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AGE-RELATED INFLUENCES ON MARKERS OF INFLAMMATION AND FIBRINOLYSIS

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AGE-RELATED INFLUENCES ON MARKERS OF INFLAMMATION AND FIBRINOLYSIS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ABSTRACT

Purpose: The primary purpose of this investigation was to determine if age is associated with blood markers of inflammation (C-reactive protein [CRP]) and fibrinolysis (fibrinogen, tissue plasminogen activator [t-PA], and plasminogen activator inhibitor – 1 [PAI-1]), independent of body fat and physical activity levels.

Methods: A total of 40 healthy males and 42 healthy females ranging in age from 21 to 89 years participated in this cross-sectional study. Blood levels of CRP, fibrinogen, t-PA, and PAI -1 were measured and compared with age, clinical characteristics, physical activity levels, and body composition. Body composition was assessed with dual-energy x-ray absorptiometry and physical activity was assessed with a StepWatch Activity Monitor.

Results: Age was not associated with concentrations of CRP ($r = -0.073$, $p = 0.519$). CRP was significantly correlated ($p < 0.05$) to HDL levels ($r = -0.255$), BMI ($r = 0.304$), percent body fat ($r = 0.221$) and trunk fat mass ($r = 0.234$). Of these, BMI was the only significant predictor ($r^2 = 0.095$, $p = 0.002$) of CRP levels. Age was not correlated with fibrinogen ($r = 0.206$, $p = 0.065$), or PAI-1 ($r = 0.084$, $p = 0.454$), but was initially correlated with t-PA ($r = 0.228$, $p = 0.042$). The markers of fibrinolysis were correlated ($p < 0.05$) with blood pressure, HDL, triglycerides, percent body fat, body fat mass, percent trunk fat, and trunk fat mass. However, none of these variables or age were independent predictors of

blood concentrations of fibrinogen ($r^2 = 0.237$, $p = 0.226$), t-PA ($r^2 = 0.079$, $p = 0.636$), or PAI – 1 ($r^2 = 0.137$, $p = 0.333$).

Conclusions: The primary finding of the current study is that age is not independently associated with blood concentrations of CRP, fibrinogen, tPA or PAI-1. However, it was observed that inflammation and fibrinolysis were associated with blood pressure, cholesterol levels, and measures of body fatness. These associations provide a possible explanation for the discrepancy between our findings and those of previous studies which did not control for such factors.

CHAPTER ONE

INTRODUCTION

Cardiovascular disease (CVD) has been and remains the leading cause of death in the United States over the last century, claiming nearly 700,000 lives annually in the United States (U.S.) (6). Over 27 million of the 71.3 million known cases of cardiovascular disease are found in adults 65 years of age or older (147), which is a reflection of the increased risk for CVD associated with aging (5,22,130). Over the last decade, evidence suggests that chronic, low-grade inflammation is an underlying component in the initial development and progression of atherosclerosis and an independent risk factor of cardiovascular disease (43,114,130). C-reactive protein (CRP), a hepatocyte-derived acute-phase protein (21,22), is the most commonly studied marker of inflammation, and is clearly related to the incidence of atherosclerosis and acute coronary syndrome (12,97,101,111,130). CRP initiates production of inflammatory cytokines such as tumor necrosis factor - alpha [TNF-α] and interleukin-6 [IL-6] (48,95) and mediates monocyte and macrophage infiltration into endothelial tissue (79). Consequently, CRP is considered to be the "golden marker" for chronic, low-grade inflammation (97).

Fibrinogen is another acute-phase protein that is often considered to be a marker of inflammation (11), but is actually a determinant of blood viscosity and an important factor in the formation of a blood clot (thrombosis) in the blood vessel (41,42). Fibrinolysis is a natural defense against development of thrombosis (45,131) and is closely linked to inflammation. Tissue plasminogen activator (t-PA) has a major role in activating the fibrinolytic system and initiating the release of plasmin, which is responsible for fibrin clot degradation (135). However, fibrinolytic activity is impaired by plasminogen activator inhibitor-1 (PAI-1), the most important inhibitor of plasma fibrinolytic activity (126). Thus, fibrinogen, t-PA, and PAI-1 are perceived as independent risk markers and prognostic indicators of cardiovascular disease (11,111,126,135).

Inflammation (6,21,22,69,130) and fibrinolysis (1,36,135) are suggested mechanistic links between cardiovascular disease (CVD) and aging (24). However, previous examinations of age-related changes in inflammation and fibrinolysis often included questionable "healthy" populations (48) that presented with confounding conditions such as hypertension (27), hypercholesterolemia (51), diabetes (37), metabolic syndrome (4,28), and smoking (37). Unfavorable changes related to inflammation and fibrinolysis are also associated with obesity, defined as a body mass index [BMI] of greater than 30.0 kg/m^2 (40), and physical inactivity (69,135). Whole body adiposity and visceral adiposity, in particular, are suspected regulators of CRP, coagulation, and fibrinolysis (149,150), and are

associated with an increase in inflammation and a decrease in fibrinolysis (34). Conversely, inflammation and fibrinolysis are improved with frequent bouts of physical activity by means of producing a subsequent decrease in blood concentrations of CRP, fibrinogen, and PAI-1, and an increase in blood concentration of t-PA (69,72,79).

Since body composition and physical activity levels change with age (24), these factors must be considered when examining the independent role of age on inflammation and fibrinolysis. Because of the inclusion of subjects with both conditions, previous epidemiologic studies have been inconclusive in identifying causative factors of age-related elevations of inflammatory and fibrinolytic markers. Therefore, the primary aim of this study was to determine if age was related to increased blood levels of inflammatory and fibrinolytic markers, independent of body composition and physical activity status.

Purposes of the Study

The purpose of this study was to determine whether:

1. Age is related to blood concentrations of CRP, fibrinogen, t-PA, and PAI-1.
2. Whole body and trunk fat mass are related to blood levels of CRP, fibrinogen, t-PA, and PAI-1.
3. Ambulatory physical activity is related to blood levels of CRP, fibrinogen, t-PA, and PAI-1.
4. The affect of age persists after controlling for ambulatory physical activity and fatness.

Research Questions

Research questions for this study include:

1. Are the concentrations of CRP, fibrinogen, t-PA, and PAI-1 in the blood related to age?
2. Is there a relationship between whole body and trunk fat mass and the concentration of CRP, fibrinogen, t-PA, and PAI-1 in the blood?
3. Is there a relationship between the amount of ambulatory physical activity and the concentration of CRP, fibrinogen, t-PA, and PAI-1 in the blood?

4. Are the concentration of CRP, fibrinogen, t-PA, and PAI-1 in the blood related to age independent of whole body fatness, abdominal fatness, and ambulatory physical activity?

Research Hypotheses

Research hypotheses for this study include:

1. The concentration of CRP, fibrinogen, t-PA, and PAI-1 will change with increasing age.
2. Whole body and abdominal adiposity will be positively related to blood concentrations of the CRP, fibrinogen, and PAI-1 and negatively related to blood concentrations of t-PA.
3. Ambulatory physical activity levels will be negatively related to blood concentrations of CRP, fibrinogen, and PAI-1 and positively related to blood concentrations of t-PA.
4. After adjusting for whole body fat mass and ambulatory physical activity, blood levels of CRP, fibrinogen, t-PA, and PAI-1 will not be related to age.

Significance of the study:

Providing evidence that aging is linked with inflammation and fibrinolysis is of high importance, as it may provide an explanation of the causes of age-related disease. There are several reports that increasing age is associated with

unfavorable changes in inflammation (5,21,22,69,130) and fibrinolysis (34,36). Inflammation and fibrinolysis have also been associated with many clinical conditions (27,100,125,150), as well as with levels of body fat (39,49,132,150) and physical activity (103,145). The increasing prevalence of clinical conditions and unfavorable changes in body composition (i.e., decreased skeletal muscle mass and increased total body fat) occurring with age (55,98) could explain previous reports of age-related changes in inflammatory and fibrinolytic activity. Thus, controlling for these confounding factors, as was done in this investigation, helps clarify that levels of inflammation and fibrinolysis are unrelated to age and, further, underscore the importance of maintaining higher levels of physical activity and lower levels of body fat mass to protect cardiovascular health.

Delimitations

Delimitations for this study included:

1. Participants were community dwelling males and females between the ages of 18 – 80 years of age.
2. Adults with physical disabilities that limited ambulatory movement were excluded from the study.
3. The participation criteria for this study were strict to exclude confounding conditions of inflammation and fibrinolysis (i.e., existing diseases,

cardiovascular risk factors, bone or joint disorders, respiratory disease, chronic or acute infections, etc).

4. Pregnant women were not allowed to participate.
5. All subjects were from the greater Oklahoma City, OK metropolitan area.

Limitations

Limitations for this study included:

1. This study used a cross-sectional design; therefore, causal relationships can not be determined.
2. The participants in this study were volunteers and, thus, potentially were not representative of the general population.
3. The StepActivity Monitor is not waterproof; therefore, water activities could not be assessed.
4. The StepActivity Monitor was worn on the ankle; therefore, upper body activity could not be quantified independent of overall body movement.
5. The dietary intake of our subjects was not monitored.

Assumptions

Assumptions for this study included that subjects:

1. Wore the Step Activity Monitor correctly throughout the entire time between visits.
2. Were in a fasted state for the blood draw and for the body composition assessment.
3. Honestly reported their medical history, age, and race.

Operational Definitions

Inflammation - a condition of a chronic, low-grade response instead of the classical form used to describe an acute bout of redness and swelling resulting from injury or infection, and is often referred to as "microinflammation" (130).

Fibrinolysis - a cascade of enzymatic reactions designed to breakdown fibrin clot formation; our body's natural defense against thrombosis (45).

Atherosclerosis - a build-up of fatty substances, cholesterol, calcium or fibrin on the inner walls of large and medium-sized arteries (114).

Hypertension - a blood pressure that is consistently higher than normal (systolic pressure ≥ 140 mmHg and a diastolic pressure ≥ 90mmHg) (125).

Hypercholesterolemia - Above normal serum levels of total cholesterol (> 200 mg/dL) (46).

Dyslipidemia - blood levels of low-density lipoproteins, high-density lipoproteins, and triglycerides that are outside of the "normal" ranges; (LDL ≥190 mg/dL; HDL < 40 mg/dL for males and < 50 mg/dL for females; Triglycerides ≥ 200 mg/dL) (46).

Abdominal obesity - a classification for a waist circumference greater than 102 centimeters in males and greater than 88 centimeters in females (46).

Body-mass index (BMI) - an index used to assess weight relative to height, and is calculated by dividing body weight in kilograms by the square of the height in meters (BMI = kg/m^2) (46).

Obesity - defined as BMI ≥ 30.0 kg/m^2 denoting an excessive accumulation of fat on the body (40).

Physical activity - any bodily movement produced by skeletal muscles resulting in energy expenditure (46).

Percent body fat (%BF) – the relative percentage of total body weight that is fat-mass, derived by dividing fat mass by total body mass and multiplied by 100 (34).

Metabolic Syndrome – a condition defined as having at least three of five risk factors such as abdominal obesity (waist circumference ≥ 102 cm or 40 inches in males, ≥ 88 cm or 36 inches in females), dyslipidemia (Triglycerides ≥ 150 mg/dL; high-density lipoprotein cholesterol < 40 mg/dL in males and < 50 mg/dL in females), raised blood pressure (systolic pressure ≥ 130 mmHg and a diastolic of ≥ 85 mmHg), and insulin resistance (fasting glucose ≥ 110 mg/dL) (46).

CHAPTER TWO

REVIEW OF LITERATURE

Introduction

Cardiovascular disease has been and remains the leading cause of death in the United States over the last century and is responsible for nearly 700,000 deaths in the U.S. a year (6). The most recent data show that there are approximately 71.3 million known cases of all cardiovascular diseases in the United States (147), with direct medical costs tallying approximately $41.2 billion (141). The predicted overall health care expenditures related to cardiovascular disease in 2006 is $403.1 billion, which underscores the prevalence and socioeconomic impact on our society (147).

Epidemiological data shows that more than 70 percent of all cardiovascular disease cases are related to atherosclerosis (141), a build-up of fatty substances, cholesterol, calcium or fibrin on the inner walls of large and medium-sized arteries. In clinical practice, the term atherosclerosis is often used interchangeably with cardiovascular disease. Several clinical conditions have been recognized as traditional risk factors for cardiovascular disease including hypertension (51), hypercholesterolemia (27), diabetes (37), obesity (defined as a body mass index [BMI] of greater than 30.0 kg/m^2) (64), physical inactivity (96) and cigarette smoking (26). Additionally, aging is recognized as an independent risk factor for cardiovascular disease (5,21,22,69,130). Still, numerous elderly patients present

with cardiovascular disease even though no co-existing risk factors (25) exist, implying that another mechanism could facilitate the development of atherosclerosis.

Chronic-low grade inflammation is now suggested as an important underlying component in the initial pathology of vascular disease, as it precipitates the breakdown of the inner walls of blood vessels (43,114,130). The fibrinolytic system is closely linked to inflammation and is directly involved in coagulation processes (45). Both of these processes have been associated with the development and risks of atherosclerosis (9,21). Is this a mechanistic link between age and cardiovascular disease? This chapter will further review the relations among age, inflammation, and fibrinolysis, as well as other influences on these processes.

Vascular Inflammation

Over the last decade, evidence has been presented that suggest inflammation is an important underlying component in the initial development and progression of atherosclerosis (43,114,130). Traditionally, inflammation has been defined as a local response to cellular injury that is marked by capillary dilatation, leukocyte infiltration, redness, heat, and pain, serving as a mechanism initiating the elimination of noxious agents and damaged tissue. Inflammation has since been considered to be a composite of many different activities brought into action in response to an environmental challenge, not a single process (130). Thus, in this context, the word inflammation implies a condition of a chronic, low-grade response instead of the

classical form used to describe an acute bout of redness and swelling resulting from injury or infection, and is often referred to as "microinflammation." (130)

Recent research has illustrated the inflammatory component in the pathology of cardiovascular disease, as well as identified conditions that induce chronic, low-grade inflammation (97). There also is a systemic response that accompanies inflammation and is known as the acute-phase response. This response stimulates the production of many hepatocyte-derived acute-phase proteins (22). The most popular, C-reactive protein (CRP), is considered to be the "golden marker" for chronic, low-grade inflammation (97).

The production and activation of CRP by hepatocytes has long been considered to be solely a response to elevated levels of inflammatory cytokines such as tumor necrosis factor- alpha and interleukin-6 (79,97), but more recently has been shown to play a role in promoting inflammatory processes (48,95). Specifically, this acute phase protein has been localized in atherosclerotic plaques and has demonstrated an increased expression in atherosclerotic arterial tissue (24,79), which led to speculation of a pro-inflammatory role of CRP.

Circulating CRP has demonstrated detrimental affects on endothelial cells by increasing adhesion molecule expression on the cell surface (79), impairing vasoreactivity by decreasing endothelial nitric oxide expression (18), and up-regulating the expression of angiotensin receptors in vascular smooth muscle (86). CRP is also known to facilitate monocyte and macrophage infiltration into endothelial tissue (79). Collectively, these actions are trademark characteristics of

inflammation that, ultimately, result in an unresponsive vessel (i.e., diminished arterial elasticity or reduced vascular reactivity), a classical feature of early atherosclerotic development (137). Furthermore, it has been shown that individuals in the highest quartile of CRP levels have a relative risk of future cardiovascular related events that is three times greater than those in the lowest quartile (112). This potentially explains the well-documented correlations between CRP and incidence of atherosclerosis or acute coronary syndrome (12,97,101,111,130), and further supports the identification of CRP as an independent risk factor for cardiovascular disease.

Inflammation and Aging

Aging has consistently been associated with increases in inflammation (8,9,23,44,68,115). Illustrating a link between the two processes is of high importance, as it may provide an explanation of the causes, or reflections, of age-related disease. Such findings would validate the use of these blood markers as an indicator of disease in clinical practice. Previous studies have shed light on this relation, but the design of these examinations (i.e., failure to control for confounding physical characteristics) leaves the relation between age and inflammation unclear.

The case of age-related changes in acute inflammatory responses has been well illustrated with *in vitro* studies using human endotoxin challenge models. For instance, Krabbe et al. (2001) used a human endotoxin challenge model, in which a wall constituent of *Escherichia coli,* a gram-negative batcteria, was injected

intravenously to induce an inflammatory state (68) to identify age-related differences in inflammatory response. While older individuals showed quicker and more prolonged inflammatory responses than younger individuals, there was no difference in peak inflammation status between the two groups. In 1999, Bruunsgaard et al. used the same type of *in vitro* stimulation in a large, population-based study (23). After the endotoxin infusion, the 80 year old adults showed a greater decrease in inflammatory response, represented by tumor necrosis factor – alpha (TNF-α), interleukin-1Beta (IL-1β), and interleukin-6 (IL-6) than the 18 to 30 year old adults. Further observation showed that the older females, while having lower inflammatory responses than the young males, did not show any difference from the young females. These findings actually suggest that there is a decline in age-related inflammatory cytokine production and that estrogen produces a suppressive effect on inflammation (23).

In earlier studies, increased age has been associated with higher levels of inflammatory cytokines than in younger adults (19,110). Specifically, one study (19) showed that the concentrations of TNF-α and IL-1β were significantly increased in older subjects. Ironically, the levels of monocytes were unchanged, which suggests that immune responses can actually be enhanced with age. The same increase in IL-1β production with age was shown by Riancho et al. (1994); however, no age-related changes occurred in TNF-α production (110).

The conflicting results found in the acute inflammatory response investigations are sustained when examining the chronic elevations of circulating

markers of inflammation in aging populations. Many studies conclude that aging is associated with increased plasma levels of CRP, TNF-α, and IL-6 (9,22,23). However, there are studies that do not support these findings (115).

In 1996, Ballou et al. studied a group of older and younger adults who were apparently healthy (9). While this study carefully excluded individuals with disorders that could affect CRP levels, they failed to take into account blood pressure, cholesterol levels (or pertaining medications), physical activity levels, or body composition. Their findings showed that elderly people had a significantly higher level of CRP (median = 3.0 μg/ml) than a younger group of people (median = 0.9 μg/ml, $p < 0.003$) (9). Within the older group (n = 131), ranging 65-94 years in age, there was no significant correlation between age and CRP levels or other acute-phase proteins (i.e., serum-amyloid A) (9). These findings weakened the claim that age is associated with inflammation.

In another cross-sectional study with a larger sample (n = 1327), Ferrucci et al. found that inflammation increased with age (48). To date, this is one of the largest age ranges (20 to 102 years) examined. CRP and IL-6 were significantly higher in the age group 65 years of age and older in both males and females even after adjusting for the existing clinical conditions. These conditions included: presence of hypertension, history of coronary artery disease or stroke, chronic heart failure, diabetes, cancer, and smokers. However, the sample also included subjects of varying body size and physical activity status. The failure to control for such

variances precluded the researchers from confidently concluding that there were age-association changes in inflammatory status.

Changes in circulating inflammatory marker levels have also been examined in large population based studies (8,44,115). In the Framingham Heart study, 711 elderly individuals did show higher production of IL-6 and IL-1 receptor antagonists as compared to a younger control group. However, no differences in circulating TNF-α and IL-1β were found between the two age groups. Unfortunately, the control group only had 21 subjects, which could have biased the conclusions. Another study examined subjects enrolled in the SENIEUR protocol, which attempted to separate the influence of morbidity and frailty from the physiologic process of aging (44). A secondary analysis found that adults from 69 to 80 years of age had higher plasma levels of TNF and IL-6 compared to young controls (22).

The studies showing a positive relation between age and inflammation support the notion that low-grade inflammatory activity in older individuals is independent of co-existing medical disorders. However, we have yet to clearly define the existence of a primary, age-associated dysregulation of inflammatory cytokine production. The findings from some of these studies could have been explained by residual confounding factors due to the high prevalence of inflammatory, non-cardiovascular conditions in the older populations. Furthermore, there may be age-cohort effects on the levels of inflammatory markers that could not be detected in these cross-sectional studies. Future research focusing on the changes

in inflammation occurring with advancing age is necessary to better define this relation.

Inflammation and Obesity

Obesity has long been associated with an increased risk of developing numerous diseases, such as hypertension, dyslipidemia, diabetes, and atherosclerosis (60,145). Several recent studies have also shown an associated between obesity and generalized, low-grade inflammation (29,86). More recently, research shows that adipocytes can produce CRP, TNF-α and IL-6, which explains the association between obesity and inflammatory activity (34,68,132). A previous report claims that 25 to 30% of circulating IL-6 may be derived from fat (87). Thus, the age-associated increase in body fat, especially abdominal adiposity, (55,98) may be an important source for increased circulating levels of inflammatory markers in the older populations (63). The remainder of this section will highlight several studies addressing the link between obesity and inflammation.

The primary basis of the suggested causal role obesity has on inflammation is supported by many studies showing an increase in circulating inflammatory markers in obesity (39,49,132,150). For example, Yudkin et al. (1999) found a close relationship of circulating CRP, TNF-α, and IL-6 concentrations with anthropometric measures of obesity in healthy subjects (150). However, participants were not excluded for smoking, hypertension, or hyperlipidemia, and the sample consisted largely of overweight individuals. Specifically, this study compared measures of

body composition to levels of CRP, TNF-α, and IL-6 in 107 individuals from 40 to 75 years of age. The initial analysis showed that all markers of inflammation were significantly related to BMI and waist-to-hip ratio. When divided into groups of "low CRP" (<1.35 μg/mL) and "high CRP" (≥ 1.35 μg/mL), the high CRP group unsurprisingly presented significantly higher values of BMI and waist-to-hip ratio. This provided an important early observation to stimulate further investigation into the link between adiposity and inflammation.

In another study, markers of inflammation were compared in an obese group of pre-menopausal women (n = 56) and a group of age-matched, normal weight controls (n = 40) (151). In the initial baseline assessments, all subjects were sedentary with no history of participating in weight-loss related diet programs in the previous six months. The obese group demonstrated significantly higher levels of TNF-α, IL-6, and adhesion molecules (ICAM-1 and VCAM-1). Additionally, the concentrations of TNF-α and IL-6 significantly correlated to anthropometric measures of "visceral" obesity, represented by waist-to-hip ratio measures ($r = 0.55$ and $r = 0.45$, respectively). After a baseline visit, the entire obese group participated in a 12 month multidisciplinary program of weight reduction, which included diet, exercise, and behavioral counseling. All of the women lost at least 10% of their original weight (9.8 ± 1.5 Kg) and, in turn, reduced the circulating blood levels of TNF-α and IL-6 ($p < 0.01$) from baseline to post-intervention. Regardless of a causal origin, this study clearly shows that obesity is related to increases in level of markers of inflammation. While it is also possible that an improved diet regimen or exercise

could be, at least partially, responsible for the results of this study, there is a strong suggestion that weight loss could be a safe method of reducing inflammatory activity.

