.1 (10.8)	11.0 (4.3)	0.34
Glucose (mmol/L)	4.8 (1.3)	4.1 (1.2)	5.1 (1.2)	0.24
HOMA-IR	1.9 (0.8)	1.7 (1.9)	2.5 (1.3)	0.15

Ctrl = controls, LAM = lifestyle modification, Exe = traditional exercise
* average number of steps from seven days

Table 2: Post-intervention subject characteristics for each group. Values are mean (SD).

	Ctrl N=4	LAM N=9	Exe N=8
Weight (kg)	94.8 (14.8)	83.1 (25.2)	78.7 (18.4)
Waist (cm)	98.0 (13.8)	86.0 (19.1)	83.3 (13.3)
BMI (kg/m^2)	35.0 (4.2)	31.3 (8.1)	29.6 (6.1)
Predicted VO_2max (ml/kg/min)	20.28 (8.37)	24.71 (6.52)	29.64 (9.54)
% Body Fat	38.3 (6.4)	31.6 (9.3)	32.1 (8.2)
Steps*	7639 (3599)	10431 (2342)	11800 (7390)
CRP (mg/L)	24.3 (4.2)	21.9 (19.4)	28.8 (17.4)
PAI-1 (ng/ml)	41.6 (29.1)	14.7 (5.6)	29.1 (22.8)
Insulin (uU/ml)	10.2 (4.1)	9.9 (9.1)	7.8 (2.7)
Glucose (mmol/L)	4.5 (1.1)	4.4 (0.8)	4.3 (1.3)
HOMA-IR	1.9 (0.4)	1.7 (1.3)	1.4 (0.4)

Ctrl = controls, LAM = lifestyle modification, Exe = traditional exercise
* average number of steps from seven days

Repeated measures ANOVA showed no overall group effects, however several time effects were found (Table 3).

Table 3: Repeated Measures ANOVA p-values for group, time and group by time interaction.

	Group	Time	Group x Time
Weight (kg)	0.53	0.33	0.20
Waist (cm)	0.52	0.54	<0.001
BMI (kg/m^2)	0.51	0.42	0.21
Predicted VO_2max (ml/kg/min)	0.43	0.01	<0.001
% Body Fat	0.60	<0.001	<0.001
Steps*	0.42	0.001	0.95
CRP (mg/L)	0.54	0.83	0.21
PAI-1 (ng/ml)	0.19	0.10	0.70
Insulin (uU/l)	0.54	0.97	0.33

* average number of steps from seven days

The control group had a significant increase in waist circumference with an average of 93 cm at baseline and 98 cm 12-weeks later (Figure 1). There was no significant difference in waist circumference in the two intervention groups, however there was a trend towards a decrease. Although there was no significant change in body weight (Figure 2) or BMI (figure 3)in the exercise group, they did have a significant decrease in percent body fat (Figure 4). The average body fat dropped from 35% to 32% (p=0.004). The control group had relatively no change in percent body fat or BMI.

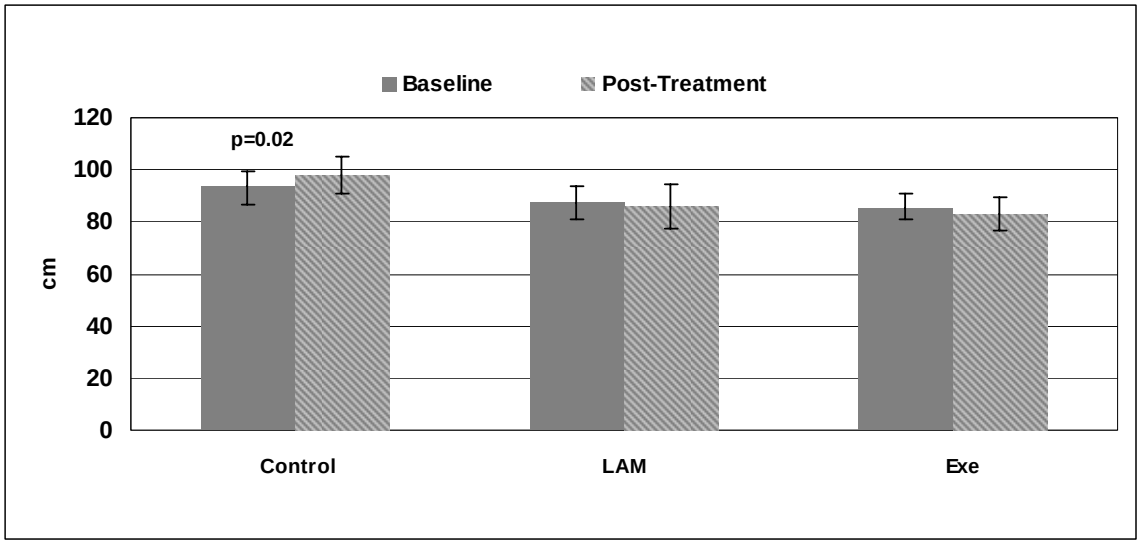


Figure 1: Waist circumference at baseline and post-intervention for each group. Values are mean (SEM). P-values compare baseline and post-treatment values within group.

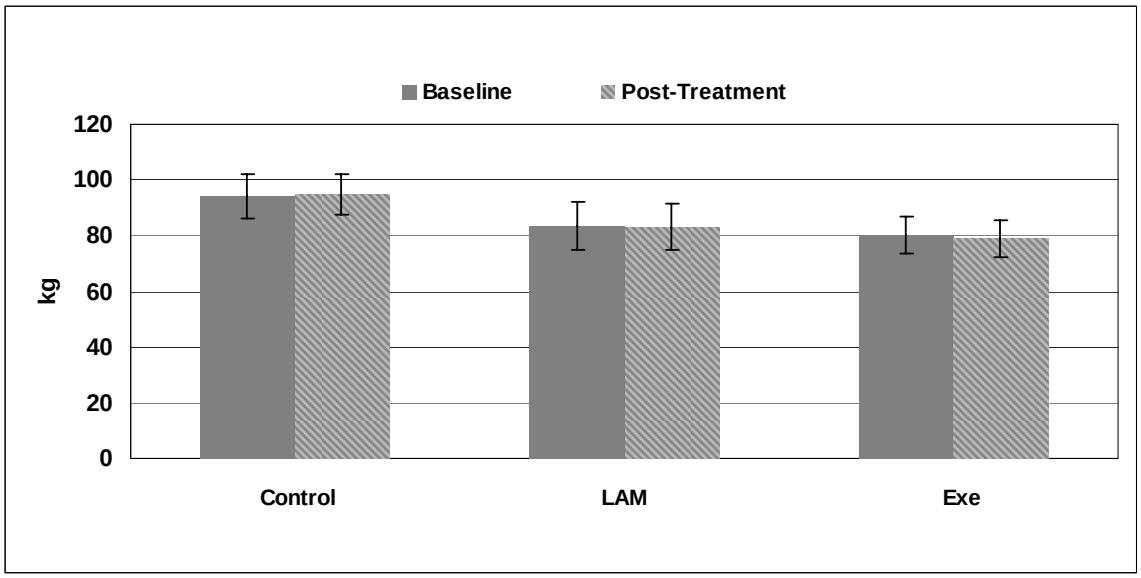


Figure 2: Body weight at baseline and post-intervention for each group. Values are mean (SEM).

