THE EFFECTS OF BODY FAT AND FITNESS ON VASCULAR HEALTH

by

MANNING J SABATIER

(Under the Direction of Kevin K. McCully)

ABSTRACT

It is currently unclear whether the negative effect of body fat on vascular health is due to low aerobic fitness. It is also unclear whether a decrease in body fat mediates improvements in vascular health that occur with aerobic exercise. The purposes of the following studies were to 1) evaluate the relationships of adiposity (percent body fat determined by dual energy x-ray absorptiometry) and aerobic fitness (VO_2peak normalized to fat-free mass) with vascular health (i.e., systemic and vascular inflammation, leptin, central arterial stiffness; and femoral artery diameter (D_{FA}), blood flow (BF_{FA}), and shear rate), 2) evaluate the relationship of leg fat and fat-free mass (FFM) with D_{FA} and BF_{FA}, and 3) determine whether the effect of aerobic exercise training on vascular health is dependent on a reduction in adiposity. Forty-six pre-menopausal women (25-40 years old) were recruited for the cross-sectional study. C-reactive protein (CRP) was a significantly correlated with adiposity, but not VO_2peak (R=0.46, P=0.002). VO_2peak reduced the increase in CRP associated with an increase in % body fat (interaction effect, P=0.01). Leptin was correlated with adiposity (R=0.77, P<0.001), as well as CRP (r=0.36, P=0.01) and ICAM-1 (r=0.56, P<0.001). Aortic pulse wave velocity (aPWV) was significantly correlated with VO_2peak, but not adiposity, (R=-0.42, P=0.02). D_{FA} was significantly correlated with VO_2peak (R=0.42, P=0.004). D_{FA} and BF_{FA} were correlated with leg FFM (r=0.28, P=0.03;

r=0.26, P=0.06). For the exercise training study, thirteen sedentary women (33±4 yrs) were tested before and after 14 weeks of cycle exercise. aPWV and sICAM-1 did not change with training, whereas CRP was reduced by 35% (P=0.05). Leptin also decreased 20% (P=0.04). Resting D_{FA} increased 11.7% (P<0.001) and shear rate decreased 28% (P=0.007). Taken together, these studies provide evidence that increased aerobic fitness is positively associated with, or positively affects, each of these measures. The results of these studies support the concept that aerobic fitness modifies the negative effect that body fat may have on vascular health.

INDEX WORDS: Body composition, Adiposity, DEXA, Doppler ultrasound, Cardiorespiratory fitness, Muscle blood flow, Artery size, Vascular inflammation, sICAM-1, CRP, Leptin

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DEDICATION

To my mother, Peggy, my wife, Jennifer, and my son, Luc.

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CHAPTER 1

INTRODUCTION

Cardiovascular disease (CVD) continues to be a significant public health problem. It accounts for a significant portion of the yearly U.S. death rate and is largely preventable. In addition, the prevalence obesity has reached record levels in recent years. The most recent estimates put the current prevalence at approximately 30% of the U.S. population. Many of these people are also inactive and have low fitness levels, which precipitates gains in body weight. Physical inactivity and low cardiorespiratory fitness are risk factors for CVD. However, it has been suggested that excess body fat that obesity often represents may also predispose people to, or even cause, CVD.

A significant portion of the obese population has no other cardiovascular or metabolic risk factors, and it is possible to be fit while also being overweight or obese. Individuals with high levels of body fat who are also physically active and fit have been found to have significantly less risk for CVD than their sedentary counterparts. Low cardiorespiratory fitness is a significant and prevalent risk factor for future cardiovascular disease and an increase in fitness through exercise training reduces CVD risk. Since fitness seems to impart a protective effect from CVD, high fitness might minimize or negate the predictive power of high body fat for CVD.

There are several biochemical markers, as well as in vivo arterial measures, that are associated with CVD progression, or atherosclerosis. Atherosclerosis is now understood to be, in

large part, an inflammatory process. Some inflammatory markers that have piqued the interest of the research community in recent years are C-reactive protein (CRP) and soluble intercellular adhesion molecule-1 (sICAM-1). CRP is a marker of systemic inflammation and sICAM-1 is a marker of vascular inflammation. Leptin, a satiety factor released by adipose cells, may exacerbate the inflammatory process that affects arteries. In vivo arterial measures include aortic pulse-wave velocity (aPWV); as well as femoral artery diameter, blood flow, and shear stress. aPWV is used to estimate central arterial stiffness and is based on the principle that stiffer arteries propagate pressure waves faster. Femoral artery size, blood flow, and shear stress have been found to be affected by fitness and physical activity and may also reflect the health of arterial linings. All of these measures have been found to be associated with CVD risk.

Statement of the Problem

Most previous studies of the ill effects of body fat have relied on imprecise physiological measures. For instance, body fat is often represented using the body mass index (BMI; weight(kg)/height(m)^2). Although it is easy to measure and does not require expensive equipment, it does not discriminate between fat and fat-free mass. When oxygen consumption has been measured, in many cases it was not normalized, or was normalized to body mass. A more precise method of normalizing oxygen consumption to compare individuals of various body sizes and fitness levels is to use fat-free mass. In many cases, maximal oxygen consumption has been estimated of based on submaximal exercise performance, rather than measured.

Whether body fat mediates the impairments in vascular health that are present with low fitness is presently unclear. Furthermore, whether a loss of body fat mediates improvements in

vascular health with exercise training and increased fitness is also unclear. Therefore, the purposes of this study were to 1) determine whether fitness offsets the effect of body fat on markers of vascular health, 2) evaluate the effects of fitness and body fat on lower extremity arterial size, blood flow, and shear stress, 3) determine whether an exercise intervention designed to improve fitness would improve markers vascular health, and 4) determine whether an exercise intervention designed to improve fitness would also reduce body fat.

Hypotheses

It was hypothesized that:

1) **fitness, but not body fat, would emerge as a significant predictor of vascular health and peripheral artery measures**
    a. Subject recruitment was organized to result in a cohort that was balanced with respect to body fat and fitness. We recruited similar numbers of subjects who had high body fat and high fitness levels, high body fat and low fitness levels, low body fat and high fitness levels, and low body fat and low fitness levels.
2) **an exercise program that improves fitness without reducing body fat would also improve vascular health**
    a. The exercise intervention was designed to maximize exposure to higher intensity aerobic cycle training and therefore maximize the potential for an increase in fitness, while minimizing subjects' energy expenditures. Subjects were also asked to maintain their starting dietary habits throughout the study.

Significance of the Study

This study will provide new information as to whether fitness modifies the potential negative effect of body fat on vascular health. Although research studies suggest that body fat is harmful, evaluation of individuals who have excess body fat but do not have other cardiovascular risk factors is rare. Furthermore, it is not clear how lower limb mass and composition affects conduit artery diameter and blood flow. This will be addressed through hypothesis one. This study will also provide information as to whether a loss of body fat is necessary to mediate an improvement in vascular health. Although frequent exercise is broadly prescribed as a corrective measure to reduce body weight and improve health, it is not clear that a loss of body weight is needed. This will be addressed through evaluation of hypothesis two. Thus, this study is significant because it will help clarify whether minimizing body weight, or achieving weight loss, is necessary in lifestyle modification efforts designed to reduce the risk of CVD.

This study will also incorporate precise measures of body fat, fitness, and vascular health. Most studies that address these issues have used BMI (kg/m^2) to describe fatness, although BMI does not discriminate between fat and fat-free mass. High BMI (e.g., obesity) often reflects low cardiorespiratory fitness. However, low BMI (e.g., normal weight) can also be found in individuals who have low cardiorespiratory fitness. In larger studies which dominate the literature on body fat and health, fitness is typically estimated using sub-maximal tests or physical activity levels are estimated using questionnaires. Maximal oxygen consumption normalized to fat-free mass will enhance both 1) the precision of our fitness measure and 2) our ability to compare individuals of different sizes and body compositions. This study also incorporates a wide range of vascular health measures, both biochemical and non-invasive, allowing for detection of possible relationships between these measures. This also allows for a

well-rounded summary of the vascular health profile of our participants. Therefore, this study has the potential to be a valuable addition to the literature.

CHAPTER 2

REVIEW OF THE RELATED LITERATURE

The literature relevant to this study is discussed in this section. The relevant literature includes information regarding all of the following: obesity, cardiovascular disease, systemic and vascular inflammation, physical activity, fitness central arterial stiffness, and peripheral artery remodeling.

Obesity and Cardiovascular Disease

Cardiovascular disease (CVD) is a chronic disease of the heart and blood vessels. It results in large part from tobacco use, diabetes, physical inactivity, poor nutrition, and excess body fat. It is responsible for approximately 40% of the yearly death rate in the U.S. and more than 64 million Americans currently live with CVD (27). The cost of heart disease and stroke alone is projected to be $368 billion for the year 2004. An active lifestyle that is complemented by a healthy diet protects against CVD. A common symptom of failure on one or both counts is excess body fat. Data suggests that in general, the more fat someone has, the higher is the risk for chronic disease. Interestingly, body fat has rarely been measured in studies that provide the data to support this notion (61). The body mass index (BMI), which is essentially a ratio of mass to height (BMI=kg/m^2), has become the standard surrogate for body fat. Although BMI is a crude measure of fatness and indiscriminant for fat and lean body mass (117), it is convenient and feasible for use in population studies and has been found to correlate fairly well with body

fatness (46, 117, 159). Classifications have been determined for underweight, overweight, obese, and morbidly obese (<18, ≥25, ≥30, and ≥35, respectively) (60). Alternatively, it has been suggested that the body composition standard that places individuals at risk for disease is 25% and 32% for men and women, respectively (55). It is estimated that approximately 20 to 30% of the American population meets the BMI criteria for obesity (43, 139).

Recommendations for body weight and/or body fat are essentially to keep both to a minimum. It was recently suggested that adults should actively attempt to maintain a BMI of 18.5-21.9 (42). This results in a body weight of 127.2 pounds (57.8 kg) for a 5'4" women and 152.2 pounds (69.2 kg) for a 5'10" man. It would appear that many people are aware that excess body weight is deleterious and are undertaking efforts to lose weight (143). Of 107,804 U.S. men and women who participated in the 1996 BRFSS, 28.8% of men and 43.6% of women were trying to lose weight. Twenty-one and a half percent and 19.4% of these men and women, respectively, reported using the recommended combination of eating fewer calories and engaging in at least 150 minutes of leisure-time physical activity per week to lose weight (143). Among men trying to lose weight, the median weight was 90.4 kg with a goal weight of 81.4 kg. Among women, the median weight was 70.3 kg with a goal weight of 59.0 kg. Therefore, it appears that a substantial portion of people who are overweight or obese are undertaking efforts to reduce weight.

The incidence of obesity was assessed at 19.5% for 1997-1998 based on the NHIS survey of 58,556 men and women (139). It was assessed at 30.5% for 1999-2000 based on NHANES survey of 4,115 men and women (43). This is an increase from 22.9% based on data from 1988-1994 using the survey from Flegal et al. (43). Therefore, between a fifth and a third of the nation's population has a BMI of at least 30 (43, 139). Despite the error inherent to a mass to

height ratio, this indicates that more Americans now have excessive fat and that efforts to quell the incidence of obesity have not been successful. The economic costs of obesity in 1995 were estimated at $99.2 billion, which was 5.7% of the total costs of illness (167). The cost rose to approximately $118 billion during the late 1990's (36). Public financing of these costs is considerable since approximately half of all health care is paid by the Federal government and state and local governments (92). It has been estimated that 280,000 to 325,000 annual deaths in the US may be attributable to obesity (4). Contrast this with other public health problems and the possible impact becomes clearer. For instance, mortality associated with tobacco, alcohol and illicit drugs is about 400,000, 100,000, and 20,000 deaths per year, respectively (28, 92). Therefore, it is in the best interest of the U.S. collective to reduce the prevalence of overfatness.

Obesity is a symptom of low physical activity and/or poor dietary habits. The excessive body fat that is obesity may also pose an independent risk to the health. In a recent analysis of direct and reinsurance business issued between 1975 and 1998, the BMI-mortality relationship was found to vary by age, gender, and smoking status (102). It was strongest for middle-aged male non-smokers. The mortality rates in relation to BMI were examined in a prospective cohort study of 48,287 Dutch men and women aged 30 to 54 years at baseline from 1974 to 1980 (142). Total mortality was increased in obese and underweight men (BMI ≥30; RR, 1.5; 95% CI, 1.1-2.0; (BMI < 18.5; RR, 2.6; 95% CI, 1.8-3.9) but not in women. The increased mortality in overweight men was mainly attributable to CHD and, in underweight men, to early mortality and especially lung cancer mortality among smokers. Similar results were found in the influential Framingham Heart Study, a prospective study of 5209 men and women. However, they found that the degree of obesity was particularly sensitive at predicting CVD in women (58). More current publications based on the Framingham cohort suggest that risk for CVD is increased with

being overweight and obese in both men (RR = 1.21 and 1.46) and women (RR = 1.20 and 1.65) (166). Also, the risk attributed to BMI≥25 for developing hypertension, angina pectoris, and CHD fell in the range of 15-28% (166).

Physical Activity and Fitness

It has been known for some time that physical activity is associated with a better health profile. As far back as the 1700's it was observed that hurrying messengers appeared more heartier and healthier than sitting sailors (128). More is currently known of the beneficial effects of regular physical activity on various dimensions of health. A great deal of attention has been given to cardiovascular disease mortality due to its large societal impact. It is currently thought to be responsible for 14% of deaths worldwide (World Health Organization) and is the leading cause of death in the U.S. (27). Cardiovascular disease has been the most significant singular cause of death in the U.S. since 1900 (36). Therefore, there have been major efforts to identify and understand the development of cardiovascular disease. The Harvard Alumni Health Study has been one of the most influential epidemiological studies that evaluated the association between physical activity (reported as kilocalories per week) and mortality (108). Of 17,000 Harvard Alumni who graduated between 1916 and 1950, those with an activity index below 2000 kilocalories per week were at 64% higher risk than classmates higher than 2000 kilokalories per week (109). Their data also suggested that the risk for a first heart attack was inversely related to energy expenditure. A great deal of data has since corroborated these findings. For example, Manson et al. found that brisk walking and vigorous exercise were associated with substantial and similar reductions in the incidence of coronary events among women (87). Interestingly, similar results have been attained using body weight as an

independent variable (88). Relative risk for mortality was 2.2 when BMI was greater than 32. Higher body weight was associated with increased mortality risk when age (but not fitness levels) was controlled for.

The American College of Sports Medicine recommends a minimum of 30 minutes or more of moderate physical activity on most or all days of the week in order to derive the health benefits of exercise (114). Only 20% of Americans achieve this minimal level of activity. Evidence from the Behavioral Risk Factor Surveillance System (BRFSS) suggests that leisure-time physical activity in adults did not decline during the 1990's, whereas the prevalence of adult obesity had increased to 20.9% as of 2001 (1, 96). On the other hand, there is also convincing evidence that inactivity has a permissive effect on obesity (56). Fitness level is often used as a proxy for physical activity levels as it can be measured more precisely. The association between these two constructs is also important when evaluating prevalence of obesity.