Piche et al. (2005) studied the association of obesity and fat distribution on inflammation in a group of 112 post-menopausal women (100). This sample was "healthy," defined as not being clinically treated for heart disease, diabetes, endocrine disorders, or additional cardiovascular risk factors. However, the sample still included (and statistically adjusted for) individuals that had a history of smoking, hypertension, and dyslipidemia. CRP concentrations were significantly correlated with BMI ($r = 0.60$) and waist circumference ($r = 0.61$). Additionally, IL-6 levels were related to BMI ($r = 0.49$) and waist circumference ($r = 0.56$), while TNF-α levels were not. A more in-depth examination of visceral adiposity, using computed tomography, showed that visceral adiposity was also correlated with CRP ($r = 0.55$) and IL-6 ($r = 0.49$). As a side note, the total sample was separated into three groups: "low CRP concentration" (< 1.0 mg/L), "normal CRP concentration" (1.0 – 3.0 mg/L), and "high CRP concentration" (>3.0 mg/L). There were no group differences in age ($p > 0.05$), but all of the body composition measures were significantly different among the groups of differing CRP concentrations. Once again, this study found elevated levels of inflammatory activity in those who were obese, while also establishing the importance of the site of fat distribution (i.e., visceral adiposity) on mediating inflammation.

Another study examined BMI, waist circumference and CRP concentrations in a population of persons older than 65 years of age (n = 1,270) (121). As expected in a sample of older people this size, there were a number of individuals who presented with clinical conditions including heart disease, chronic heart failure, history of stroke, and diabetes. In general, the individuals presenting with global obesity (defined as a BMI $\geq$ 30.0 kg/m^2) demonstrated higher levels of CRP concentrations than the non-obese individuals. The subjects considered to have central obesity, defined as being in the upper sex-specific tertile of waist circumference (121), had an even higher concentration of CRP than subjects classified as non-obese or obese without central distribution of fat. The group of subjects presenting with global and central obesity yielded the highest CRP concentrations, suggesting an additive affect of the two conditions on inflammation.

In further examination, Schrager et al. (2007) found that individuals with global obesity had higher concentrations of TNF-α than those with central adiposity (121). The highest concentrations of TNF- α were found, however, in the group presenting with both, global and central obesity. In contrast, IL-6 concentrations were highest in the central obesity group, while those with the lowest concentrations of IL-6 were in the global obesity group. The presence of global and central obesity did, however, appear to have an additive affect on cytokine concentration that was similar to that shown with CRP. Even with the slight conflict in results between inflammation and fat distribution, this study continues to claim that overall obesity is related to inflammation.

Overall, the studies showing a link between obesity and inflammation are consistent, regardless of other potentially confounding clinical conditions. With further evidence that adipose tissue secretes several inflammatory proteins activity (34,68), it is probable that excessive amounts of fat are a major source of inflammation in many populations (132). This body of research suggests that the changes in body composition that normally occur with age (i.e., decreased skeletal muscle mass and increased total body fat) (55,98) could explain some of the aforementioned associations between age and inflammation. In any case, the inflammatory response stemming from increasing whole body and, even more specific, abdominal adiposity has justified the classification of obesity as an independent risk factor of cardiovascular disease.

Inflammation and Physical Activity

Increasing physical activity level is accepted as a means to decrease risk of many health problems, especially cardiovascular disease (72,140). Additionally, it is well documented that increasing amounts of physical activity can result in a decrease in the amount of adipose tissue (55,98). Because of the common connection between physical inactivity and inflammation as risk factors for cardiovascular disease, it is a logical next step for research to examine the influence of physical activity level on inflammatory activity.

Generally, studies show that more frequent bouts of activity lower blood levels of inflammatory and fibrinolytic markers (96,135). For instance, one study

showed that individuals with higher levels of interview based, self-reported physical activity had lower concentrations of CRP and IL-6 (109). Specifically, this study examined community-dwelling subjects enrolled in the MacArthur study. When the group was divided into tertiles by amounts of activity, the most active tertile had concentrations of CRP and IL-6 that were about 30% lower than the other two-thirds of the sample. Furthermore, individuals incorporating higher amounts of house or yard work activities had an additional protective effect beyond that of recreational activity on CRP levels, but not IL-6 levels. These findings are greater than a previous report of the group in the top quartile of physical activity the Cardiovascular Health Study, a large population based study, presented with up to 19% lower concentrations of CRP (57).

Another cross-sectional study examined the association between physical activity and markers of inflammation in a cohort of the Health, Aging and Body Composition Study (n = 3,075) (30). Again, the sample was split into three groups based upon amounts of activity: No Activity (0 minutes of exercise per week), Low Activity (between 0 and 180 minutes of exercise per week), and High Activity (≥ 180 minutes of exercise per week). The concentrations of CRP, IL-6 and TNF-α were the lowest in the high activity group. It should be noted that BMI and the prevalence of hypertension, cerebrovascular disease, peripheral vascular disease, respiratory disease and diabetes mellitus were also significantly lower in this sample. Significance in inflammatory marker concentration remained after adjusting for all clinical conditions, but the concentrations of CRP and TNF-α were no longer

significantly different between activity groups after adjusting for body composition measures. Although there may be an additive affect, these data suggest that body composition may have a greater influence on inflammatory status than physical activity.

In an examination between leisure time physical activity and markers of chronic inflammation, Verdaet et al. (2004) found conflicting results with the previously discussed studies (138). This cross-sectional study found that CRP levels were not associated with leisure time physical activity in a cohort of the BELSTRESS study. It should be noted that any affect of activity might have been lost due to the fact that the sample included many subjects that smoked, were diabetic, or had other co-existing conditions. Leisure time activity was, however, negatively associated with BMI, waist to hip ratio, and the lipid profile. This suggests that these conditions may have more of an influence on inflammatory activity than the level of leisure time activity. This study also suggests that the intensity of the activity (or exercise) may have a determining role on inflammation.

A study by Pischon et al. in 2003 shed light on this particular question. A group of 859 people was separated into five groups depending on activity status (102). This study extends previous findings that CRP and other markers of inflammation are lower in men and women that are more active. As expected, the groups with higher activity (in both men and women) showed an inverse relation between CRP, IL-6, and TNF-α. Specific to CRP, the blood concentrations linearly declined through each quintile of activity level, which may suggest a dose response

exists on the protective effect of activity on inflammation. Furthermore, the groups of greater activity also demonstrated lower BMIs and a lower prevalence of smoking. Adjusting for these factors weakened the association between activity levels and inflammation. Again, this implies that the beneficial association between physical activity and inflammation is partially due to less body fat in subjects with higher levels of physical activity.

Overall, there is a general notion that physical activity and exercise is inversely related to chronic levels of inflammatory markers (30,57,102,105). There is a re-occurring observation among all of these studies that allege the link between physical activity and inflammation may be mediated by the paralleling changes in body composition. Regardless, these preliminary claims further evidence that increasing physical activity can decrease cardiovascular disease risk. Continued research in this area, which is gaining in popularity, will provide the necessary clarity to define the relation between physical activity and inflammation.

Inflammation and other clinical conditions

Numerous clinical conditions (or co-morbidities) are showing an instigative role for inflammation. While it is traditional and logical to associate inflammation with acute infections, many investigators are linking low-grade inflammation with an increased prevalence of chronic, asymptomatic infections (22,107). Interestingly, aging is associated with an increased prevalence of many of the same types of infections, such as dental infections (85), urinary tract infections (2), and bacterial

infections (59,85). This is a growing concern because bacterial infections can spread from the respiratory tract throughout the body via circulating monocytes (113) and, in turn, induce systemic immune responses by stimulating inflammatory cytokine production (i.e., TNF-α, IL-1β, and IL-6) (108,113).

Similarly, a rise in inflammatory markers or fibrinolytic activity can be caused by other existing clinical conditions or diseases. Diabetes (37,90) and components of metabolic syndrome (4,28) are shown to elevate inflammatory activity. Additionally, individuals presenting with known cardiovascular disease (22), especially atherosclerosis (97), and pulmonary disease (123) consistently have higher levels of inflammatory markers than those who are disease free.

Hypertension, which has a higher prevalence in older adults, also has been linked to chronic, low-grade inflammation (22). Typically, isolated hypertension is defined as a systolic blood pressure ≥ 140 mmHg and a diastolic blood pressure ≥ 90 mmHg (6^{th} Report). Yudkin et al. (1999) and Piche, et al. (2005) both found systolic and diastolic blood pressure levels were significantly related to markers of inflammation (CRP, IL-6, TNF-α) (100,150). Piche also found that individuals in the top two tertiles of CRP concentrations had significantly higher systolic and diastolic pressures (100). While these associations are becoming more popular, it is unclear if increased inflammatory activity is a product of sustained high blood pressure, or if it instigates hypertension. Evidence that angiotension II, a potent vasoconstrictor, causes monocytes activation would support the idea of an inflammatory response to elevated blood pressure (99,120).

Another common clinical condition that is associated with higher levels of circulating inflammatory markers is hyperlipidemia (27,51). In a study by Piche et al. (2005), triglycerides levels were positively related ($p < 0.05$) to levels of CRP ($r = 0.33$) and IL-6 ($r = 0.32$) (100). As expected, HDL levels were inversely related to the same markers of inflammation, demonstrating that higher HDL levels are associated with decreased inflammatory activity (100). These findings parallel previous cross-sectional observations linking lipid profiles to inflammation (84,111,150).

Furthermore, lifestyle characteristics, such as cigarette smoking and alcohol intake (139), have been linked to inflammation (27). In an early study, smokers had higher circulating levels of IL-6 compared to their non-smoking counterparts (122) Another adjusted analysis showed that CRP and IL-6 (as well as levels of adhesion molecules) were independently related to smoking status, which would suggest that smoking lead to increases in inflammatory activity (13).

There are a couple of additional biological factors that are speculated to influence inflammatory activity in humans. For instance, in 1996, Ballou et al. found that black people had slightly higher CRP levels than whites (7.0 vs. 3.0 μg/ml, $p = 0.019$) (9). More so, Native Americans have been shown to have higher levels of inflammatory markers than other ethnic groups (84,92). However, these investigations also note that there is a high prevalence of diabetes and metabolic syndrome, which could have additive effects on CRP levels. The genetic role of regulating inflammation has recently gained attention in research. Previous studies

have failed to directly link polymorphisms to inflammatory activity (35,142). There are known gene polymorphisms, however, that are related to several diseases, including insulin resistance, Alzheimer's disease, and arthritis, which could assuredly increase inflammatory activity (22,31,33).

The chronic inflammation resulting from these conditions can explain a mechanistic link between the traditional risk factors and atherosclerotic development. Furthermore, the associations between clinical conditions and inflammatory, while significant, may only explain a small part of the low-grade inflammatory activity (22,23). Regardless, excluding subjects presenting with these characteristics can remove any confounding influence on inflammation in future research studies.

Fibrinolysis

Fibrinolysis is a cascade of enzymatic reactions, bringing together plasminogen and its activators (tissue plasminogen activator, and is designed to breakdown fibrin clot formation (45,150). Specifically, tissue plasminogen activator (t-PA) has a major role in activating the fibrinolytic system and initiating the release of plasmin, the protein responsible for lysing fibrin clots (135). The essential balance in plasma is largely between t-PA and the inhibitor, plasminogen activator inhibitor - 1 (PAI-1) (88). PAI-1 is the most important inhibitor of plasma fibrinolysis (126). An increase of plasma t-PA and PAI-1 are both indicative of an increase in fibrinolytic activity (126,135) and can result in a pro-thrombotic state. When PAI-1

concentrations reach a level four or five times greater than the activators, haemostatic conditions favor fibrin clot formation (i.e., hypofibrinolysis) (88).

Fibrinogen, another acute-phase protein, is often considered as a marker of inflammation (11). However, fibrinogen is more of a determinant of blood viscosity, an important factor in blood clot formation (41,42). In addition to serum concentrations of CRP, fibrinogen is not only associated with the risk of developing cardiovascular disease, but is also indicative of the severity of existing disease (107,111). Therefore, fibrinogen is perceived as another independent marker of cardiovascular disease risk as well as a prognostic indicator (11).

Clot formation is an essential protector from bleeding, but persistent conditions can ultimately lead to thrombosis. The fibrinolytic system is our body's natural defense against thrombosis. Thrombosis exacerbates the risk of a catastrophic acute coronary episode in the presence atherosclerotic plaques, especially if the plaques rupture. The increase in fibrinolytic activity is capable of independently predicting catastrophic cardiovascular disease related events (52). Thus, t-PA and PAI-1 are recently being considered additional, independent risk markers of cardiovascular disease.

The role of fibrinolysis in the early development of atherosclerosis is a rather new idea. It is suggested that excessive amounts or fragments of fibrin can be deposited in the vessel wall, contributing to the progression of atherosclerotic lesions. Specifically, recent clinical evidence suggests that low fibrinolytic activity is a determinant of major CHD events in young and middle-aged men (82).

Furthermore, high PAI-1 activity combined with a low plasminogen activator capacity were risk factors for re-infarction in young, male survivors of myocardial infarction (62). Another study, also carried out in persons with severe CHD, found that tissue plasminogen activator antigen, but not plasminogen activator inhibitor activity, was predictive of total mortality during a 7 year follow-up. Thus, there is still a need for clarification of the determinants of coagulation and fibrinolytic activity.

Fibrinolysis and Aging

Age-related changes in coagulation and fibrinolytic factors are speculated to be contributors to the increase in risk of thrombotic events in older adults (61,67). Increases in fibrinogen and other coagulation factors, without a proportional increase in anticoagulant factors, likely contribute to this risk (61). Over the past couple of decades, a positive correlation between age and the occurrence of thrombosis has been presented (36,148). Hashimoto et al. presented some of the first evidence of a relationship between age and fibrinolytic activities; namely levels of tissue plasminogen activator (t-PA) antigen, plasminogen activator inhibitor (PA inhibitor) activity, and plasminogen activator activity (PA activity). A dramatic increase in both t-PA antigen and the plaminogen activator inhibitors was shown in persons with increasing age, while t-PA activity decreased with age.

DeSouza et al. (1998) also examined the age differences in coagulations and fibrinolytic factors in pre- and post-menopausal women, as well as identifying the

impact of habitual exercise on these hemostatic factors in the two age groups (36). To do so, they set up four groups: (1) pre-menopausal sedentary, (2) pre-menopausal active, (3) post-menopausal sedentary, and (4) post-menopausal active. They measured resting levels of plasma fibrinogen, tissue-type plasminogen activator (t-PA) antigen and activity, and plasminogen activator inhibitor-1 (PAI-1) activity as indicators of coagulation and fibrinolytic factors. The post-menopausal had significantly higher ($p < 0.05$) levels of fibrinogen, t-PA antigen, and PAI-1 lower t-PA activity than the pre-menopausal women, indicating that fibrinolysis is impaired with age. Furthermore, the active post-menopausal women demonstrated lower ($p < 0.01$) plasma fibrinogen, t-PA antigen, and PAI-1 activity and higher ($p < 0.01$) t-PA activity levels than the sedentary post-menopausal women (36). Even more importantly, the fibrinolytic profile of Post-PA did not differ from that of the active premenopausal women. This suggests that the adverse age-associated differences, however great they may be, in plasma fibrinogen concentrations and the endogenous fibrinolytic system in sedentary healthy women are either attenuated or absent in highly physically active women.

Changes in the endothelium, including early development of atherosclerotic plaques, have been proposed as potential contributors to the increase in thrombotic cardiovascular disease found in the elderly (148). The capacity of the vascular endothelium locally to release t-PA is critical for effective endogenous fibrinolysis. Using both cross-sectional and intervention approaches, Smith et al determined the influence of ageing and regular aerobic exercise on the net release of t-PA across the

human forearm in vivo (127). They studied the endothelial release rates of t-PA in 62 healthy men aged 22-35 or 50-75 years of age who were either sedentary or endurance exercise-trained. Similar to DeSouza's findings (36), net endothelial t-PA release was significantly blunted (approximately 35% less, $p < 0.05$) with age in the sedentary men, while no age-related declines in net release of t-PA antigen were found in the endurance-trained men. Again, these results illustrate that endothelial t-PA release declines with age in sedentary men, but the decline could be prevented, or even reversed with regular aerobic exercise. Perhaps the tendency of decreased PA activity with increasing age may be related to the high incidence of thrombosis in older persons. The influence of physical activity and exercise on fibrinolysis will be later discussed in this chapter.

In 2005, Yamamoto et al. examined if gene expression of PAI-1, as the principal inhibitor of fibrinolysis, varied across increasing age (148). The expression of PAI-1 is not only elevated in the elderly but also significantly induced in a variety of pathologies associated with the process of aging, such as obesity, insulin resistance, and vascular sclerosis. Furthermore, the gene expression of PAI-1 was found to be positively regulated by several cytokines and hormones. These included tumor necrosis factor-alpha, a marker of systemic inflammation, and angiotensin II, a mediator of vasoconstriction. This raises suspicion that impaired fibrinolysis may result from an inflammatory interaction (148). Additionally, it could explain the heightened risk for thrombotic cardiovascular disease in obesity with aging, as adipose is a known source of inflammatory cytokines. None the less, future studies

on the genetic regulators of aging-associated PAI-1 induction will be necessary to define the basis for cardiovascular aging in relation to thrombosis.

Overall, these studies show that increasing age is consistently associated with a decrease in fibrinolytic activity (36,61,148). While this could be a primary affect of aging, the data showing that increasing physical activity levels negate this affect and introduce new factors that dilute the clarity of this relation (36,127). Is this an age effect? Or is this more of a result of age-related declines in physical activity patterns? Other conditions that can affect fibrinolysis, and coincidentally change with age, will be discussed in the following sections. But regardless of why or how, further studies will be warranted to define the mechanisms for thrombosis in the elderly as the population ages.

Fibrinolysis and Obesity

Obesity has repeatedly been shown to be associated with PAI-1, the major fibrinolysis inhibitor (78,81,134). PAI-1 is directly correlated with BMI (133) and waist-to-hip ratios, indicating an influence from abdominal fat (134). More specifically, adipocytes have recently been shown to produce and mediators of fibrinolysis (126), which explains the link between high levels of fibrinolytic markers with excessive adiposity (i.e., high percent body fat) (34).

In a two-part study, a group of investigators examined a group of 66 sedentary adults, which included 28 normal-weight, 22 overweight, and 16 obese adults (136). In the first, cross-sectional observation, the amount of t-PA antigen

released from endothelial was significantly lower ($p < 0.05$) in the overweight and obese adults, compared to their normal weight peers. In a bivariate analysis, the total amount of t-PA antigen release was inversely related ($p < 0.05$) to body mass ($r = -0.47$), percent fat ($r = -0.29$), BMI ($r = -0.36$), and waist circumference ($r = -0.28$). The impaired endothelial t-PA release found in overweight individuals further support the notion that impaired fibrinolysis is a consequence of obesity, with specific emphasis on abdominal adiposity.

Mavri et al. (1999) enrolled 52 obese, pre-menopausal women in a 10-12 week body weight reductions program, with 19 age-matched lean women as a control group (78). PAI-1 concentrations were measured at the time of entry, one week after the start of the program, at time of program completion, and five months after program completion. At the start of the program, PAI-1 activity concentrations were positively correlated with BMI ($r = 0.35$), waist-to-hip ratio ($r = 0.30$), and an equation derived percent body fat ($r = 0.43$). At the end of the program, women reduced their body weight by 17% and their percent body fat by 13%, while PAI-1 activity had decreased by 74%. However, five months after program ended, 16 women regained over 25% of their original body weight. As a result, PAI-1 activity levels significantly increased, paralleling the increase in BMI. Thus, this study suggests that the elevated PAI-1 levels seen in obesity are closely linked to the amount of adipose tissue stored (78).

For three months, 55 subjects participated in a weight loss program using meal replacements or a structured eating plan (29). At the end of the study, mean

weight loss was 6.3 ± 3.7 kg with no differences between diet groups. As a whole, PAI-1 concentration significantly decreased from baseline to after weight loss (70.84 ± 30.91 ng/ml vs. 55.55 ± 31.64 ng/ml, respectively, $p < 0.001$). Furthermore, t-PA levels dropped by 7% ($p < 0.05$) from baseline. As a side note, individuals presenting with CRP concentrations higher than 10 mg/L at baseline showed significant reductions after the three month diet intervention (3.65 ± 2.15 mg /L vs. 3.01 ± 2.19 mg/L, $p < 0.01$). This supports the previous findings showing that weight loss intervention leads to a reduction in fibrinolytic activity (78).

Bouchard et al examined if there were polymorphism variations the PAI-1 gene related to body fat and abdominal fat mass (20). In women, BMI and body fat mass was associated with the PAI-1-675 4G/5G, while abdominal visceral fat mass was related to the same polymorphism and the c43G>A variant within the exon 1. Furthermore, carriers of the -675 5G and the 43A allele had nearly 50% more visceral fat (assessed by computed tomography) compared to the -675 4G allele. None of these associations were found in the men. These findings support previous claims that PAI-1 polymorphisms may be associated with BMI (65). However, this is the first study to observe an association between the PAI-1-675 polymorphisms and measures of whole body and abdominal fat mass. This implies that the association between PAI-1 activity and fat distribution may be influenced by more than lifestyle characteristics, particularly in women.

There is overwhelming evidence showing that obesity is associated with impaired fibrinolysis (78,81,134). With specific emphasis on abdominal adiposity,

these studies provide a mechanism that bridges obesity with cardiovascular disease (136). As previously mentioned, the changes in body composition occurring with age (i.e., decreased skeletal muscle mass and increased total body fat) (55,98) could possibly explain prior observations of a relation between age and fibrinolysis. At any rate, the rationale for classifying obesity as an independent risk factor for cardiovascular disease continues to grow with this evidence that decrease in fibrinolytic activity decreases with increasing whole body and, even more specific, abdominal adiposity.

Fibrinolysis and Physical Activity

Over the last few decades, it has become quite clear that regular aerobic exercise is associated with a reduction in cardiovascular events in overweight and obese adults, independent of weight changes (14,136). As a contributing mechanism, the affects of physical conditioning on fibrinolysis were examined more closely (7,32,47). By comparing 60 healthy men in three physical fitness categories (sedentary, joggers, and marathoners), Ferguson et al. discovered that fibrinolytic activity increased with exercise early on (47). More recently, physical activity is consistently understood to be inversely correlated with blood levels of fibrinolytic markers (96,135), a suggested protective mechanism of physical activity against cardiovascular disease. The general notion is that more frequent bouts of activity can lower blood levels of fibrinolytic activity.