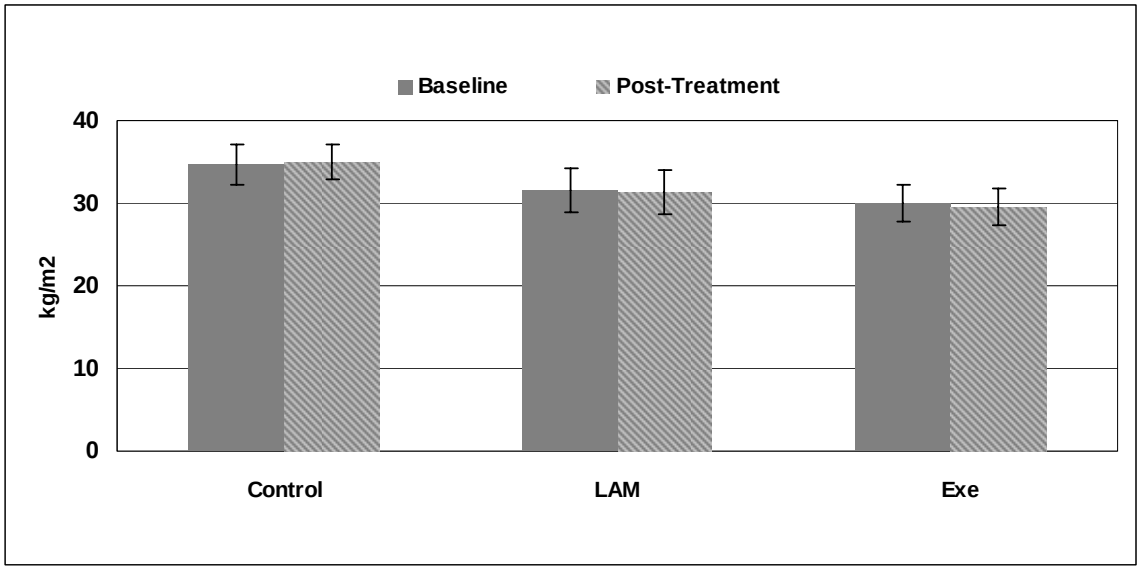


Figure 3: Body mass index at baseline and post-intervention for each group. Values are mean (SEM).

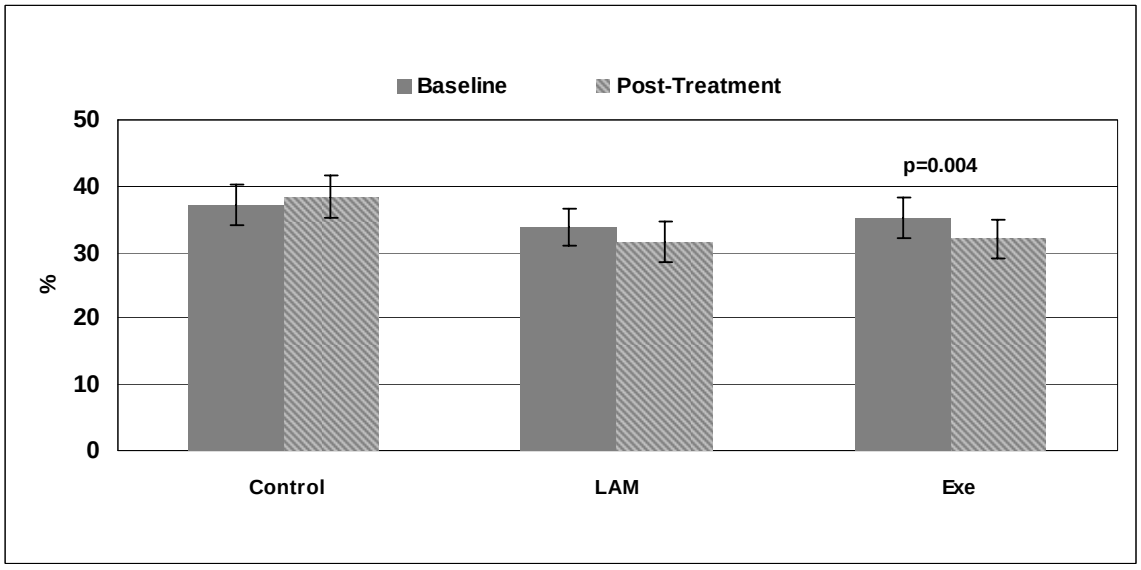


Figure 4: Percent body fat at baseline and post-intervention for each group. Values are mean (SEM). P-values compare baseline and post-treatment values within group.

Both the LAM and exercise group had a significant increase in predicted VO_2max, while the control group had a non-significant decrease (Figure 5).

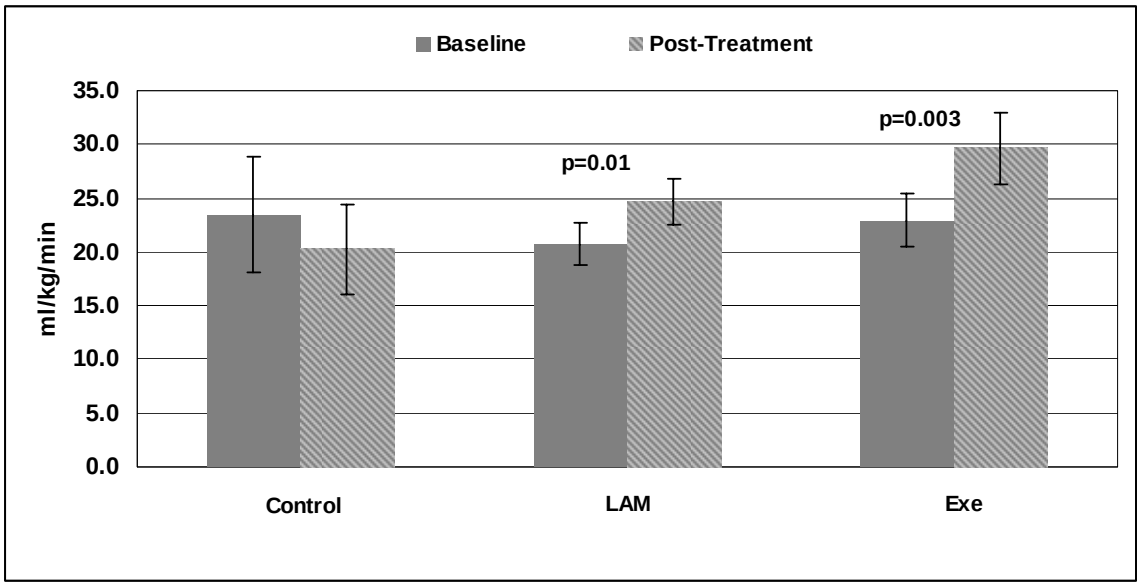


Figure 5: Predicted VO_2max at baseline and post-intervention for each group. Values are mean (SEM). P-values compare baseline and post-treatment values within group.

In addition, both of these groups had a significant increase in their average number of daily steps. The LAM group increased their average steps by 3487 steps and the exercise group increased by 6376 steps (Figure 6).

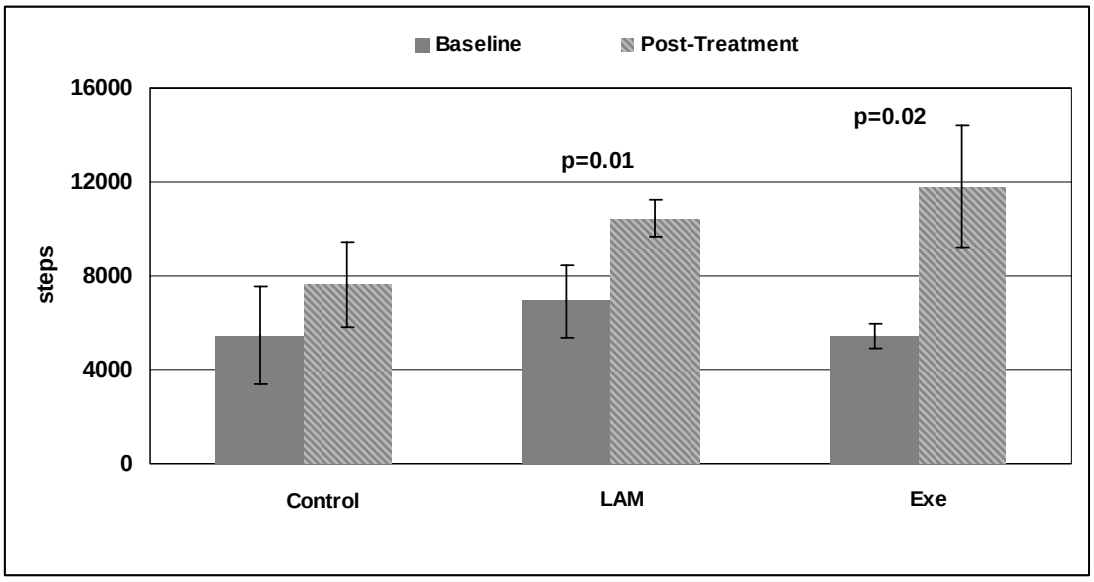


Figure 6: Average steps at baseline and post-intervention for each group. Values are mean (SEM). P-values compare baseline and post-treatment values within group.