Physical activity and cardiorespiratory fitness are inextricably linked. It was previously thought that upwards of 90% of physical fitness could be accounted for by genetics (68). However, it is now recognized that activity levels have a much more substantial impact (21, 116). Previous survey studies have found favorable relationships between the number of times subjects exerted themselves enough to sweat and VO_2max (107) and only one-third to one-half of the variation in VO_2max is now thought to be attributable to genetics (20, 21).

Results from the Cooper Institute for Aerobics Research have highlighted the impact that measurement of physical of activity can have on the corresponding relationships between physical activity and physical fitness (107). Participants inputted information pertaining to their exercise bouts into computer terminals and these data were stored in a computer data base so that an aggregate record of physical activity resulted. They found strong associations between

average weekly aerobic points and maximal treadmill time for adults younger and older than 50 years of age (r values of 0.66, 0.71, 0.78, and 0.83 for younger men, older men, younger women, and older women). Considering that maximal time on the treadmill test was strongly correlated with VO_2max (i.e., r=0.92 for men and r=0.94 for women), this strongly suggests that physical activity has a powerful effect on cardiorespiratory fitness (120, 121). Since cardiorespiratory fitness has an effect on all-cause mortality at a range of BMI's (73), it is worthwhile to maximize the accuracy of our assessments of physical activity.

Beyond the relationships between physical activity, cardiorespiratory fitness, and obesity, energy deficits created by exercise alone have been found to be equally as effective as diet alone in creating changes in body weight, subcutaneous fat, and visceral fat (105) and therefore potentially reversing obesity per se. Therefore, physical activity appears to have a restrictive effect on obesity and obesity's effects on health (73). Taken together, these data point towards a mediating effect of physical activity that may supersede obesity as a determinant of health (73, 108, 115, 163).

Vascular Inflammation

Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis and mediate many of the stages of atheroma development, from initial leukocyte recruitment to eventual rupture of the unstable atherosclerotic plaque. Elevation of several markers of the inflammatory cascade has been shown to predict future risk of plaque rupture. Some of these are P-selectin, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), soluble intercellular adhesion molecule-1(sICAM-1), and C-reactive protein (CRP) (15). Evidence is mounting that

high-sensitivity C-reactive protein (hsCRP) has the potential to play an important role as an adjunct for global risk assessment in the primary prevention of cardiovascular disease (131).

The immune system is a coordinated association of molecules, cells, tissues and organs that act in a coordinated manner to defend the body against several potential aberrations to the body's internal milieu; including foreign substances, mutated and 'out of control' cells, and deleterious effects of tissue damage. The last case is referred to as inflammation and is a product of innate immunity. It is a process whereby damage to vascularized tissue is repaired through the delivery of defensive materials to the site of injury (85). Three primary effects of inflammation are to 1) prevent the dispersion of damaging agents to surrounding tissue, 2) dispose of cellular debris and pathogens, and 3) set the stage for the repairs (85).

Atherosclerosis is increasingly understood as an inflammatory phenomenon. First, with atherosclerosis the conduits by which sustenance is delivered to typical sites of damage (e.g., muscle tissue) are now the sites of damage and therefore the process of manipulating fluid delivery is not as pertinent. Second, debris accumulates and is incorporated into the site of insult (e.g., the fibrous cap). Thirdly, the repair process is continuous and perpetuates itself such that an end-state of healing is never achieved.

Atherosclerosis is a form of arterial disease characterized by thickening and hardening of the vessel walls that is caused by soft deposits of intra-arterial fat and fibrin that harden over time (24). The fatty streak is the first sign of atherosclerosis. Outward signs of endothelial dysfunction may precede the fatty streak and provide opportunity for early recognition (26). The fatty streak is a site of damage and essentially represents a hole in the intima. It is quickly infiltrated by T lymphocytes and then by monocytes. T lymphocytes are believed to actively participate in lesion formation (40). They release cytokines, particularly interferon gamma

(INFγ), that potentiate monocyte invasion, promote proliferation of smooth muscle cells, and regulate matrix metalloproteinases (67). Cytokines are peptides that participate in intercellular signaling through autocrine, paracrine, and endocrine mechanisms (25). Some important cytokines that are integral to the inflammatory process are IL-1 and TNF-alpha (25).

IL-6 released from macrophages present in the fatty streak is the principal stimulus for CRP synthesis with vascular inflammation, although IL-1 and TNF-α also play a role (129, 158). CRP is a major acute phase reactant and mediates its effects through activation of the complement system in order to lyse antigens. With respect the atherosclerosis, CRP may facilitate phagocytosis of injured biological membranes (129). In general, data suggests that it undermines the structural and functional integrity of the endothelial lining. A few other mechanisms by which it may accomplish this end is by increasing secretion of IL-1 and TNF-α (47), IL-6 (158), MCP-1 (112, 158); and increasing expression of ICAM-1 (113).

Whereas neutrophils typically infiltrate the fatty streak with immediacy, monocytes follow after approximately 24 hours (161). Monocytes are large leukocytes (up to ~18μm) that travel in the blood and are attracted by chemokines released from damaged tissue (85). When imbedded in a damage site, monocytes evolve into very active macrophages that engulf lipids, damaged tissue and other debris and evolve into foam cells. This begins a positive feedback mechanism that creates the calcified, collagenous, lipid-laden plaque that intrudes into the vessel lumen. The damage appears to be accelerated by elevated hematocrit, blood glucose, and homocysteine (which increases hydrogen peroxide production, which damages endothelial cells); as well as smoking to name just a few vascular insults.

Once the leukocyte adheres to the endothelium and imbeds itself in the wall, it is able to dissolve the basement membrane by way of the dissolving enzymes collagenase and elastase

(85). This process is called diapedesis. The adhesion molecules are integral to this process. That is, adhesion molecules enable the slowing down and binding of leukocytes to the endothelium. They include the selectins, integrins, and the adhesion molecules (e.g., ICAM-1 and VCAM-1); which in combination allow for marginalization and removal of the leukocyte from the axial stream of blood flow through slowing and then firm binding to the endothelium. ICAM-1 is thought to be the most sensitive adhesion molecule for up-regulation signals and the most pertinent for polymorphonuclear neutrophil attraction and adhesion to endothelial cells (161). Without ICAM-1 expression (ICAM-1 knockout mice), the inflammatory response to high lipid levels is reduced (161).

The eventual layer of plaque that covers the necrotic core (composed of proteolytic enzymes, macrophages, and lymphocytes) results from platelet-derived growth factors, including transforming growth factor β, interleukins, and TNF-α (80). These plaques have been characterized as stable or unstable, the former of which is characterized by a well-preserved lumen and a thick fibromuscular cap (40). The unstable plaque is characterized by large lipid core, fraught with inflammatory cells, covered by a thin cap (40, 81). An imbalance of matrix metalloproteinases and clot dissolving substances (plamsin, tPA-1, etc.) can quickly cause a thrombus or embolus to develop, the former of which can be especially dangerous if it travels to the lungs or brain (79, 80).

Oxidized low-density lipoprotein (LDL) in the lesion is an important component because its oxidation stimulates the production of monocyte chemoattractant protein-1 (MCP-1) (134). Oxidized LDL can inhibit macrophage mobility, exacerbating foam cell formation, and is also independently toxic to endothelial cells (125). Statin drugs have a beneficial effect by reducing

LDL production by the liver and therefore lipid accumulation in the lesion and inflammation (82, 140).

Foam cells are part of the positive feedback system that increases LDL receptor concentration and affinity on both macrophages and endothelial cells. This process also increases the concentration of angiotensin II receptors, which facilitates smooth muscle contraction and therefore narrows the lumen and stiffens the vessel. In addition to its potent constriction effects, angiotensin II also encourages smooth muscle growth and lypo-oxygenase activity (increasing synthesis of leukotriene B4, another potent chemokine and growth factor) (85).

Cardiovascular Disease

Several markers of vascular inflammation have been found to correlate with overt CVD. The adhesion molecules have been used as an index of vascular inflammation in large-scale case-control studies. ICAM-1 was found to predict risk for development of CHD in the Atherosclerosis Risk in Communities (7) study (59). Higher levels of E-selectin and ICAM-1 were observed for the patients with CHD and those with coronary artery atherosclerosis compared with control subjects. Similar results were found in the Physician's Health Study (PHS) (133). The relative risk (RR) for future myocardial infarction was 1.8 (P=0.03) for those in the highest quartile for sICAM-1 levels. Vascular adhesion molecule was not associated with increased cardiovascular risk in either of these studies. This underscores what may be important mechanistic distinctions between these two classes of adhesion molecules. ICAM-1 is highly expressed in endothelial cells and macrophages. In contrast, VCAM-1 is found in only one third of lesions and it is primarily found on endothelial cells (33).

CRP has been evaluated most extensively as a marker of inflammation in clinical studies. Baseline levels of CRP, as well as serum amyloid A, IL-6 and sICAM-1, were significantly elevated among postmenopausal women who subsequently developed cardiovascular events compared with those who did not (132). CRP was most associated with development of CVD. The RR for the highest compared to lowest quartile of CRP was 4.4 (P<0.001) (132). Another significant finding from this investigation was that increased levels of markers of vascular inflammation also significantly increased the risk of future cardiovascular events in women with LDL below 130 mg/dL. This effect was also strongest for CRP. This suggests a strong relationship of vascular inflammation with overt CVD that is independent of other risk factors.

Body Fat

It has become clear that adipose tissue plays a much greater physiological role than simple energy storage. Adipose is now known to interact and communicate with a variety of other bodily tissue and organ systems through the metabolism of and response to a variety of hormones and cytokines (65, 144). There are several potential biological mechanism(s) whereby adipose tissue may increase the rate of atherosclerosis independent of the lifestyle factors that contribute to overweight and obesity. There are also epidemiological data to support a role of body fat in promotion of vascular inflammation.

The identification and characterization of leptin in 1994 helped to establish the role of adipose tissue as an endocrine organ. Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight. Leptin receptors are also found on T lymphocytes, macrophages/foam cells and vascular endothelial cells (111). Leptin exerts pro-inflammatory, pro-angiogenic effects through simulation of vascular endothelial growth

factor (VEGF) and fibroblast growth factor (FGF)-2 by activating a specific receptor (Ob-Rb) which is expressed in human endothelial cells (52). Critical steps in early atherosclerosis are endothelial infiltration by macrophages and evolution to foam cells, as well as vascularization of immature plaques. A recent study found that leptin receptors were expressed in human atheromatous plaques and that leptin increased VEGF, human umbilical vein endothelial cells (HUVEC's), and matrix metalloproteinases (111). These findings suggest a link between leptin and degradation of subendothelial basement membrane and the surrounding extracellular matrix, as well as endothelial cell migration and vessel proliferation. Experimental work with rat corneas demonstrated a co-localization of Ob-R and VEGFR-1 receptors (111). Other data suggest that leptin regulates the osteoblastic differentiation and calcification of vascular cells and that the artery wall may be an important peripheral tissue target of leptin action (110).

Data is mounting to suggest that adipose tissue is a significant site of IL-6 production (9, 84, 95). For instance, Maachi et al. (84) found strong positive correlations between adipose and circulating levels of TNFα, IL-6, and CRP. This is consistent with the role of human adipose tissue in the regulation of blood circulating CRP concentrations via IL-6 production in obesity. Obesity has been found to be directly associated with increased plasma levels of hsCRP (130, 131) and CRP is thought to play a direct role in promoting vascular inflammation (113).

Recent data from the Third National Health and Nutrition Examination Survey (1988-1994) support the notion that body fat levels impact CRP levels. A total of 16,573 subjects 20 years or older were evaluated for CRP, BMI, and diabetes status. CRP was lowest among individuals with a BMI < 18.5 and increased with increasing BMI categories. After adjusting for age, sex, race or ethnicity, and education, OR for an elevated CRP (≥ 85th percentile of the sex-specific CRP distribution) among participants with a BMI of 25 to 29.9, 30 to < 35, 35 to < 40,

and ≥40 were 1.51 (95% CI 1.23-1.86), 3.19 (2.60-3.91), 6.11 (4.67-7.98), and 9.30 (6.43-13.46), respectively, compared with participants with BMI < 25.

IL-6 may serve a similar function as leptin (i.e., negative feedback for body fat levels and energy intake. In addition to mediating inflammation, it also increases energy expenditure (Wallenius, 2003 #351). Injection of IL-6 in rats was found to increase resting energy expenditure and decrease body fat (160). Subcutaneous IL-6 administration induced synchronized dose-dependent increases in the resting metabolic rate and hypothalamic-pituitary-adrenal axis activity in humans (153). IL-6 is also a potent stimulus for ICAM-1 up-regulation, although it is also up-regulated by other cytokines such as IL-1 and TNF-α (161).

A practical illustration of some of these relationships is found in the Pima Indians of Arizona. They have higher incidence of obesity and higher concentrations of humoral markers of inflammation than normal (Weyer, 2002 #349). CRP, secretory phospholipase A2 (sPLA2), and sICAM-1 have all been found to be positively correlated with percent body fat in this group ($r=0.71$, 0.57, and 0.51, $P<0.01$). Similar results were found for obese hypertensive and non-hypertensive men as compared to matched controls (41). Obese versus normal-weight groups (i.e., BMI<25), for both hyper- and normotensive groups, showed higher ICAM-1 and VCAM-1 with obesity ($P<0.001$).

Weight Loss

Since excessive body fat seems to result in excessive concentrations of markers of vascular inflammation, it stands to reason that a loss of body weight should reduce these concentrations. In fact, there is data to support this notion. Twelve weeks of increased activity in obese women lead to a reduction in body weight and fat and concurrent reductions in sICAM-

1 and sE-selectin (61). Reductions in markers of vascular inflammation were only weakly associated with reductions in body fat ($r=0.34$, $p=0.06$ and $r=0.46$, $p=0.06$, for sICAM-1 and sE-selectin, respectively). Similar results were found in obese men who lost weight through diet alone (VCAM-1 $P=0.04$, ICAM-1 $P=0.003$, E-selectin $P<0.0001$, and vWF $P<0.0001$) (41). In another study, reductions in body weight and in total body fat mass in obese postmenopausal women were induced through caloric restriction alone. Reductions in both were positively associated with reductions in plasma CRP (150). Interestingly, the cohort was still nearly obese as the group BMI after the weight loss was 29.7±4.2.