Acute fibrinolytic responses to exercise are also being examined and are of high clinical relevance, as numerous catastrophic events occur from an occlusive thrombus (58). With previous evidence of an increased potential for blood coagulation during physical exertion (7,32), there is an urgency to learn if a body can counter by increasing fibrinolytic activity (144,145). Womack et al. examined the acute changes in fibrinolytic activity occurring with exercise (146). Specifically, 15 healthy males performed three different cycle ergometer tests: (1) a VO_{2max} and lactate threshold test, (2) a one hour exercise session over lactate threshold, and (3) a one hour exercise session below lactate threshold. t-PA activity was significantly higher immediately following the exercise above lactate threshold, but not in the exercise below the lactate threshold. The concentrations of t-PA antigens were significantly higher immediately following both exercise conditions, but were still significantly higher in the exercise session above lactate threshold. PAI-1 activity significantly decreased during both exercise conditions; however, the decrease occurring in the exercise below lactate threshold only occurred when post-exercise values were corrected for plasma volume changes. All marker concentrations, except t-PA antigen levels in the above lactate threshold exercise, returned to baseline within the one hour post measurement. Overall, this study demonstrates that intensity is a more important determinant on acute fibrinolytic responses to exercise, showing that exercising below the lactate threshold elevates t-PA antigen levels but not t-PA activity (146). Thus, a higher intensity exercise can heighten fibrinolytic activity.

In a previously discussed study, Van Guilder et al. enrolled 17 overweight or obese individuals (11 males, 6 females) of the total 66 initially examined subjects in a three month, home based aerobic exercise training program (136). During their participation, subjects were asked to exercise 5-7 days per week, 40-50 minutes a day, at 60-75% of their maximum heart rate. After the training program was completed, there were no changes observed in basal release rates of t-PA or PAI-1 antigens. However, t-PA release rates were markedly increased after bradykinin infusions, increasing net t-PA antigen amount by nearly 55%. This evidences that there is, indeed, an improved capacity of the endothelium to release t-PA in overweight and obese adults. Because these changes occurred without a paralleled shift in body size or composition, there is a much stronger notion that aerobic activity may have a primary modulatory effect on endothelial fibrinolytic regulation (136).

Another report showed that leisure-time physical activity was not an independent predictor of fibrinogen (138). In a sample of 892 male subjects, leisure-time physical activity (LTPA) was related to a number of cardiovascular risk factors, such as BMI ($p = 0.01$), waist to hip ratio ($p < 0.001$), and HDL levels ($p = 0.003$). Unadjusted means across the four categories of LTPA ("No LTPA," "Low LTPA," "Moderate LTPA," and "High LTPA") showed a significant inverse relationship with CRP ($p = 0.02$) and fibrinogen ($p = 0.02$). However, in a multivariate analysis adjusting for BMI, smoking status, presence of diabetes, and alcohol consumption, these relationships were no longer significant ($p > 0.05$). This indicates that there is

no independent association between LTPA and markers of inflammation or fibrinolysis and further strengthens the previously discussed claims that intensity has a greater influence than duration (146).

Exercise interventions have also been shown to have beneficial effects in clinical populations, especially those with cardiovascular disease (91,100). Killewich et al. (2004), studied 21 patients with peripheral arterial disease (PAD) (70). After completing a six month training program of treadmill exercise three times a week, fibrinolytic activity significantly improved, expressed as a 23% reduction in PAI-1 activity and a 28% increase in t-PA activity. No changes were noted in a control group of 20 age-matched men presenting with claudication. Additionally, it was noted that the greatest improvements in fibrinolytic activity occurred in individuals with the lower amounts of fibrinolysis (i.e., highest levels of PAI-1 activity). This adds to the growing body of literature evidencing a beneficial effect of exercise on fibrinolysis. Combined with the other studies of similar outcomes, these data suggest that serious consideration should be given to exercise interventions as a treatment option in cardiovascular patients.

In 2002, Wannamethee et al. examined the relations between physical activity and hemostatic variables in a large population based study (140). In 3810 adult men, physical activity showed a significant inverse dose response relationship with many hemostatic variables, including fibrinogen, t-PA antigen, and CRP. In this case, physical activity was collected by interview with a score assigned and used to categorize partcipants into one of six groups: (1) “No activity,” (2) “Occasional

Activity," (3) "Light Activity," (4) "Moderate Activity," (5) "Moderate to Vigorous Activity," and (6) "Vigorous Activity." As expected, the diseased men presented consistently higher values of these blood markers in every activity group, but the inverse relationships were similar between men with and without cardiovascular disease. This supports the notion that the benefit of physical activity on cardiovascular disease may be, at least partly, due to the positive effect on fibrinolysis (140).

In the past twenty years, research has illustrated the positive influence of regular aerobic exercise in fibrinolysis (14,37,136). Although more research can more clearly define a dose-response effect of exercise, the benefits on cardiovascular disease may be, at least partly, due to the positive effect on fibrinolysis (140). Moreover, the tendency of decreased PA activity with increasing age may be related to the high incidence of thrombosis in older persons.

Fibrinolysis and other clinical conditions

There is a well defined association between fibrinolysis and known diseases, such as cardiovascular disease (75), diabetes (37), and components of metabolic syndrome (4,28,140). Early development of atherosclerotic plaques has also been shown to impair fibrinolytic activity (119). In 1995, a cross-sectional case-control study with 457 pairs of subjects found that t-PA and PAI-1 were higher ($p \leq 0.001$) in patients above the 90^{th} percentile of intima-media thickness of carotid arteries compared to those having a thickness below the 75^{th} percentile of the Atherosclerotic

Risk in Communities (ARIC) Study (119). These findings led to the notion that thrombosis and fibrinolysis may play a role at the early stage of the atherosclerotic process.

Hypertension is an established risk factor for acute coronary events that is also being associated with impaired fibrinolysis (25,53,103,128). In a cross-sectional investigation, the relations of systolic and diastolic blood pressures (SBP and DBP) to levels of plasminogen activator inhibitor antigen, tissue plasminogen activator antigen, fibrinogen, were examined in 1193 men and 1459 women of the Framingham Offspring Study (103). In both sexes, levels of plasminogen activator inhibitor and tissue plasminogen activator antigen were positively related to systolic and diastolic blood pressures, even after adjusting for age, body mass index, smoking, diabetes, total cholesterol, HDL, triglycerides, alcohol intake, and estrogen use in women ($p < 0.001$). However, there was no association between systolic and diastolic blood pressure in either sex. These data provides another potential mechanism by which hypertension contributes to the pathogenesis of cardiovascular disease in hypertensive patients.

The negative influences of hypertension on fibrinolysis have been alleviated by exercise (36). Specifically, hypertensive subjects had a 172% increase in t-PA activity and a 25% decrease in PAI-1 activity after an acute bout of exercise (36). There are pharmacological treatments of hypertension that have an interfering role on fibrinolysis as well (103). As it is known that angiotension II increases PAI-1

activity (89,148), angiotension converting enzyme (ACE) inhibitors decrease PAI-1 levels and increase t-PA levels (103).

Numerous reports have also shown a link between impaired fibrinolysis and high cholesterol levels, another established risk factor of cardiovascular disease (93,105,106). Puccetti et al. compared individuals with hypercholesterolemia, hypertriglyceridemia and low HDL cholesterol levels in 75 males and females (105). Hypertriglyceridemia patients were found to have higher PAI-1 serum levels compared to hypercholesterolemia and control subjects ($p < 0.001$). Impaired fibrinolysis in subjects with hypertriglyceridemia or low HDL-cholesterol is associated with increased serum levels of PAI-1 whereas enhanced thrombin generation was the main finding in hypercholesterolemia. Such data may suggest the opportunity of evaluating several fibrinolytic factors when studied as prognostic factors in varying lipid profiles. Furthermore, Orem et al. showed that, after successful lipid lowering therapy, statin use improved global fibrinolytic capacity ($p = 0.003$), increased tissue plasminogen activator levels ($p = 0.04$) and decreased plasminogen activator inhibitor type-1 levels ($p = 0.02$) in dyslipidemia patients (93). Thus, pharmacological treatment may negate the negative effect of hypercholesterolemia on the fibrinolytic system.

The association between fibrinolysis and cigarette smoking, an established risk factor for cardiovascular disease, is less apparent than the relation between smoking and inflammation (35,38). One study showed that non-smokers had significantly higher ($p < 0.03$) levels of t-PA activity than current or former smokers

(91). Another study showed that there were no differences in fibrinogen levels between smokers and non-smoker (35). In contrast, a third investigation found that smokers had fibrinogen levels 0.34 g/L higher than non-smokers, showing a correlation between number of cigarettes smoked and plasma fibrinogen levels ($r = 0.21$, $P = 0.006$) (38). Collectively, these studies fail to clarify the association, if any, between fibrinolysis and smoking.

Another lifestyle factor that can influence fibrinolysis is alcohol consumption, showing that regular moderate alcohol intake can reduce coagulation and increase fibrinolysis (118,139). More specifically, Salem et al. found that individuals drinking 240 mL of red wine a day, showed increased levels of t-PA antigen and PAI-1 antigen, and lower levels of fibrinogen (118). The greatest benefit of alcohol consumption on fibrinolysis occurred in people following a low fat diet. Those ingesting diets of high fat content did not have improved fibrinolytic activity. Perhaps, this is another mechanism bridging a high fat diet to cardiovascular disease.

Impairments in fibrinolysis resulting from these clinical conditions present another potential mechanism that links traditional risk factors to the development of cardiovascular disease. Such claims support the notion that thrombosis and fibrinolysis may contribute to atherosclerotic development. However, traditional treatments (i.e., pharmaceutical or physical activity interventions) for these conditions do show a beneficial affect on fibrinolytic activity (36,93,103).

Summary

Chronic-low grade inflammation is becoming a well-recognized component of vascular disease pathology, as it instigates the breakdown of the inner walls of blood vessels (43,114,130). Components of the fibrinolytic system, more commonly associated with the coagulation process, are suggested to not only exacerbate existing vessel disease, but also to facilitate atherosclerotic development (45,131). As discussed throughout this chapter, much attention has been placed on identifying factors that affect inflammation or fibrinolytic activity.

In the past several years, the age-related changes occurring with these two conditions have been suspected to bridge aging and the increased prevalence of cardiovascular disease. However, paralleling changes in physical and clinical characteristics that occur with age (e.g., decreased physical activity levels, increased body fat, and an increased prevalence of other clinical conditions) make examining age-related changes in inflammation and fibrinolysis difficult. In fact, the failure to exclude, or at least statistically adjust for, these confounding conditions dilute the previous claims regarding the association between advancing age and hemostatic changes. Thus, we have yet to clearly define the existence of a primary, age-associated dysregulation of inflammatory cytokine production and thrombolytic degradation systems. Maybe there is no sole connection between these instigators of vascular disease and the unavoidable aging process. In order to clarify this relationship, it is crucial that such factors are considered when examining the age-related changes in inflammation and fibrinolysis.

CHAPTER THREE

METHODOLOGY

The primary aim of this study was to examine the independent relationship between age and blood levels of inflammatory and fibrinolytic markers. To isolate this relationship, the relationship between additional confounding factors (i.e., body composition and physical activity levels) and the concentrations of inflammatory and fibrinolytic markers were examined. The specific procedures and methods of this study to evidence these relationships are described in detail throughout this chapter.

Study Population

Males and females of any race between 18 and 90 years of age were recruited for this examination. The ultimate goal for recruitment into the study was set at 80 subjects with equal division among males and females. Recruitment of women and minorities into this protocol were a high priority to assess inflammatory markers, physical activity, and body composition among various demographic groups. This study was conducted on an outpatient basis, in the General Clinical Research Center (GCRC) on the University of Oklahoma Health Sciences Center (OUHSC) campus. The protocol was approved by the

GCRC Advisory Committee (Protocol #0103) (Appendix C) and the OUHSC Institutional Review Board (#11841) (Appendix D), and was supported by the OUHSC GCRC grant (M01-RR-14467) sponsored by the National Center for Research Resources from the National Institutes of Health.

To participate in this study, the inclusion criteria states that an individual must be between 18 and 90 years of age, ambulatory, and living independently at home. Subjects were excluded from the study if they presented with any of the following criteria:

1. Unable to perform physical activity: Non-ambulatory or other neuro-musculoskeletal limitations.
2. Non-independent living as determined by Impairments in Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL)
3. History of atherosclerotic cardiovascular diseases (ACVD):
    a. Coronary artery disease (CAD): Angina pectoris, current or history of unstable angina, history of myocardial infarction, history of coronary angioplasty or stents, history of coronary artery bypass graft, acute coronary syndrome
    b. Peripheral arterial disease (PAD): Ankle-brachial index (ABI) = 0.9 or less, history of intermittent claudication, history of aorto-femoral or any other lower extremity bypass surgery for occlusive PAD, history of ischemic gangrene or amputation,

c. History of aortic aneurysm (thoracic or abdominal)
d. History of cerebral vascular disease: History of carotid stent, carotid endarterectomy, stroke or transient ischemic attack (TIA)
e. History of atherosclerotic renal artery disease
f. History of congestive heart failure (CHF)

4. Atherosclerotic cardiovascular risk factors (ACVRF):
    a. Having metabolic syndrome, defined as three or more of the following:
        i. Abdominal obesity: waist circumference > 40 in (102 cm) in men or > 35 in (88 cm) in women
        ii. Blood pressure ≥130/85 mm Hg
        iii. Fasting blood glucose ≥ 110 mg/dL
        iv. Fasting plasma high-density lipoprotein (HDL) cholesterol < 40 mg/dl (men) or < 50 mg/dL (women)
        v. Fasting plasma triglycerides ≥ 150 mg/dL
    b. Smoking: current or within past 12 months
5. Pulmonary diseases: chronic obstructive lung disease (COPD), asthma, reactive airway disease, bronchitis, pneumonia, pulmonary fibrosis,
6. Active renal disease
7. Rheumatologic diseases: Rheumatoid arthritis, systemic lupus erythematosis (SLE), scleroderma, or any other autoimmune disease

8. Endocrine diseases: Diabetes, hypo- or hyper-thyroidism, Cushing's syndrome, Adrenal diseases
9. Gastro-intestinal disease: Peptic ulcer disease, gastro-esophageal reflux disease, inflammatory bowel disease (Crohn's disease or Ulcerative colitis),
10. Liver diseases
11. Active Cancer or active chemo- or radio-therapy
12. Psychiatric impairments: Cognitive impairment (mini-mental status examination score = 23 or less), depression, psychosis, delusions
13. Substance abuse: Alcohol abuse current or within past 10 days, any other substance abuse
14. Recent vaccination: influenza, pneumovax, or others
15. Infectious diseases: Common cold or any other acute infection within 14 days
16. Medications: regular use of non-steroidal anti-inflammatory drugs (NSAIDs)
17. Surgery within past 3 months
18. Strenuous physical activity within 48 hours.

Prior to enrolling in the study, each subject underwent a brief telephone interview to reveal any potential exclusionary conditions. During this screening interview, over 80 individuals were excluded from participation due to regular medication use (e.g. anti-hypertensives, anti-inflammatory, cholesterol lowering drugs) or pre-existing medical conditions (e.g. known cardiovascular disease, arthritis, gastro-intenstinal disorders). If subjects declined to wear the physical

activity monitors, their participation in the study would have concluded at the end of the first visit. Additionally, study participation would have been terminated early if the subject declined to participate in any of the remaining tests, or if the test administrator believed that it was in the best interest of the subject not to participate. However, all subjects who qualified for participation in the study completed all tests.

Recruitment

Subjects of any race from 18 to 90 years of age were recruited for this examination. The goal for recruitment into the study was set at a minimum of 60 subjects and a maximum of 80 subjects with equal division among males and females. The subjects were recruited using newspaper advertisements, email announcements to OUHSC and OU faculty and staff, and media flyers. Recruitment of women and minorities into this protocol was a high priority to obtain measures of inflammatory markers, physical activity, vascular function, and body composition among various demographic groups. This study was conducted on an outpatient basis, in the General Clinical Research Center (GCRC) on the OUHSC campus. At the end of each visit, the subjects received a small meal. A report and an explanation of the results obtained was prepared for and given to all subjects after the tests were completed.

Research Design

This was a three-visit, cross-sectional study in which the levels of inflammatory and fibrinolytic markers were measured via blood samples in apparently healthy subjects. Additionally, tests were administered to assess body composition and monitors were provided to assess physical activity. The independent variable of this study is age of the participant. The dependent variables of this study are the blood levels of the inflammatory and fibrinolytic markers. The descriptive characteristics of the subjects were considered as potential covariates in the final analysis of the study.

Research Protocol

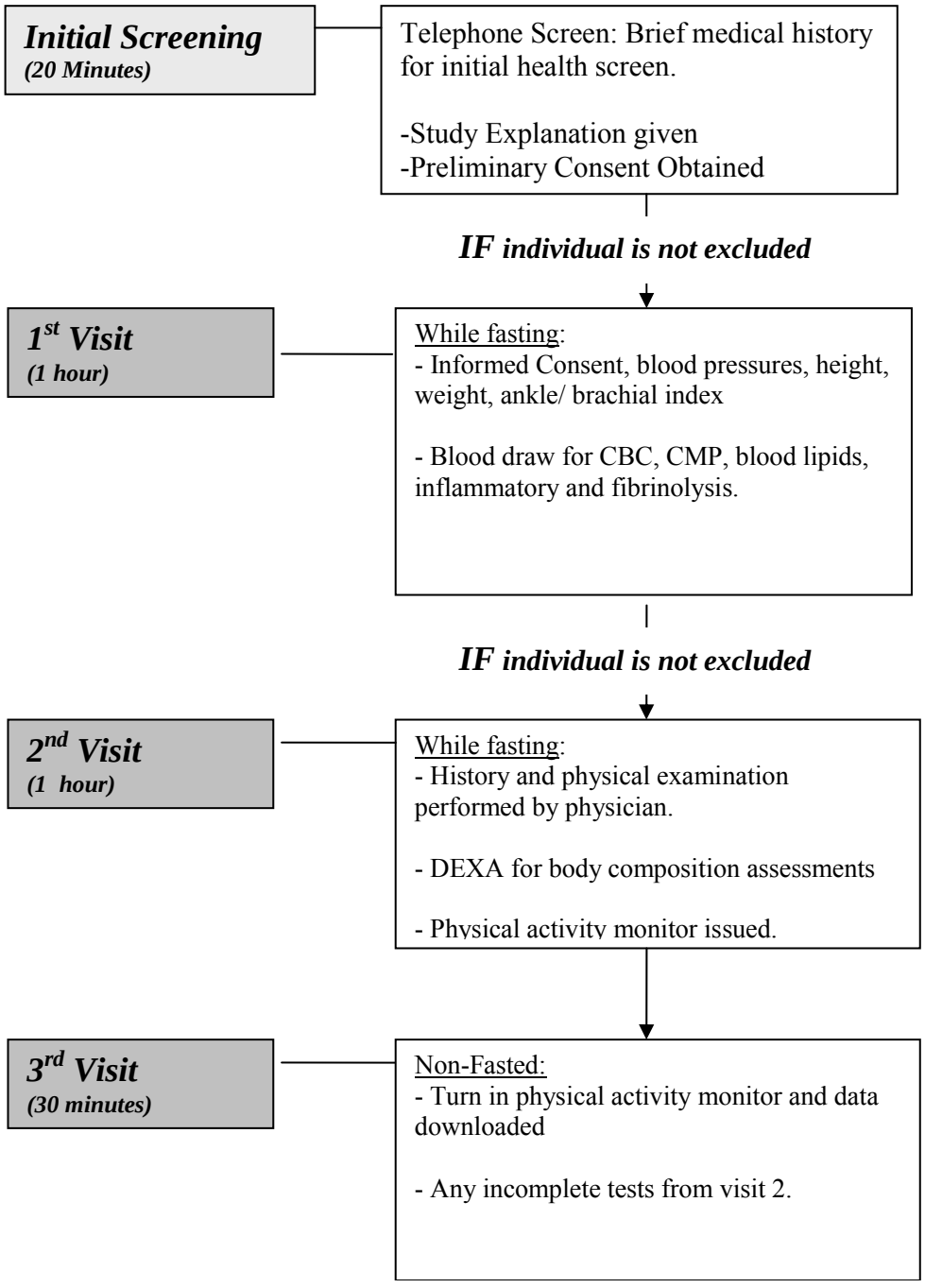
During this study, the subject completed three visits within one week, illustrated in Figure 1. During the first visit, the subject completed the informed consent (Appendix A) and HIPAA forms (Appendix B). Each subject also completed an initial screening to evaluate his or her health and medical history. Additionally, a blood draw was performed to obtain specimens for all blood tests. The first visit lasted approximately one hour.

On the second visit, the subjects completed a physician conducted health screening to ensure the subjects were healthy and free on all confounding conditions highlighted in the exclusion criteria. This screening was completed on the second visit so the attending physician could examine the blood chemistry

reports to identify any underlying exclusionary conditions. Physical activity monitors were given to the subject and they were instructed how to properly wear the device. Additionally, a dual energy X-ray absorptiometry (DEXA) scan was performed to assess body composition. The second visit lasted about one hour.

On the third and final visit, the subjects returned the physical activity monitor and any tests that were not conducted in the first two visits were completed. For example, if a subject was not fasting on the second visit, the testing was completed during the third visit. This visit could have lasted up to about one hour.

Figure 1. **Flow Chart of Subject Participation.**



Instruments and Measures

Health Screening Measurements

Prior to completing any of the tests in this study, preliminary information regarding health status was obtained. This included age, height, weight, blood pressure, sex, race, complete blood count (CBC), comprehensive metabolic profile (CMP), and blood lipid panels. In addition, a medical history and a physical examination was performed by the GCRC site physician to confirm that all participants in the study were free of any conditions in the exclusion criteria.

Physical Characteristics of the Subjects

The age and race of the subjects were obtained by self-report. The age was recorded to the nearest year. The race was recorded as: Caucasian, Asian, African American, American Indian, Hispanic, or Other.