Although there were no significant differences in any of the glycemic measures, there were several notable trends. The glucose for both the control and LAM groups stayed the same, while there was a slight decrease in the exercise group (Figure 7). The control and LAM group had a slight increase in insulin levels, while the exercise group had a decrease (Figure 8). Finally, the control and LAM group had no change in insulin resistance measured via HOMA-IR, but the exercise group had a decrease (Figure 9).

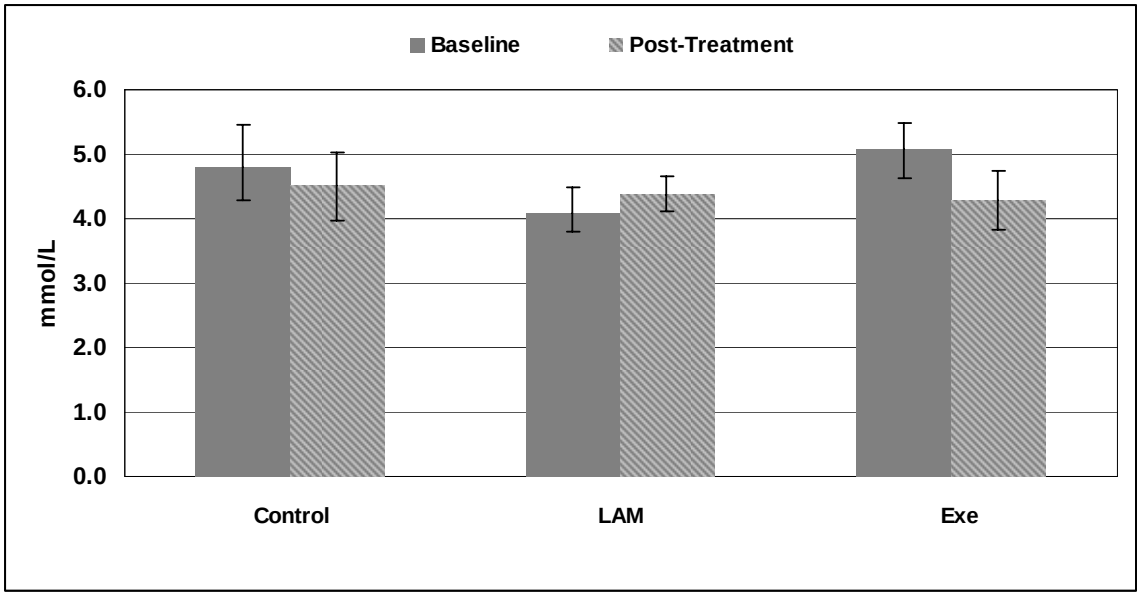


Figure 7: Glucose at baseline and post-intervention for each group. Values are mean (SEM).

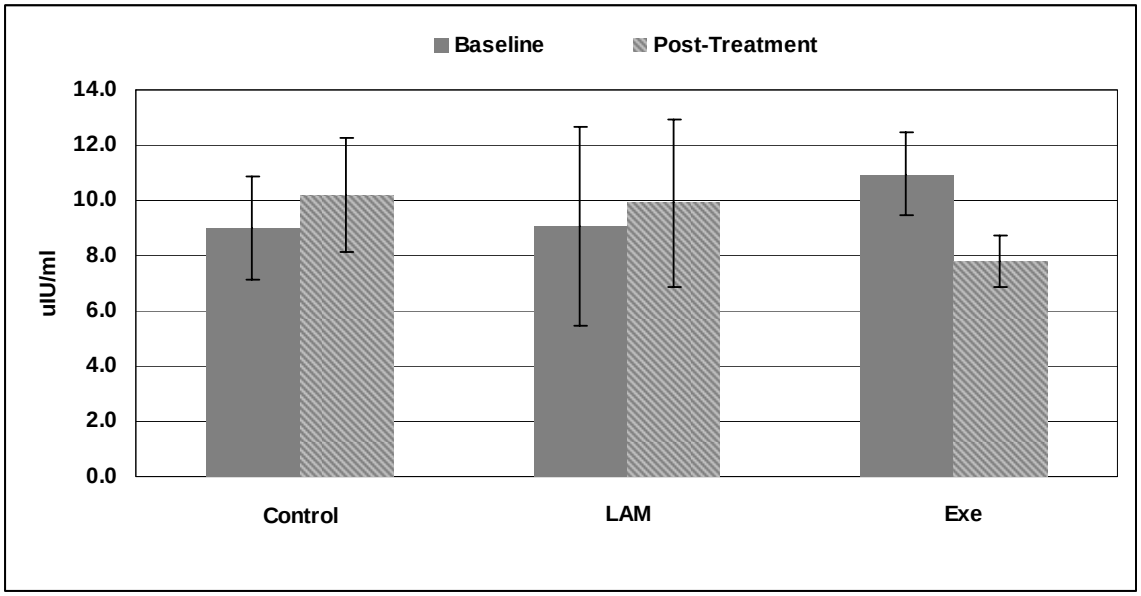


Figure 8: Insulin at baseline and post-intervention for each group. Values are mean (SEM).

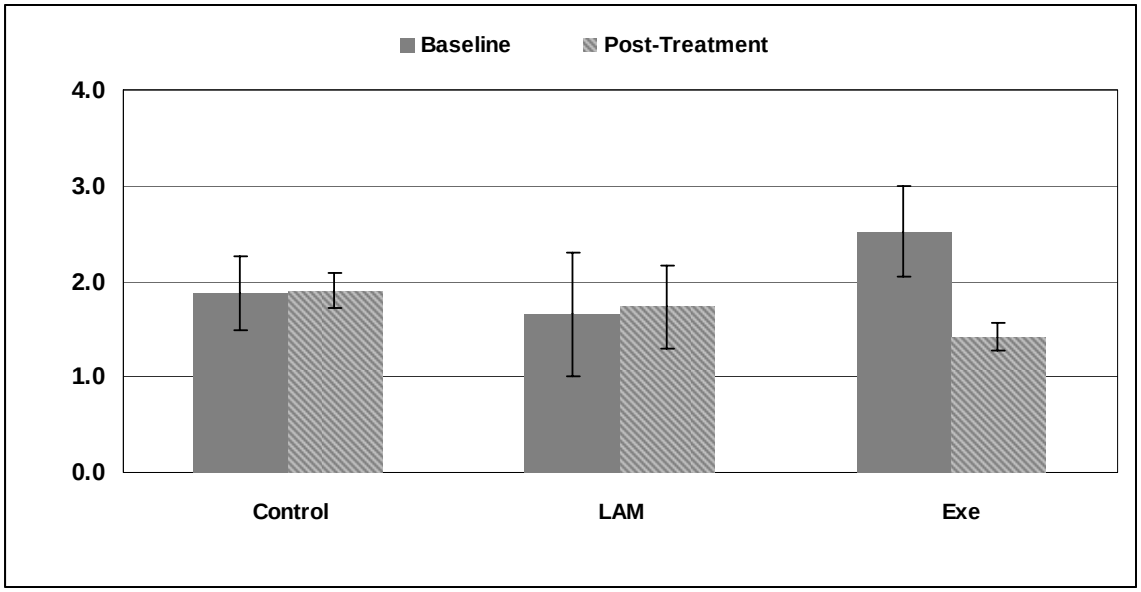


Figure 9: HOMA-IR values at baseline and post-intervention for each group. Values are mean (SEM).

There were no significant changes for PAI-1 or CRP and given the large variation in these measurements, there were no notable trends (Figure 10 and 11).

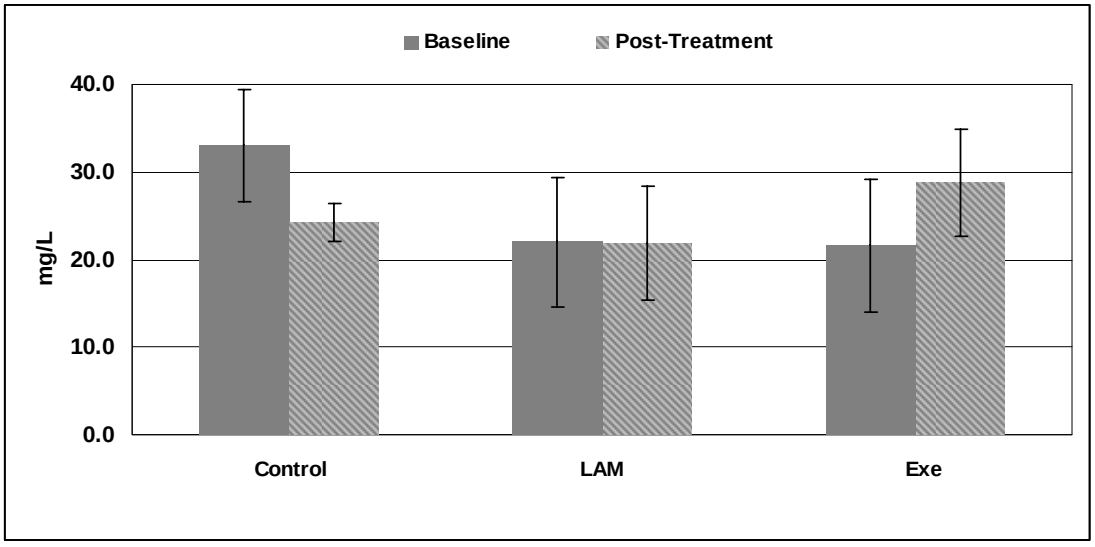


Figure 10: CRP at baseline and post-intervention for each group. Values are mean (SEM).