Physical Activity and Fitness

Approximately 28.7% of the American population is estimated to be inactive, and 25.4% is estimated to be active (at recommended levels of physical activity), as of 1998 (1). Unfortunately, this data is limited in some respects. For one, there is error involved in recalling physical activity. Another limitation is that the 2 most prominent activities over the previous month were reported. However, this data does suggest that a very large portion of the American population engages in no leisure-time physical activity. The literature suggests that as little as 30 minutes per day of moderate-intensity physical activity (e.g., brisk walking) reduces the incidence of clinical cardiovascular events in men and women (8). This is supported by several large-scale prospective trials, including the Multiple Risk Factor Intervention Trial (76), the Lipid Research Clinics Mortality Follow-up Study (38), and the Harvard Alumni Study (108). Cardioprotective mechanisms of physical activity include reducing adiposity, blood pressure, diabetes incidence, dyslipidemia, and inflammation, and enhancing insulin sensitivity, glycemic control, fibrinolysis, and endothelial function (8). Regular exercise may also retard the

progression of asymptomatic coronary and peripheral arteriosclerosis. Only a quarter of the American population engages in sufficient leisure-time physical activity to confer protection against chronic disease. With that, it is obvious why the study of physical activity and risk for chronic disease attracts a great deal of attention.

Several investigations of vascular inflammation support the notion that physical activity has evolved into a necessity for humans. In a recent prospective investigation 405 healthy men and 454 healthy women were assessed for physical activity, soluble tumor necrosis factor receptor-1 (sTNF-R1), sTNF-R2, IL-6, and CRP (118). Physical activity was inversely associated with plasma levels of sTNF-R1, sTNF-R2, IL-6, and CRP ($P=0.07$, $P=0.004$, $P=0.04$, and $P=0.009$) independent of other predictors of inflammation. However, after adjustment for BMI and leptin, most of these associations were no longer significant. Physical activity was also inversely related to insulin and C-peptide levels ($P=0.008$ and $P<0.001$). Other data generally corroborates these findings (2, 48). For example, vascular inflammation was evaluated in recent study of 3638 apparently healthy US men and women (age≥40 yrs) conducted in coordination with the Third National Health and Nutrition Examination Survey (2). After controlling for BMI and diet, a 23% reduction in the odds ratio for elevated CRP (≥0.70 mg/dL) was found for those who engaged in physical activity 4 to 21 times per month (2). In a recent report, data from the National Health and Nutrition Examination Survey III on 13,748 participants (age ≥ 20 years) was used to evaluate the relationship between physical activity and CRP (44). After adjusting for confounders, the OR for elevated CRP (dichotomized at the ≥85th percentile of the sex-specific distribution) were 0.98 (95% CI = 0.78-1.23), 0.85 (0.70-1.02), and 0.53 (0.40-0.71) for participants who engaged in light, moderate, and vigorous physical activity, respectively, during the previous month compared with participants who did not engage in any leisure-time physical

activity (44). Although these results support the concept that physical activity reduces inflammation, they are inconsistent as to whether physical activity mediates its effects on inflammation through its effect on body fat levels.

Data also suggests that the extent of vascular inflammation is modifiable within the context of a training intervention. In a recent longitudinal study, six months of individualized exercise was found to decrease the atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease (146). Mononuclear cell production of atherogenic cytokines fell by 58.3 % ($P<0.001$) following the exercise program. Also, the production of atheroprotective cytokines rose by 35.9% ($P<0.001$).

Cardiorespiratory fitness has also been shown to be correlated with vascular inflammation. This is considered a representative surrogate as a substantial portion of the variation in cardiorespiratory fitness is determined by physical activity (21). There was an inverse association of CRP across fitness levels, with the highest adjusted CRP value in the lowest fitness quintile (1.64 (1.27 to 2.11) mg/L) and the lowest adjusted CRP value in the highest fitness quintile (0.70 (0.60 to 0.80) mg/L) (29). Similar results were found for the prevalence of elevated CRP across fitness quintiles.

Does Physical Activity or Cardiorespiratory Fitness Modify the Effect of Body Fat on Vascular Inflammation or CVD Risk?

Several well-controlled studies suggest that physical activity and higher cardiorespiratory fitness diminish the health risks associated with overweight and obesity. The following conclusions were drawn in a recent review (11).

1) regular physical activity clearly attenuates many of the health risks associated with overweight or obesity

2) physical activity appears to not only attenuate the health risks of overweight and obesity, but active obese individuals actually have lower morbidity and mortality than normal weight individuals who are sedentary, and

3) inactivity and low cardiorespiratory fitness are as important as overweight and obesity as mortality predictors

Convincing data has come from the Cooper Institute in Dallas as to the net conflicting effects of fitness and fatness. After adjustment for age, examination year, cigarette smoking, alcohol intake, and parental history of ischemic heart disease, unfit (low cardiorespiratory fitness as determined by maximal exercise testing), lean men had double the risk of all-cause mortality of fit, lean men (RR: 2.07; 95% CI: 1.16, 3.69; P = 0.01) (72). Unfit, lean men also had a higher risk of all-cause and CVD mortality than did men who were fit and obese. Similar results were found for fat and fat-free mass in relation to mortality. Unfit men had a higher risk of all-cause and CVD mortality than did fit men in all fat and fat-free mass categories. Similarly, unfit men with low waist girths (<87 cm) had greater risk of all-cause mortality than did fit men with high waist girths (> or =99 cm). These data comprise some of the most convincing evidence that the health benefits of leanness are limited to those who are fit, and especially that being fit may reduce the risk associated with obesity.

Another study from the Cooper Institute evaluated the effect of cardiorespiratory fitness on mortality in 25,714 men stratified by BMI (163). In this prospective cohort study, low fitness was an independent predictor of mortality in all body mass index groups after adjustment for

other mortality predictors. Approximately 50% of obese men had low fitness, which led to a population-attributable risk of 39% for CVD mortality and 44% for all-cause mortality. Low cardiorespiratory fitness was a strong and independent predictor of CVD and all-cause mortality and of comparable importance with that of diabetes mellitus and other CVD risk factors. This supports the notion that higher fitness lessens the risk that being over-fat imposes on health. Similar findings have also come from studies utilizing female cohorts. In women with suspected myocardial ischemia, BMI, waist circumference, waist-hip ratio, and waist-height ratio were not independently associated with angiographic CAD or adverse CV events (164).

The veracity of these outcomes is strengthened by an autopsy study designed to control for the effects of body fat and factors related to excess body fat that may mediate the effects of obesity on CVD. Obesity did not stand out as a risk factor for atherosclerosis as assessed by mean coronary wall thickness, coronary calcification, incidence of raised lesion in the abdominal aorta, and incidence of raised lesions in the coronary arteries (115).

The next question is whether physical activity quells or precludes the effect of excess body fat on vascular inflammation. The literature is not completely clear as there are data to suggest that physical activity does not modify the deleterious effect of body fat on vascular inflammation. The effects of BMI and both current and previous-year physical activity on hsCRP was examined in healthy men and women (N = 109). Average hsCRP was significantly correlated with average BMI ($r = 0.50$; $P < 0.001$) but was not related to previous-year physical activity levels ($r = 0.02$; $P = 0.89$). Current physical activity was similar between the three BMI groups (i.e., <25, 25-29.9, ≥30) at all times, and was unrelated to hsCRP in all groups throughout the study period. Similarly, data from Pischon et al. (118) suggest that physical activity mediates its effects on vascular inflammation through reductions in body fat. After adjusting for body fat,

the relationships between increased physical activity and reduced vascular inflammatory markers were no longer significant.

In contrast, data also exist to suggest that physical activity may reduce vascular inflammation independent of body fat. An inverse dose-response relationship between physical activity and vascular dysfunction and several markers of inflammation (including CRP) was maintained even after adjustment for BMI (162). The relative odds of having high CRP (i.e., ≥4.27mg/dL) was 0.37 and 0.44 in the moderate to vigorous and vigorous activity groups, respectively. These data also suggest that regardless of fatness, it is beneficial to take up physical activity. Similar results were reported from the Cardiovascular Health Study (48). In a cohort of 5,888 men and women aged ≥65 years of age, the highest quartile of self-reported physical activity had 19%, 6%, 4%, and 3% lower concentrations of C-reactive protein, white blood cells, fibrinogen, and Factor VIII activity, respectively. This was after adjustment for gender, the presence of cardiovascular disease, age, race, smoking, body mass index, diabetes, and hypertension. Both of these studies evaluated elderly men and long-term prospective cohort studies, as well as data from female cohorts, are not available. However, it is interesting to note that a reduction in vascular inflammation in overweight and obese groups who take up exercise occurs despite maintenance of overweight or obese status.

In a randomized control trial, twelve weeks of increased activity in obese women lead to a reduction in body weight and fat and concurrent reductions in sICAM-1 and sE-selectin (61). Reductions in markers of vascular inflammation were weakly associated with reductions in body fat ($r=0.34$, $p=0.06$ and $r=0.46$, $p=0.06$, for sICAM-1 and sE-selectin, respectively). BMI was 27.2±1.9 at baseline and 25.3±2.1 after training. Since a 'normal weight' control group was not utilized, it is not possible to determine whether the experimental group was in a 'normal' range

for sICAM-1 and sE-selectin. In a group of obese postmenopausal women in whom reductions in body weight and in total body fat mass were induced through caloric restriction alone, significant reductions in plasma CRP occurred (150). However, the cohort was still overweight to obese (BMI: 29.7±4.2).

Cardiorespiratory Fitness

The term fitness, or physical fitness, is used holistically to describe how well an individual is suited to engage in physical activity as well as their extent of general health (3). The term encompasses at least 4 physical domains, including body composition, flexibility, muscular fitness and cardiorespiratory fitness (122, 154). The concept of physical fitness was promoted as early as 3000 B.C. in India in the Ajur Veda, which eventually evolved into Yoga (154). Due to the prevalence of chronic behavior-related disease (e.g., cardiovascular disease), fitness continues to receive a great deal of attention in terms of research (91) and public advocacy. Cardiorespiratory fitness has probably received the most attention in recent decades.

Cardiorespiratory fitness is typically quantified as maximal oxygen consumption (i.e., VO_2max), the greatest oxygen utilization rate of which an individual is capable (3). It is typically measured using a graded exercise test performed on either a treadmill or cycle ergometer. The treadmill has the advantage that the burden of the physical effort is distributed over a larger proportion of muscle mass and therefore the rate of oxygen utilization is greater during the maximal effort. On the other hand, the cycle ergometer removes body weight from the challenge but relies proportionately more on the thighs. A disadvantage is cardiorespiratory fitness measured in this way is sometimes limited by thigh fatigue. In the case of obesity, higher

body weights may impose a larger limitation for exercise where body weight must be maneuvered as compared to cycle ergometry where the effect of body weight is removed.

Methods of assessing of VO_2max have been called into question over the years (53). In order to make an objective determination that VO_2max has been reached a plateau in VO_2 must occur, meaning that with an increase in exercise intensity, there is not a concomitant increase in VO_2. If a plateau does not occur, then the term VO_2peak is used to express that the value is not necessarily maximal but rather the highest attained during the test (90). Since VO_2peak occurs during the highest intensity attained, the test is also in large part an effort-dependent performance test rather than a physiologic index. Furthermore, it cannot be objectively determined whether the intensities reached during training are maximal, and therefore the adaptations may not be accurately reflected by the typical results of a *maximal* test. Therefore, assessment of VO_2 at a given high but sub maximal heart rate may have merit and even more accurately reflect global adaptations to a cardiorespiratory training program. Since test results are not excluded based on VO_2max test criteria (e.g., <2ml change in VO_2 with increase in intensity, heart rate <85% maximal predicted heart rate, RER > 1.1) there is less likelihood that resources are wasted due to data exclusion.

Although it is estimated that genetics account for 20 to 30% of cardiorespiratory endurance (20, 89), it is nevertheless a trainable attribute (3). Sedentary adults trained for 20 weeks on a cycle ergometer, progressing to 75% of the heart rate associated with VO_2max for 50 minutes for the last 6 weeks (20). The mean increase in VO_2max was 400 ml/min, with values as high as 1.01 L/min. Another study found that adaptations to training at 45% of VO_2max were comparable to training at 80-85% of VO_2max when overall energy expenditure was standardized (45). Therefore, cardiorespiratory fitness appears to be modifiable by lifestyle habits that include

at least moderate-intensity aerobic exercise for the minimum recommendation of 3 days per week. These findings in combination with data that show that cardiorespiratory fitness is inversely related to risk for mortality (13) suggest that behavioral habits associated with better fitness also result in reduced risk for cardiovascular deterioration and mortality.

Central Arterial Stiffness

Arterial stiffness has been described as the degree to which an artery expands which each pulse under a given pressure stimulus and is inversely related to arterial compliance and distensibility. Arterial stiffness is affected by vascular smooth muscle tone (22, 156), arterial pressure (71), and elastin and collagen content of the vessel wall (30, 31, 100). Aberrant changes in arterial structure and function associated with atherosclerosis manifest in an increase in arterial stiffness (100). Arterial stiffness is reflected in the speed at which the pulse wave traverses the vascular tree (e.g., aortic pulse wave velocity or aPWV). Pulse wave analysis has experienced significant use as non-invasive indicator of arterial stiffness (32, 39, 98). Increased aPWV is associated with increased stiffness and central arterial plaque accumulation (10, 32, 157).

Aortic pulse wave velocity is influenced in large part by intrinsic properties of artery walls such that arteries that are stiffer and non-elastic transmit the pulse faster. In general, more compliant arteries (e.g., the aorta) are more prone to an increase in stiffness with age and/or disease than more muscular peripheral arteries (23, 104). The aorta serves a major damping function for the vascular tree as it is the primary vessel that converts cardiac pulsations into a more steady flow pattern for the smaller, more distal vessels (62). For instance, a less compliant aorta does not distend as well and experiences a larger increase in systolic pressure with

ventricular ejection, manifesting in a heightened systolic blood pressure. Downstream changes that reflect increased central arterial stiffness include increased pressure wave amplitude and blood flow velocity. These attributes reflect structural elements of arteries, notably smooth muscle, elastin, and collagen (62). The accumulation of other material is thought to be secondary to, and reparative for, a loss of elastin (98, 103). Elastin is the most inert material in the human body and its physical characteristics make it vulnerable to the repetitive bending and stretching that constitute circulatory function (98). Therefore, its degeneration over time is somewhat predictable based on fundamental engineering principals (103) and is reflected by the strong and independent effect of age on central artery stiffness (6, 141, 148, 149).

The effect of a stiffened vasculature has particular significance for cardiac function. A loss of elastin and increased stiffness of the aorta is positively related to ventricular hypertrophy (100). Although cardiac contractile efficiency is maintained in the face of a stiff receptacle, energy demand is increased (64). This might have little effect on the risk for an adverse cardiovascular event at rest. However, during times of increased exertion this may pose a significant limitation on performance and a significant risk for adverse myocardial events (66). Prospective studies have, in fact, revealed a positive association between elevated aPWV and mortality (10, 93).

Arterial stiffness changes throughout the lifespan and this is often reflected in increased arterial blood pressure. Age has most consistently been found to be associated with elevated central arterial stiffness (6, 141, 148, 149, 155). Even in normotensive, rigorously screened volunteers in whom systolic blood pressure increased an average of 14% between ages 20 and 90 years, major age-associated increases of arterial stiffness occur (155). Although they reflect

similar components of physiological function, data suggest that blood pressure and measures of arterial stiffness are sufficiently independent to justify the measurement of aPWV.