Height was measured using a wall-mounted stadiometer (Accustat™ Genentech® Inc.; San Francisco, USA). All subjects were asked to remove their shoes and stand up straight against the stadiometer, with their feet flat on the floor. The height was measured as the distance from the floor to the top of the head and recorded to the nearest 0.1 cm. After removing any excess clothing, jewelry, and objects from their pockets, the subjects were then weighed by an electronic scale (Seca, Vogel & Halke; Germany). Weight was measured to the

nearest 0.1 kg. Body mass index (BMI) was calculated from the height and weight measurements, as follows: BMI = weight (kg) / height $(m)^2$.

Waist circumference was measured to ensure that all male subjects less than 102 cm and that all female subjects were less than 88 cm. The waist circumference was defined as the narrowest part of the torso (above the umbilicus and below the xiphoid process). A horizontal Gulick measuring tape (Creative Health Products; Plymouth, USA) was used to determine circumference, and the measurement was recorded to the nearest 0.1 cm.

Blood pressure and Pulse

Blood pressure and heart rate were measured with a Critikon automated Dinamap sphygmomanometer (Welch Allyn; Arden, USA) following 10 minutes of supine rest. Blood pressure readings were obtained in both arms to determine if systolic blood pressure (SBP) was less than 130 mmHg and diastolic blood pressure (DBP) was less than 85 mmHg. The resting heart rate was recorded as number of beats per minute.

Ankle-Brachial Index

An ankle-brachial index (ABI) test was performed using the Doppler ultrasound technique to rule out the presence of peripheral arterial disease, defined as an ABI of ≤ 0.90 (54). The brachial systolic pressures were obtained

as described above. The ankle systolic pressures were taken simultaneously with each brachial pressure. Specifically, a hand-held biodirectional Doppler machine (Hokanson; Bellevue, USA) was used to determine systolic pressure of the posterior tibialis and the dorsalis pedis arteries. After finding the resting pulses at each location, a blood pressure cuff (Hokanson; Bellevue, USA) was secured around the ankle and inflated to approximately 200 mmHg. The pressure in the cuff was slowly released at a rate of 2-4 mmHg per second. The pressure corresponding with the first sound of a pulse was recorded as the systolic pressure. The higher blood pressure of the two arteries was used as the ankle SBP. The ABI was then calculated by the following equation: ABI = Ankle SBP ÷ Brachial SBP.

Blood Markers

Sample Preparation

On the first visit of the study, venapuncture was used to obtain the blood specimen for analysis of the blood markers. Specifically, venous blood samples were collected from a vein in the antecubital space of either arm. The blood was ejected into two types of vacutainers: (1) a non-additive (red top) vacutainer for the CRP analysis and (2) a citrate anti-coagulant additive (1:9 vol of sample) siliconized (black top) vacutainer for the fibrinogen, t-PA, and PAI-1 analyses.

After the whole blood was collected into the vacutainer, the samples were centrifuged (Marathon 3200R; Fisher Scientific, USA) at 3000 X g for 15 minutes. The serum from the red top tubes (~10 mL of whole blood) was drawn and distributed into 1.0 ml aliquots in plastic microcentrifuge tubes. The plasma from the black top tubes (~9 mL of whole blood) was distributed into 0.5 ml aliquots in plastic microcentrifuge tubes. Care was taken to ensure a "platelet free" preparation, since platelets can release PAI-1. The samples were then stored in an ultra-low freezer at -80°C, where they were batched until all samples were collected for analysis.

C-Reactive Protein

A high-sensitivity Near Infrared Particles Immunoassay was used to quantify the concentration of C-reactive protein (CRP) from a serum sample of 300 µl, the optimum sample volume for this specific assay. A commercially available device, the SYNCHRON LX-20 (Beckman-Coulter; California, USA), was used to automatically perform the assay. Prior to performing each assay, the SYNCHRON system was calibrated and a calibration curve was established. To start the high-sensitivity assay, the SYNCHRON LX-20 combined one part of serum sample with 26 parts of the CRPH reagent, a pre-made solution consisting of an anti-CRP antibody-coated particle that causes a cloudy reaction when binding to CRP. The change in absorbance, directly proportional to the

concentration of CRP, was monitored by the SYNCHRON system at 940 nanometers and was used to calculate the CRP concentration based upon the pre-determined calibration curve. The "normal" reference range for concentrations of CRP using this high-sensitivity assay is 0.0 – 3.3 mg/L (16,76,129).

Fibrinogen

The ZYMUTEST Fibrinogen (DiaPharma) kit is a two site enzyme-linked immunosorbent assay (ELISA) for measuring human Fibrinogen in plasma, or in any fluid where Fibrinogen can be present. This specific ELISA is designed with rabbit polyclonal antibodies and is affinity purified. A standard curve, using the concentrations of 0, 5, 10, 25, and 50 ng/ml, was constructed on each microplate and was used for the assays. At the start of the process, 200 µl of the standard solutions or the tested samples were introduced into the corresponding microplate well. The microplate was mixed gently on a plate shaker and set to incubate for one hour at room temperature (18-25 °C). All plate shakes for each ELISA were set to 600 rpm, unless otherwise noted. After this first incubation period, the wells were emptied and subjected to five successive washings with the provided washing solution (20 fold diluted in distilled water).

Next, 200 µl of the Anti-Fibrinogen HRP immunoconjugate was introduced into the plate wells. Again, the plate was gently mixed on a plate shaker and allowed to incubate for 1 hour at room temperature (18-25 °C). The

microplate wells were washed another five times, as previously described. Immediately following the second wash, 200 µl of a tetramethylbenzidine (TMB) with hydrogen peroxide (H_2O_2) substrate was introduced into the wells. This was allowed to develop a blue color in the wells, and should take approximately five minutes. In the exact same fashion the TMB/H_2O_2 substrate was added, 50 µl of 0.45 M sulfuric acid was added to the wells to stop the color development. After letting the last step stabilize for 10 minutes, the microplate spectrophotometer analyzed the samples at 450 nm. Fibrinogen concentration in normal human plasma is in the range 1.5 to 5 mg/ml, in which greater than 5 mg/ml is associated with inflammation.

Tissue Plasminogen Activator

Biopool's Chromolize™ t-PA (DiaPharma Group, Inc; Ohio, USA) is a commercially available bio-functional immunosorbent assay (BIA) intended for the quantitative determination of human tissue plasminogen activator activity in plasma. All of the steps involved with this assay were performed at room temperature (18-25 °C). A standard curve was included with each plate analysis, using known concentrations of 0, 0.5, 1.0, 1.5, 2.0 IU/ml for the standards. A total of 100 µl of t-PA standards or 100 µl of sample to the microplate wells and the positions were recorded. The plate was allowed to incubate for 20 minutes on a plate shaker at ambient temperature. After this incubation period, the

supernatant was discarded by tapping onto an absorbent towel. Following this step, the wells were filled completely with Phosphate, NaCl, EDTA, and Tween 20 (PET) concentrate buffer and emptied to wash the wells 4 times.

After the first washes, 50 µl of Lyophilised H-D-But-CHT-Lys-pNA and poly-D-lysine (substrate reagent) were added to each well using a repeating pipette. Immediately following, 50 µl Lyophilised plasminogen and FDP (Plasminogen Reagent) were added to each well using the same technique, which was completed within 2 minutes. Again, the plate incubated for 90 minutes at ambient temperature on a microtest plate shaker at 600 rpm. Finally, 50 µl a stop solution (1.7 M acetic acid) was added to each well and allowed to mix on a microtest plate shaker for at least 15 seconds, forming a yellowish color.

The absorbance was set at 405 nm on the spectrophotometer, and a "blank" was run against air. Then, the absorbance in all wells was measured at 405 nm. A second measurement may be made at 492 nm and these subtracted from the readings at 405 nm. Paranitroanilide absorbs light at 405 nm, whereas the absorbance due to turbidity is approximately equal at 405 nm and 492 nm. Therefore, absorbance at 492 nm was measured and subtracted to correct for background due to turbidity. For healthy humans the basal level is between 0.2-2 IU/ml (38).

Plasminogen Activator Inhibitor – 1

Biopool Chromolize™ PAI-1 (DiaPharma Group, Inc; Ohio, USA) is a bio immunoassay (BIA) for the quantitative determination of active human plasminogen activator inhibitor, type 1 (PAI-1) in human plasma. With each assay, the PAI-1 standards were made for the PAI-1 concentrations of 0, 15, 30, and 50IU/ml and were used in the standard curve, which is used in the interpolation of PAI-1 activity in the patient's plasma specimen. After the standards were prepared, the wells in each microplate were reconstituted with 25 µl of the PET buffer. The plate was allowed to incubate on a microplate shaker at a speed of 600-800 r.p.m. for 2 minutes. Exactly 25 µl of plasma samples or the PAI-1 Standards was added to each well, and the positions were recorded. Immediately following (not to exceed 1 minute), 25 µl of Conjugate was added to each well. The plate then incubated for 30 minutes on a microplate shaker set at a speed of 600-800 r.p.m. Then the contents were discarded, washed with the PET buffer and emptied onto an absorbent towel. This washing process was repeated four times.

Next, 100 µl of the phosphate / citrate buffer with hydrogen peroxide (HRP) substrate to each well and the tray incubated for exactly five minutes on a shaker as described above. The reaction was stopped by adding 100 µl of 1.6 M sulphuric acid to each well in the same order and speed as the substrate was added. To quantify the PAI-1 in the specimen samples, the absorbance was set at

492 nm on the microplate spectrophotometer, with a "blank" against air being run first. Following, the absorbance in all wells were measured at 492 nm. The detection range for this specific assay is 2.0 to 50 IU/ml. Using this assay, the expected concentration of PAI-1 in plasma samples seen healthy adults should be around 5.15 ± 7.13 (SD) IU/ml (38).

Body Composition Assessment

Dual-energy X-ray absorptiometry (DXA) was used to measure body fat mass and fat-free mass during the second visit of the study. This DXA technique provides a body composition measurement, dividing the body into fat tissue, fat-free tissue, and bone mineral.

The Hologic QDR 4500 (Hologic Inc; Massachusetts, USA) DXA scanner is a fan beam scanner. Fan beam scanners perform one or more sweeps across the patient instead of the two-dimensional scan required by pencil beam geometry, resulting in decreased scan times and higher image resolution (15). There is an exposure to radiation during a whole body DXA scan is approximately 1.0 mrem. The x-ray tube is equipped with a filter to convert the polychromatic X-ray beam into low (100 keV) and high-energy peaks (140 keV). The scanning arm passes over the DXA bed and detects the x-ray energy that passes through the body in a posterior to anterior direction. The attenuation of the x-ray has a direct relationship with the mass of the tissue through which it passes. The QDR4500A

scanner has shown to be a highly reliable an accurate assessment of percent body fat (%BF), with a precision of <1 - 1.2 %BF (10,80).

Prior to each scan, all subjects were instructed to remove any metal or other interfering objects, such as jewelry, from their body. For all females, a human chorionic gonadotropin (HCG) test was completed to ensure they were not pregnant. Subjects underwent a total body scan with a fan beam X-ray in the array mode, after being properly situated in the supine position on the DXA bed. All scan analyses were performed by the same qualified technician. Quality Assurance and control phantom calibration scans were completed at the beginning of each test day.

Ambulatory Physical Activity Assessment

Ambulatory physical activity level was monitored for two to seven consecutive days (4.8 ± 1.0 days) by a StepWatch activity monitor (Cyma; Seattle, Washington) wrapped around the ankle using elastic straps above the lateral malleolus of the right leg. The StepWatch activity monitor measured strides and minutes of ambulatory activity accumulated per day for activity at a lower cadence (<15 strides per minute), activity at a moderate cadence (≥15 and < 30 strides per minute) and activity at a higher cadence (≥ 30 strides per minute), and total activity (the sum of activity at a lower, moderate, and a higher cadence).

Subjects were instructed to wear the device in this exact position, during their waking hours and to remove them before retiring to bed. Additionally, the subjects were instructed to carry out their usual activities through the monitored period. The activity monitor was issued on the first visit and returned on the second visit, at which time the data was downloaded and recorded. The step activity monitor is a highly reliable and validated method of quantifying of ambulatory physical activity (77,124).

Statistical Analysis

In an initial analysis of the study data, a summary description of the population was made by presenting the gender specific mean, standard deviation, maximum, and minimum values for each variable. All values were reported as the mean ± standard deviation (SD). The data were graphed as a first examination of relationships between variables to reveal any outliers in the data set. Outliers were checked as possible data entry errors. Errors were corrected or omitted. Outliers that were not errors were examined to determine their influence on the statistical results, by running the analyses with and without the outlier. Influential outliers were omitted from final analyses but noted and discussed.

To fully address the age associated changes in inflammation and fibrinolysis, subjects were grouped into one of three categories according to age: "Younger" = 20 – 39 years, "Middle Age" = 40 – 59 years, and "Older" = 60

years and greater. An analysis of variance was used to detect mean differences among the three groups for all measured variables.

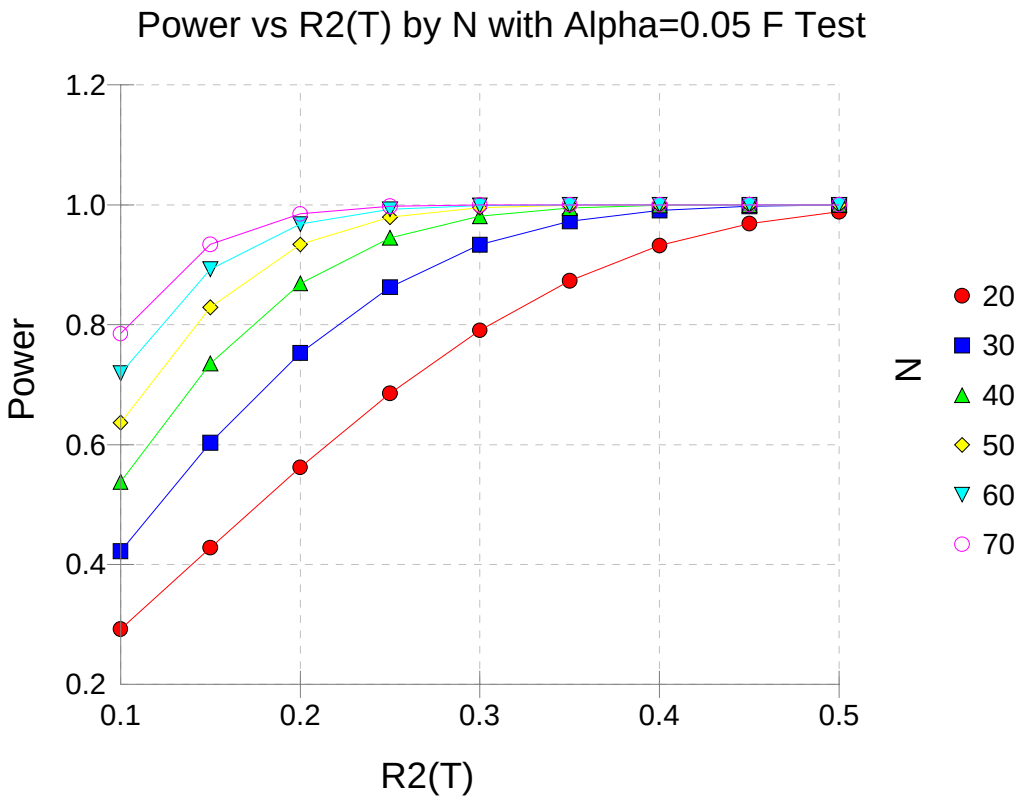
A correlation matrix correlating each variable with each other variable was constructed to examine pairwise relationships and dependencies between physical characteristics of the subjects, the blood concentrations of the inflammatory and fibrinolytic markers. In particular, correlations between the independent variables identified redundant characteristics. For example, if BMI and percent body fat are highly correlated, then we used the more descriptive variable (the larger absolute correlation) as a covariate in further statistical analyses.

Following these summary and investigational analyses, the primary analysis used a general linear model (GLM) to determine the significant predictors of levels for each marker of inflammation and fibrinolysis. The initial independent variable was age and the outcome (dependent) was the serum concentration of inflammatory or fibrinolysis marker. Physical characteristics (i.e., body composition, gender, and physical activity) found to significantly correlate with the concentration of each blood marker were entered as covariates into the statistical model to isolate the affect of age on inflammation and fibrinolysis. A p value was provided for each hypothesis test and an alpha = 0.05 was used to define significance. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, v. 11.5, Chicago, IL) software.

Statistical Power and Sample Size

Typically a relationship between an independent variable and a dependent variable is of interest if at least 25% of the variation in the dependent variable is attributable to variation in the independent variable, that is, $r^2 \geq 0.25$. A power analysis was performed using the NESS-PASS statistical software package to determine the number of subjects needed to detect relationships at the significance level (alpha) of 0.05. As shown in figure 2, it was determined that about 30 subjects were necessary to detect a relationship of $r^2 \geq 0.25$, ensuring a power of 80% ($\beta = 0.20$). Because there are two gender groups, at least 30 males and 30 females were needed. The study aimed to recruit and complete testing on a total of 80 subjects (40 males, 40 females) to allow for loss of participants and/or data from the study.

Figure 2. Results of the power calculations.



CHAPTER FOUR

RESULTS

The purpose of this study was to investigate the relationship between age, whole body and trunk fat mass, physical activity level and blood levels of CRP, fibrinogen, t-PA, and PAI-1. In order to accomplish this, 88 participants were enrolled in the study. Of the total enrolled, six individuals were excluded from participating, as one person reported regular use of non-steroidal anti-inflammatory drug, one person did not show up for the second visit, one person reported a recent influenza vaccination, and three people had hypercholesterolemia. Results of the remaining 82 participants are presented throughout the remainder of this chapter.

Group Characteristics and Comparisons

Comparing Men and Women Subjects. The means and standard deviations of the physical characteristics were calculated for the men and women and are presented in Table 1. Independent t-tests between gender groups showed that the men were taller than the women ($p < 0.001$) and weighed more than the women ($p < 0.001$). The females had a higher resting pulse compared to men ($p = 0.004$) and a higher high-density lipoprotein (HDL) level than men ($p = 0.013$). There were no differences ($p > 0.05$) between men and women on any other physical characteristic.

Table 2 presents the body composition data for men and women. The men had a greater waist circumference than the women ($p < 0.001$), but they had a lower percent fat in the trunk ($p < 0.001$) compared to the women. Additionally, the percent of whole body fat was lower in men than in women ($p < 0.001$), as was the whole body fat mass ($p < 0.001$). No differences were found between men and women in body mass index (BMI) or trunk fat mass ($p > 0.05$).

The ambulatory activity measurements were compared between men and women and are presented in Table 3. Overall, the women accumulated more total minutes of daily ambulatory activity than the men ($p = 0.046$), as well as more minutes of activity at a lower cadence ($p = 0.008$). The men were more sedentary than the women ($p = 0.046$). The remaining ambulatory activity measurements were not significantly different ($p > 0.05$) between the men and women.

The blood marker concentrations in the men and women are compared in Table 4. There was no difference in CRP values between the men and the women ($p = 0.504$). Additionally, no significant differences were found between men and women in blood levels of fibrinogen ($p = 0.700$), tissue plasminogen activator (t-PA) ($p = 0.984$), or plasminogen activator inhibitor – 1 (PAI-1) ($p = 0.453$).

Comparing Age Groups. To examine age-related differences in measurements, subjects were categorized into three groups according to age: "Younger" = 20 – 39 years, "Middle Age" = 40 – 59 years, and "Older" = 60 years and greater. The number of men and women in each group is shown in Figure 3, and the physical characteristics of each age group are presented in Table 5. One-way

ANOVAs revealed significant differences among the three age groups in systolic and diastolic blood pressure, total cholesterol, and low-density lipoprotein (LDL) level. More specifically, the older adults had a higher systolic blood pressure than the younger adults ($p < 0.001$) and middle age adults ($p < 0.001$), and a higher diastolic blood pressure than the younger adults ($p = 0.004$). The older adults also had a higher total cholesterol ($p<0.001$) and LDL level ($p<0.001$) than the younger and middle age adults.

Body composition measurements were also examined by age group, as shown in Table 6. Significant differences were found among the groups in waist circumference ($p = 0.012$), percent body fat ($p = 0.001$), body fat mass ($p = 0.016$), and the percent trunk fat ($p < 0.001$). The younger adults had a smaller waist circumference than the middle age ($p = 0.024$) and older adults ($p = 0.046$). Additionally, the older adults had a higher percent body fat ($p = 0.001$), higher body fat mass ($p = 0.014$), and a higher percentage of trunk fat ($p < 0.001$) compared to the younger adults. No significant differences were found in weight ($p = 0.442$), BMI ($p = 0.125$), or trunk fat mass ($p = 0.054$).

Table 7 shows the ambulatory activity measurements by age group. Overall, there were no significant differences in activity measures among the younger, middle age, and older adults ($p > 0.05$).

The blood marker concentrations among the younger, middle age, and older adults are presented in Table 8. Results from one-way ANOVA shows that the older age group had a significantly higher level of t-PA than the younger group ($p =$

0.042). No significant differences were found among age groups in the concentrations of CRP ($p = 0.413$), fibrinogen ($p = 0.231$), and PAI-1 ($p = 0.489$).

Correlations Among Blood Markers and Physical Characteristics

Age and Physical Characteristics. Pairwise correlations between age and physical characteristics are presented in Table 9. As shown, age was positively related to systolic blood pressure ($r = 0.573$, $p < 0.001$), diastolic blood pressure ($r = 0.406$, $p < 0.001$), total cholesterol ($r = 0.333$, $p = 0.002$), LDL level ($r = 0.286$, $p = 0.009$), and HDL level ($r = 0.231$, $p = 0.036$). The scatter plots examining age with physical characteristics to identify potential outliers in the data set are shown in Appendix I.

Age and Body Composition. Pairwise correlations between age and body composition also are presented in Table 9. Age was positively related to waist circumference ($r = 0.295$, $p = 0.007$), percent of whole body fat ($r = 0.401$, $p < 0.001$), whole body fat mass ($r = 0.335$, $p = 0.002$), percent trunk fat ($r = 0.430$, $p < 0.001$), and trunk fat mass ($r = 0.248$, $p = 0.025$). Body composition measurements were plotted against age (Appendix J) to identify potential outliers in the data set.

Age and Ambulatory Activity Measures. Pairwise correlations between age and ambulatory activity measures also are shown in Table 9. The only activity measures that were related to age were the number of strides per day at a moderate cadence ($r = 0.268$, $p = 0.016$) and the number of minutes per day at a moderate cadence ($r = 0.232$, $p = 0.037$). Physical activity measures were plotted against age

(Appendix K) to identify potential outliers in the data set. The data for age vs. total strides per day, strides of activity at a lower cadence, and strides of activity at a moderate cadence all showed one outlier in the scatter plots. After confirming that this was not a data entry error, the data point was removed from the data set and the correlations with age were repeated. The correlation coefficient remained consistent with our initial analyses, therefore the outlying data point was included in the data set. Later analyses for each blood marker were also done with and without the outlier to determine the influence, if any, on the blood marker concentrations.