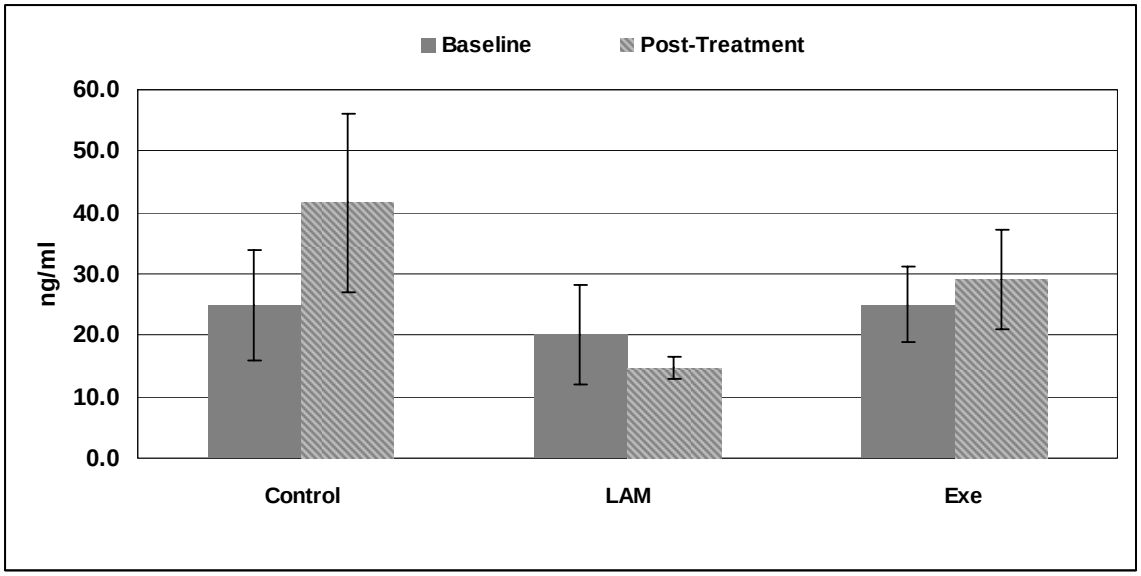


Figure 11: PAI-1 at baseline and post-intervention for each group. Values are mean (SEM).

Correlational statistics showed several significant relationships (Table 4). Both PAI-1 and CRP were positively correlated with body weight, percent body fat, and BMI and PAI-1 was positively correlated with waist circumference. CRP was also negatively correlated with predicted VO_2max. Additionally, PAI-1 was positively correlated with insulin and HOMA-IR. Insulin measures were positively correlated with waist circumference, BMI and percent body fat. Spearman correlations revealed a positive, significant correlation with percent change in body weight, BMI, and percent body fat with change in insulin and HOMA-IR. Additionally, change in body weight was positively correlated with change in predicted VO_2max.

Table 4: Significant correlations for all variables, pre- and post-intervention measures combined. R and p-values shown.

	Weight (kg)	Waist (cm)	BMI	VO_2max	%BF	CRP	PAI-1	Insulin	Glucose
Waist (cm)	.8335 p<.001								
BMI	.9331 p<.001	.919 p<.001							
VO_2max	-.3420 p=.041	-.3457 p=.039	-.3737 p=.025						
%BF	.7951 p<.001	.8605 p<.001	.8465 p<.001	-.6200 p<.001					
CRP	.3383 p=.044		.4081 p=.013	-.3322 p=.048	.3465 p=.038				
PAI-1	.5856 p<.001	.4952 p=.002	.4599 p=.005		.4087 p=.013				
Insulin		.3782 p=.023	.3393 p=.043		.3486 p=.037	.3220 p=.055	.3671 p=.028		
HOMA-IR		.3454 p=.039	.3294 p=.050				.3360 p=.045	.8762 p<.001	.4034 p=.015

CHAPTER V

DISCUSSION

Anthropometric and Body Composition:

Based on previous research, we anticipated a decrease in anthropometric measures and an improvement in body composition. Our results do not show a decrease in body weight, BMI, or waist circumference in either intervention group, however, the control group did have an increase in waist circumference. Additionally, the exercise group had a significant decrease in percent body fat and the LAM group showed a non-significant decrease.

Dunn *et al* (1999) found a similar decrease in percent body fat for both LAM and exercise groups after 24 months of intervention and Schmidt et al (2001) reported a decrease in body weight, BMI, percent body fat and waist circumference after a twelve week intervention consisting of a continuous exercise group, a group that participated in two bouts of 15 minutes per day and a third group with three, 10 minute bouts per day, all of whom participated in their assigned protocol 3-5 days per week at 75% of their heart rate reserve. However, this study also contained a diet component that contributed to their improvement in body composition, even though there were no differences in calorie intake between groups. Donnelly *et al* (2000) saw improvement in anthropometric measures and/or body composition in both a continuous and intermittent intervention at

nine months in an eighteen month study. However, the intermittent group returned to baseline body weight and percent body composition at eighteen months.

There were few changes in anthropometric measures in the two intervention groups in the current study however, there was a significant increase in waist circumference in the control group, which suggests that the increase in activity level prevented an increase in waist circumference in the exercise groups.

The LAM and exercise group both had a significant increase in number of steps and both increased their average steps to > 10,000 per day as recommended (Tudor-Locke and Bassett, 2004). Cross-sectional studies have shown that individual who accumulate 10,000 step or more a day have a significantly lower BMI, waist circumference, and percent body fat (Krumm *et al*, 2006, Thompson *et al*, 2004) and this has been shown to be true in a group of African-American females as well (Hornbuckle *et al*, 2005). In the current study, only the structured exercise group had a significant decrease in percent body fat, suggesting the LAM group did not participate in either enough activity, the intervention was not long enough to produce significant changes or the intensity was not great enough to illicit a change in body composition. Swartz *et al* (2003) conducted a walking study in which participants were instructed to accumulate 10,000 steps per day and after eight weeks, subjects showed improved glucose tolerance, but no changes in body weight or composition were made. These findings suggest that perhaps a longer intervention period is necessary to see changes in body composition, even when significant alterations in activity level have occurred for short durations.

A limitation to this study is that adherence for the LAM group was calculated based on attendance to the group meetings and not adherence to a specified amount of

activity completed per day (i.e. # of days that 10,000 steps were achieved). Additionally, the number of steps were calculated as an average over the course of one week at baseline and at 12 weeks. The number of steps taken each day over the course of the 12 week study is not known.

Cardiovascular Fitness

There was a significant increase in predicted VO_2max in both activity groups. These results are consistent with other findings. In studies ranging from 8 weeks to 24 months, for both men and women, VO_2max significantly increased with intermittent and/or continuous exercise interventions (Dunn *et al*, 1999, Schmidt *et al*, 2001, Donnelly *et al*, 2000, Jakicic *et al*, 1995, DeBusk *et al*, 1990, Murphy and Hardman, 1998). Additionally, there was a significant correlation between percent body fat, body weight, BMI, waist circumference and predicted VO_2max.