Systolic hypertension, highly reflective of central arterial stiffness, is the most important pressure-related determinant of ventricular hypertrophy (78) and is a significant correlate of premature mortality (50). However, although the increase in central arterial stiffness is related to arterial blood pressure (147), it has also been found to increase independent of blood pressure and peripheral arterial stiffness with age (147). In addition, aPWV is an independent predictor of cardiovascular risk in older adults with (10) and without (93) essential hypertension, and individuals with hypertension have been found to have aPWV similar to matched controls (39). These data suggest that the aPWV effect attributed to blood pressure is an artifact of age (39, 101).

Although the association between aging and arterial stiffness has been realized for some time, more recent evidence suggests that it may also be affected by body habitus and fitness. In one study of patients with sustained essential hypertension, BMI and aPWV were significantly correlated ($r=0.85$) and aPWV was significantly elevated in those who were obese (151). In addition, fitness has the opposite effect on aPWV. Vaitkevecius et al. (155) found an inverse correlation between cardiorespiratory fitness (treadmill VO_2max). In endurance trained male athletes 54 to 75 years old, aPWV was significantly reduced (26% lower) relative to their sedentary age-matched peers, despite similar blood pressures (155).

Arterial Remodeling and Blood Flow

Lower extremity conduit arteries participate in the transmission of blood flow from the central circulation to the microcirculation of the appendages. The major one is the femoral artery

as it supplies the larger part of the lower extremities. It is a continuation of the external iliac artery and bifurcates into the superficial femoral artery (SFA) and the deep (profunda) femoral artery (PFA). The femoral artery transmits blood from the iliac artery to the smaller more distal continuations of the femoral, such as the popliteal artery (continuation of the SFA). The popliteal in turn trifurcates into the anterior and posterior tibial arteries and peroneal artery supplying blood to the calf and foot. The PFA provides circumferential blood supply to the deep muscles of the upper thigh and femur and also supplies important collateral flow to the lower thigh and leg when the SFA is narrowed or occluded. The SFA has experienced relatively more study as its location is more superficial and it is easier to accurately image using current technology. It is referred to below as 'femoral artery'.

Femoral artery size has been found to be related to age, sex, body size and physical activity or fitness level (35, 94, 136). There is also data to suggest that femoral artery size is proportional to leg muscle mass (106, 126). However, the extent to which arterial size and blood flow are attributable to fat mass has not been clarified.

The process by which conduit arteries change in size is commonly referred to as arteriogenesis or arterial remodeling (35, 123, 124). Modeling of arterial diameter is largely mediated by the effect of blood flow on the endothelium and involves the release of nitric oxide and prostacyclin (70, 75, 99). It has been proposed that in situ arterial remodeling (at least in peripheral conduit arteries) is a teleological phenomena that occurs in order to accommodate periodic increases in blood flow and/or oxygen demand that occur during exercise (35). Thresholds of blood flow and shear stress appear to be necessary to maintain both endothelial health (86) and arterial size (70). Blood flow and shear stress increase during physical activity in order to meet the metabolic demands of skeletal muscle that is contracting. This may help

explain the connection between cardiorespiratory fitness and femoral artery size as regular physical activity accounts for a significant portion of cardiorespiratory fitness.

One model that has been used to study arterial size is spinal cord injury (SCI). Extreme disuse, or a complete lack of physical activity in the lower extremities, is a regression from normal daily activity and is embodied in the condition of SCI. Studies have reported smaller femoral arteries in subjects with SCI as compared to size-matched controls. Olive et al. (106) reported a 36% reduction in femoral artery size as a result of chronic SCI. However, the ratio of artery size to limb volume was similar between an SCI group and a matched AB group. Interestingly, we have shown in a subset of the subjects with SCI from that study (106) that a 35% increase in muscle size is not associated with a concurrent increase in either femoral artery diameter or blood flow (in review). This suggests that any continuity in the relationship between artery size and limb volume that may exist is not completely resistant to change.

Previous research in AB subjects also suggests that resting femoral artery diameter and blood flow are primarily determined by leg fat-free mass and resting oxygen consumption (34). However, that resting blood flow does not seem to change after a training intervention that changes sedentary subjects' status to 'active', and increases fitness (35), suggests that resting blood flow may be a somewhat static physiological feature or that the Doppler ultrasound method of blood flow measurement is insensitive to changes in blood flow. It also leaves muscle size as a primary determinant of resting blood flow.

Other studies have attempted to re-create similar effects to SCI in AB subjects through bed rest or limb immobilization. Fifty-two days of bed rest in AB subjects resulted in a 13% decrease in femoral artery diameter (17). Blood flow was unchanged (17). In another study, femoral artery diameter decreased by 12% after 4 weeks of unilateral lower limb suspension

(16). Therefore, the magnitude of decrease in femoral artery diameter with short-term disuse does not appear to reach the same magnitude as that found in cross-sectional studies evaluating the effects of SCI.

The effects of cardiorespiratory fitness on positive arterial remodeling, or increases in arterial caliber have also been studied. For instance, femoral artery diameter increases in response to short-term training (35), correlates with oxygen uptake (127), and is larger in people who are endurance-trained (138, 165). Dinneno et al. (35) used 3 months of lower body aerobic training (i.e., walking) in sedentary men to induce a 9% increase in femoral artery diameter. In a cross-sectional study, they found femoral artery diameter to be 7% greater in endurance exercise-trained versus sedentary men (35). Although a precise cause-and-effect relationship is not available, consideration of the evidence presented would support the notion that heightened cardiorespiratory fitness mediates an increase in arterial caliper, at least in part, through physical activity required to increase and/or maintain increased cardiorespiratory fitness. The data up to now do not permit a specific determination of whether the same genetic determinants that affect cardiorespiratory fitness also determine femoral artery diameter.

Shear stress has been implicated in arterial remodeling (49, 168). Interestingly, blood flow does not appear to be affected by training status in AB subjects (35) or by disuse associated with SCI (106). However, one study found a statistical trend for a drop with training (35). Two studies have found elevated resting shear stress with SCI (19, 137). The clinical implications of reduced resting shear rate are not completely clear as considerations of shear stress have traditionally been approached from the perspective that elevations are of benefit to the endothelium and arteries in general, which has overwhelming experimental support (70, 86, 99, 152). Therefore, the role of reduced resting shear rate in people with higher physical activity or

fitness has yet to be clarified. Longitudinal studies have not yet determined whether shear rate is decreased in sedentary individuals who take up exercise. It is also not clear whether this is a consistent effect of increased exercise or what type of exercise is required to generate this effect.

Summary

The preceding review of literature highlights some of the research on obesity; physical activity and fitness; and cardiovascular health. There is enough data currently to justify the need for humans to meet minimal standards of physical activity and fitness in order to maintain health and improve resistance to CVD (154). However, there is much left to clarify regarding how fitness and body fat interact to affect health. The vascular health indicators highlighted in this review have been successfully developed and are suitable candidates to help in research efforts aimed at clarifying the fat and fitness controversy.

CHAPTER 3

THE RELATIONSHIP OF VASCULAR FUNCTION TO FITNESS AND ADIPOSITY IN HEALTHY WOMEN[1]

[1] Sabatier, M.J., E. Marinik, E. Schwark, S. Reffett, M. Bergeron, R. Lewis, G. Sloan, J. Cannon, & K.K. McCully. To be submitted to the Journal of Applied Physiology.

ABSTRACT

Physical activity can increase aerobic fitness and reduce adiposity, resulting in decreased risk for cardiovascular disease (CVD). The primary aim of this cross-sectional study of 46 healthy adult women (31 ± 5 years old) was to determine if functional measures of vascular health, namely aortic pulse wave velocity (aPWV, an index of arterial stiffness), femoral artery diameter (D_{FA}), and femoral artery blood flow (BF_{FA}); and serum concentrations of proteins associated with risk for cardiovascular disease, including C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and leptin, were related directly to adiposity (percent body fat determined by dual energy x-ray absorptiometry) or aerobic fitness (VO_2peak normalized to fat-free mass). aPWV and D_{FA} significantly correlated with VO_2peak (r=-0.42, P=0.02 and r=0.42, P=0.004, respectively), but none of the vascular measures significantly correlated with % body fat. In addition, D_{FA} significantly correlated with leg fat free mass (r=0.28, P=0.03). CRP, sICAM-1, and leptin significantly correlated with % body fat (r=0.46, P=0.002; r=0.29, P=0.03; r=0.84, P<0.001), but not VO_2peak. However, VO_2peak reduced the increase in CRP associated with an increase in % body fat (interaction effect, P=0.01). A secondary aim of this investigation was to determine if functional measures of vascular health were related to CRP, sICAM-1, and leptin. sICAM-1 significantly correlated with aPWV (r=0.30, P=0.02), whereas CRP and leptin did not significantly correlate with any of the vascular measures. The inverse relationship of arterial stiffness to aerobic fitness, without a concomitant relationship to adiposity, as well as the moderating effect of aerobic fitness on the positive CRP-adiposity relationship, suggest that improving aerobic fitness may deserve a higher priority than reducing total body fat in an attempt to reduce CVD risk in healthy adult women. Moreover, a circulating protein originating

from vascular cells (sICAM-1) may be a more specific indicator of vascular function than a protein originating from a distal source, such as CRP.

Key words: percent body fat, sICAM-1, CRP, leptin, femoral artery, pulse wave velocity

INTRODUCTION

Cardiovascular disease (CVD) has proven to be a severe and persistent public health problem (8). At the same time, the prevalence of obesity, or elevated adiposity, has reached record levels and is associated with elevated risk for CVD mortality (6, 40). Reducing body fat, or maintaining low body fat, is recommended to minimize CVD risk. Since both CVD and elevated adiposity typically reflect poor aerobic fitness, it is increasingly important to understand whether adiposity and aerobic fitness are independently associated with CVD risk. Although elevated aerobic fitness has been found to reduce the risk for CVD associated with elevated adiposity (28, 31), there remains some question as to how this is accomplished. Evaluation of functional measures of vascular health, as well as proteins associated with inflammation and CVD risk, may help explain the beneficial effects of physical activity and aerobic fitness.

Aberrant changes in arterial structure and function associated with atherosclerosis manifest in an increase in arterial stiffness (35) and have been evaluated non-invasively using aortic pulse-wave velocity (aPWV) (10). Aortic PWV is the speed at which compression waves generated by cardiac contractions travel the length of the aorta (26) and is typically measured between a central location (the heart, aorta, or carotid artery) and a distal location (the femoral artery). Increased aPWV is associated with increased stiffness and central arterial plaque accumulation (48), and prospective studies have revealed a positive association between elevated aPWV and mortality (32). It is also well-established that aging has a significant and independent impact on arterial stiffness (2). More recent studies show aPWV to be inversely associated with fitness (47) and positively associated with adiposity (46) as well.

Conduit arteries have been found to adapt to increased circulatory demands through an increase in size (i.e., arterial remodeling). For instance, recent studies have shown significant

effects of fitness and physical activity (presumably increased circulatory demands) on lower extremity arterial diameter and blood flow in normal weight adults (13, 14, 16). However, lower extremity artery diameter is also associated with lower extremity mass (16). At this time it is not clear how aerobic fitness and adiposity interact with limb size to determine lower extremity artery structure and blood flow.

At present it is understood that the formation of an atherosclerotic lesion begins with the receptor-mediated uptake of low density lipoproteins (LDL) by endothelial cells (21). The LDL accumulates in the intima and is modified by oxidation and glycation. These products are recognized by scavenger receptors on resident macrophages, which in turn are activated to produce cytokines including IL-1β, TNFα and IL-6. These cytokines initiate a vicious cycle by activating endothelial cells to express more LDL receptors as well as adhesion molecules that promote recruitment of monocytes and T cells. These cytokines also promote the proliferation of T cells and vascular smooth muscle cells (22), further increasing intimal thickness.

The circulating concentrations of several proteins that may originate from, or influence the development of, atherosclerotic plaques are currently under investigation as adjuncts to cholesterol/lipoprotein measurements in assessment of risk for cardiovascular disease. Among those reported to be independent predictors of cardiovascular disease risk are C-reactive protein (CRP) (30), leptin (49), and soluble intercellular adhesion molecule-1 (sICAM-1) (25).

During acute infection, hepatic production of CRP is stimulated by IL-6 and other cytokines produced by activated leukocytes, resulting in circulating CRP concentrations that can increase >1,000-fold. In this context, CRP binds pathogens and damaged cells, facilitating their clearance by phagocytic cells (3). In epidemiological studies involving hundreds or thousands of subjects, increased cardiovascular risk has been associated with 2- or 3-fold increases in serum

CRP. These small, but presumed chronic elevations are thought to perhaps reflect the release of low levels of cytokines from atherogenic lesions that stimulate hepatic CRP synthesis. In vitro studies have also raised the possibility that CRP might have direct effects on vascular cells that may promote plaque development (reviewed in (4)).

Leptin is an adipocyte-derived, 16 kDa hormone/cytokine that influences energy balance, thermoregulation and reproductive function (24). Leptin receptors have been identified on vascular cells (43), and several in vitro and animal studies have indicated that leptin may influence atherosclerotic plaque development (reviewed in (9)).

Endothelial membrane-associated ICAM-1 binds integrins expressed on circulating leukocytes and provides an anchoring point for their transendothelial migration. Expression of ICAM-1 on the membrane is upregulated by IL-1 and TNFα. Shear stress and TNFα can also activate metalloproteases that cleave ICAM-1 from the endothelial surface, liberating a soluble form (sICAM-1) (33, 44). However, using the circulating concentration of sICAM-1 as an index of vascular endothelial cell activation is complicated by factors such as IL-2 or thrombin that can induce ICAM-1 expression on the membrane without significant shedding of the soluble form (42).

The epidemiological associations of CRP, leptin and sICAM-1 with cardiovascular disease risk are based primarily on study populations of relatively advanced age that may include numerous individuals with comorbid conditions. Therefore, we sought to determine if any of these risk factors were related to vascular function in the relatively younger and healthier women involved in the present study.

It remains unclear how aerobic fitness reduces the risk for CVD in the face of excessive adiposity (28, 31) and whether this effect is found in healthy adults before advanced age. In

addressing this issue, it is important that efforts are made to use precise measures of adiposity and aerobic fitness, and to study cohorts that do not demonstrate a trend for poor aerobic fitness with elevated adiposity. Therefore, the purpose this study was to determine if functional measures of vascular health, as well as serum concentrations of proteins associated with risk for cardiovascular disease, are related directly to adiposity (percent body fat determined by dual energy x-ray absorptiometry) or aerobic fitness (VO_2peak normalized to fat-free mass). A secondary aim of this investigation was to determine if the functional measures of vascular health are related to serum concentrations of proteins associated with risk for CVD.