Predictors of C-Reactive Protein. CRP values for the entire group ranged from 0.2 mg/L to 10.4 mg/L, and are presented across age in Figure 4. Upon reviewing the C-reactive protein (CRP) levels, one subject had a CRP value of 77.0 mg/L. This value lies nearly nine standard deviations above the mean CRP concentration for the sample (2.3 ± 8.5 mg/L). This extremely high value is indicative of an underlying infection that went undetected during screening (129), which is part of the exclusion criteria of the study. Thus, the data point was removed from the remaining analyses.

As shown in Table 9, CRP was not significantly correlated to age ($r = -0.073$, $p = 0.519$), but was correlated to total cholesterol ($r = -0.338$, $p = 0.002$) and HDL levels ($r = -0.255$, $p = 0.022$). CRP also was significantly correlated to the body composition measures: BMI ($r = 0.304$, $p = 0.006$), waist circumference ($r = 0.279$, $p = 0.012$), percent body fat ($r = 0.221$, $p = 0.048$), percent trunk fat mass ($r = 0.243$, p

= 0.029), and trunk fat mass (r = 0.234, p = 0.036). None of the ambulatory activity measures were related to CRP levels in the sample.

To reduce the number of covariates to be considered in a subsequent model, a correlation analysis was done between biologically correlated variables. This secondary correlation analysis showed that HDL and total cholesterol were significantly correlated (r = 0.449, $p < 0.001$). Therefore, HDL was used as a covariate in the GLM to assess the relationship between age and CRP. All of the body composition measures were significantly related ($p < 0.05$) with one another. BMI, percent body fat and trunk fat mass were selected as covariates between body composition and CRP, as they were the strongest correlates with CRP. All variables were entered one at a time into the GLM assessing the relation between age and CRP to determine which combination has the greatest confounding influence of CRP levels.

A general linear model (GLM) showed that CRP levels did not change with age ($R^2 = 0.011$, p = 0.641). BMI, percent body fat, trunk fat mass, and HDL were all entered separately into the general linear model (Appendix L) examining whether age was an independent predictor of CRP. Individually, BMI was the most powerful predictor (Sum of Squares [SS] = 28.896; $R^2 = 0.095$, p = 0.013) of all of the body composition measures. The model was not changed after adding trunk fat mass (SS = 28.926; $R^2 = 0.083$, p = 0.031) and percent body fat (SS = 29.344; $R^2 = 0.085$, p = 0.029). Thus, BMI and HDL were the covariates in the final CRP model. While the final model was significant (SS = 30.22; $R^2 = 0.077$, p = 0.050), BMI, not age or

HDL, was found to be the only significant predictor ($p = 0.036$) of CRP levels. Lastly, there was no significant age by gender interaction in the model.

Predictors of Fibrinogen. Fibrinogen values for the entire group averaged 4.2 ± 1.7 IU/mL and ranged from 2.16 IU/mL to 13.35 IU/mL. The fibrinogen value of 13.35 IU/ml was 5.4 standard deviations above the mean, and was removed from the data for the remaining analyses. After removing this outlier, the fibrinogen values for the group averaged 4.1 ± 1.4 IU/mL and ranged from 2.16 IU/ml to 9.72. These data are presented across age in Figure 5.

Fibrinogen was not significantly correlated to age ($r = -0.010$, $p = 0.951$) (Table 9). In contrast, fibrinogen concentration was related to strides per day of activity at a higher cadence ($r = -0.242$, $p = 0.029$), minutes per day of activity at a higher ($r = -0.223$, $p = 0.045$), and percent trunk fat ($r = 0.238$, $p = 0.031$). After removing the outlier, fibrinogen levels also were correlated to resting heart rate ($r = 0.306$, $p = 0.005$), percent body fat ($r = 0.257$, $p = 0.020$), body fat mass ($r = 0.239$, $p = 0.026$), percent trunk fat ($r = 0.307$, $p = 0.004$), trunk fat mass ($r = 0.297$, $p = 0.007$), and strides per day of activity at a higher cadence ($r = -0.225$, $p = 0.045$).

A secondary correlation analysis showed the percent body fat correlated with body fat mass ($r = 0.949$, $P < 0.001$) and percent fat correlated with trunk fat mass ($r = 0.761$, $p < 0.001$). To reduce the number of covariates, resting heart rate, percent body fat, percent trunk fat, and strides per day of activity at a higher cadence were selected as covariates for the GLM examining the relation between age and fibrinogen.

In the initial GLM, age was not an independent predictor of fibrinogen concentrations (SS = 117.15; R^2 = 0.131, p = 0.319) (Appendix M). There was no significant age by gender interaction in the model. Resting heart rate, percent body fat, percent trunk fat, and strides per day of activity at a higher cadence were all entered separately into the GLM (Appendix M). Individually, heart rate was the most influential covariate (Sum of Squares [SS] = 125.91; R^2 = 0.230, p = 0.117), however it was not significant. The model only slightly changed after adding percent body fat, percent trunk fat and strides per day of activity at a higher cadence (SS = 128.62; R^2 = 0.237, p = 0.226). The final GLM showed that age was not an independent predictor of fibrinogen levels (p = 0.236), and that there was no significant age by gender interaction (p = 0.825) in the model.

Predictors of Tissue Plasminogen Activator, Activity. The t-PA concentrations for the entire group (0.72 ± 0.44 IU/mL) ranged from 0.14 IU/mL to 2.61 IU/mL. Upon further review, one individual had a t-PA level of 2.61 IU/mL (4.3 standard deviations above the mean) and another had a t-PA level of 2.43 IU/ml (3.9 standard deviations above the mean). Therefore, these outliers were excluded from the analyses. The corrected t-PA concentrations for the entire group then averaged 0.68 ± 0.34 IU/mL and range from 0.14 IU/mL to 1.64 IU/mL. They are presented across age in Figure 6.

Initially, t-PA was correlated with age (r = 0.228, p = 0.042) (Table 9), and was negatively correlated with triglyceride level (r = -0.279, p = 0.012). Consequently, triglyceride concentration was used as a covariate in the GLM that

assessed the relationship between age and t-PA. None of the other physical characteristics, measures of body composition or measures of ambulatory activity were significantly related to t-PA concentrations ($p < 0.05$). These results were the same when examined with and without the outliers.

In contrast to the correlation analysis, the initial GLM showed that age was not a predictor of t-PA concentrations (SS = 6.38; $R^2 = 0.030$, $p = 0.567$) (Appendix N). The model was unchanged after adding triglyceride levels to the GLM (SS = 6.38; $R^2 = 0.079$, $p = 0.636$). There was no age by gender interaction ($p = 0.525$).

Predictors of Plasminogen Activator Inhibitor -1, Activity. The PAI-1 concentrations for the entire group (6.47 ± 9.39 IU/mL) ranged from 0.00 IU/mL to 40.03 IU/mL. The subject with a PAI-1 level of 40.03 IU/mL was removed from the analysis, as the value was 4.0 standard deviations above the mean. Thus, the corrected PAI-1 values for the group averaged 5.91 ± 7.58 IU/mL and ranged from 0.00 IU/mL to 29.13 IU/mL. The PAI-1 concentrations across age for the entire group are presented in Figure 7.

Before removing the outlier, PAI-1 concentrations were significantly correlated to triglyceride levels ($r = 0.310$, $p = 0.005$), HDL ($r = -0.218$, $p = 0.049$), BMI ($r = 0.277$, $p = 0.012$), waist circumference ($r = 0.53$, $p = 0.022$), and percent trunk fat ($r = 0.245$, $p = 0.026$). Once the outlier was removed, PAI-1 was significantly correlated to weight ($r = 0.262$, $p = 0.018$), systolic blood pressure ($r = 0.221$, $p = 0.047$), diastolic blood pressure ($r = 0.275$, $p = 0.013$), triglyceride level ($r = 0.334$, $p = 0.002$), HDL level ($r = -0.280$, $p = 0.011$), BMI ($r = 0.377$, $p = 0.001$),

waist circumference ($r = 0.334$, $p = 0.002$), percent body fat ($r = 0.236$, $p = 0.034$), body fat mass ($r = 0.248$, $p = 0.026$), percent trunk fat ($r = 0.307$, $p = 0.005$), and trunk fat mass ($r = 0.264$, $p = 0.017$). PAI-1 concentrations were not significantly correlated ($p > 0.05$) with age or any of the ambulatory activity measures.

A secondary correlation analysis between biologically correlated variables was done to further reduce the number of covariates to be considered in a subsequent model. Systolic blood pressure was highly correlated with diastolic blood pressure ($r = 0.715$, $p < 0.001$) and triglyceride level correlated with HDL level ($r = -0.440$, $p < 0.001$). BMI was significantly correlated with waist circumference ($r = 0.864$, $p < 0.001$), percent body fat ($r = 0.449$, $p < 0.001$), body fat mass ($r = 0.596$, $p < 0.001$), percent trunk fat ($r = 0.642$, $p < 0.001$), trunk fat mass ($r = 0.699$, $p < 0.001$). Percent body fat was correlated with body fat mass ($r = 0.932$, $p < 0.001$) and percent trunk fat was correlated with trunk fat mass ($r = 0.761$, $p < 0.001$). Therefore, diastolic blood pressure, triglyceride levels, body fat mass, and percent trunk fat were selected as covariates for the GLM, as they were the strongest correlates with PAI-1.

Age was not an independent predictor of PAI-1 concentrations in the initial GLM ($SS = 3199.94$; $R^2 = 0.158$, $p = 0.736$). Diastolic blood pressure, triglyceride levels, body fat mass, and percent trunk fat were all entered separately into the GLM (Appendix O). Diastolic blood pressure ($SS = 388.92$; $R^2 = 0.105$, $p = 0.012$), and percent trunk fat ($SS = 247.11$; $R^2 = 0.001$, $p = 0.051$) were the most influential factors in the GLM. Therefore, these variables were entered as covariates in the final

model to determine if age is an independent predictor of PAI-1 levels. The final model showed that age was not an independent predictor of PAI-1 concentrations (SS = 3654.78; R^2 = 0.137, p = 0.333). There was no significant age by gender interaction (p = 0.836) in the model.

Table 1 displays the physical characteristics of the male and female subjects. The p value represents the significance between the two groups.

	Males (n = 40)			*Females (n = 42)*			
	Mean ± s.d.	***Min.***	***Max.***	***Mean ± s.d.***	***Min.***	***Max.***	***p value***
Age (Years)	45.9 ± 18.2	22	89	47.9 ± 17.3	21	81	0.611
Height (cm)	179 ± 5.9	166	196	166 ± 6.6	153	185	< 0.001
Weight (Kg)	81.4 ± 11.5	57.1	111	66.6 ± 10.9	49.2	93.1	< 0.001
Systolic Pressure (mmHg)	120 ± 14	98	177	115 ± 13	94	150	0.104
Diastolic Pressure (mmHg)	72 ± 8	54	84	72 ± 8	52	86	0.863
Resting Heart Rate (bpm)	55 ± 7	41	68	61 ± 11	42	92	0.004
Total Cholesterol (mg/dL)	181 ± 34	104	256	191 ± 49	64	331	0.247
Triglyceride (mg/dL)	81 ± 44	22	245	76 ± 34	31	154	0.598
Low-Density Lipoproteins (mg/dL)	114 ± 28	53	178	120 ± 39	69	251	0.415
High-Density Lipoproteins (mg/dL)	50 ± 13	26	80	58 ± 17	38	111	0.013
LDL / HDL Ratio	2.4 ± 1	1.1	6.4	2.1 ± 0.8	0.5	4.2	0.108

“s.d.” = standard deviation; “min.” = Minimum value; “max.” = Maximum value

Table 2 displays the body composition measures of the male and female subjects. The p value represents the significance between the two groups.

	Males (n = 40)			*Females (n = 42)*			
	Mean ± s.d.	***Min.***	***Max.***	***Mean ± s.d.***	***Min.***	***Max.***	***p value***
Body Weight (Kg)	81.4 ± 11.5	57.1	111.0	66.6 ± 10.9	49.2	93.1	< 0.001
Body Mass Index ***(Kg/m^2)***	25.5 ± 3.2	20.3	32.6	24.2 ± 4.0	18.6	33.9	0.1
Waist Circumference ***(Centimeters)***	87.0 ± 9.5	72.6	112.0	78.6 ± 10.7	64.0	104.3	< 0.001
Whole Body Fat (%)	19.1 ± 6.8	9.3	36.8	31.1 ± 7.5	15.3	46.2	< 0.001
Whole Body Fat (g)	16512 ± 7498	6413	36380	24581 ± 7913	12736	45063	< 0.001
Trunk Fat (%)	19.0 ± 8.3	7.2	41.8	27.8 ± 9.8	10.7	45.2	< 0.001
Trunk Fat Mass (g)	8506 ± 5888	2431	27656	9277 ± 5046	3031	20301	0.525

“s.d.” = standard deviation; “min.” = Minimum value; “max.” = Maximum value

Table 3 displays the ambulatory activity measurements of the male and female subjects. The p value represents the significance between the two groups.

	Males (n = 40)			***Females (n = 42)***			
	Mean ± s.d.	***Min.***	***Max.***	***Mean ± s.d.***	***Min.***	***Max.***	***p value***
Total Activity							
Strides / Day	6049 ± 3342	1760	20411	5924 ± 1893	2545	11245	0.836
Minutes / Day	344.0 ± 117.2	103.6	572.9	394.9 ± 108.8	201.8	650.7	0.046
Lower Activity							
Strides / Day	1427 ± 914	279	5774	1589 ± 544	664	3062	0.335
Minutes / Day	207.3 ± 72.8	40.5	374.7	253.9 ± 80.8	103.6	496.6	0.008
Moderate Activity							
Strides / Day	1776 ± 1078	391	6371	1892 ± 780	783	4007	0.580
Minutes / Day	78.6 ± 39.4	17.5	200.8	87.7 ± 34.8	36.5	181.1	0.275
Higher Activity							
Strides / Day	2846 ± 1948	529	9363	2443 ± 1162	441	5341	0.260
Minutes / Day	63.2 ± 42.5	19.9	199.9	53.3 ± 24.0	11.7	124.5	0.199
Sedentary Time							
Minutes / Day	1096.0 ± 117.2	867.1	1336.4	1045.1 ± 108.8	789.3	1238.2	0.046

"s.d." = standard deviation; "min." = Minimum value; "max." = Maximum value.

Table 4 displays the blood marker concentrations in the male and female subjects.

The p value represents the significance between the two groups.

	Males (n = 40)			***Females (n = 42)***			
	Mean ± s.d.	***Min.***	***Max.***	***Mean ± s.d.***	***Min.***	***Max.***	***p value***
C-Reactive Protein *(mg/L)*	1.20 ± 1.40	0.20	6.80	1.50 ± 1.90	0.20	10.40	0.504
Fibrinogen *(IU/mL)*	4.30 ± 2.08	2.16	13.35	4.15 ± 1.30	2.55	9.72	0.700
tPA Activity *(IU/mL)*	0.67 ± 0.35	0.14	2.61	0.68 ± 0.32	0.18	1.30	0.984
PAI-1 Activity *(IU/mL)*	7.04 ± 9.08	0.00	40.03	5.60 ± 7.80	0.00	29.13	0.453

Figure 3 is the number of participants in each age group (by decade).

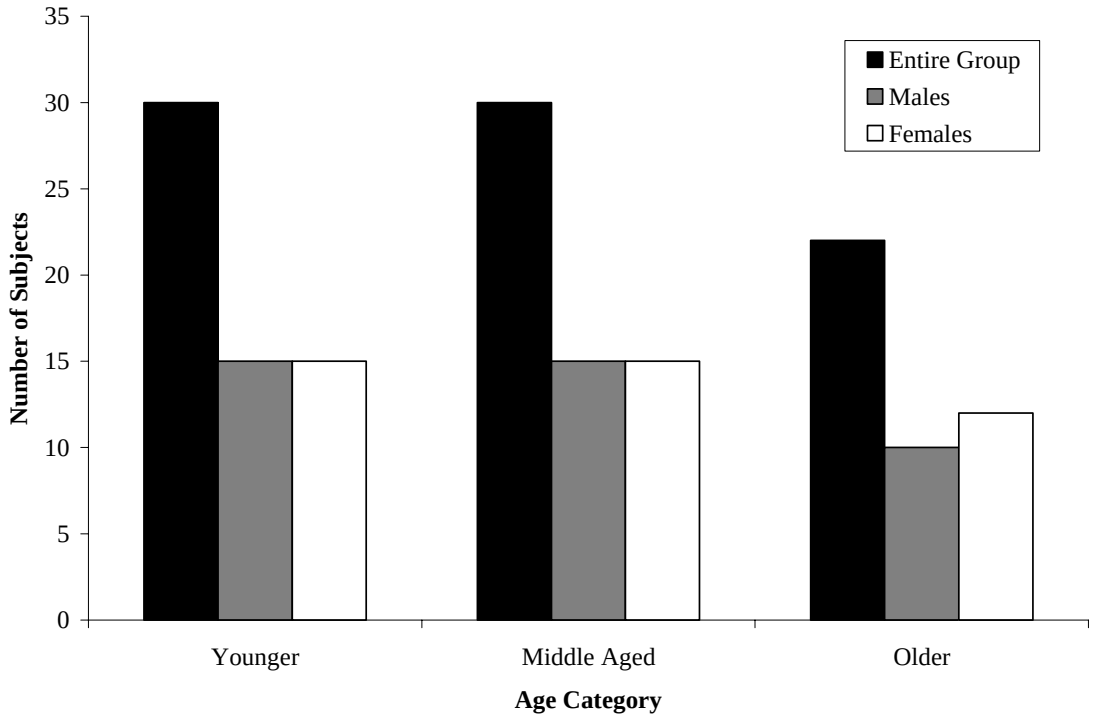


Table 5 displays the physical characteristics of the younger, middle age, and older adults. The p value represents the significance between the three age groups.

	Age Group			
	Younger	***Middle Age***	***Older***	***p value***
Height (cm)	173.0 ± 9.3	171.7 ± 7.9	171.2 ± 9.8	0.743
Weight (kg)	71.4 ± 12.3	75.9 ± 14.4	74.2 ± 13.7	0.442
Systolic BP (mmHg)	112 ± 7	116 ± 11	129 ± 16*†	< 0.001
Diastolic BP (mmHg)	69 ± 7	72 ± 8	76 ± 7*	0.006
Resting Heart Rate (bpm)	58 ± 11	56 ± 9	62 ± 10	0.092
Total Cholesterol (mg/dL)	173 ± 40	178 ± 30	215 ± 47*†	< 0.001
Triglyceride (mg/dL)	83 ± 47	72 ± 38	81 ± 28	0.528
LDL (mg/dL)	110 ± 31	107 ± 23	140 ± 40*†	< 0.001
HDL (mg/dL)	50 ± 11	56 ± 18	59 ± 16	0.098

LDL = low density lipoproteins; HDL = high-density lipoproteins; BP = blood pressure. * denotes a significant difference from the younger adults. † denotes a significant difference from the middle age adults.

Table 6 displays the body composition measures of the younger, middle age, and older adults. The p value represents the significance between the two groups.

	Age Group			
	Younger	***Middle Age***	***Older***	***p value***
Weight (kg)	71.4 ± 12.3	75.9 ± 14.4	74.2 ± 13.7	0.442
BMI (kg/m^2)	23.8 ± 3.4	25.6 ± 4.0	25.2 ± 3.2	0.125
Waist Circumference (cm)	78.1 ± 9.3	85.4 ± 11.7*	85.4 ± 10.2*	0.012
Whole Body Fat (%)	21.1 ± 9.1	25.4 ± 8.1	30.5 ± 8.8*	0.001
Body Fat Mass (kg)	17.4 ± 8.3	21.3 ± 7.7	24.2 ± 9.2*	0.016
Trunk Fat (%)	18.5 ± 9.7	23.9 ± 9.3	29.6 ± 8.2*	< 0.001
Trunk Fat Mass (kg)	7.3 ± 5.8	8.9 ± 5.4	11.0 ± 4.6	0.054

BMI = body mass index. * significantly different from younger adults ($p < 0.017$)

Table 7 displays the ambulatory activity measurements of the younger, middle age, and older adults. The p value represents the significance between the three age groups.

	Age Group			
SAM Data averaged per day	***Younger***	***Middle Age***	***Older***	***p value***
Total Activity				
Strides / Day	5697 ± 2421	6116 ± 1918	6207 ± 3793	0.760
Minutes / Day	371 ± 114	379 ± 117	356 ± 118	0.794
Lower Activity				
Strides / Day	1396 ± 479	1547 ± 678	1613 ± 1085	0.560
Minutes / Day	234 ± 69	238 ± 85	217 ± 88	0.636
Moderate Activity				
Strides / Day	1670 ± 832	1795 ± 692	2111 ± 1271	0.238
Minutes / Day	77 ± 38	82 ± 31	92 ± 43	0.362
Higher Activity				
Strides / Day	2631 ± 1719	2774 ± 1261	2484 ± 1876	0.818
Minutes / Day	59 ± 35	59 ± 24	56 ± 45	0.952
Sedentary Time				
Minutes / Day	1069 ± 114	1061 ± 117	1083 ± 118	0.794

Table 8 displays the blood marker concentrations among the younger, middle age, and older adults. The p value represents the significance among the three age groups.

	Age Group			
	Younger	***Middle Age***	***Older***	***p value***
CRP (mg/L)	1.68 ± 2.41	1.14 ± 1.16	1.20 ± 0.77	0.413
Fibrinogen(IU/mL)	3.87 ± 1.44	3.92 ± 1.48	4.70 ± 1.03	0.231
tPA (Activity)(IU/mL)	0.590 ± 0.261	0.657 ± 0.342	0.825 ± 0.387	0.042
PAI-1 (Activity)(IU/mL)	4.95 ± 6.43	6.45 ± 8.09	6.49 ± 8.52	0.489

CRP = C-reactive protein; t-PA = tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor-1.

Table 9 is the bivariate correlations between age, the blood marker concentrations and all measured variables for the entire group.