Glycemic Measures

There were no significant changes in glucose, insulin or HOMA-IR measures. However, there was a tendency for the exercise group to lower their insulin levels and reduce their HOMA-IR values. It should be noted that for a study population that included overweight or obese women, their glycemic indicators were extremely good. Glucose measures were in the normal range (mean 4.6 mmol/L), however insulin levels were very low. There is no set standard for insulin levels, but in general measures from 6-26 uIU/ml are considered normal (Pagna and Pagna, 2003). Normal HOMA-IR values are reported around 1 and values greater than 4 are considered insulin resistant (Quon *et al*, 2001). Osei *et al* (2005) reported average (SD) insulin levels of 13.01 (±9.32) uIU/ml in normal glucose tolerant African-Americans. The average HOMA-IR value for this

group was reported at 2.81 (± 2.06). Additionally, Patel *et al* (2006) found that mean (SD) levels of insulin in African-American females was 13.5 (±9.4) uIU/ml and the mean HOMA-IR value was 3.0 (±2.4). HOMA-IR values for African-American women have been reported has high as 15.8, compared to 8.6 in Caucasian women (Lamonte *et al,* 2005).

The results of this study showed no significant improvements in glycemic measures, however, since the subjects baseline characteristics classified them as very healthy and extremely insulin sensitive (average insulin = 9.8 (± 7.5) uIU/ml and average HOMA-IR = 2.0 (±1.5)), it is possible that no significant improvements could be made.

As expected, change in insulin and HOMA-IR measured were correlated with changes in body weight, body mass index, and body composition.

Plasminogen Activator Inhibitor-1

The current study did not show a change in PAI-1 antigen in any of the groups. It was hypothesized that PAI-1 would decrease, specifically in association with an improvement in body composition and an increase in activity level. Our results included a significant increase in number of steps and predicted VO_2max in both the LAM and exercise group. Given this, the hypothesis that PAI-1 would decrease in conjunction with an increase in activity level is rejected. The exercise group had a significant decrease in percent body fat, but no other anthropometric changes. Therefore, it is likely that the change in percent body fat was not strong enough to illicit a change in PAI-1.

Past research is controversial. Some studies show a decrease in PAI-1 with a decrease in body weight changes, independent of activity level and vice versa. PAI-1 is not only expressed in the liver, but also in adipose tissue (Shimomura *et al*, 1996) and

PAI-1 has been shown to have a dose dependent relationship with a decrease in weight loss (Folsom *et al*, 1993). Vague *et al* (1986) found a positive association between BMI and PAI-1 levels.

Individuals that are sedentary tend to have higher levels of both PAI-1 antigen and PAI-1 activity (DeSouza *et al*, 1998). Studies with exercise or lifestyle interventions that had concomitant reductions in body composition, consistently report reductions in PAI-1 (Folsom *et al*, 1993, Lindahl *et al*,1999, Marvi *et al* 1999). Interestingly, Marvi *et al* (1999) found a decrease BMI, percent body fat and PAI-1 activity and antigen levels with only two, ten minute bouts of exercise per day for twelve weeks. However, this exercise intervention was done in conjunction with a low-calorie diet. In support of the role of change in body composition for reductions in PAI-1, Bodary *et al* (2003), found no change in PAI-1 activity or antigen with an intense exercise program that resulted in no change in body weight. Conversely, only one study (Hayase *et al* 2003) found a decrease in PAI-1 antigen in a 10 week training study that was not correlated with change in total fat mass.

In the current study, the exercise group saw a significant decrease in percent body fat, however, there were no other anthropometric improvements seen in either the LAM or exercise group. Both the exercise and LAM group did see a significant improvement in activity level (measured via number of steps). It is possible that the small change in body composition and improvement in activity level was not enough to decrease PAI-1. Furthermore, decreases in PAI-1 are often associated with decreases in insulin measurements and these values did not change significantly in our population. More specifically in African-American females, PAI-1 changes are more strongly related to

changes in insulin resistance (HOMA-IR) than in changes in fat mass (Solano *et al*, 2003).

Studies show that PAI-1 is significantly associated with insulin levels and often decrease with a decrease in insulin. Vague *et al* (1986) found a positive association between insulin levels and Eliasson *et al* (1994) found that insulin levels are a predictor of PAI-1 activity. Furthermore, PAI-1 is also positively associated with insulin resistance, specifically measured via HOMA-IR measures (Lindahl *et al*, 1996, Vague *et al*, 1986). This association between insulin and PAI-1 remains even after adjustment for age, sex, ethnicity, BMI and glucose tolerance (Festa *et al,* 1999). In addition, Potter can Loon *et al* (1993) showed an association of insulin resistance and PAI-1 via a hyperinsulinemic euglycemic clamp. This specifically shows that peripheral and hepatic insulin actions are associated with PAI-1. The current study did not see any significant changes in insulin, glucose, or HOMA-IR measures and there was a significant, positive correlation between insulin and PAI-1 levels, as expected. In addition, this group of African-American females was extremely insulin sensitive as measured by their insulin levels and HOMA-IR values at baseline. Solano *et al* (2003) compared PAI-1 in Caucasian (CC) and African-American (AA) women. Results showed that fasting insulin levels and HOMA-IR levels were independently and positively associated with PAI-1 levels in AA women, but not in CC women.

Since there was no change in body weight, BMI, waist circumference or insulin, the lack of change in PAI-1 levels is not surprising. Our findings corroborate previous studies that indicate that changes in PAI-1 levels are positively correlated with changes in anthropometric measures and insulin levels.

There was large variability in PAI-1 measures in the current study, with levels ranging from 0.32 to 82 ng/ml. Some subjects showed extreme variability in their pre- and post-treatment measurements of PAI-1 and there are many factors that may have contributed to this. PAI-1 fluctuates with circadian rhythm, with the highest measures seen in the early morning (Kluft *et al,* 1988, Juhan-Vague *et al*, 1992). It is possible that blood was collected at different times for some of these subjects. One limitation to this study was that the time of day for blood collection was not recorded, it can not be determined with this subject pool if time of day was correlated with PAI-1 measures.

The mean for PAI-1 for all subjects at baseline was 22.9 ng/ml, which is slightly higher than the normal range of 10-20 ng/ml (Kluft *et al,* 1988, Juhan-Vague *et al*, 1992). However, this normal range is affected by body composition. African-American women have higher rates of obesity than African-American men and Caucasian men and women (Ogden, 2006). African-American women have significantly higher BMIs than Caucasian women (Nelson *et al,* 2002, Appel *et al*, 2002, Morrison *et al*, 2006). This elevated prevalence of overweight and obesity probably contributes to elevated PAI-1 measures. Solano *et al* (2003) reported mean (SD) values of PAI-1 antigen at 48.1 (± 32) in a group of overweight or obese African-American women. The average BMI in the current study was 32 kg/m^2, which explains our higher values of PAI-1. Moreover, compared to Solano *et al* (2003) whose average BMI was 34 kg/m^2, our PAI-1 values were much lower.

C-Reactive Protein

CRP was also expected to decrease in both treatment groups, under the assumption that there would be improvements in body composition and an increase in activity level in both groups. Again, the lack of change in body composition may help explain the lack of change in CRP.

Most studies show a significant correlation between body composition and CRP levels (Hak *et al* 1999, Tchernof *et al* 2002, Rawson *et al* 2003). Body weight (Tchernof *et al* 2002), waist circumference (Hak *et al* (1999) and BMI (Rawson *et al* 2003) have all been shown to be independent predictors of CRP levels. Recently, gender differences in the association between body weight and CRP were investigated and results indicate that this relationship holds only for women (Marcell *et al* 2005) and that CRP values in women were 2-fold greater than in men.

Some studies have reported an association between activity level and CRP. Maximal oxygen uptake was an independent factor for CRP levels (Kondo *et al* 2005) and higher CRP levels were associated with lower levels of physical activity in a group of subjects with type 2 diabetes (MaGavock *et al* 2004) and elderly patients (Colbert *et al*, 2004). However, Colbert *et al* (2004) reported that the association was lost after adjusting for body composition.

However, some intervention studies show no decrease in CRP with exercise. Marcell *et al* (2005) had subjects participating in either a moderate or intense exercise protocol for 16 weeks. Overall, there were no changes in CRP levels in either group. Even when subjects were divided into tertiles based on change in VO_2max, there was still no change with CRP levels. Several studies have shown that a decrease in CRP is seen

with weight loss alone, without an exercise component, suggesting that any CRP decrease seen with an exercise intervention is likely due to the change in body weight and not exercise per se (Tchernof *et al,* 2002, Heilbronn *et al*, 2001).