METHODS

Subjects

The study population included forty-six pre-menopausal females with a wide range of body fat and cardiorespiratory fitness levels (Tables 3.1 and 3.2). Thirty-three were Caucasian, 10 were African American, and 3 were of other descent. All subjects were screened to exclude for self-reported cardiovascular or other inflammatory disease, neuromuscular disease, hypertension (arterial blood pressure > 140/90 mmHg), and smoking. Subjects were studied on 2 days within a 7-day span. For all subjects who reported regular menstruation (n=43), testing occurred within 10 days of the cessation of menstrual bleeding (52). We found a significant relationship between progesterone and CRP (r=0.28, P=0.03). However, progesterone was not a significant independent variable when entered into regression models and did not correlate with either fitness or adiposity.

Data were collected at 2 sites: the Medical College of Georgia (site 1, n=20), and the University of Georgia (site 2, n=26). Maximal exercise tests and body composition

measurements were performed with equipment and investigators specific to each site. Measurements of the femoral artery were made with different ultrasound units at site 1 and 2 (the General Electric LOGIQ Book and the General Electric LOGIQ 400 CL, respectively). All other data were collected and analyzed by one investigator with the same equipment. All procedures were approved by the Human Assurance Committee at the Medical College of Georgia and the Institutional Review Board at The University of Georgia. All subjects were informed of the procedures involved in the project and gave written informed consent before undergoing testing.

Cardiorespiratory Fitness

Maximal exercise testing was performed using a friction-braked Monark cycle ergometer (12). The stationary cycle was used to avoid the possible effects of larger body sizes on maximal testing with protocols using ambulation, even though maximal oxygen consumption measured using the stationary cycle is 10% lower than that using a treadmill (11). The test began with 4 minutes of cycling without resistance followed by an increase of 15 W every minute (12). Heart rate (EKG) and blood pressure (auscultation) were assessed during each stage and subjects provided subjective ratings of perceived exertion during every other stage of the test. Peak oxygen consumption (VO_2peak) was taken as the maximal 30-second average.

Oxygen consumption was calculated based on measures of pulmonary ventilation and mixed expired oxygen and carbon dioxide using the Sensormedics Vmax 229 (site 1) or a Vmax Spectra (site 2) (Yorba Linda, CA). A separate group of investigators conducted tests at each site. This has the potential to be a confounding element of the study. However, we took several steps to help minimize this possibility. A calibrated Monark cycle ergometer, and the exact same protocol and data collection procedures were used for all tests at each site. The protocol was

rehearsed in full with investigators from both sites together in order to standardize testing procedures prior to subject recruitment and testing. A comparison of VO_2peak from the inactive groups from each site (n=13 and n=20 at site 1 and 2, respectively) using an independent t-test did not reveal a significant difference (25.8±6.3 ml · kgFFM^{-1} · min^{-1} vs. 25.9±4.3 ml · kgFFM^{-1} · min^{-1}, P=0.98). This suggests there was not consistent bias resulting from testing site. We were unable to make the same comparison for active females because only one fit female was tested at site 2.

Body Composition

Body composition was measured using DEXA whole body scan. The Hologic QDR 4500W Elite was used at site 1, and Hologic Delphi A (Bedford, MA) was used at site 2. One investigator at each site performed all scans and analyzed all data. Clothing was standardized (t-shirts and shorts) to minimize the inflation of body density due to added clothing and all metal was removed from the body including metal clothing and jewelry. The subject rested in the supine position with arms away from their sides and leg straight with ankles at least 2 inches apart. Each scan was compartmentalized and analyzed using Hologic whole body software for bone mineral density, fat free soft tissue and percent body fat. Inter-machine variability for DEXA scanners by the same manufacturer, with respect to percent body fat, has previously been found to minimal (-1.7±1.0%) (45). This has also been supported using DEXA scanners of different manufacturers (18). Furthermore, a soft-tissue phantom was used to calibrate the DEXA scanner at both sites as previously recommended (37).

Laboratory Measurements

Blood samples were obtained by venipuncture after a 12-hour fast to assay for CRP, ICAM-1, and leptin. Blood was collected into vacutainer tubes containing EDTA (1 mg/ml blood) as an anticoagulant and aprotinin (500 KIU/ml of blood) to limit proteolytic degradation. Blood was centrifuged at 300g for 10 minutes after which the plasma was collected and centrifuged at 10,000g for one minute to remove platelets. The platelet-free plasma was stored in a -70 °C freezer for later analysis. Serum CRP, leptin and ICAM-1 were assayed using ELISAs constructed with separately-purchased reagents. Primary antibodies were polyclonal rabbit anti-human C-reactive protein (A0073, DAKO, Carpintera, CA), monoclonal mouse anti-human leptin (MAB398, R, & D Systems, Minneapolis, MN), and monoclonal mouse anti-human ICAM-1 (MAB720, R & D Systems). Standards curves were prepared by 2-fold serial dilutions of purified human serum CRP (#236603, Calbiochem-Novabiochem, San Diego, CA), recombinant human leptin (398-LP, R & D Systems), and recombinant human ICAM-1 (ADP4, R & D Systems). For all assays, capture antibodies were coated on 96-well polystyrene plates (Corning #3590, Corning, NY). Samples and standards were incubated in duplicate in plates overnight at 4ºC. For CRP, the plates were washed and then horseradish peroxidase-conjugated polyclonal rabbit anti-human C-reactive protein detection antibodies (P0227, DAKO) were applied for two hours at room temperature. Color was developed using the substrate 2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma #1888, St Louis, MO). Absorbance at 405nm was measured with a Labsystems Multiskan MCC/340 plate reader (Needham Heights, MA). For leptin and ICAM-1, biotinylated mouse anti-human detection antibodies (R & D Systems BAM398 and BAF720, respectively) were applied for 2 hours followed by streptavidin-conjugated horseradish peroxidase (Pierce, Rockford, IL) for 30 minutes. Color was developed

and absorbance measured as described for CRP. Detection limits, inter- and intra-assay variabilities for each assay were: CRP - 0.3 ng/ml, 12%, 3%; leptin - 0.06 ng/ml, 5%, 2%; ICAM-1 - 0.14 ng/ml, 9%, 6%.

Aortic Pulse Wave Velocity and Arterial Blood Pressure

Aortic pulse wave velocity was used to evaluate central arterial stiffness (36). A Biopac MP100 physiological data acquisition system (Biopac Systems Inc., Goleta, CA) with AcqKnowledge software (v3.7.3) was used to evaluate aPWV. Biopac EKG electrodes were interfaced with a MEC110C transducer and an ECG100C electrocardiogram amplifier. One electrode was placed over the superior and another over the distal-most boundary of each scapulae, with a third placed on the lower back. A 40.6-66.0 cm Biopac blood pressure cuff (RX120F) was interfaced with a TSD120 transducer and DA100C general purpose transducer amplifier. The cuff was inflated to 60 mmHg for real time assessment of pulse and EKG waves. All recordings were made after 30 minutes of supine rest. Peripheral arterial blood pressure was measured during this period over the brachial artery of the left arm with a semi-automated blood pressure machine (Datascope, Mahwah, NJ) using an appropriate-sized cuff (39). The average of 3 to 4 blood pressure readings was used for subsequent analysis.

Distance from the sternal notch to the thigh cuff was measured with a standard tape measure maintaining a parallel orientation of the tape measure to the examination table without conforming to body topography. The data were converted to standard text format, and retrieved and graphed using spreadsheet software. The time difference (23) between the inflection point of the systolic pulse wave and the peak of the R-wave of the EKG signal was calculated for 30 cardiac cycles for which the pulse wave inflection point was most distinct. A time constant of

0.05 was subtracted from this difference to correct for isovolumetric contraction phase that precedes ejection (15). The calculation was performed as follows: aPWV = distance (cm) ÷ [time difference (sec) – 0.05 (sec)]. Day-to-day reproducibility with 2 testing days was evaluated in 17 subjects (11 females, 6 males; 22±2 yrs of age) using this method of evaluating aPWV with an average day-to-day difference of 8.1%.

Femoral Artery Diameter and Blood Flow

In all tests the subject rested quietly in the supine position for 15 minutes before data acquisition began. One investigator performed all Doppler ultrasound measurements for this study. The imaging site was located 1 - 5 cm distal to the femoral bifurcation. Pulsed Doppler ultrasound was recorded in the longitudinal view using an insonation angle between 45^{o} and 60^{o}. The velocity gate was set to include the entire lumen area. Resting flow velocity and B-mode images were saved electronically.

A portable Doppler ultrasound unit (GE LOGIQ Book, Rainbow City, AL) with a linear probe (4-11 mHz) was used at the site 1, and a the LOGIQ 400 CL (GE, Rainbow City, AL) with a 7-13 MHz linear-array ultrasound transducer was used at site 2 to measure arterial diameter (millimeters) and blood velocity (cm/second). Blood velocity was auto calculated by both units every cardiac cycle by GE advanced vascular program software at both sites. We acquired approximately one flow velocity number per cardiac cycle as the time-averaged maximal velocity (TAMAX) and took the average of these values as our resting flow velocity estimate for each subject over a 5-minute collection period. The TAMAX is calculated as a function of the upper boundary of the velocity signal divided by the duration of the cardiac cycle. Image files were opened using semi-automated software specially coded for use with NI LabVIEW (Austin,

TX). Arterial diameter was measured manually by applying straight lines conforming to the wall-lumen interface of two-dimensional images captured during data collection. Blood flow (mL/min) is the product of blood velocity and artery cross-sectional area and was calculated as follows: blood flow = $[\pi(\text{diameter}/2)^2]$*[velocity*60seconds/minute].

Day-to-day reproducibility with 2 testing days was evaluated in 19 subjects (10 females, 8 males; 22±2 yrs of age) for the GE LOGIQ Book portable ultrasound unit. Average day-to-day difference for diameter, blood velocity, and blood flow was 3.4%, 32.4%, and 32.9%, respectively. Day-to-day reproducibility with 2 testing days was also evaluated in 15 subjects (9 females, 6 males; 21±1 yrs of age) for the GE 400CL ultrasound unit. Average day-to-day difference for diameter, blood velocity, and blood flow was 1.7%, 30.0%, and 32.1%, respectively.

In addition, we evaluated inter-unit agreement with 15 subjects (9 females, 6 males; 21±1 yrs of age), each on 2 separate days. Each subject was evaluated with both units simultaneously, with probes (one interfaced with each unit) within 1 cm of each other along the long axis of the femoral artery, arranged with the femoral artery in view. Average inter-unit difference was 2.6% for femoral artery diameter. Flow velocity was collected with the 2 units simultaneously and averaged into 30-second epochs. There was a systematic difference in blood velocity between the LOGIQ Book as compared to the 400CL which was characterized by the following equation:

$$y = -0.01x^3 + 0.33x^2 - 1.66x + 8.30$$

y = predicted GE 400CL TAMAX

x = measured GE LOGIQ Book TAMAX

Eighty-five percent of the variance in velocities measured with the GE 400CL was accounted for using the equation above to correct velocities measured using the LOGIQ Book. This equation was applied to all blood velocities measured with the GE LOGIQ Book to standardize all blood velocities to that which would have theoretically been measured with the GE 400CL. The corrected blood velocity was used in subsequent calculations of blood flow. After correction of blood velocities estimated with the LOGIQ Book, average inter-unit difference for blood velocity and blood flow was 23.4% and 23.1%, respectively

Statistical Analysis

Statistical analyses were performed with the SPSS (version 13.0) statistical package. Multiple linear regression analyses related each dependent variable (CRP, sICAM-1, leptin, D_{FA}, and BF_{FA}) with percent body fat (adiposity) and VO_2peak (fitness). Adiposity and fitness were evaluated for a linear relationship using the Pearson product moment correlation coefficient (r). We evaluated the linear relationship of resting femoral artery diameter and blood flow on leg mass, leg fat-free mass, and leg fat mass using Pearson product moment correlation coefficients. The Grubb's Test was used to detect outliers in data sets that were determined to be normal (20). In the event of non-normal data we performed a logarithm transformation and confirmed normality before proceeding with parametric analysis. Data are summarized as mean and standard deviation or median and interquartile range. Results of statistical tests were considered significant if P was less than 0.05.

RESULTS

Aortic PWV was significantly correlated with fitness (r=-0.41, P=0.002), but not adiposity. A scatter-plot illustrates the relationship between fitness and aPWV in Figure 3.1. In the regression model with adiposity and fitness entered as independent variables, only fitness was significant (β=-7.572, P<0.004). Aortic PWV was also significantly correlated with sICAM-1 (r=0.30, P=0.02) with exclusion of one subject (Figure 3.2). Grubb's test was used to determine that this data point is a significant outlier (z-score=3.8; Grubb's Test, P<0.001) (20). Exclusion of this data point did not significantly affect the result of any other statistical analysis involving aPWV.

Femoral artery diameter significantly correlated with fitness (r=0.42, P=0.002), but not adiposity. A scatter plot illustrates the fitness-D_{FA} relationship in Figure 3.3. In the regression model with adiposity and fitness entered as independent variables, only fitness was significant (β=0.003, P=0.006). Femoral artery diameter significantly correlated with fat-free mass (r=0.28, P=0.03). The correlation between leg mass and D_{FA} approached statistical significance (r=0.24, P=0.07). Femoral artery blood flow did not correlate with either adiposity or fitness and the regression models were not statistically significant. The correlation between leg fat-free mass with BF_{FA} approached statistical significance (r=0.26, P=0.06).

Log-transformed CRP did not follow the same pattern with regard to adiposity and fitness (test for interaction, P=0.01). The amount by which the mean of CRP increases for a one-unit increase in adiposity, holding fitness fixed, depends on the level of fitness. Scatter-plots illustrate the relationships of CRP with adiposity and fitness in Figure 3.4. Log-transformed sICAM-1 was also significantly correlated with adiposity (r=0.29, P=0.03). The regression models were not statistically significant.

Log-transformed leptin was also significantly correlated with adiposity (r=0.84, P<0.001). In the regression model with adiposity and fitness entered as independent variables, only adiposity was significant (β=0.047, P<0.001). Leptin was also significantly correlated with both CRP (r=0.35, P=0.01) and sICAM-1 (r=0.56, P<0.001).

DISCUSSION

To address the question of whether vascular health is correlated with fitness or fatness, women who were apparently healthy but varied widely in aerobic fitness and adiposity were tested. The subject population was assembled to minimize the potential spurious effect of high body fat through poor fitness on vascular health. We studied dependent measures that have previously been found to be associated with poor health, atherosclerosis, and/or elevated risk for CVD mortality. The results of our analysis showed an inverse relationship between aPWV and fitness. This is in keeping with previous studies as aPWV has been found to correlate with VO_2max in women who vary significantly in age (r=-0.49, P=0.01) (47). In addition, a recent study found that cardiorespiratory fitness mediated the reduction in aPWV associated with elevated physical activity in men and women (5). To our knowledge, the current study is the

first to show a significant relationship between fitness and aPWV in women who also vary in adiposity.

Another finding unique to this study was a significant correlation between sICAM-1 and aPWV. Soluble ICAM-1 primarily reflects vascular activation associated with inflammatory stimuli (29), (reviewed in (51)). On the other hand, aPWV is a physiological measure that reflects alterations in aortic structure and function associated with atherosclerosis (35) and central arterial plaque accumulation (48). Our results support a role for ongoing inflammation-related vascular activation in central artery degeneration.