	Age	*CRP*	*Fibrinogen*	*t-PA*	*PAI-1*
Age (Years)	--	-0.073	0.206	0.228*	0.084
Height (cm)	-0.126	-0.103	0.022	-0.010	-0.038
Weight (Kg)	0.073	0.162	0.114	-0.145	0.262*
Systolic Pressure (mmHg)	0.573**	-0.066	0.116	-0.009	0.221*
Diastolic Pressure (mmHg)	0.406**	-0.077	0.083	-0.110	0.275*
Resting Heart Rate (bpm)	0.149	0.091	0.306**	0.043	0.147
Total Cholesterol	0.333**	-0.338**	-0.02	0.156	-0.082
Triglyceride	0.000	0.136	0.038	-0.279*	0.334**
Low-Density Lipoproteins	0.286**	-0.155	0.030	0.181	-0.049
High-Density Lipoproteins	0.231*	-0.255*	-0.066	0.117	-0.280*
LDL / HDL Ratio	0.079	0.074	0.006	0.033	0.136
Body Mass Index (Kg/m^2)	0.184	0.304**	0.125	-0.214	0.377**
Waist Circumference (cm)	0.295**	0.279*	0.152	-0.146	0.334**
Whole Body Fat (%)	0.401**	0.221*	0.257*	-0.065	0.236*
Whole Body Fat (g)	0.335**	-0.073	0.239*	-0.102	0.248*
Trunk Fat (%)	0.430**	0.243*	0.307**	-0.105	0.319**
Trunk Fat Mass (g)	0.248*	0.234*	0.297**	-0.142	0.264*
Total Steps per Day	0.122	-0.125	-0.167	0.051	-0.116
Total Minutes per Day	0.022	-0.003	-0.106	0.078	-0.187
Low Steps per Day	0.164	-0.030	-0.043	0.132	-0.205
Low Minutes per Day	0.138	0.074	-0.039	0.072	-0.200
Moderate Steps per Day	0.268*	-0.094	-0.062	0.108	-0.113
Moderate Minutes per Day	0.232*	-0.089	-0.058	0.117	-0.123
High Steps per Day	-0.027	-0.141	-0.225*	-0.039	-0.034
High Activity Minutes per Day	-0.002	-0.136	-0.203	-0.040	-0.042
Sedentary Minutes per Day	-0.022	0.003	0.106	-0.078	0.187

CRP = C-reactive protein; t-PA = tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor – 1; LDL = low density lipoprotein; HDL = high density lipoprotein. * $p < 0.05$, ** $p < 0.01$.

Figure 4 is a scatter plot figure for CRP (C-reactive protein) values (y axis) across age (x axis) for the entire group.

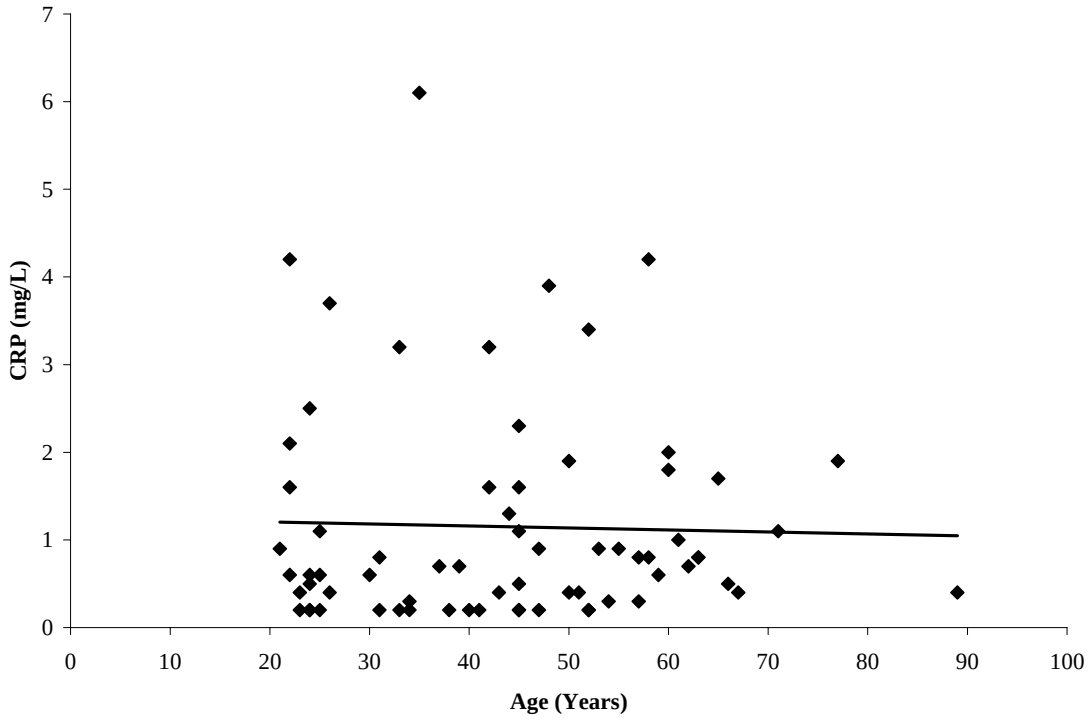


Figure 5 is a scatter plot figure for fibrinogen concentrations (y axis) across age (x axis) for the entire group.

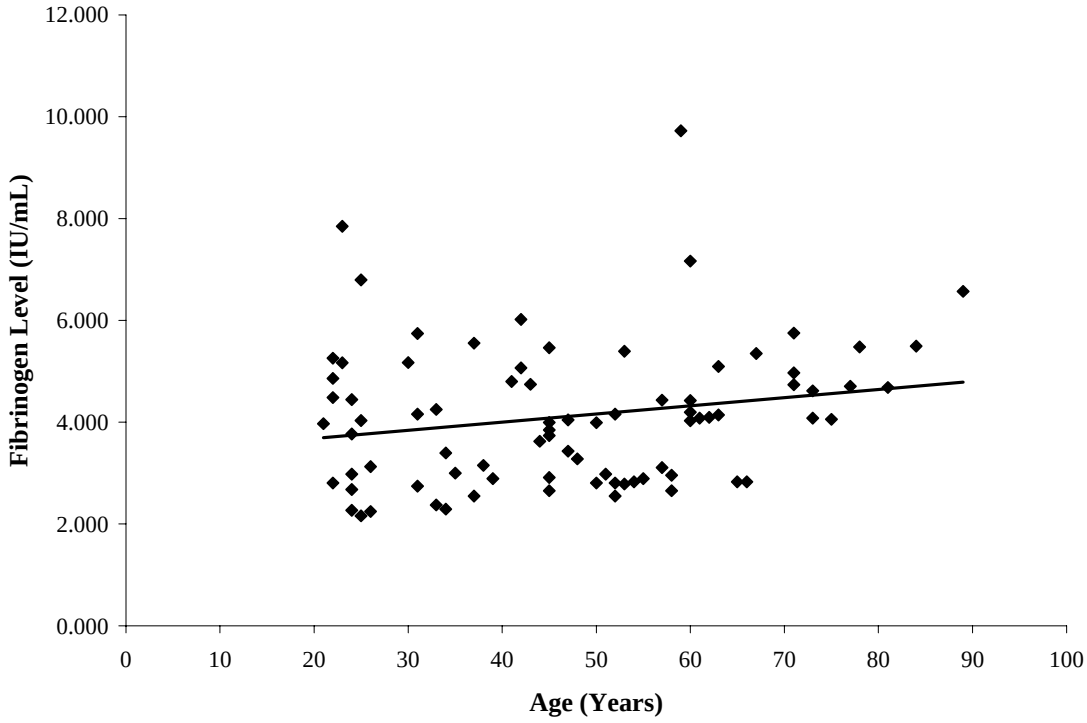


Figure 6 is a scatter plot figure for tissue plasminogen activator (t-PA) concentrations (y axis) across age (x axis) for the entire group.

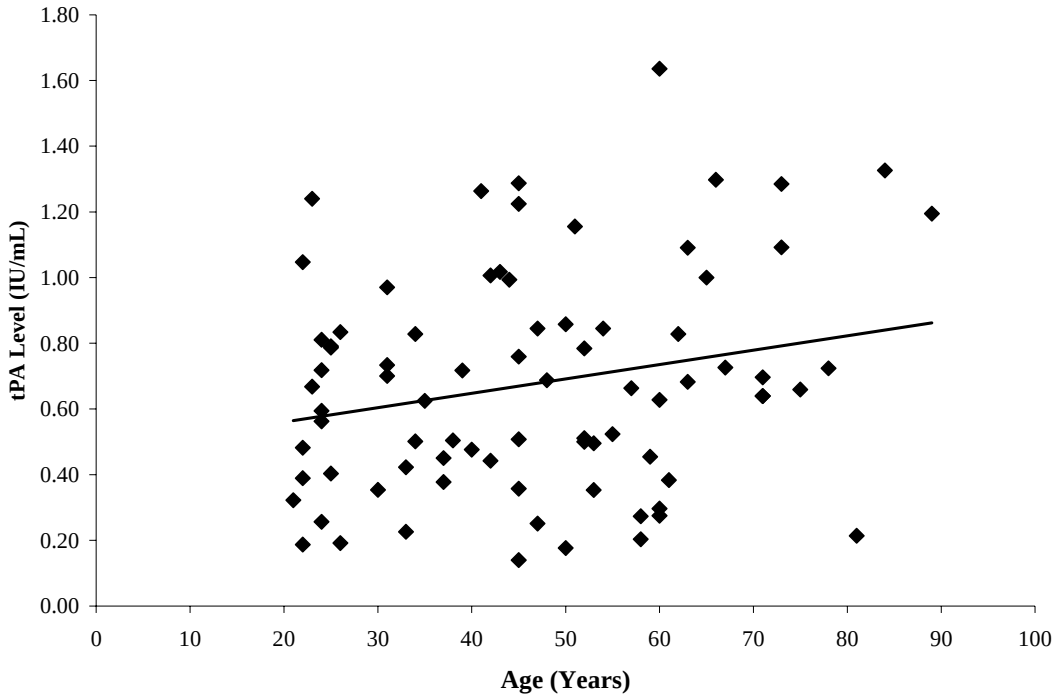
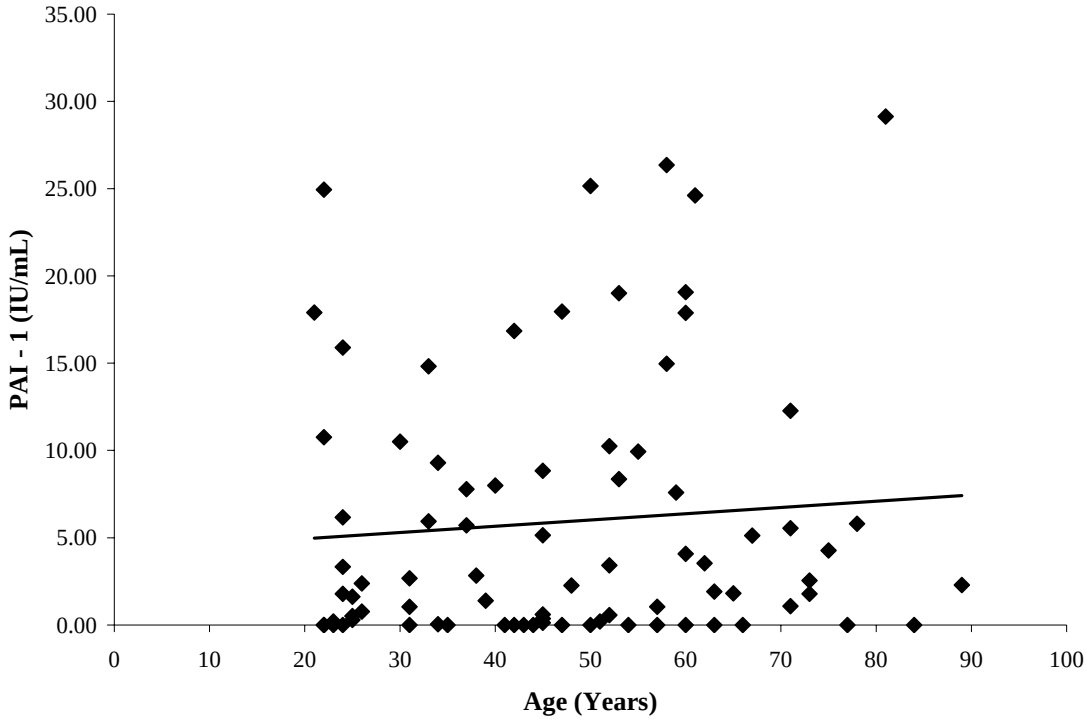


Figure 7 is a scatter plot figure for plasminogen activator inhibitor – 1 (PAI-1) concentrations (y axis) across age (x axis) for the entire group.



CHAPTER 5

DISCUSSION AND CONCLUSIONS

The main purpose of this investigation was to determine if age is associated with blood concentrations of C-reactive protein (CRP), fibrinogen, tissue plasminogen activator (t-PA), and plasminogen activator inhibitor – 1 (PAI-1), independent of body fat and physical activity levels. The primary finding of the current study shows that age is not independently associated with blood concentrations of CRP, fibrinogen, t-PA or PAI-1. Additionally, this study revealed that BMI is a predictor of CRP levels, but not of markers of fibrinolysis.

Aging and Inflammation

These results show that CRP concentrations did not increase with advancing age, which conflicts with previous claims. Many studies conclude that aging is associated with increased plasma levels of CRP, TNF-α, and IL-6 (9,21,23). In one of the largest age ranges examined (20 to 102 years of age), CRP and IL-6 were found to be significantly higher in the age groups 65 years of age and older in both, males and females even after adjusting for the existing clinical conditions (i.e., hypertension, hypercholesterolemia) (48). But, the researchers failed to account for varying body size and physical activity status.

Another study comparing older and younger adults who were apparently healthy (9) carefully excluded for any disorders that could affect CRP levels. However, they did not account for blood pressure, cholesterol levels (or pertaining medications), physical activity levels, or body composition. The failure to control for such potential covariates limits the conclusion that there are age-association changes in inflammatory status. This study not only excluded subjects presenting with any co-existing clinical conditions, but also further adjusted for varying body size, body compositions, and physical activity levels. To date, this is one of the most complete examinations of the independent association between age and inflammation.

There also have been previous reports that did not shown an increase in inflammation with age. In the Framingham heart study, no differences were found in circulating TNF-α and IL1β between elderly adults and a younger, control group (115). The Framingham findings are consistent with the findings of this study that inflammatory activity does not change with age. Furthermore, the lack of an age-related change in inflammatory markers suggests that inflammation does not independently increase the risk of cardiovascular disease as people age. Instead, the prevention of atherosclerotic development should focus more on the increasing prevalence of co-morbid conditions (e.g. diabetes and hypertension) that occur with advancing age.

Aging and Fibrinolysis

The analysis between age groups did reveal significantly higher levels of t-PA in the older adults compared to younger adults ($p = 0.042$). Additionally, this study found a correlation between age and t-PA ($r = 0.228$, $p = 0.042$). Two previous reports show that t-PA release is blunted in older adults (36,127); however, they also reported the potential impact of habitual exercise on hemostatic conditions.

Age is associated with decreased physical activity, decreased t-PA levels, and a higher incidence of thrombosis (36). In this study, the older group had higher t-PA levels than expected and it was found that the strides and minutes of ambulatory activity at a moderate cadence per day increased with age. Higher levels of physical activity have the potential to blunt the decrease in t-PA that is normally associated with age (36,127). The higher levels of ambulatory physical activity measured among the older adults in this study also may help explain why the expected associations between age and inflammation and fibrinolysis were not present. Thus, the adverse age-associated impact on fibrinolysis, however great it may be, appears to be either attenuated or absent in highly physically active individuals.

A more in-depth analysis did not show a change in concentrations of fibrinogen, t-PA or PAI-1 with increasing age. These results contradict the positive correlation between age and the occurrence of thrombosis that has been presented over the past couple of decades (36,148). These studies showed a dramatic increase in PAI-1 and a decrease in t-PA activity with increasing age. As previously suggested, the lack of change in fibrinolytic activity with age that was found in this

study may be a consequence of the higher physical activity levels that were maintained by the older adults in this study.

While there could be a primary affect of aging, the data showing that increasing physical activity levels negate this affect introduce new factors that, at minimum, call this relation into question (36,127). These studies in combination with the findings of this study raise the question if there is truly an age effect on fibrinolytic activity, or whether fibrinolysis is more of a result of typical age-related declines in physical activity patterns.

Other Factors and Inflammation

Lipids. There were several factors found to be significantly related to CRP levels in this investigation. First, CRP levels were inversely related to total cholesterol ($r = -0.338$, $p = 0.002$) and HDL levels ($r = -0.255$, $p = 0.022$). HDL levels have previously been shown to be negatively correlated to CRP concentrations (100). Most likely, the correlation between HDL and CRP explain the negative association between total cholesterol and CRP, as HDL is a component of total cholesterol levels. This suggests that higher total cholesterol levels are probably not associated with lower levels of inflammation. However, this negative correlation between HDL & CRP may help explain the link between high HDL levels and the associated decrease in risk for cardiovascular disease.

Obesity and Body Composition. Obesity has been consistently associated with generalized, low-grade inflammation (29,86). In the current study, CRP levels

were also related to several measures of body composition. BMI and waist circumference were positively related to CRP concentrations. Overall, it was found that BMI is an independent predictor of CRP levels ($p = 0.038$) in healthy adults. Similar investigations have also shown CRP concentrations were significantly correlated with BMI, waist circumference, and waist-to-hip ratio (100,121,150,151).

Percent body fat, a more precise measure of body composition, also correlated positively with levels of CRP ($r = 0.221$, $p = 0.048$), as did percent trunk fat and trunk fat mass. These results are not surprising, as previous research has shown that adipocytes can produce CRP, TNF- and IL-6 (34,68,87,132). Furthermore, the age-associated increase in body fat (55,98) may be an important source of increased circulating levels of inflammatory markers in the older population (63). More importantly, this adds to the growing body of literature showing a link between obesity and inflammation, and strengthens the notion that inflammation may be the mechanistic link between obesity, and specifically abdominal obesity, and cardiovascular disease.

Other Factors and Fibrinolysis

Lipids. Individuals with high triglyceride and low HDL levels were found to have high PAI-1 serum levels (93,106). The findings of this study support these results, showing that triglyceride levels were positively related to PAI-1 levels ($r = 0.334$, $p = 0.002$) and negatively related to t-PA levels ($r = -0.279$, $p = 0.012$). Additionally, HDL levels were negatively related to PAI-1 levels ($r = -0.280$, $p =$

0.011). As shown by Orem et al (93) , successful lipid lowering therapy can improve fibrinolytic activity by increasing t-PA and decreasing PAI-1, suggesting that correcting dyslipidemia can reduce the risk of thrombosis formation.

Blood Pressure. PAI-1 concentration was also positively related to systolic ($r = 0.221$, $p = 0.047$) and diastolic blood pressure ($r = 0.275$, $p = 0.013$). Hypertension, an established risk factor for acute coronary events, has been linked to impaired fibrinolysis in men and women (25,53,103,128). As impaired fibrinolysis can lead to thrombosis (25,53), these data collectively provide another potential mechanism by which hypertension contributes to the pathogenesis of cardiovascular disease in hypertensive patients.

Obesity and Body Composition. As expected, BMI was directly related to PAI-1 levels ($r = 0.377$, $p = 0.001$), which indicates a decrease in fibrinolytic activity with increasing BMI. Furthermore, these results show that fibrinogen and PAI-1 were elevated with increases in more specific measures of whole body fatness, such as percent body fat and body fat mass. The results of the current study agree with numerous previous studies (78,81,133), suggesting that the bridge between obesity and cardiovascular disease is becoming better defined. From a clinical perspective, it is encouraging to learn that acute weight loss in obese individuals has been shown to improve fibrinolytic activity (29,78) and, in turn, decrease the risks of thrombus related accidents.

PAI-1 levels were also positively associated with waist circumference ($r = 0.334$, $p = 0.002$). More specifically, it was found that fibrinogen and PAI-1 levels

were positively related to percent trunk fat and trunk fat mass. This suggests that abdominal adiposity may contribute to increased levels of PAI-1, the major inhibitor of fibrinolysis. While fat distribution has been suggested to influence fibrinolytic activity (78,81,134), more studies are needed to clarify to the association between abdominal adiposity and fibrinolytic activity.

Study Limitations

This study is not without its limitations. This study used a cross-sectional design which does not allow for a causal link to be established between aging and inflammation or fibrinolysis. The participants in this study, particularly the older adults, were extremely healthy volunteers and, thus, may not be representative of the general population around the Oklahoma City metropolitan area, which in other studies may be considered a limitation. However, the rigor of the exclusion criteria was necessary to isolate the effect of age rather than other confounding factors commonly associated with age. The sample was largely Caucasian. Because of this, the results can not be generalized to adults from minority populations.

Another limitation is that the StepActivity Monitor is not waterproof and it is worn on the ankle, so water and upper body activity were not quantified throughout the monitoring period. All subjects were questioned about their physical activity regimen, however, and only one participant engaged in water activity during the monitoring time period. The most serious limitation related to the measurement of physical activity is that it is likely that a selection bias exists in this study, as only the

high-active elderly may have agreed to participate whereas low/moderate active younger subjects may not have volunteered.

Also, the dietary intake of subjects was not monitored in this study. It has been shown that people on low fat diets have significantly improved fibrinolytic activity (118). All of our subjects were tested in a fasted state, but the long-term dietary habits, including regular fat intake, is unknown. By excluding for high cholesterol and high blood pressure, we also may have excluded for poor dietary habits as well.

Conclusions

In summary, study results show that age is not independently associated with blood concentrations of CRP, fibrinogen, t-PA or PAI-1. It can therefore be concluded that aging is not associated with inflammation and impaired fibrinolysis in subjects carefully screened for co-morbid conditions and cardiovascular risk factors. While blood pressure, cholesterol levels and measures of body composition were significantly related to markers of inflammation and fibrinolysis, BMI was found to be an independent predictor of only CRP, not of fibrinolytic markers. Finally, it is speculated that previous observations of age-related changes in inflammation (9,21,22,23) and fibrinolysis (36,148) resulted from the variance in body composition, physical activity and co-existing clinical conditions that occur with aging.

Clinical Significance

This study indicates that inflammatory and fibrinolytic activity does not change with increasing age. However, it was observed that inflammation and fibrinolysis were associated with high blood pressure, high cholesterol levels, and measures of body fatness, which provides a possible explanation for the discrepancy between our findings and those of previous studies which did not control for such factors.