The current study did not see any significant changes in CRP levels. However the exercise group did see a non-significant increase whereas the other two groups decreased. It has been hypothesized that exercise has an inflammatory component that may actually cause CRP levels to increase during an exercise program (Okita *et al*, 2004, Marcell *et al*, 2005). Additionally, it has been found that acute bouts of aerobic exercise increase muscle inflammation and oxidative stress (Bloomer *et al*, 2005, Pittaluga *et al*, 2006). This may explain the slight increase in CRP seen in the exercise group. As expected, we did see a significant, positive correlation with CRP and body weight, BMI and percent body fat, which is consistent with previous studies reported above.

The women's health study showed that African-American women had a significantly higher mean CRP than Caucasian women, however, they also had a significantly higher BMI. When this association was adjusted for BMI, the difference in CRP levels dropped from 44.6% to 10.6%, but remained significantly different (Albert *et al*, 2004). Additionally, Lamonte *et* al (2005) reported that elevated CRP levels were found in 22.7% of African-American women compared to 4.3% of Caucasian women. These studies suggest that there may be an independent ethnic factor associated with levels of CRP. However, the median CRP level in the women's health study was 2.96 mg/L in the African-Americans, dramatically and significantly lower than found in the current study.

Since CRP is an acute phase reactant, which is produced in the liver in response to infection, inflammation and trauma, there are many variables that make this protein difficult to measure. CRP levels in these subjects were extremely high. The normal range for CRP is 1-3 mg/L. CRP levels above 3 mg/L are considered a high risk factor for cardiovascular disease (Pearson *et al*, 2003). Measurements above 10 mg/L are rarely reported in healthy subjects. In general, elevated levels of CRP are seen with high blood pressure, high BMI, smoking, metabolic syndrome and/or diabetes, low HDL, high triglycerides, hormone replacement therapy, chronic infection and inflammation (Pearson *et al,* 2003). Two of our subjects reported taking birth control. Nearly all of the subjects had a high BMI. Some subjects reported swelling of the legs and/or arms and asthma, which could be considered a state of chronic inflammation. All of these could have affected the abnormal CRP values in this study.

Limitations

There are several limitations to this study. The subject size was very small, with only four in the control group, nine in the LAM group and eight in the exercise group. If repeated, the subject size should be increased and equally distributed among the groups. Although subjects were asked to report all medications that they were taking and any current medical conditions the might have, more specific monitoring needs to be done given the variables that were used in this study. It is known that three subjects were taking aspirin on a daily basis and one subject was taking Bextra, an anti-inflammatory. Both aspirin and anti-inflammatory medications effect the production of CRP (Backes *et al*, 2003). Generally, these medications reduce inflammation and should reduce CRP measures. However, subjects taking these medications in this study did not have

significantly lower CRP values than anyone else. Two subjects were asthmatic, which is a chronic inflammatory condition, thus affecting CRP levels.

Other possible extraneous variables that may affect PAI-1 and/or CRP were not controlled for and include; time of day for blood sampling, non-prescription medications such as Vitamin E and C and fish oil supplements that can affect both CRP and PAI-1 levels.

Future Research and Recommendations

The current study had 4 control subjects, 9 LAM subjects, and 8 structured exercise subjects. In order to increase the power of the study and decrease variability, more subjects should be included in future research. The original goal of this study was to have 10 in each group. Although both the LAM and exercise group were close to this, the control group had less than half. This could be due to lack on interest in this group. More contact with this group may be beneficial for improving adherence, as well as monetary compensation.

The current study found no changes in body weight, BMI, insulin, HOMA, CRP, and PAI-1. The above mentioned low subject numbers could have contributed to this. However, it is possible that the intervention was not long enough. Twelve weeks may not be long enough to see necessary changes in body weight that may illicit changes in insulin, CRP and PAI-1. In addition, the prescribed exercise intensity for the structured exercise group may not have been high enough to cause the changes we expected. The intensity was prescribed at 50-65% maximum heart rate. Several other published studies have used higher intensities and a rate closer to 75% may be needed. Furthermore, diet

was not controlled for in this study. There could have been a kilocalorie change within and between the groups that may have affected all the variables measured.

Results showed a significant increase in the number of steps in both the LAM and exercise group. The number of steps were calculated and averaged over the course of one week at baseline and again at 12 weeks. This likely does not reflect the true nature of the physical activity level of individuals, since it does not reflect the actual steps taken each day over the course of the study. In addition, the number of steps for the exercise group may be lower since the pedometer does not record activity done on a bicycle or elliptical.

Furthermore, there was a trend for the control group to increase their number of steps. This could be due to effect of wearing a pedometer and may explain why there was no difference between the number of steps in the control group at 12 weeks compared to both the LAM and exercise group. Future studies should have the participants record their number of steps each day so that both the daily and weekly averages can be compared between the two groups throughout the duration of the study. This could help tease out differences seen between the LAM and exercise group.

One issue that affects PAI-1 levels is the time of day of blood collection. The current study did not record the time of day for blood collection. Although most blood samples were collected in the morning, it is impossible to tell if the times were consistent across a given subject. Since PAI-1 levels are known to be highest early in the morning, this may alter the findings. Subjects should be required to come in between a set time such as 7:00-10:00 am and the pre- and post-treatment blood collection should be done at the same time.

Finally, subjects were required to disclose all medical conditions and medication they were currently taken. In addition to medications, supplements such as vitamins and anti-oxidants should be controlled for in future studies. It is known that such supplements can lower CRP levels. Moreover, subjects taking birth control, aspirin, and anti-inflammatories or those with chronic conditions such as asthma, should likely be excluded from future studies.

Conclusions

In conclusion, twelve weeks of LAM or structured exercise significantly improved daily activity level and cardiovascular fitness and prevented an increase in waist circumference seen in the control group. Only structured exercise significantly improved body composition. Neither LAM or structured exercise improved nontraditional cardiovascular risk factors PAI-1 or CRP.

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APPENDIX A

MEDICAL QUESTIONNAIRE

The purpose of this questionnaire is to determine if you have any physical limitations that may exclude you from participation in this investigation. All information will be kept completely confidential.

A. Personal Information

Name/Last: _____ First: _____ Middle Initial: _____ Date of Birth: _____ Gender: _____
Address/Street: _____
City: _____ State: _____ Zip Code: _____
Phone (H): _____ Phone (w): _____
Phone (cell): _____ E-mail: _____
Best way to reach you and when?_____

1. When was your last physical exam? _____
2. Please list any serious or chronic illnesses of which you are aware._____
3. Please list any allergies to medications, foods, or other substances.
_____
4. Please list any medication you have been on or presently take

Type	Dosage/Frequency	How Long?	Why?

_____

B. Medical History

1. Illnesses---Please check if you have had any of the following:

Illness	Present	Past	Dates
Heart attack			
Anemia			
Asthma			
Epilepsy			
Lung disease			
Stroke			
Gout			
Diabetes			
Hypoglycemia			

Rheumatic fever
Heart murmur
Hernia

2. Symptoms---During the last 12 months, have you experienced:

Condition	Yes	No
High blood pressure		
Swelling of hands and feet		
Pain or cramps in legs		
Orthopedic problems		
Musculoskeletal problems		
ECG abnormalities		
Blurred vision		
Chest pain/pressure		
Shortness of breath		
Unusual fatigue		
Dizziness/light headed		
Significant weight change		
High cholesterol		
Numbness in limbs or face		

3. Is there any chance you are pregnant?

4. Hospitalizations---List the dates and the reasons for hospitalizations for any significant illness.

	Date	Diagnosis
1.		
2.		
3.		

C. Family History

1. Is your father living? Yes No If not, age at death and cause.
2. Is your mother living? Yes No If not, age at death and cause.
3. Has you father, mother, grandparents, or siblings had:

Condition	*Yes*	*No*	*Who?*
High blood pressure			
Stroke			
Heart attack (<50 yrs)			
Heart attack (>50 yrs)			
Diabetes			

Cardiovascular disease

Other

APPENDIX B: PHYSICAL ACTIVITES READINESS QUESTIONAIRE

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

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

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

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

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

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

If you answered

YES to one or more questions

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

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

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

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

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

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

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

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

NAME _____

SIGNATURE _____ DATE _____

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

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

Supported by: Health Canada Santé Canada

continued on other side...

APPENDIX C: CONSENT FORM

THE UNIVERSITY OF NORTH CAROLINA
GREENSBORO

CONSENT TO ACT AS A HUMAN SUBJECT

Project Title: The Comparison of Lifestyle Activity Modification (LA) and Structured Exercise on Obesity-related Risk Factors in African-American Women.