In this study we found that resting femoral artery size correlated with both fitness and leg FFM, but not with leg fat mass. Previous research has shown that an increase in fitness is associated with an increase in femoral artery diameter (14, 34). Additionally, a recent cross-sectional study found that femoral artery diameter was significantly associated with peripheral lean mass and that the strength of this relationship was significantly reduced when adjusted for aerobic fitness (16). Our results are in agreement with these studies and support the concept that femoral artery diameter reflects to a large extent exercise capacity. In future studies femoral artery size should be equated to lean leg mass rather than total leg mass, as total leg mass includes fat mass which was not correlated with artery size.

We also calculated resting blood flow in the femoral artery and found that it correlated with leg FFM (approached statistical significance, $r=0.28$, $P=0.06$). A relationship between FFM and BF_{FA} is consistent with previous studies (13, 14). Whether lower extremity blood flow is determined to any significant extent by fat mass has not previously been addressed to our knowledge. Although the correlation between leg FFM and BF_{FA} was modest, this finding

supports the concept that resting blood flow is primarily directed to muscle tissue and that adipose may be inert in this regard.

C-reactive protein (CRP) is a significant acute-phase reactant, principally of hepatic origin (41). Caution is currently warranted in interpreting CRP as work is still needed to establish the accuracy and reliability of CRP, as well as whether there is value in reducing CRP levels (27). Nevertheless, CRP has been found to be associated with elevated adiposity (17, 50) and CVD (38). This study showed that fitness restrained the increase in CRP that was associated with an increase in adiposity. A previous study found a significant inverse relationship between fitness and CRP, especially in subjects whose BMI exceeded thirty (1). However, in this previous study the cohort under investigation was significantly older than that of the present study (50±10 vs. 31±5 years of age) and body fat was not measured. Our results extend these findings to younger adults without cardiovascular risk factors other than obesity.

In this study peak oxygen consumption was normalized to FFM. It is important to pay special attention to normalization of maximal oxygen consumption when evaluating aerobic fitness in a subject population that varies in adipose levels. The method of expressing fitness used in this study reflects the ability of the cardiorespiratory system to meet the oxidative demands of the body and is minimally influenced by adiposity (19). We did not find a correlation between % body fat and VO_2peak (ml/kgFFM/min). To evaluate whether the cohort was balanced with respect to fitness, we divided the group based on the median value for VO2peak (*'low-fit'* < median VO2peak < *'high-fit'*). The groups compared well with sedentary and trained, non-athletic, females tested for VO_2peak in other studies (7, 12) and our high-fit group had a mean VO_2peak approximately 40% greater than the low-fit group. The groups were not different on adiposity (t(0.71), P=0.48). Therefore, the subject population represented a wide

range of aerobic fitness levels and did not exhibit a significant clustering of high adiposity with low aerobic fitness.

Limitations

A limitation of this study was the use two testing sites, specifically for evaluation of blood flow and body composition. To help account for the use of two ultrasound units, we corrected blood velocity measurements based on data collected with both units simultaneously. Nevertheless, this introduces error to our blood flow measurements and may have reduced our ability to detect relationships between resting blood flow and other measures. In addition, two DEXA scanners were used for this study. However, both scanners were made by the same manufacturer, both use the fan beam scanning method, and both were calibrated daily. Nevertheless, we acknowledge this as a potential limitation to the interpretations of this study.

Conclusion

In conclusion, these data suggest that aerobic fitness is more important than adiposity with regard to functional aspects of vascular health, and that aerobic fitness may restrain some of the inflammation associated with increasing levels of adiposity. Since the only well-established method to consistently improve or maintain aerobic fitness is regular aerobic exercise training, the results of this study support its use to promote vascular health. There is an abundance of data to suggest that adiposity be kept to a minimum in order to minimize CVD risk. However, in those with high adiposity, aerobic exercise training (independent of fat loss) may help ease their physiological burden.

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Table 3.1. Subject Characteristics (n=46)

	Mean (SD)	range
Age (yr)	31 (5)	25, 40
Height (cm)	163 (5)	152, 175
Weight (kg)	72.1 (16.3)	48.1, 121.8
BMI (kg/m^2)	27.2 (6.5)	18.3, 48.8
Heart Rate (bpm)	65 (9)	47, 84
Systolic BP (mmHg)	113 (9)	97, 132
Diastolic BP (mmHg)	69 (7)	53, 85

Table 3.2. Independent and Outcome Measures.

	Mean (SD) or Median (interquartile range)	range
Outcome Measures		
CRP (mg/L)	1.2 (0.6, 2.4)	0.2, 10.3
sICAM-1 (ng/mL)	150 (133, 172)	94, 311
Leptin (ng/mL)	20.7 (12.5, 40.8)	2.8, 85.0
aPWV (cm/sec)	841 (151)	542, 1409
Femoral Artery Diameter (mm)	5.2 (0.6)	3.7, 6.7
Femoral Artery Blood Flow (mL/min)	205 (100) *	53, 469
Independent Measures		
Adiposity (% body fat)	36.4 (6.4)	21.0, 49.7
VO_2peak (ml/kgFFM/min)	44.5 (8.6)	29.8, 62.0

* We were not able to acquire blood velocity data for some subjects due to an inability to acquire velocity signals (n = 38).

FIGURE LEGENDS

Figure 3.1. Scatter-plot of fitness (VO2peak) and central arterial stiffness (aPWV). Fitness significantly correlated with aPWV (R=-0.42, P=0.004). Subjects who were above the median for percent body fat are displayed as dots, and those who were above the median for percent body fat are displayed as triangles.

Figure 3.2. Scatter-plot of log-transformed sICAM-1 and central arterial stiffness (aPWV). aPWV significantly correlated with sICAM-1. Subjects who were above the median for percent body fat are displayed as dots, and those who were above the median for percent body fat are displayed as triangles. The data point excluded from the correlation analysis is displayed as a star.

Figure 3.3. Scatter-plot of fitness (VO2peak) and femoral artery diameter (Diameter). Femoral artery diameter significantly correlated with fitness. Subjects who were above the median for percent body fat are displayed as dots, and those who were above the median for percent body fat are displayed as triangles.

Figure 3.4. Scatter-plots illustrating the relationships between adiposity (Body fat %) and CRP (A), and fitness (VO2peak) and CRP (B).

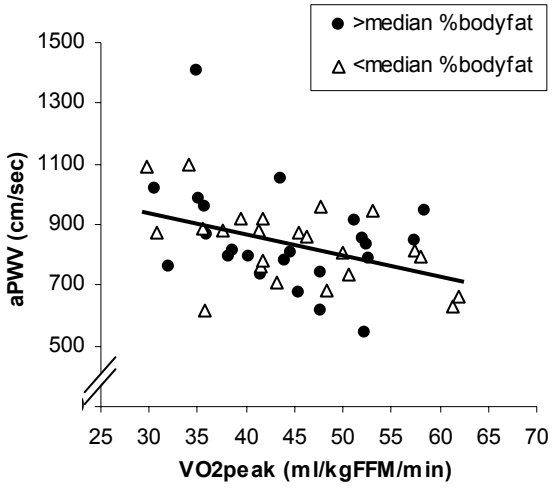
>median %bodyfat
<median %bodyfat
aPWV (cm/sec)
1500
1300
1100
900
700
500
25
30
35
40
45
50
55
60
65
70
VO2peak (ml/kgFFM/min)

Figure 3.1

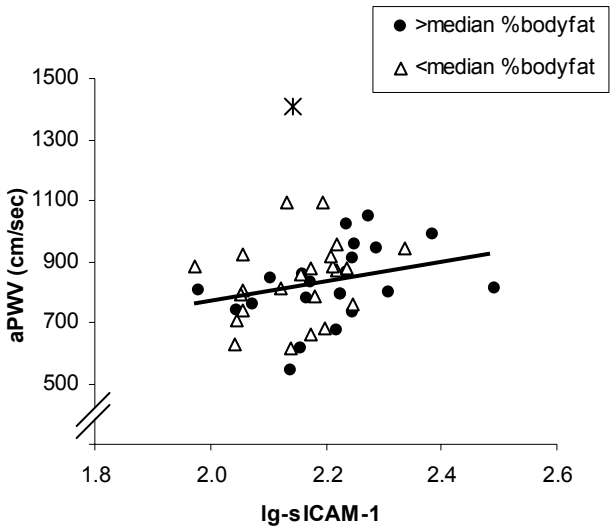
>median %bodyfat
<median %bodyfat
1500
1300
1100
900
700
500
aPWV (cm/sec)
1.8
2.0
2.2
2.4
2.6
lg-sICAM-1

Figure 3.2

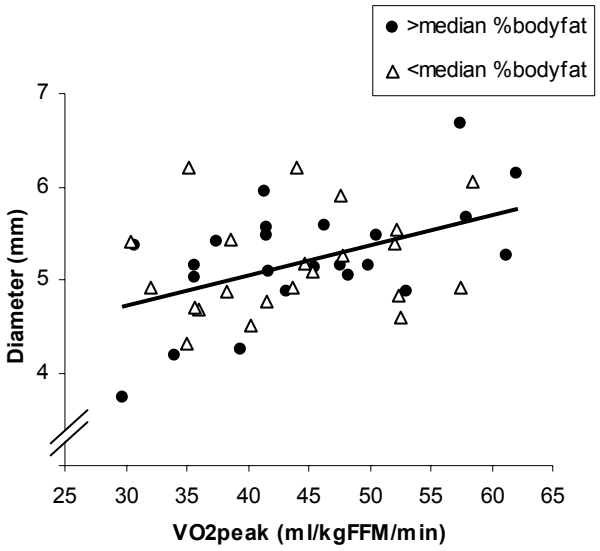
● >median %bodyfat
△ <median %bodyfat
Diameter (mm)
7
6
5
4
25
30
35
40
45
50
55
60
65
VO2peak (ml/kgFFM/min)

Figure 3.3

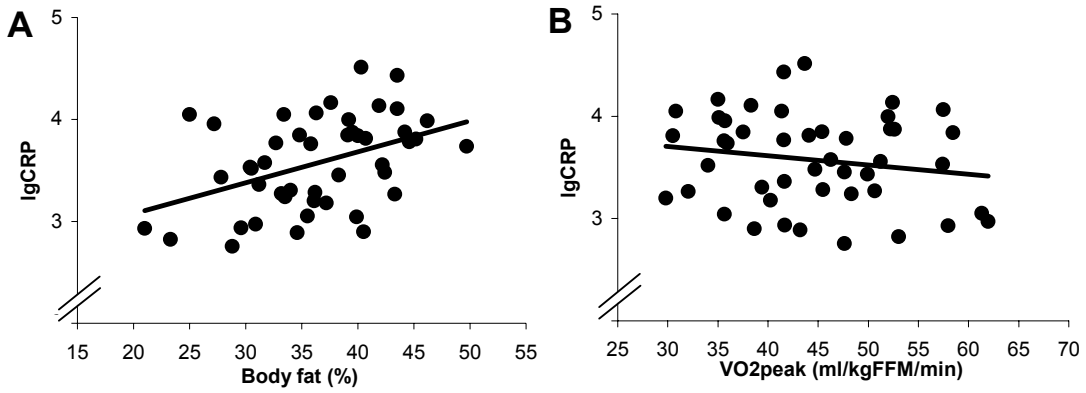
A
B
lgCRP
lgCRP
5
4
3
5
4
3
15 20 25 30 35 40 45 50 55
Body fat (%)
25 30 35 40 45 50 55 60 65 70
VO2peak (ml/kgFFM/min)

Figure 3.4

CHAPTER 4

AEROBIC EXERCISE TRAINING REDUCES CRP AND INCREASES FEMORAL ARTERY DIAMETER IN WOMEN WITHOUT SIGNIFICANTLY REDUCING ADIPOSITY[2]

[2]Sabatier, M.J., E. Schwark, R. Lewis, G. Sloan, J. Cannon, and K.K. McCully. To be submitted to the Journal of Applied Physiology.

ABSTRACT

It is currently unclear whether reductions in adiposity mediate improvements in vascular health that occur with aerobic exercise. The purpose of this longitudinal study of 13 healthy women (33 ± 4 years old) was to determine whether 14 weeks of aerobic exercise would alter functional measures of vascular health, namely aortic pulse wave velocity (aPWV, an index of arterial stiffness), femoral artery diameter (D_{FA}), and femoral artery blood flow (BF_{FA}); and serum concentrations of proteins associated with risk for cardiovascular disease, including C-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and leptin, independent of adiposity. Aerobic fitness (VO_2peak normalized to fat-free mass) and adiposity (percent body fat determined by dual energy x-ray absorptiometry) were also measured. Subjects cycled for 50 minutes, 3 times per week. Aerobic fitness increased 5.8% (P=0.03) whereas adiposity did not change. aPWV and sICAM-1 did not change with training, whereas CRP was reduced by 35% (P=0.05). Leptin also decreased 20% (P=0.04). Resting D_{FA} increased 11.7% (P<0.001) and resting shear rate decreased 28% (P=0.007). Significant reductions in adiposity are not necessary for aerobic exercise training to bring about improvements in fitness and vascular function, as well as potential improvements in CVD-related inflammation.

Key words: body fat, stationary cycle, femoral artery, sICAM-1, CRP, leptin, pulse wave velocity

INTRODUCTION

Low aerobic fitness is a significant and prevalent risk factor for future cardiovascular disease (CVD) (30, 43). An increase in aerobic fitness through exercise training reduces CVD risk (3, 4). In keeping with this concept, increased fitness has been shown to improve functional measures of cardiovascular health, such as aortic stiffness measured using aortic pulse wave velocity (aPWV) (7) and femoral artery size (12, 44, 45). In addition, elevated resting shear stress in the femoral artery has been found in the extreme case of complete disuse of the lower extremities found in those with spinal cord injury (6, 48). Considering that SCI imparts a substantial reduction in physical activity and increase in CVD risk as compared to uninjured population (reviewed in (24)), elevated resting shear stress may be associated with increased CVD risk.

Elevated adiposity also increases the risk for CVD and has increased in prevalence over the last several decades (8, 38, 47). Therefore, reducing the prevalence of obesity is a major public health initiative. Elevated adiposity is also associated with low physical activity and weight reduction typically involves an increase in physical activity. It is not entirely clear whether elevated adiposity is an independent risk factor for CVD or merely a proxy for low physical activity, low fitness, and poor diet. Prospective studies have shown that aerobic fitness reduces the negative effect of adipose tissue on CVD mortality (55). Additionally, weight loss programs have been associated with repetitive weight loss and weight gain, net increases in weight and increased CVD mortality (5, 25, 31). This suggests that programs designed to improve cardiovascular health may do better to focus on improved aerobic fitness rather than weight loss.