Furthermore, the maintenance of physical activity levels across the age range found in this sample suggests that physical activity may provide a protective effect against the age-related changes in inflammatory and fibrinolytic activity previously shown. Regardless, blood pressure, cholesterol, body composition and physical activity levels are all manageable. These results suggest that improving these problems will reduce the risk of developing cardiovascular disease by reducing inflammation and increasing fibrinolytic activity. Combined with existing research, this study ultimately suggests that the risks associated with atherosclerotic disease are modifiable, not inevitable.

REFERENCES

1. Abbate R, D Prisco, C Rostangno, M Boddi, and GF Gensini. Age-related changes in the hemostatic system. *Int J Clin Lab Res* 1993; 23:1-3.

2. Abrutyn E, J Mossey, M Levison, J Boscia, P Pitsakis, D Kaye. Epidemiology of asymptomatic bacteriuria in elderly women. *J Am Geriatr Soc* 1991;39:388-393.

3. Anand SS, F Razak, Q Yi, B Davis, R Jacobs, V Vuksan, E Lonn, K Teo, McQueen, S Yusuf. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. *Arterioscler Thromb Vasc Biol* 2004; 24:1509-1515.

4. Anand SS, Q Yi, H Gerstein, E Lonn, R Jacobs, V Vuksan, K Teo, B Davis, P Montague, and S Yusuf. Relationship of metabolic syndrome and fibrinolytic dysfunction to cardiovascular disease. *Circulation* 2003; 108:420-425.

5. Andersson SE, ML Edvinsson, L Edvinsson. Cutaneous vascular reactivity is reduced in aging and in heart failure: association with inflammation. *Clin Sci* 2003; 105:699-707.

6. Anderson RN, and BL Smith. Deaths: leading causes for 2002. *Nat Vital Stat Report* 2005; 53(17):1-7.

7. Andrew M, C Carter, H O'Brodovich, G Hiegenhause. Increases in factor VIII complex and fibrinolytic activity are dependent on exercise intensity. *J Appl Physiol* 1986;60:1917-1922.

8. Baggio G, S Donazzan, D Monti, D Mari, S Martini, C Gabelli. Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors. *FASEB J* 1998; 12: 433-437.

9. Ballou SP, GB Lozanski, S Hodder, DL Rzewnicki, LC Mion, JD Sipe, AB Ford, and I Kushner. Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. *Age Aging* 1996; 25:224-230.

10. Barthe N, Braillon P, Ducassou D, Basse-Cathalinat B Comparison of two Hologic DXA systems (QDR 1000 and QDR 4500/A). *Br J Radiol* 1997;70:728–739.

11. Bennermo M, C Held, A Hamsten, LE Strandberg, CG Ericsson, LO Hansson, P Tornvall. Prognostic value of plasma C-reactive protein and fibrinogen determinations in patients with acute myocardial infarction treated with thrombolysis. *J Intern Med* 2003; 254:244-250.

12. Berk BC, WS Weintraub, RW Alexander. Elevation of C-reactive protein in "active" coronary artery disease. *Am J Cardiol* 1990; 65:168-172.

13. Bermudez EA, N Rifai, JE Buring, JE Manson, PM Ridker. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol* 2002;89:1117-1119.

14. Blair SN, S Brodney. Effects of physical inactivity and obesity on morbidity and mortality: current evidence and research issues. *Med Sci Sports Exerc* 1999;31:S646-S662.

15. Blake GM, Parker JC, Buxton FM, Fogelman I. Dual X-ray absorptiometry: a comparison between fan beam and pencil beam scans. *Br J Radiol* 1993;66:902–906.

16. Blake GJ, and PM Ridker. Inflammatory bio-markers and cardiovascular risk prediction. *J Int Med* 2002; 252:283-294.

17. Blake GJ, and PM Ridker. Novel clinical markers of vascular wall inflammation. *Circ Res* 2001; 89:763-771.

18. Bossaller C, GB Habib, H Yamamoto, C Williams, S Wells, PD Henry. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J Clin Invest* 1987; 79(1):170-174.

19. Born J, D Uthgenannt, C Dodt, D Nunninghoff, E Ringvolt, T Wagner, HL Fehm. Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. *Mech Ageing Dev*. 1995; 84(2):113-26.

20. Bouchard L, P Mauriege, MC Vohl, C Bouchard, L Perusse. Pasminogen-activator inhibitor-1 polymorphisms are associated with obesity and fat distribution in the Quebec Family Study: evidence of interactions with menopause. Menopause 2005;12(2):136-43.

21. Bruunsgaard H, S Ladelund, AN Pedersen, M Schrolls, T Jorgensen, BK Pedersen. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin Exp Immunol* 2003; 132:24-31.

22. Bruunsgaard H, BK Pedersen. Age-related inflammatory cytokines and disease. *Immunol Allergy Clin N Am* 2003; 23:15-39.

23. Bruunsgaard h, AN Pederson, M Schroll, P Skinhog, BK Pedersen. Impaired production of proinflammatory cytokines in response to LPS stimulation in elderly humans. *Clin Exp Immunol* 1999; 118:235-41

24. Burke GL, AM Arnold, DE Bild, M Cushman, LP Fried, A Newman, C Nunn, and J Robbins. Factors associated with healthy aging: The Cardiovascular Healthy Study. *JAGS* 2001; 49:254-262.

25. Castelli WP. Lipids, risk factors and ischaemic heart disease. *Atherosclerosis* 1996; 124 suppl:S1-S19.

26. Centers for Disease Control and Prevention (CDC). Centers for Disease Control and Prevention (CDC). Cigarette smoking among adults–United States, 2003. *MMWR.* 2004; 54: 1121–1124.

27. Centers for Disease Control and Prevention (CDC). Trends in cholesterol screening and awareness of high blood cholesterol–United States, 1991-2003. *MMWR.* 2005; 54; 865–870.

28. Chaudhuri A, JL Izzo. Insulin resistance and hypertension in the absence of subcutaneous fat. *Rev Cardiovasc Med* 2000; 1(2):120-124.

29. Clifton PM, JB Keogh, PR Foster, and M Noakes. Effect of weight loss on inflammatory and endothelial markers and FMD using two low-fat diets. *Int J Obesity* 2005; 29:1445-1451.

30. Colbert LH, M Visser, EM Simonsick, RP Tracy, AB Newman, SB Kritchevsky, M Pahor, DR Taaffe, J Brach, S Rubin, TB Harris. Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study.
J Am Geriatr Soc. 2004;52(7):1098-104.

31. Collins JS, RT Perry, B Watson Jr., LE Harrell, RT Acton, D Blacker. Association of haplotype for tumor necrosis factor in siblings with late-onset Alzheimer disease: the NIMH Alzheimer Disease Genetics Initiative. *Am J Med Genet* 2000;96:823-830.

32. Davis GL, CF Abildgaard, EM Bernauer, M Britton. Fibrinolytic and hemostatic changes during and after maximal exercise in males. *J Appl Physiol* 1976;40:287-292.

33. Day CP, J Grove, AK Daly, MW Stewart, PJ Avery, M Walker. Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. *Diabetologia* 1998;41:430-434.

34. De Lorenzo F, M Mukherjee, Z Kadziola, S Suleiman, VV Kakkar. Association of overall adiposity rather than body mass index with lipids and procoagulant factors. *Thromb Haemost* 1998; 80:603-606.

35. De Maat MP, A Trion. C-reactive protein as a risk factor versus risk marker. *Curr Opin Lipidol* 2004;15(6):651-7.

36. DeSouza CA, PP Jones, and DR Seals. Physical activity status and adverse age-related differences in coagulation and fibrinolytic factors in women. *Arterioscler Thromb Vasc Biol* 1998; 18:362-368.

37. Diabetes Surveillance Report, 1999. *CDC, USDHH*S.

38. Eliasson M, K Asplund, PE Evrin, and D Lundblad. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. *Atherosclerosis* 1995 Feb; 113(1):41-53.

39. Engstrom G, B Hedblad, L Stavenow, P Lind, L Janson, F Lindgarde. Inflammation-sensitive plasma proteins are associated with future weight gain. *Diabetes* 2003; 52(8): 2097-2101.

40. Eriksson P, S Reyniskottir, F Lonngvist, V Stemme, A Hamsten, P Arner. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia* 1998; 41:65-71.

41. Ernst E, and W Koenig. Fibrinogen and cardiovascular risk. *Vascular Med* 1997; 2:115-125.

42. Ernst E and KL Resch. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. *Ann Intern Med* 1993; 118: 956-963.

43. Erren M, H Reinecke, R Junker, M Fobker, H Schulte, JO Schurek, J Kropf, S Kerber, G Breithardt, G Assmann, P Cullen. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. *Arterioscler Thromb Vasc Biol* 1999; 19:2355-2363.

44. Ershler WB, ET Keller. Age-associated increased interleukin-6 gen expression, late-life diseases, and frailty. *Annu Rev Med* 2000; 51: 245-270.

45. Esmon CT, FB Taylor Jr, and TR Snow. Inflammation and coagulation: linked processes potentially regulated through a common pathway mediated by protein *C. Thromb Haemost* 1991; 66(1):160-165.

46. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Executive summary of the third report of The National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486–2497.

47. Ferguson EW, LL Bernier, GR Banta, J Yu-Yahiro, EB Schoomaker. Effects of exercise and conditioning on clotting and fibrinolytic activity in men. *J Appl Physiol*. 1987;62(4):1416-21.

48. Ferrucci L, A Corsi, F Lauretani, S Bandinelli, B Bartali, DD Taub, JM Guralnik. The origins of age-related proinflammatory state. *Blood* 2005; 105(6):2294-2299.

49. Festa A, R D'Aostino, K Williams, AJ Karter, EJ Mayer-Davis, RP Tracy, SM Haffner. The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes Relat Metab Disord* 2001; 25(10):1407-1415.

50. Flegal KM, MD Carroll, CL Ogden, CL Johnson. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; 288:1723-1727.

51. Fields LE, VL Burt, JA Cutler, J Hughes, EJ Roccella, P Sorlie. The burden of adult hypertension in the United States, 1999–2000: a rising tide. *Hypertension.* 2004; 44: 398–404.

52. Folsom AR, N Aleksic, E Park, V Salomaa, H Juneja, KK Wu. Prospective study of fibrinolytic factors and incident coronary heart disease. *Arterioscler Thromb Vasc Biol* 2001;21:611-617.

53. Fuster V, L Badimon, JJ Badimon, JH Chesebro. The pathogenesis of coronary artery diseases and the acute coronary syndromes. *N Engl J Med* 1992;326:242-250.

54. Gardner AW, Simeminski DJ, Killewich LA. The effect of cigarette smoking on free-living daily physical activity in older claudication patients. *Angiology* 1997;48:947-955.

55. Gallagher D, E Ruts, M Visser, S Heshka, RN Baumgartner, J Wang. Weight stability masks sarcopenia in elderly men and women. *Am J Physiol Endocrinol Metab* 2000; 279: E366-E375.

56. Gensini GF, M Comeglio, and A Colella. Classical risk factors and emerging elements in the risk profile for coronary artery disease. *Eur Heart J* 1998; 19(suppl A):A53-A61.

57. Geffken DF, M Cushman, GL Burke, JF Polak, PA Sakkinen, RP Tracy. Association between physical activity and markers of inflammation in a healthy elderly population. *Am J Epidemiol.* 2001;153(3):242-50.

58. Giri S, PD Thompson, FJ Kiernan, J Clive, DB Fram, JF Mitchel, JA Hirst, RG McKay, DD Waters. Clinical and angiographic characteristics of exertion-related acute myocardial infarction. *JAMA* 1999;282(18):1731-6.

59. Grayston JT. Background and current knowledge of Chlamydia pneumoniae and atherosclerosis. *J Infect Dis* 2000;181(suppl):S402-S410.

60. Guzik TJ, D Mangalat, R Korbut. Adipocytokines – Novel link between inflammation and vascular function. *J Physiol Pharmocol* 2006; 57(4): 505-528.

61. Hamsten A. The hemostatic system and coronary heart disease. *Thromb Res* 1993;70:1-38.

62. Hamsten A, G Walldius, A Szamosi, M Blombäck, U de Faire, G Dahlen, C Landou, B Wiman. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987;2:3-8.

63. Hawkins MA. Markers of increased cardiovascular risk: are we measuring themost appropriate parameters? *Obes Res* 2004; 12: 1075-1148.

64. Hedley AA, CL Ogden, CL Johnson, MD Carroll, LR Curtin, KM Flegal. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA.* 2004; 291: 2847–2850.

65. Hoffstedt J, L Andersson, L Persson, B Isaksson, P Amer. The common-675 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is strongly associated with obesity. *Diabetologia* 2002;45:584-587.

66. Jankord R and B Jemiolo. Influence of physical activity on serum IL-6 and IL-10 levels in healthy older men. *Med Sci Sports Exerc* 2004; 36(6):960-964.

67. Kannel WB, MC Hjortland, PM McNamara, T Gordon. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med* 1976;85(4):447-452.

68. Kern PA, S Ranganathan, C Li, L Wood, C Ranganathan. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol* 2001; 280:E745-E751.

69. Kiecolt-Glaser JK, KJ Preacher, RC MacCallum, C Atkinson, WB Malarkey, R Glaser. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci U S A* 2003; 100(15):9090-9095.

70. Killewich LA, RF Macko, PS Montgomery, LA Wiley, AW Gardner. Exercise training enhances endogenous fibrinolysis in peripheral arterial disease. *J Vasc Surg* 2004;40:741-745.

71. Krabbe KS, H Bruunsgaard, CM Hansen, K Moller, L Fonsmark, J Qvist, PL Madsen, G Kronborg, HO Andersen, P Skinhoj, BK Pedersen. Ageing is associated with a prolonged fever response in human endotoxemia. *Clin Diagn Lab Immunol*. 2001;8(2):333-8.

72. Lakka TA, JM Vanalainen, R Rauramaa, R Salonen, J Tumilehta, and JT Salonen. Relationship of leisure-time physica activity and cardiorespiratory fitness to the risk of actue myocardial infarction in men. *N Engl J Med* 1994; 330:1549-1554.

73. Rosito GA, FD Fuchs, BB Duncan. Dose-dependent biphasic effect of ethanol 24-h blood pressure in normotensive subjects. *Am J Hypertens.* 1999;12(2 Pt 1):236-40.

74. Liuzzo G, M Santamaria, LM Biasucci, M Narducci, V Colafrancesco, A Porto, S Brugaletta, M Pinnell, V Rizzello, A Maseri, F Crea. Persistent activation of nuclear factor kappa-B signaling pathway in patients with unstable angina and elevated levels of C-reactive protein. *J Am Coll Cardiol* 2007; 49(2):185-194.

75. Loscalzo J. The relation between atherosclerosis and thrombosis. *Circulation* 1992;86(Suppl III):III95-III99.

76. Liuzzo G, M Santamaria, LM Biasucci, M Narducci, V Colafrancesco, A Porto, S Brugaletta, M Pinnelli, V Rizzello, A Maseri, F Crea. Persistent activation of nuclear factor kappa-B signaling pathway in patients with unstable angina and elevated levels of C-reactive protein evidence for a direct proinflammatory effect of azide and lipopolysaccharide-free C-reactive protein on human monocytes via nuclear factor kappa-B activation. *J Am Coll Cardiol* 2007;49(2):185-94.

77. Macko RF, Haeuber E, Shaughness M, Coleman KL, Boone DA, Smith GV, Silver KH. Microprocessor-based ambulatory activity in stroke patients. *Med Sci Sports Exerc* 2002;34(3):394-399.

78. Mavri A, M Stegnar, M Krebs, JT Sentocnik, M Geiger, BR Binder. Impant of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. *Arterioscler Thromb Vasc Biol* 1999;19:1582-1587.

79. Mazer SP, and LE Rabbani. Evidence for C-reactive protein's role in (CRP) vascular disease: Atherothrombosis, Immuno-regulation and CRP. *J Throm and Thrombol* 2004; 17(2):95-105.

80. Mazess RB, Barden HS, Bisek JP, Hanso, J. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990; 51:1106-1112.

81. McGill JB, DJ Schneider, CL Arfken, CL Lucore, BE Sobel. Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. *Diabetes* 1994;43:104-109.

82. Meade TW, V Ruddock, Y Stirling, R Chakrabarti, GJ Miller. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 1993;342:1076-1079.

83. Megraud F, RM Brassens, F Denis, A Belbouri, DQ Hoa. Seroepidemiology of Campylobacter pylori infection in various populations. *J Clin Microbiol* 1989;27:1870-1873,

84. Mendall MA, DP Strachan, BK Butland, L Ballam, J Morris, PM Sweetnam, PC Elwood. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J* 2000;21(19):1584-90.

85. Meurman JH, H Pajukoski, S Snellman, S Zeiler, R Sulkava. Oral infections in home-living elderly patients admitted to an acute geriatric ward. *J Dent Res* 1997;39:388-393.

86. Miller GE, KE Freedland, RM Carney, CA Stetler, WA Banks. Pathways linking depression, adiposity, and inflammatory markers in healthy young adults. *Brain Behav Immun* 2003; 17:276-285.

87. Mohamed-Ali V, S Goodrick, A Rawesh, DR Katz, JM Miles, JS Yudkin. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinolo Metab* 1997; 82:4196-4200.

88. Mutch NJ, HM Wilson, NA Booth. Plasminogen activator inhibitor-1 and haemostasis in obesity. *Proc Nutr Soc* 2001;60:341-347.

89. Nakamura S, I Nakamura, L Ma, DE Vaughan, AB Fogo. Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo. *Kidney Int* 2000;58(1):251-9.

90. Nesto R. C-reactive protein, its role in inflammation, Type 2 diabetes and cardiovascular disease, and the effects of insulin-sensitizing treatment with thiazolidinediones. *Diabet Med* 2004;21:810-817.

91. Newby DE, AL McLeod, NG Uren, L Flint, CA Ludlam, DJ Webb, KA Fox, NA Boon. Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: direct link between endothelial dysfunction and atherothrombosis. *Circulation* 2001;103(15):1936-41.

92. Okin PM, MJ Roman, LG Best, ET Lee, JM Galloway, BV Howard, RB Devereux. C-reactive protein and electrocardiographic ST-segment depression additively predict mortality. *JACC* 2005; 45(11):1787-1793.

93. Orem C, HA Uydu, R Yilmaz, M Gokce, M Baykan, S Eminagaoglu, A Orem. The effects of atorvastatin treatment on the fibrinolytic system in dyslipidemic patients. *Jpn Heart J* 2004 Nov;45(6):977-87.

94. Paramo JA, I Olavide J Barba R Motes, C Panizo, MC Munoz. Long-term cardiac rehabilitation program favorably influences fibrinolysis and lipid concentrations in acute myocardial infarction. *Haematologica* 1998;83:519-524.

95. Pasceri V, JT Willerson, ET Yeh. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; 102:2165-2168.

96. Pate RR, M Pratt, SN Blair, WL Haskell, CA Macera, C Bouchard, D Buchner, W Ettinger, GW Heath, AC King. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 1995; 273: 402–407.

97. Patel VB, MA Robbins, EJ Topol. C-reactive protein: a 'golden marker' for inflammation and coronary artery disease. *Clev Clin J Med* 2001; 68(6):521-534.

98. Pedersen M, H Bruunsgaard, N Weis, HW Hendel, BU Andreassen, E Eldrup. Circulating levels of TNF-alpha and IL6: relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. *Mech of Aging Dev* 2003; 124(4):495-502.

99. Peeters AC, MG Netea, BJ Kullberg, T Thien, JW Van der Meer. The effect of rennin-angiotensin system inhibitors on pro- and anti-inflammatory cytokine production. *Immunology* 1998;94:376-379.

100. Piche ME, S Lemieux, SJ Weisnagel, L Corneau, A Nadeau, J Bergeron. Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and triglyceride levels in healthy postmenopausal women. *Am J Cardiol.* 2005;96(1):92-7.

101. Pietila KO, AP Harmoinen, J Jokiniitty, AI Pasternack. Serum C-reactive protein concentration in actue myocardial infarction and its relationship to mortality during 24 months of follow-up in patients under thrombolytic treatment. *Eur Heart J* 1996; 17:1345-1349.

102. Pischon T, SE Hankinson, GS Hotamisligil, N Rifai, EB Rimm. Leisure-time physical activity and reduced plasma levels of obesity-related inflammatory markers. *Obes Res*. 2003;11(9):1055-64.

103. Poli KA, GH Tofler, MG Larson, JC Evans, PA Sutherland, I Lipinska, MA Mittleman, JE Muller, RB D'Agostino, PW Wilson, D Levy. Association of blood pressure with fibrinolytic potential in the Framingham offspring population. *Circulation* 2000;101(3):264-9.

104. Ponjee GA, EM Janssen, J Hermans, JW van Wersch. Regular physical activity and changes in risk factors for coronary heart disease: a nine months prospective study. *Eur J Clin Chem Clin Biochem* 1996;34:477-483.

105. Puccetti L, F Bruni, AL Pasqui, M Pastorelli, G Bova, M Cercignani, A Palazzuoli, A Auteri. Dyslipidemias and fibrinolysis. *Ital Heart J* 2002 Oct;3(10):579-86.

106. Puccetti L, AL Pasqui, M Pastorelli, G Bova, M Cercignani, A Palazzuoli, A Auteri, F Bruni. Different mechanisms of fibrinolysis impairment among dyslipidemic subjects. *Int J Clin Pharmacol Res* 2001;21:147-55.

107. Radar DJ. Inflammatory markers of coronary risk. *N Engl J Med* 2000; 343:1179-1182.

108. Redecke V, K Dalhoff, S Bohnet, J Braun, M Maass. Interaction of Chlamydia pneumoniae and human alveolar macrophages: infection and inflammatory responses. *Am J Respir Cell Mol Biol* 1998;19:721-727.

109. Reuben DB, L Judd-Hamilton, TB Harris, TE Seeman. The associations between physical activity and inflammatory markers in high-functioning older persons: MacArthur studies of successful aging. *J Am Geriatr Soc* 2003; 51:1125-1130.

110. Riancho JA, MT Zarrabeitia, JA Amado, JM Olmos, MJ Gonzalez. Age-related differences in cytokine secretion. *Gerontol* 1994; 40:8-12.

111. Ridker PM, M Cushman, MJ Stampfer, RP Tracy, and CH Hennekens. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 1998; 97:425-428.

112. Ridker PM, CH Hennekens, JE Buring, N Rifai. C-reactive protein and other markers of inflammation in the prediction of cardiovascular in women. *N Engl J Med* 2000; 342: 836-843.

113. Rosenfeld ME, E Blessing, TM Lin, TC Moazed, LA Campbell, C Kuo. Chlamydia, inflammation, and atherogenesis. *J Infect Dis* 2000;181(suppl):S492-S497.