Project Director: Laurie Wideman, PhD

Subject's Name: _____

Date of Consent: _____

DESCRIPTION AND EXPLANATION OF PROCEDURES:

The purpose of this study is to compare the effectiveness of two different exercise programs on the health status of sedentary African-American women between the ages of 18 and 55.

As a subject, you will be asked to report to the Exercise Physiology Laboratory in the Department of Exercise and Sport Science at UNC-Greensboro for testing on 3 different occasions. Prior to participation, you will fill out a medical questionnaire and a physical activities readiness questionnaire (PARQ), to determine if you have any limitations that may exclude you from the investigations. If you are pregnant, or think you might be, you will be excluded from the study. If you are eligible for the study, you will go through a familiarization session. During the session you will have an opportunity to learn how to use the testing bicycle and it will be adjusted to your individual dimensions. At this time you will become accustomed to the use of the oxygen consumption equipment. To measure the amount of oxygen that you consume while you exercise, we will use a facemask that covers your nose and mouth and has a free flow valve in the front and allows you to easily breathe in and out. When you exhale, a small air sample is taken from the valve and analyzed by the computer to assess how many calories you are using. This familiarization visit should take about 1 hour.

At least 24 hours after your familiarization session, you will return to the lab for a sub-maximal cycling test, body composition assessment and blood sample collection. The total amount of time you will be at the Exercise Physiology Lab this testing will be approximately 45 minutes (this includes measurements, set-up time and data collection). A certified phlebotomist will draw blood samples, the amount of which is comparable to approximately 4 tablespoons. Prior to the exercise test, several measurements will be taken to assess the amount of body fat you have. These measurements will include circumferences of your waist and hips and we will measure using a bioelectrical impedance (BIA) device. You will lay flat on a non-conductive table and rest quietly for 5 minutes. After this time, four gel electrodes will be placed on the hand, wrist, ankle and foot of the right side of the body. A very small (5 kHz and 1 MHz) alternating current, that cannot be felt, will be applied for approximately 5 minutes. For the cycling tests, you will warm-up for 2-3 minutes at a low intensity, you will then pedal for 6 minutes at an increasing intensity, after which you will cool down for 2 minutes, again at a low intensity to allow you to recover from your activity. During the test your heart rate will be monitored at all times. The cycling test will last about 2 minutes. You are asked to refrain from drinking alcoholic beverages within 48 hours of testing and you should abstain from eating and drinking caffeinated beverages for the 2 hours prior to testing. You should also abstain from exercise the day of your testing.

After the testing session, you will be randomly assigned to one of three groups: 1) a non-exercise control group; 2) a traditional exercise group; or 3) a lifestyle modification group. The control group will be asked to maintain current activity patterns throughout the course of the 12 week study, but will participate in 8 meetings at the Corbett Center on the campus of North Carolina A&T State University during this time, as well as all testing sessions. The traditional exercise group will meet four times per week at the North Carolina A&T Fitness Lab in Moore Gym for cardiovascular exercise sessions using cycling, stair-stepping or walking on stationary exercise equipment. Each session will consist of a warm up and cool-down period, as well as at least 40 minutes of moderate intensity exercise. We will use heart rate monitors to help you work out at the proper intensity. The lifestyle modification group will meet each week for the first 6 weeks of the exercise program and every other week thereafter, for educational sessions at the Corbett Center on the campus of A&T. Participants will be instructed to incorporate short bouts of exercise into their normal daily routines in order to accumulate at least 30 minutes of moderate activity each day for minimum of 5 days per week. All participants will be asked to continue with current dietary patterns throughout the duration of the study. Activity level will be recorded for all groups using daily activity logs, 7-day recall questionnaires and occasional use of pedometer and/or heart rate monitors. After the 12-week intervention, you will return to the Exercise Physiology Laboratory at UNC-Greensboro and repeat the same testing that you did prior to starting the

intervention. The total amount of blood that you will give is approximately equal to 8 tablespoons, which is approximately ¼ of the amount you would give for a blood donation.

RISKS AND DISCOMFORTS:

The risks during exercise testing include: fainting, abnormal heart beat, abnormal blood pressure response, muscle and joint strains and in rare instances, heart attack. The risk of death is less than one in 10,000 tests, which includes patients with a history of coronary heart disease. This risk is minimized by allowing time for proper warm-up and cool-down, by monitoring heart rate during testing, and by using healthy subjects with no known cardiovascular risk factors. Personnel will be trained in CPR and the use of the AED (automated external defibrillator). There is an AED located in the Exercise Physiology Laboratory. Orthopedic injury is also remotely possible, but is not likely since you will be instructed on proper treadmill walking technique at your familiarization session.

Your privacy will be maintained at all times and you will be assigned a number when you enter the study. This number will be used on all data and only the principal investigator will have a copy of the list of subjects. You will not be identified in any way in any publication that results from this study. After publication or release of data for destruction, the data from the study will be destroyed by shredding.

POTENTIAL BENEFITS:

Exercise testing will provide you with information regarding your physical fitness status. The information gained from this investigation can also be utilized to help you design an individualized aerobic program that will be tailored to your personal needs. Increases in activity levels have been implicated in the reduction of risk associated with obesity, cardiovascular disease, diabetes and other chronic health problems.

COMPENSATION/TREATMENT FOR INJURY:

If any injury occurs during the testing sessions, subjects will be provided with on-site first aid and will be advised to seek further medical attention if necessary. Neither the institution at which you are being tested [UNC-Greensboro or North Carolina A&T State University], the investigators, nor those individuals assisting in the administration of the programs will be responsible for any medical care that may be required due to an injury resulting from your participation in this investigation.

CONSENT:

By signing this consent form, you agree that you understand the procedures and any risks and benefits involved in your participation in this study. You are free to refuse to participate or withdraw your consent to participate at any time without penalty or prejudice; your participation is entirely voluntary and any decision you make will not effect your participation in future studies. Your privacy will be protected because you will not be identified by name as a participant in this study.

The research and this consent form have been approved by the University of North Carolina at Greensboro Institutional Review Board and the North Carolina A&T State University Institutional Review Board, which insures that research involving people follows federal regulations. Questions regarding your rights as a participant in this study can be answered by calling Dr. Beverly Maddox-Britt at (336) 334-5878. Questions about the research itself will be answered by Dr. Laurie Wideman at (336) 334-3234 or Brenda Swearingin at (336) 334-7712. Any new information that develops during the study will be provided to you if the information might affect your willingness to continue participation in the study.

By signing this form, you are agreeing to participate in the project described to you by

_____.

_____
Subject's Signature*

_____
Witness to Signature

APPENDIX D: ASSAY PROCEDURES

GLUCOSE ASSAY:

1. Reconstitute calibrator with 3 ml from 4 ml vial of diluent supplied and gently mix for 15 minutes (concentration is 167 mg/dl).
2. Take 100 µl of calibrator and add to small tubes with 100 µl of diluent to make 83.5 mg/dl.
3. Take 100 µl of the 83.5 mg/dl and add to another small tube with 100 µl of diluent to make 41.75 mg/dl.
4. Take 100 µl of 41.75 mg/dl and add to another small tubes with 100 µl of diluent to make 20.88 mg/dl.
5. First set of wells to be filled with 92 µl of distilled water to act as blanks.
6. Second set to be filled with 2 µl of diluent and 90 µl of distilled water.
7. Pipette 2 µl of each calibrator and sample into appropriate well and add 90 µl of distilled water into each (except as note below)
    a. Seventh set to be filled with 4 µl of 167 mg/dl of calibrator and add 88 µl of distilled water.
    b. Eight set to be filled with 8 µl of 167 mg/dl of calibrator and add 84 µl of distilled water.
8. Place on shaker for 20 seconds.
9. Add 150 µl of glucose reagent to all wells.
10. Read immediately.
11. Incubate plate at 37ºC for 3 minutes on plate warmer.
12. Read plate again.

INSULIN ELISA:

1. Pipette 25 µl of each calibrator and samples into appropriate wells.
2. Add 200 µl of enzyme conjugate to each well.
3. Incubate on plate shaker for 1 hour at room temperature.
4. Hand wash plate 3 times with wash buffer. After final wash, invert and tap plate firmly against absorbent paper.
5. Add 200 µl of substrate TMB into each well.
6. Incubate for 15 minutes at room temperature on plate shaker.
7. Add 50 µl of stop solution to each well.
8. Place on plate shaker for 5 seconds and read at optical density at 450 nm.