The circulating concentrations of several proteins that may originate from, or influence the development of, atherosclerotic plaques are currently under investigation as adjuncts to cholesterol/lipoprotein measurements in assessment of risk for cardiovascular disease. Among those reported to be independent predictors of cardiovascular disease risk are C-reactive protein (CRP) (33), soluble intercellular adhesion molecule-1 (sICAM-1) (20), and leptin (54).

Elevated levels of CRP and sICAM-1 have been associated with CVD (32), as well as with adiposity (17, 56). Adiposity is also associated with elevations in leptin, an adipocyte-derived, 16 kDa hormone/cytokine that influences energy balance, thermoregulation and reproductive function (19). Leptin receptors have been identified on vascular cells (49), and several in vitro and animal studies have indicated that leptin may influence atherosclerotic plaque development (reviewed in (9)). A reduction in these biological proteins might confer protection against the progression of atherosclerosis.

Previous studies that have evaluated the interaction between aerobic fitness and adiposity have commonly used body mass index (BMI) as an index of adiposity. BMI is not a measure of the amount of adipose tissue an individual has and its relationship to adiposity, or percent body fat, may vary considerably between individuals (28). Furthermore, without an accurate measure of adiposity, maximal oxygen consumption as an index of aerobic fitness is compromised. For example, total body weight is typically used to normalize oxygen consumption. Therefore, increased adiposity will automatically lower aerobic fitness values irrespective of the maximal capacity of the individual's muscles to utilize, and cardiorespiratory system to deliver, oxygen. Dual x-ray absorptiometry (DEXA) yields accurate estimates of fat free mass (23) which can be used to normalize maximal oxygen consumption and help minimize this limitation (18). The purpose of this study was to determine whether a 14-week aerobic exercise program would 1)

improve aerobic fitness, 2) reduce adiposity, 3) improve functional measures of vascular health, and 4) reduce serum concentrations of proteins associated with risk for cardiovascular disease. A secondary purpose was to determine whether changes in functional measures of vascular health and serum concentrations of proteins associated with risk for cardiovascular disease were dependent upon changes in aerobic fitness.

METHODS

Subjects

Thirteen sedentary pre-menopausal females (33±4 years old) were studied on 2 days within a 7-day span, before and after a 14-week cycle training program. Subjects were screened to exclude for self-reported cardiovascular or other inflammatory disease, neuromuscular disease, hypertension, and smoking; and testing occurred within 10 days of the cessation of menstrual bleeding (58). All procedures were approved by the Human Assurance Committee at the Medical College of Georgia and the Institutional Review Board at The University of Georgia. All subjects were instructed as to the procedures involved in the project and gave written informed consent before undergoing testing procedures.

Cardiorespiratory Fitness

Maximal exercise testing was performed using a friction-braked Monark cycle ergometer as described previously (11). Although maximal oxygen consumption measured using the stationary cycle is on the order of 10% lower than that generated from performance of a treadmill graded exercise test (10), the stationary cycle was used to avoid the possible effects of larger body sizes on maximal testing with protocols involving ambulation. The test began with 4

minutes of cycling without resistance followed by an increase of 15 W every minute (11). Oxygen consumption was calculated based on measures of pulmonary ventilation and mixed expired oxygen and carbon dioxide using the Vmax Spectra (Yorba Linda, CA). Heart rate (EKG) and blood pressure (auscultation) were assessed during each stage and subjects provided subjective ratings of perceived exertion during every other stage of the test. Peak oxygen consumption (VO_2peak) was taken as the maximal 30-second average. and normalized to fat-free mass (18).

Body Composition

Body composition was measured using DEXA (Delphi A, Hologic Inc., Bedford, MA) whole body scan. Clothing was standardized (t-shirts and shorts) to minimize the inflation of body density due to added clothing and all metal was removed from the body including metal clothing and jewelry. The subject laid in a supine position with arms away from their sides and leg straight with ankles at least 2 inches apart. All scans were compartmentalized and analyzed by one investigator using Hologic whole body software for bone mineral density, fat free soft tissue and percent body fat.

Laboratory Measurements

Blood samples were obtained by venipuncture after a 12-hour fast to assay for CRP, ICAM-1, and leptin. Blood was collected into vacutainer tubes containing EDTA (1 mg/ml blood) as an anticoagulant and aprotinin (500 KIU/ml of blood) to limit proteolytic degradation. Blood was centrifuged at 300g for 10 minutes after which the plasma was collected and centrifuged at 10,000g for one minute to remove platelets. The platelet-free plasma was stored

in a -70 °C freezer for later analysis. Serum CRP, leptin and ICAM-1 were assayed using ELISAs constructed with separately-purchased reagents. Primary antibodies were polyclonal rabbit anti-human C-reactive protein (A0073, DAKO, Carpintera, CA), monoclonal mouse anti-human leptin (MAB398, R, & D Systems, Minneapolis, MN), and monoclonal mouse anti-human ICAM-1 (MAB720, R & D Systems). Standards curves were prepared by 2-fold serial dilutions of purified human serum CRP (#236603, Calbiochem-Novabiochem, San Diego, CA), recombinant human leptin (398-LP, R & D Systems), and recombinant human ICAM-1 (ADP4, R & D Systems). For all assays, capture antibodies were coated on 96-well polystyrene plates (Corning #3590, Corning, NY). Samples and standards were incubated in duplicate in plates overnight at 4ºC. For CRP, the plates were washed and then horseradish peroxidase-conjugated polyclonal rabbit anti-human C-reactive protein detection antibodies (P0227, DAKO) were applied for two hours at room temperature. Color was developed using the substrate 2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma #1888, St Louis, MO). Absorbance at 405nm was measured with a Labsystems Multiskan MCC/340 plate reader (Needham Heights, MA). For leptin and ICAM-1, biotinylated mouse anti-human detection antibodies (R & D Systems BAM398 and BAF720, respectively) were applied for 2 hours followed by streptavidin-conjugated horseradish peroxidase (Pierce, Rockford, IL) for 30 minutes. Color was developed and absorbance measured as described for CRP. Detection limits, inter- and intra-assay variabilities for each assay were: CRP - 0.3 ng/ml, 12%, 3%; leptin - 0.06 ng/ml, 5%, 2%; ICAM-1 - 0.14 ng/ml, 9%, 6%.

Aortic Pulse Wave Velocity and Arterial Blood Pressure

Aortic pulse wave velocity was used to evaluate central arterial stiffness (41). A Biopac MP100 physiological data acquisition system (Biopac Systems Inc., Goleta, CA) with AcqKnowledge software (v3.7.3) was used to evaluate aPWV. Biopac EKG electrodes were interfaced with a MEC110C transducer and an ECG100C electrocardiogram amplifier. One electrode was placed over the superior and another over the distal-most boundary of each scapulae, with a third placed on the lower back. A 40.6-66.0 cm Biopac blood pressure cuff (RX120F) was interfaced with a TSD120 transducer and DA100C general purpose transducer amplifier. The cuff was inflated to 60 mmHg for real time assessment of pulse and EKG waves. All recordings were made after 30 minutes of supine rest. Peripheral arterial blood pressure was measured during this period over the brachial artery of the left arm with a semi-automated blood pressure machine (Datascope, Mahwah, NJ) using an appropriate-sized cuff (42). The average of 3 to 4 blood pressure readings was used for subsequent analysis.

Distance from the sternal notch to the thigh cuff was measured with a standard tape measure maintaining a parallel orientation of the tape measure to the examination table without conforming to body topography. The data was converted to standard text format, and retrieved and graphed using spreadsheet software. The time difference between the inflection point of the systolic pulse wave and the peak of the R-wave of the EKG signal was calculated for 30 cardiac cycles for which the pulse wave inflection point was most distinct. A time constant of 0.05 sec was subtracted from this difference to correct for isovolumic contraction phase that precedes ejection (13). The calculation was performed as follows: aPWV = distance (cm) ÷ [time difference (sec) – 0.05 (sec)]. Day-to-day reproducibility with 2 testing days was evaluated in 17

subjects (11 females, 6 males; 22±2 yrs of age) using this method of evaluating aPWV with an average day-to-day difference of 8.1%.

Femoral Artery Diameter, Blood Flow, and Shear Rate

In all tests the subject rested quietly in the supine position for 15 minutes before data acquisition began. One sonographer performed all tests for this study. B-mode imaging was used to visualize the artery 1 - 5 cm distal to the femoral bifurcation using a LogiQ 400CL (General Electric, Rainbow City, AL) with a 7-13 MHz linear-array ultrasound transducer. B-mode images were recorded during diastole for off-line diameter (mm) measurements. Resting blood velocity (cm/second) was assessed using pulsed Doppler ultrasound recorded in the longitudinal view using an insonation angle between 45^{o} and 60^{o}. The velocity gate was set to include the entire lumen area. Time averaged maximum velocity (TAMAX) was auto calculated every cardiac cycle by the GE 400CL advanced vascular program. TAMAX values were acquired and saved directly to a computer using specially coded optical character recognition software (NI LabVIEW 6i, Austin, TX), allowing data acquisition on a beat-by-beat basis. Image files were opened using semi-automated software specially coded for use with NI LabVIEW. Arterial diameter was measured manually by applying straight lines conforming to the wall-lumen interface of two-dimensional images captured during data collection. Blood flow (mL/min) is the product of blood velocity and artery cross-sectional area and was calculated as follows: blood flow = $[\pi(\text{diameter}/2)^2]$*[velocity*60seconds/minute]. We used shear rate as an estimate of shear stress. Shear rate was calculated as blood velocity ÷ diameter (46).

Day-to-day reproducibility for diameter, blood velocity, blood flow, and shear rate was evaluated between 2 testing days in 15 subjects (9 females, 6 males; 21±1 yrs of age) that were

not part of the training study. Average day-to-day difference for diameter, blood velocity, blood flow, and shear rate was 1.7%, 30.0%, 32.1%, and 19.9%, respectively.

Training Intervention.

The training program was conducted at the University of Georgia's Adult Fitness Center. Subjects were scheduled to exercise three times per week for 14 weeks under direct supervision of one of the study's investigators but were permitted to conduct exercise sessions on their own on an infrequent basis in the event of extenuating personal circumstances. Any subject who utilized this option had to: 1) have access to a stationary cycle, 2) be thoroughly familiar with the protocol, and 3) be proficient at monitoring their own heart rate while cycling. This was infrequent for 11 of the 13 subjects. Although two subjects conducted 50% of their exercise sessions away from our direct supervision, their results were not markedly different from the remainder of the group.

Airdyne bikes were utilized for training with minimal or no use of handles. Cycling was performed at a comfortable, self-chosen pace during the initial 3-4 sessions of the training program. After this induction phase, ten 2-minute alternating intensity bouts (high and low) followed the warm-up. Each session included 5 minutes of warm up, 40 minutes of intermittent intensity cycle training (2 minutes high, 2 minutes low), and 5 minutes of cool down, for a total of 50 minutes of pedaling. High intensities were chosen to elicit a heart rate of 75-90% of heart rate reserve and were achieved through an increase in pedaling speed. Low intensities were chosen to elicit 55-65% of heart rate reserve. Heart rate responses were monitored to ensure that proper intensities were achieved. Figure 4.1 illustrates results of one session for one subject while heart rate and expiratory gases were measured using EKG and open circuit spirometry.

Percent heart rate reserve and percent VO_2 reserve were closely matched during the training session (r=0.92), supporting our use of heart rate to control and monitor training intensity.

Statistical Analysis

Statistical analyses were performed with the SPSS (version 13.0) statistical package. Pre vs. post measurements were evaluated using paired Student's t-tests. Non-normal data were logarithm transformed and confirmed for normality before proceeding with parametric analysis. Results are reported as mean (SD) unless otherwise noted and statistical significance was determined at $P \leq 0.05$.

RESULTS

Pre-post results for clinical characteristics and aerobic fitness are shown in Table 4.1. There were small and insignificant changes in body weight, BMI, percent body fat, fat mass, and fat-free mass. Resting heart rate decreased significantly with training (4.6%) while arterial blood pressure did not change.

VO_2peak (aerobic fitness) increased 6% with training. In addition, at level 6 (115 W) of the maximal exercise test, there was a decrease in heart rate after training that approached statistical significance (155 vs 150 bpm, P=0.07; 95% C.I. on the mean difference = -9.4, 0.9); and there was a significant reduction in the rate pressure product ($2.5 \cdot 10^4$ vs $2.2 \cdot 10^4$ mmHg*bpm, P=0.02; 95% C.I. on the mean difference = $-0.02 \cdot 10^4$, $-0.3 \cdot 10^4$).

Pre-post results for biological proteins associated with CVD are shown in Table 4.2. CRP and leptin decreased with training (35% and 20%, respectively). The pre and post training results for central arterial stiffness (aPWV); and femoral artery diameter, blood flow, and shear

rate are illustrated in Figure 4.2, A-D, respectively. aPWV did not change with training (886±196 vs. 903±227, P=0.30). Femoral artery size increased from 5.1±0.5 to 5.7±0.5 mm (P<0.001), whereas resting femoral artery blood flow did not change (167±89 vs. 160±68, P=0.15). Resting shear rate decreased from 24.9±9.5 to 17.6±6.1 (P=0.007).

DISCUSSION

The exercise program used in this study resulted in a 5.8% increase in VO_2peak, less than that reported in previous studies that used comparable training programs and similar measurements in the middle aged women (50). Our subjects did reach the same end of test criteria (perceived exertion, heart rate, RER) in the VO_2peak test before and after training. We also had comparable rates of adherence to the training program, and we found no evidence that adherence to the training program explained any of the variance in our outcome variables. The improvements in aerobic fitness, in combination with the reduction in rate-pressure product during maximal exercise testing, suggest a significant physiological adaptation on the whole.

The reduction in CRP with exercise training suggests that systemic inflammation may have been reduced. This is consistent with previous studies that tested young athletic populations and found increased competitive activity to result in a decrease in plasma CRP (15, 35). In a previous study that tested a similar cohort to that of the present study, two years of a multidisciplinary program that incorporated walking decreased CRP by 1.6 mg/L (P=0.008) (14). The group in the previous study started at a higher CRP concentration (i.e., 3.2 mg/L) and the intervention was over 6 times longer than that of this study, which may explain the 2-fold effect size discrepancy between our study and the former study (0.8 vs 1.6 mg/L, respectively). Fitness has also been found to be negatively correlated with CRP across levels of BMI in cross-sectional

studies (1, 29), especially across fitness levels in obese subjects (1). Our results extend previous findings by demonstrating that little more than 3 months of cycle training (with a relatively small investment of time) is sufficient to reduce CRP in healthy, normotensive, pre-menopausal women. It should be noted that at this time work is still needed to establish the accuracy and reliability of CRP, as well as whether there is value in reducing CRP levels (27). Therefore, it is not clear that reducing CRP levels reduces the risk for CVD. However, a reduction in CRP that is associated with aerobic exercise training, considering the substantial body of literature that supports its beneficial effects on cardiovascular health, is consistent with a reduction in vascular inflammation and atherosclerosis.

Exercise training resulted in a decrease in leptin concentrations in the absence of a significant reduction in adiposity, extending previous research that has shown acute, exercise-related reductions in leptin (21, 22). Previous research has further suggested that the amount of circulating leptin, as a percentage of fat mass, may also be modifiable. This is also consistent with the current study as the reduction in leptin/fat mass was 21% for the group as a whole (not statistically significant) and 32% (P=0.02) for the 8 subjects who were ≥overweight (n=8, BMI = 33.3±9.3). It is reasonable to expect that this effect would be limited to subjects who have more adipose tissue, which is the primary source of leptin. At least two other studies found leptin/fat mass to be reduced with exercise training. One of these studies evaluated men and the 1-year training protocol used by these investigators resulted in significant changes in body weight and fat (37). The second study evaluated older women (60±4 yrs) with a BMI of 26.4±2.3 kg/m^2 (26). Therefore, this study extends these findings to younger women. These results suggest that reductions in leptin are not dependent upon reductions in adiposity. This may suggest further

improvement in arterial status since leptin may influence atherosclerotic plaque development (reviewed in (9)).

Exercise training did not decrease arterial stiffness (i.e., aPWV). Although cross-sectional studies suggest that higher fitness is associated with lower aPWV, age is an important factor to consider. Aortic PWV was found to increase with age in a group of 480 Chinese citizens (ages 3 to 89) in whom atherosclerosis is known to be rare and who did not have CVD (2). In a cross-sectional study of elderly adults, those who were endurance-trained athletes had significantly lower aPWV than those who were sedentary (53). In another cross-sectional study, there was an absence of age-related increases in aPWV in highly physically active women (51). These studies suggest that age is a significant and independent predictor of central arterial stiffness and that its effects are lessened when accompanied by years of aerobic training. It is possible that the morphological aspects of the aorta the aPWV is thought to reflect, namely atherosclerotic plaque accumulation, loss of elastin, and collagen build-up, are not significantly reversible (39). However, it seems more likely that central arterial stiffness may was not sufficiently elevated in this study for exercise and increased fitness to evoke a measurable reduction.

One of the significant findings from this study is that femoral artery diameter increased significantly. Physical activity (12, 36) and aerobic fitness (16) have previously been found to be positively associated with femoral artery diameter. This study demonstrates a positive effect of exercise training on the femoral artery, independent of adiposity. In addition, resting femoral artery shear rate decreased significantly with training (Figure 4.2D). Elevated shear rates during exercise induce positive adaptations in arterial health (34, 40, 52). These adaptations result in larger arteries that subsequently have reduced shear rates at rest. An extreme example of the

effect of shear rates is found in patients with spinal cord injury (SCI). Patients with SCI are unable to increase shear rates in their affected limbs due to paralysis, and subsequently have smaller arteries and elevated resting shear rates compared to able-bodied control groups (6, 48). Our results suggest that in previously sedentary able-bodied subjects, exercise training results in a reduction in resting shear rate.

Limitations

One potential limitation of the present study is the heterogeneity of our group with respect to adiposity. Similarly, the group was composed of women who were normal weight, overweight, and obese (as defined by the World Health Organization (57)), as it is possible that the vascular response to exercise training would be change with adiposity. We did not have a larger enough sample size to answer this question, although visual inspection of our data did not suggest that adiposity influenced our results. In addition, we feel that the limitation of not having a homogeneous cohort (i.e., all within a small range of body fat levels) is balanced by the increase in statistical power afforded by more subjects and that the majority of our subjects were overweight and above (i.e., BMI≥25, n=8).

Conclusion

In this study we found that 14 weeks of aerobic exercise increased aerobic fitness, reduced serum CRP and leptin, and favorable altered resting femoral artery diameter and shear rate. These results support the hypothesis that aerobic fitness influences vascular health independent of adiposity, and also supports a prominent focus on aerobic fitness rather than weight loss when developing interventions to reduce CVD risk.

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Table 4.1. Clinical characteristics and aerobic fitness before and after training.

	Pre Mean (SD)	Post Mean (SD)	95% C.I.	P-value
Age (yr)	33 (4)	---	---	---
Height (cm)	160 (6)	---	---	---
Weight (kg)	74.4 (21.6)	74.3 (21.4)	-1.0, 0.8	0.43
BMI (kg/m^2)	29.1 (9.1)	29.1 (9.0)	-0.4, 0.3	0.38
Body fat (%)	37.3 (7.3)	36.5 (7.0)	-1.9, 0.2	0.08
Fat mass (kg)	29.0 (13.8)	28.3 (13.8)	-1.8, 0.4	0.12
Fat-free mass (kg)	45.4 (8.4)	46.0 (8.2)	-0.2, 1.4	0.08
Heart Rate (bpm)	65 (9)	62 (9)	-1.0, -6.4	0.01
Systolic BP (mmHg)	110 (9)	108 (11)	-6.6, 1.7	0.14
Diastolic BP (mmHg)	70 (7)	68 (7)	-5.1, 0.9	0.10
VO_2peak (ml/kgFFM/min)	44.5 (6.8)	47.1 (5.7)	0.1, 4.8	0.03

Data in Pre and Post columns are shown as mean (SD).
95% C.I. are for the mean difference between Pre and Post and are reported in the following format: lower boundary, upper boundary.
P-values were calculated based on paired Student's t-tests.

Table 4.2. Biological proteins associated with CVD, before and after training.

	Pre	Post		
	Mean (SD) or Median (interquartile range)	Mean (SD) or Median (interquartile range)	95% C.I.	P-value
CRP (mg/L) †	1.7 (0.9, 3.1)	1.1 (0.4, 2.5)	-1.7, 0.0	0.03
ICAM-1 (ng/mL)	169 (37)	172 (74)	-14, 18	0.40
Leptin (ng/mL)	31 (22)	25 (21)	-12, 0	0.04

Data in Pre and Post columns are shown as mean (SD), unless otherwise indicated.
† results reported as geometric mean (25^{th} percentile, 75^{th} percentile).
95% C.I. are for the mean difference between Pre and Post and are reported in the following format: lower boundary, upper boundary.
P-values were calculated based on paired Student's t-tests.

FIGURE LEGENDS

Figure 4.1. Heart rate and oxygen consumption during one training session for one subject. Heart rate and oxygen consumption, as percentages of reserve, changed concurrently throughout the session (r=0.92) and both remained above 60% for the entire 40-minute body of the training session. The upper and lower edge of the box super-imposed on the graph corresponds with 90% and 75% of HR reserve, respectively.

Figure 4.2. aPWV (A) did not change with training. Femoral artery diameter (B) increased 12%, femoral artery blood flow (C) did not change, and femoral artery shear rate (D) decreased 29% with training. Data are illustrated as mean ± 95% C.I.

FIGURES

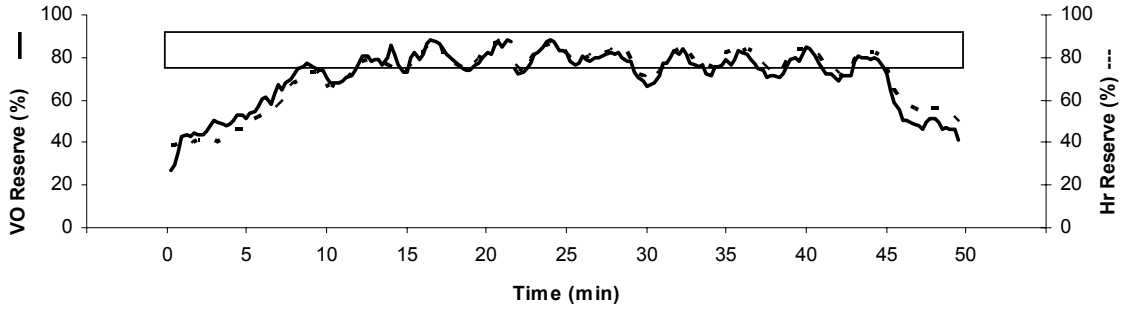

Figure 4.1

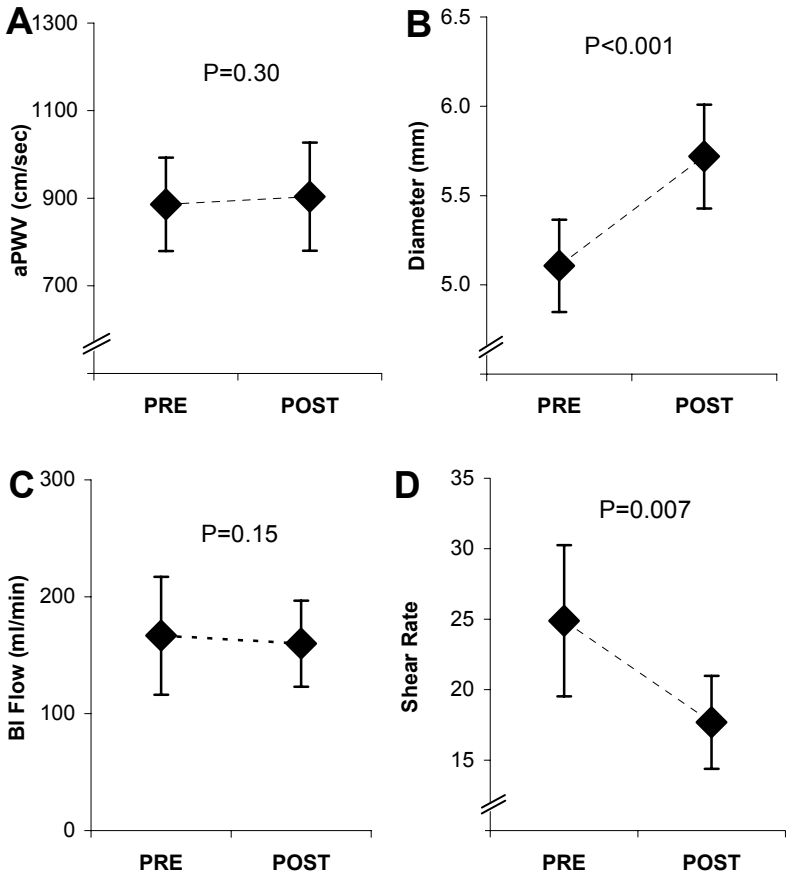
A
1300
1100
900
700
aPWV (cm/sec)
P=0.30
PRE
POST
B
6.5
6.0
5.5
5.0
Diameter (mm)
P<0.001
PRE
POST
C
300
200
100
0
BI Flow (ml/min)
P=0.15
PRE
POST
D
35
30
25
20
15
Shear Rate
P=0.007
PRE
POST

Figure 4.2

CHAPTER 5

SUMMARY AND CONCLUSIONS

Adipose tissue (body fat) is an essential component of the human body. In addition to its role in insulation and energy storage, it is now known to function as an endocrine organ and secretes factors that may facilitate chronic arterial damage. Whether body fat independently mediates meaningful physiological dysfunction continues to be investigated and the current study falls under this umbrella of research.

Although an excessive accumulation of body fat (e.g., obesity) generally results from a lack of physical activity and/or excess energy intake (77), it may not accurately reflect the metabolic or vascular health of an individual (63, 145). Increased body fat and low physical activity are independent predictors of pre-mature mortality (57) and higher levels of physical activity are beneficial at all levels of body fat in both men (72) and women (57).

Although the prevalence of overweight and obesity has clearly increased in recent years (54), it is presently controversial as to whether an abundance of body fat causes, or is merely associated with, the deterioration of health that often accompanies excessive body fat (63, 72, 73, 145). The public health question is whether weight loss or increased fitness should be the primary goal of interventions designed to reduce the risk for CVD (97). While weight loss and increased fitness are commonly prescribed together, the success of an exercise program is often defined primarily by weight loss. These controversies have not yet been fully resolved in the literature.

As demonstrated in this study, it is possible to be fit without having low body fat, and for an intervention to influence fitness more than fatness. A number of other studies have shown a dissociation of good fitness and low body weight. Also, the relationship between body fat and health is imperfect. A significant portion of obese humans (estimated at ~20%) do not demonstrate a clustering of metabolic or cardiovascular risk factors (18). Not everyone who succeeds in meeting the current guidelines for exercise or physical activity loses significant weight or reaches the CDC's recommendations for 'normal-weight'. Just as the population varies in physical and mental attributes, there is a great deal of population variance in the response to energy expenditure. In fact, the literature demonstrates a low success rate for maintaining weight loss (5, 69, 119, 169) and even achieving weight loss to begin with (119, 169). Paradoxically, weight loss attempts are also associated with elevated risk of further weight accumulation (69) and recurrent weight loss and gain is associated with significantly increased mortality from all causes and from coronary heart disease (14, 74). In addition, improvements in the metabolic profile of obese diabetics occur without a change in body fat (37, 145). A recent report shows a drop in CVD risk factors across BMI groups in the last 40 years (51). In consideration of these results, increasing fitness and maintaining a physically active lifestyle should be prioritized (119).

We focused on fitness, as opposed to physical activity, as the primary independent measure in this study. Although fitness is a physiological attribute, it has a high degree of correlation with physical activity (20, 21) and is also well-supported as a primary correlate of premature CVD-related mortality (13, 72). In consideration of the literature as a whole, one or the other does not stand out as the more important determinant of health (12). An advantage of using fitness, as in our study, is that fitness measurements have greater precision than self-

reported measures of physical activity (135). This was important given that the sample sizes in our study were relatively small.

Although we did not report measures of physical activity in the preceding studies, the Baecke Questionnaire of Physical Activity was administered to assess recent physical activity levels. Physical activity provided similar although generally weaker relationships than our measurements of fitness. This is consistent with previous studies that have found similar effects of fitness and physical activity (12).

Overall, the results of this study suggest that the beneficial effects of greater, or improved, fitness are not wholly dependent upon minimizing body weight and/or fat. This conclusion is based on results from healthy middle-aged women. While this is a relatively understudied population, it will be important to extend this research to individuals who are less healthy and not solely overweight and/or obese.

In the cross-sectional study, fitness was associated with better vascular health as accounted for with non-invasive measures of (i.e., central arterial stiffness and femoral artery diameter). Fitness was also had a restraining effect on the increase in CRP associated with an increase in body fat. In the longitudinal study, CRP and leptin levels were reduced, femoral artery diameter was increased, and femoral artery shear rate was decreased. Therefore, there is a degree of inconsistency between the two studies. For example, it would be expected that a 14-week exercise stimulus that increases fitness should also alter parameters that were found to correlate with fitness in the cross-sectional study. There are several possible explanations for this discrepancy. The measures that were resistant to change in the training study might be 1) relatively more dependent upon body fat stores than on physical activity and fitness, 2) relatively more dependent upon dietary status, which may have influenced the two studies differently,

and/or 3) heavily dependent upon a clustering of risk factors and/or significant aging, neither of which were evaluated with this study. Between the two studies, however, evidence is available to suggest that increased fitness is related to, or affects, all measures that might represent links between body fat and poor arterial health. Therefore, the results of this study are further support for the concept that fitness modifies the independent and negative effect that body fat may have on vascular health (83, 145).

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