PAI-1 ELISA:

1. Samples and controls must be diluted five fold with F-sample diluent.
2. Prepare standards.
3. Add 100 µl of conjugate to each well.
4. Add 100 µl of standard and samples to appropriate wells.
5. Mix gently on plate shaker and incubate for 1 hour at room temperature.
6. Wash plate 5 times with wash solution.
7. Add 200 µl of TMB substrate into each well.
8. Incubate for 5 minutes at room temperature.
9. Add 50 µl of stop solution (sulfuric acid) into each well.
10. Wait 10 minutes then read plate at absorbance at 450 nm.

CRP ELISA:

1. Samples must be diluted 1:100 with standard A.
2. Add 100 µl of calibrator and samples into appropriate wells.
3. Incubate for 1 hour on plate shaker at room temperature.
4. Wash plate 5 times with 250 µl of wash buffer.
5. Add 100 µl of prediluted peroxidase-labeled CRP antibody.
6. Incubate for 1 hour on plate shaker at room temperature.
7. Decant content of plate and wash plate 5 times with 250 µl of wash buffer.
8. Add 100 µl of TMB substrate solution into all wells.
9. Incubate 5-10 minutes at room temperature in the dark.
10. Add 50 µl of stop solution and mix shortly.
11. Immediately read plate at absorbance at 450 nm.

APPENDIX E: RAW DATA

Subject #	group	kg1	kg2	waist1	wasit2	bmi1	bmi2	vo1	vo2	BF1	BF2	step1	step2
12	Ctrl	73	74	77	79.6	28.6	29.4	15.8	15.4	31.2	34.4	2507	5611
26	Ctrl	110	107.7	104	108	40.3	39.4	18.3	16.1	45.8	47.4	8292	7550
34	Ctrl	99.5	102.3	90.8	95.2	35.2	36.2	20.3	16.8	37.7	38	9648	12728
73	Ctrl	94	95	102	109	34.5	35	39.3	32.8	34	33.4	1381	4666
2	LAM	127	125	127.5	127	46.4	45.9	14.7	15.7			1536	10263
8	LAM	98	100	98.4	97.7	36.5	37.2	18.7	23.6	39.3	39.4	5655	9829
10	LAM	72	72	81.1	79.5	28.8	28.4	18.2	18.7	31.9	29.4	2717	10032
14	LAM	58	59	68	68	23.3	23.8	18.3	28.1	25	25.3	5794	9334
20	LAM	55	54	73	72.4	23.3	21.9	18.7	26.7	33	28.5	16182	14580
29	LAM	83	83	87	83.2	31	30.9	20.9	23.3	40.2	37.2	5563	7042
40	LAM	80	78	76	70.5	30	29.2	31.8	38.8	24.8	18.3	4953	8086
61	LAM	62	62	75	75.9	24.4	24.4	28.9	25.1	27.8	26.9	12553	12866
74	LAM	117	115	100.5	99.8	40.2	39.8	16.4	22.4	48.4	47.9	7453	11846
9	EXE	60.5	61.4	77.6	78	23.2	23.5	18.2	20	33.6	29.7		
13	EXE	90	86.8	99.8	97	40	38.6	20.8	29.6	40.2	38.3	4715	11038
16	EXE	86.4	87.7	94.5	91.4	33.3	34.2	20.7	22.7	41.4	35.1		
18	EXE	73	71	84	85	28.2	27.5	20	23.7	35.9	32.6	3667	9710
49	EXE	57	54	64	60.3	24.2	23.2	25.4	39.4	24.8	22.3	5634	7934
57	EXE	57	84	94.5	89	20.2	29.9	24.6	37.2	36.7	33.3	5049	26242
72	EXE	72	72	66.5	69	23.5	23.5	38.3	44.4	21.6	20	7970	5221
75	EXE	114	113	102	96.8	36.7	36	15.5	20.1	47	45.2	5507	10652

	kg1	**kg2**	**waist1**	**wasit2**	**bmi1**	**bmi2**	**vo1**	**vo2**	**BF1**	**BF2**	**step1**	**step2**
Mean Ctrl	94.13	94.75	93.45	97.95	34.65	35.00	23.43	20.28	37.18	38.30	5457.00	7638.75
SEM Ctrl	7.78	7.39	6.21	6.88	2.40	2.08	5.37	4.18	3.17	3.19	2059.89	1799.48
Mean LAM	83.56	83.11	87.39	86.00	31.54	31.28	20.73	24.71	33.80	31.61	6934.00	10430.89
SEM LAM	8.58	8.40	6.24	6.36	2.68	2.70	1.92	2.17	2.77	3.11	1550.22	780.70
MEAN EXE	76.24	78.74	85.36	83.31	28.66	29.55	22.94	29.64	35.15	32.06	5423.67	11799.50
SEM EXE	6.99	6.50	5.22	4.69	2.55	2.17	2.47	3.37	2.99	2.89	506.54	2612.79

Subject #	group	CRP1	CRP2	PAI1	PAI2	Ins1	Ins2	Gluc1	Gluc2	HOMA1	HOMA2
12	Ctrl	45.6	24.0	1.907	19.959	5.177	7.274	4.347	5.278	1.000	1.706
26	Ctrl	32.2	27.3	39.555	41.147	6.608	6.666	6.520	5.111	1.915	1.514
34	Ctrl	38.5	27.3	19.687	22.413	13.2	11.316	4.926	4.659	2.890	2.343
73	Ctrl	15.8	18.4	38.544	82.9	11.096	15.491	3.451	2.954	1.702	2.034
2	LAM	54.6	36.9	58.779	21.992	37.401	22.712	3.915	4.649	6.507	4.693
8	LAM	37.4	52.0	14.012	13.814	6.605	8.459	5.111	5.111	1.500	1.921
10	LAM	31.7	23.6	3.109	16.635	1.895	4.873	4.618	4.824	0.389	1.045
14	LAM	52.4	39.8	1.34	2.861	5.54	5.299	2.553	4.524	0.629	1.066
20	LAM	5.5	5.1	7.272	12.042	7.578	5.39	3.571	4.751	1.203	1.138
29	LAM	10.4	6.5	32.561	13.839	8.976	6.271	6.112	4.436	2.438	1.236
40	LAM	0.60	0.04	4.744	12.835	4.368	2.624	4.639	4.916	0.900	0.573
61	LAM	0.6	0.5	0.328	17.915	5.177	5.069	3.900	3.777	0.897	0.851
74	LAM	4.6	32.3	58.8	20.396	4.128	28.424	2.387	2.413	0.438	3.048
9	EXE	2.4	30.3	18.089	21.409	4.691	6.484	6.003	6.581	1.252	1.897
13	EXE	58.4	53.3	12.785	23.119	15.648	8.52	5.988	4.177	4.164	1.582
16	EXE	53.5	37.4	27.371	5.579	15.146	13.419	5.954	3.190	4.008	1.902
18	EXE	9.1	7.0	36.315	20.294	15.266	4.934	6.013	4.447	4.080	0.975
49	EXE	14.6	16.3	5.416	14.168	10.971	5.54	4.835	4.225	2.357	1.040
57	EXE	6.2	14.7	4.806	18.085	8.711	7.361	4.502	3.476	1.743	1.137
72	EXE	14.0	19.5	45.549	62.791	11.504	6.953	2.693	5.522	1.377	1.706
75	EXE	14.5	51.8	49.198	67.12	5.729	9.244	4.669	2.674	1.189	1.099

	CRP1	CRP2	PAI1	PAI2	Ins1	Ins2	Gluc1	Gluc2	HOMA1	HOMA2
Mean Ctrl	33.03	24.25	24.92	41.60	9.02	10.19	4.81	4.50	1.88	1.90
SEM Ctrl	6.36	2.10	8.93	14.56	1.88	2.05	0.65	0.53	0.39	0.18
Mean LAM	21.98	21.86	20.11	14.70	9.07	9.90	4.09	4.38	1.66	1.73
SEM LAM	7.40	6.48	8.01	1.87	3.61	3.04	0.40	0.28	0.64	0.44
MEAN EXE	21.58	28.79	24.94	29.07	10.96	7.81	5.08	4.29	2.52	1.42
SEM EXE	7.67	6.15	6.17	8.07	1.52	0.95	0.41	0.45	0.48	0.14