114. Ross R. Atherosclerosis in an inflammatory disease. *Am Heart J* 1999; 138:S419-S420.

115. Roubenoff R, TB Harris, LW Abad, PF Wilson, GE Dallal, CA Dinarello. Moncyte cytokine production in an elderly population: effect of age and inflammation. *J Gerontol A Biol Sci Med Sci* 1998; 53: M20-M26.

116. Rudin E and N Barzilai. Inflammatory peptides derived from adipose tissue. *Immun Aging* 2005; 2:1.

117. Saito I, K Yonemasu, and F Inami. Association of body mass index, body fat, and weight gain with inflammation markers among rural residents in Japan. *Circ J* 2003; 67:323-329.

118. Salem RO and M Laposata. Effects of alcohol on hemostasis. *Am J Clin Pathol* 2005;123(Suppl):S96–105.

119. Salomaa V, V Stinson, JD Kark, AR Folsom, CE Davis, KK Wu. Association of fibrinolytic parameters with early atherosclerosis. The ARIC Study. Atherosclerosis Risk in Communities Study. *Circulation*. 1995;91(2):284-290.

120. Savoia C, EL Schiffrin. Vascular inflammation in hypertension and diabetes: molecular mechanisms and therapeutic interventions. *Clin Sci (Lond)* 2007;112(7):375-384.

121. Schrager MA, EJ Metter, E Simonsick, A Ble, S Bandinelli, F Lauretani, L Ferruci. Saropenic obesity and inflammation in the InCHIANTI study. *J Appl Physiol* 2007; 102: 919-925.

122. Schroll M, T Jorgensen, J Ingerslev. The Glostrup Population Studies, 1964-1992. Dan Med Bull 1992;39:204-207.

123. Seemungal T, R Harper-Owen, A Bhowmik, I Moric, G Sanderson, S Message, P Maccallum, TW Meade, DJ Jeffries, SL Johnston, JA Wedzicha. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164(9):1618-23.

124. Shepherd EF, E Toloza, DC McClung, TP Schmalzried. Step activity monitor: increased accuracy in quantifying ambulatory activity. *J Orthop Res* 1999;17(5): 703-708.

125. Sixth Report of the Joint Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNCVI), Public Health Service, National Institutes of Health, National Heart, Lung and Blood Institute, Bathesda, MD *NIH Publication* 1997; 98.

126. Skurk T and H Hauner. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Obes* 2004; 28:1357-1364.

127. Smith DT, L GL Hoetzer, JJ Greiner, BL Stauffer, CA DeSouza. Effects of ageing and regular aerobic exercise on endothelial fibrinolytic capacity in humans. *J Physiol* 2003;546:289-298.

128. Stamler J, JD Neaton, DN Wentworth. Blood pressure and risk of fatal coronary heart disease. *Hypertension* 1989;13(Suppl 1):1-2 – 1-12.

129. Torres JL, PM Ridker. High sensitivity C-reactive protein in clinical practice. *Am Heart Hosp J* 2003;1(3):207-211.

130. Tracey RP. Emerging relationships of inflammation, cardiovascular disease and chronic diseases of aging. *Int J Obesity* 2003; 27:S29-S34.

131. Tracy R, E Macy, E Bovill. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vas Biol* 1997; 17:2167-2176.

132. Trayhurn P, LS Wood. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans* 2005; 33(5):1078-81.

133. Urano T, Y Kohima, M Takahashi, K Serizawa, Y Takada, A Takada. Impaired fibrinolysis in hypertension and obesity due to high plasminogen activator inhibitor-1 level in plasma. *Jpn J Physiol*. 1993;43:221-228.

134. Vague P, I Juhan-Vague, V Chabert, MC Alessi, C Atlan. Fat distribution and plasminogen activator inhibitor activity in nondiabetic obese women. *Metabolism* 1989;38:913-915.

135. Van Den Burg PJM, JEH Hospers, WL Mosterd, BN Bouma, and IA Huisveld. Aging, physical conditioning, and exercise-induced changes in hemostatic factors and reaction products. *J Appl Physiol* 2000; 88:1558-1564.

136. Van Guilder GP, GL Hoetzer, DT Smith, HM Irmiger, JJ Greiner, BL Stauffer, CA DeSouza. Endothelial t-PA release is impaired in overweight and obese adults, but can be improved with regular aerobic exercise. *Am J Physiol Endocrinol Metab* 2005;289:E807-E813.

137. Venopugal KS, S Devaraj, I Yuhanna, P Shaul, I Jialal. Demonstration that C-reactive protein decrease eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 2002; 106:1439-1441.

138. Verdaet D, P Dendale, D De Bacquer, J Delanghe, P Block, G De Backer. Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis* 2004;176:303-310.

139. Volpato S, M Pahor, L Ferrucci, EM Simonsick, JM Guralnik, SB Kritchevsky, R Fellin, TB Harris. Relationship of alcohol intake with inflammatory markers and plasminogen activator inhibitor-1 in well-functioning older adults: the Health, Aging, and Body Composition study. *Circulation* 2004;109(5):607-12.

140. Wannamethee SG, GD Lowe, PH Whincup, A Rumley, M Walker, L Lennon. Physical activity and hemostatic and inflammatory variables in elderly men. *Circulation* 2002;105(15):1785-90.

141. Wang G, M Pratt, CA Macera, ZJ Zheng, G Heath. Physical activity, cardiovascular disease, and medical expenditures in U.S. adults. *Ann Behav Med*. 2004; 28(2):88-94.

142. Wang XY, M Hurme, M Jylha, A Hervonen. Lack of association between human longevity and polymorphisms of IL-1 cluster, IL-6, IL-10 and TNF-α genes in Finnish nonagenarians. *Mech Ageing Dev* 2001;123:29-38.

143. Weiss R, J Dziura, TS Burgert, WV Tamborlane, SE Taksali, CW Yeckel, K Allen, M Lopes, M Savoye, J Morrison, RS Sherwin, S Caprio. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004;350(23):2362-74.

144. Weiss C, G Seitel, P Artsch. Coagulation and fibrinolysis after moderate and very heavy exercise in healthy male subjects. *Med Sci Sports Exerc* 1998;30:246-251.

145. Weiss C, B Welsch, M Albert, B Briedmann, G Strobel, J Jost. Coagulation and thrombomodulin in response to exercise of different type and duration. *Med Sci Sports Exerc* 1998;30:1205-1210.

146. Womack CJ, JM Rasmussen, DG Vickers, CM Paton, PJ Osmond, GL Davis. Changes in fibrinolysis following exercise above and below lactate threshold. *Thromb Res* 2006;118(2):263-8.

147. Writing Group. Heart Disease and Stroke Statistics – 2006 Update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006; 113:85-151.

148. Yamamoto K, K Takeshita, T Kojima, J Takamatsu, H Saito. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. *Cardiovasc Res* 2005;66(2):276-85.

149. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. *Int J Obesity* 2003; 27:S25-S28.

150. Yudkin JS, DC Stehouwer, JJ Emers, SW Coppack. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol.* 1999;19(4):972-978.

151. Ziccardi P, F Nappo, G Giugliano, K Esposito, R Marfella, M Cioffi, F D'Andrea, AM Molinari, D Giugliano. Reduction of inflammation cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 2002; 105: 804-809.

APPENDIX A

Informed Consent Form

Version 4, October 26, 2006

INFORMED CONSENT FORM
for research being conducted at the
University of Oklahoma Health Sciences Center

Study Title: The age-related influences on inflammatory markers: a pilot study.

Principal Investigator: Andrew W. Gardner, Ph.D.

This is a research study. Research studies involve individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Is This Study Being Done?

This study will determine if natural substances in your blood stream, involved in blood clotting and fighting off infections, are related to age. Secondly, this study will see how physical activity, health status, body composition, and physical function affect this relationship.

How Many People Will Take Part In The Study?

A total of 100 people will complete this study in the General Clinical Research Center at the University of Oklahoma Health Sciences Center.

What Is Involved In The Study?

MEASUREMENTS

Health Status. We will obtain some information regarding your health. This will include recording your age, height, weight, sex, race, smoking habits, education level, physical ailments, and your self-assessment of your overall level of health. It will take approximately 20 minutes to obtain this information.

Questionnaires. Your physical activity level will be measured with a questionnaire, and your health-related quality of life will be measured with a questionnaire that asks about various aspects pertaining to your physical and mental health. The questionnaires will take approximately 15 minutes to complete.

Blood Tests. We will measure the levels of natural substances in your blood stream. These substances are made by cells in the body to fight off infections or injuries, and help in clotting of the blood. A small amount of blood, about 20 ml, will be taken from a vein in your forearm by trained personnel. This procedure will take approximately five minutes to complete.

Body Composition. A total body scan to determine body composition will be performed using dual energy x-ray absorptiometry (DEXA). This test is similar to an X-ray but only exposes you to approximately 1/10 of the radiation of a normal X-ray. This test is non-invasive and requires only that you lie still for the test to be completed. The DEXA scan lasts approximately 15 minutes.

Vascular Tests. You will be measured on these tests to assess your blood flow, blood pressure, and heart rate. These tests will require approximately 30 minutes to perform and will include the following:

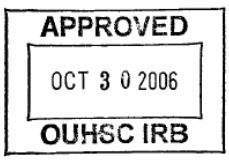
You will lie down for 10 minutes, after which leg blood flow, blood pressure in your arms and legs will be assessed at rest. The measurement of leg blood flow will be done by placing a small rubber band object around your leg calf muscle and inflating blood pressure cuffs around your thigh and ankle. Both blood pressure cuffs will be deflated after the resting measurement is obtained. Your arm blood pressure and heart rate will be measured automatically by a blood pressure machine. At the same time, your ankle blood pressure will be measured in the arteries in your foot by a Doppler machine.

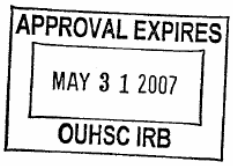
Following the resting measurements, we will perform a reactive hyperemia test. This test assesses the increase of blood flow occurring in the leg after the thigh blood pressure cuff is fully inflated for three minutes while you are lying down. After three minutes, the thigh blood pressure cuff is deflated and blood flow, blood pressure, and heart rate measurements will be measured as described above.

Following this test, a thigh blood pressure cuff will be fully inflated while you stand and perform heel-raises. The thigh blood pressure cuff will then be immediately deflated and you will lie down. Maximal leg blood flow, arm blood pressure, and heart rate will then be measured.

Functional Tests. You will perform functional tests which consist of walking, balancing, standing from a chair, and daily physical activity level. These tests will take approximately 30 minutes to complete. Your walking will be evaluated by a 4-meter walk test (about 12 feet). Your balance will be assessed by attempting to maintain your feet in a side-by-side, semi-tandem (heel of one foot beside the big toes of the other foot), and tandem (heel of one foot directly in front of the other foot) positions for 10 seconds each while you stand. Additionally, the time that you can stand on one foot with the other foot several inches off of the ground and your arms held loosely at your sides will be measured. Your ability to rise from a chair will be measured by having you fold your arms across your chest and stand up from a chair and sit down five times as quickly as possible.

To measure your physical activity level, you will wear two small (about the size of a pager) activity monitoring devices for at least two days. One device will be worn close to your waist (i.e., attached to your belt) and will measure the number of calories that you burn, while the other device will be fastened by elastic bands around your ankle and will measure the number of steps that you take. These devices will not restrict you from any activity; however, they should not be worn while sleeping or bathing.





How Long Will I Be In The Study?

During this study, you will make three visits within about two weeks. The visits will be the following:

- Visit 1: You will complete an initial screening to evaluate your health and medical history and a blood sample will be collected. This visit will take about one hour.
- Visit 2: Your second visit will be within one week after visit 1. You will complete a physician screening, questionnaires, body composition and vascular measurements described above. You will also have physical activity monitors placed around your waist and ankle for you to wear until Visit 3. This visit will take about two hours.
- Visit 3: Your third visit will be within one week after Visit 2. You will return the monitors, and complete the functional tests described above. This visit will take about one hour.

You have the option to decline any test or questionnaire that is part of the evaluation in this study. If you chose to not wear the activity monitors, then you will only have one visit during this study.

What Are The Risks of The Study?

RISKS / DISCOMFORTS There are minimal risks and discomforts involved during your participation in this research study. All research procedures will be conducted by trained clinical research personnel. The potential risk and discomforts associated with each test in this research study are listed below:

Health Status. There are no risks or discomforts associated with obtaining this information through an interview.

Questionnaires. There are no risks associated with obtaining information through the questionnaires.

Radiation. If you participate in this research you will receive radiation exposure from a DEXA scan (a type of x-ray) that you will not receive if you choose not to participate. The amount of additional radiation to which you will be exposed is approximately 1/5000th of the amount of radiation that an x-ray technologist is permitted to receive in one year.

Blood Draws. There is a minimal risk of having local discomfort, bruising, swelling, and in rare instances infection and fainting from the needle stick to obtain about 10 tablespoons of blood to measure the blood profiles.

Vascular Tests. There are no risks associated with the leg blood flow test. However, several minutes of discomfort can be expected during the leg blood flow test.

due to inflation of the blood pressure cuffs on your leg. Furthermore, there may be minor discomfort during the measurement of arm blood pressure due to the inflation of the blood pressure cuff on your arm.

Functional Tests. There are minimal risks associated with performing the functional tests. The test administrator may decide to not conduct any test that you may have trouble completing.

Physical Activity Monitors. There are no risks associated with wearing the physical activity monitors, nor will they affect your day-to-day activities. The only potential discomfort is the inconvenience of wearing a small device around your ankle.

Pregnancy / Reproductive Risks For Women and Men:

Pregnant women should not be exposed to radiation. If you become pregnant or suspect that you are pregnant during this study, you should immediately inform the study personnel and a pregnancy test will be done. If pregnancy is confirmed, you may be withdrawn from the study. As previously stated, there is a minimal risk for exposure to radiation. However, besides pregnancy, this risk is not detrimental to the reproductive system in either males, or females.

Are There Benefits to Taking Part in The Study?

If you agree to take part in this study, there may or may not be direct medical benefit to you. You will receive information about your cardiovascular function, health status, physical activity, physical function, and body composition at the end of the testing. Additionally, your participation will make a contribution to science because the information obtained will help us understand what factors are related to cardiovascular function in men and women of various ages. This information may be used in future studies aimed at improving cardiovascular function. Finally, you will have the opportunity to interact with investigators and ask questions regarding your measurements.

What Other Options Are There?

Instead of being in this study, you have the option to not participate.

What About Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the General Clinical Research Center (GCRC), the US Food & Drug administration, the National Institutes of Health, and the OUHSC Institutional Review Board. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

What Are the Costs?

Transportation will be your only cost. Parking is free, as are all medical tests.

Will I Be Paid For Participating in This Study?

You will not be paid for participating in this study.

What if I am Injured or Become Ill While Participating in this Study?

In the case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company may be expected to pay the usual charge for this treatment. No funds have been set aside by The University of Oklahoma Health Sciences Center or the Department of Health and Exercise Science to compensate you in the event of injury.

What Are My Rights As a Participant?

Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. If you agree to take part and then decide against it, you can withdraw for any reason. Please be sure to discuss leaving the study with the principal investigator or your regular physician. Leaving the study will not result in any penalty or loss of benefits that you would otherwise receive.

We will tell you about any new information that may affect your health, welfare or willingness to stay in this study.

You understand that you have the right to access the medical information that has been collected about you as a part of this research study. However, you agree that you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?

If you have questions about the study, Muhammed Firdaus, M.D. is available to be contacted 24 hours a day at 405-271-3050. For questions about your rights as a research subject, contact the OUHSC Director, Human Research Participant Protection Program at 405-271-2045.

Signature:

In the future, researchers involved with this project may need more information about you, or may ask you if you are willing to participate in other research studies. Please check below to indicate whether or not you may be contacted within the next few years by the investigators conducting this study. If you agree to be re-contacted, you may still change your mind about providing additional information, or in participating in other research studies in the future.

_____ I may be re-contacted for information or a future study.

_____ I may not be re-contacted for information or a future study.

Subject Signature:_____ Date: _____

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or institution from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

Research Subject: _____ Date:_____

Subject's Printed Name _____

Person Obtaining
Informed Consent:_____ Date:_____

APPENDIX B

Health Insurance Portability and Accountability Act (HIPAA)
Participant Form

AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required. Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **The age-related influences on inflammatory markers: a pilot study.**

Leader of Research Team: **Andrew W. Gardner, Ph.D.**

Address: **General Clinical Research Center, University of Oklahoma Health Sciences Center**

1122 N.E. 13th Street, ORI-W 1400. Oklahoma City, OK 73117

Phone Number: **(405)325-1371**

If you decide to join this research project, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private Information To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to help evaluate the most important factors that are related to chronic inflammation and physical activity.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research sponsor, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS). The researchers may also share your private information with family members.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information

based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OUHSC.

Revoking Permission. If you give the OUHSC researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will never end. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma Health Sciences Center
PO Box 26901, Oklahoma City, OK 73190
If you have questions call: (405) 271-2511

APPROVED
JUN 2 6 2006
OUHSC IRB

APPROVAL EXPIRES
MAY 3 1 2007
OUHSC IRB

Version: 6/02/03
IRB Office Version: 010605

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Andrew W. Gardner, Ph.D., permission to share your private information for the research project called The age-related influences on inflammatory markers: a pilot study.

Patient/Subject Name: _____

_____ _____
Signature of Patient-Subject Date
or Parent if subject is a child

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Subject, provide a description of the relationship to the Patient-Subject and the Authority to Act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB No.: 11841

APPROVED
JUN 2 6 2006
OUHSC IRB

APPROVAL EXPIRES
MAY 3 1 2007
OUHSC IRB

APPENDIX C

Institutional Review Board
Approval Letters

The University of Oklahoma
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 11841
Meeting Date: October 25, 2004
Approval Date: February 02, 2005

February 07, 2005

Andrew Gardner, Ph.D.
Univ of Oklahoma, Dept of HSS
1401 Asp Avenue, Room 110
Norman, OK 73019

RE: The Age-Related Influences on Inflammatory Markers: A Pilot Study

Dear Dr. Gardner:

The University of Oklahoma Health Sciences Center's Institutional Review Board (IRB) reviewed the above-referenced research protocol at its regularly scheduled meeting on October 25, 2004. It is the IRB's judgement that the rights and welfare of the individuals who may be asked to participate in this study will be respected; that the proposed research, including the process of obtaining informed consent, will be conducted in a manner consistent with the requirements of 45 CFR 46 or 21 CFR 50 & 56, as amended; and that the potential benefits to participants and to others warrant the risks participants may choose to incur.

On behalf of the IRB, I have verified that the specific changes requested by the convened IRB have been made. Therefore, on behalf of the Board, I have granted final approval for this study.

This letter documents approval to conduct the research as described:

Survey Instrument Dated: October 05, 2004 Physical Activity Scale
Survey Instrument Dated: October 05, 2004 MOS-36 Questionnaire
Radiation Safety CA Ltr Dated: January 18, 2005
IRB Application Dated: December 14, 2004
Consent form - Subject Version : 3 Dated: January 28, 2005
Priv - Research Auth 1 Dated: January 06, 2005
Protocol Version : 3 Dated: January 28, 2005

As principal investigator of this protocol, it is your responsibility to make sure that this study is conducted as approved by the IRB. Any modifications to the protocol or consent form, initiated by you or by the sponsor, will require prior approval, which you may request by completing a protocol modification form.

It is a condition of this approval that you report promptly to the IRB any serious, unanticipated adverse events experienced by participants in the course of this research, whether or not they are directly related to the study protocol. These adverse events include, but may not be limited to, any experience that is fatal or immediately life-threatening, is permanently disabling, requires (or prolongs) inpatient hospitalization, or is a congenital anomaly, cancer or overdose. For multi-site protocols, the IRB must be informed of serious adverse events at all sites.

The approval granted expires on September 30, 2005. Should you wish to maintain this protocol in an active status beyond that date, you will need to provide the IRB with an IRB Application for Continuing Review (Progress Report) summarizing study results to date. The IRB will request a progress report from you approximately three months before the anniversary date of your current approval.

If you have questions about these procedures, or need any additional assistance from the IRB, please call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu. Finally, please review your professional liability insurance to make sure your coverage includes the activities in this study.

Sincerely yours,

Martina Jelley, M.D., M.S.P.H.
Chair, Institutional Review Board

Ltr_Prot_Fappv_B

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73190 • (405) 271-2045 • FAX: (405) 271-1677

The University of Oklahoma
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 11841
Approval Date: August 29, 2005

August 31, 2005

Andrew Gardner, Ph.D.
O'Donoghue Rehabilitation Institute
1122 N.E. 13th, ORI 1400
Oklahoma City, OK 73117-1039

RE: The Age-Related Influences on Inflammatory Markers: A Pilot Study

Dear Dr. Gardner:

Thank you for completing and returning the IRB Application for Continuing Review (Progress Report) for the above-referenced study. You have indicated that the study is still active. At the meeting held August 29, 2005 the Institutional Review Board (IRB) reviewed and approved the Progress Report and determined that this study was appropriate for continuation.

This letter documents approval to conduct the research as described in:
Cont Review Form Dated: July 14, 2005
Protocol Dated: July 14, 2005
Consent form - Subject Version : 3 Dated: January 28, 2005
Priv - Research Auth 1 Dated: January 06, 2005

Please remember that any change in the protocol, consent document or other recruitment materials (adverstisements, etc.) must be approved by the IRB prior to its incorporation into the study procedures. Submit a completed Protocol Modification form to the IRB office. Any serious, unanticipated adverse events involving participants enrolled in this study at OUHSC must be reported within four working days on the IRB Adverse Event Report form. Any event which involves the death of a participant must be reported no later than the next working day. All other adverse events (from outside sites) must be forwarded to the IRB office within 14 working days of receipt.

Approximately three months prior to the expiration date of this approval, you will be contacted by the IRB staff about procedures necessary to maintain this approval in an active status. Although every attempt will be made to notify you when a study is due for review, it is the responsibility of the investigator to assure that their studies receive review prior to expiration.

The approval of this study expires on July 31, 2006 and must be reviewed by the convened IRB prior to this time if you wish to remain in an active status. Federal regulations do not allow for extensions to be given on the expiration date.

If we can be of further assistance, please call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu.

Sincerely yours,

Martina Jelley, M.D., M.S.P.H.
Chair, Institutional Review Board

Ltr_Prog_Appv_Active

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73190 • (405) 271-2045 • FAX: (405) 271-1677

The University of Oklahoma
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 11841
Amendment Approval Date: October 30, 2006

October 31, 2006

Andrew Gardner, Ph.D.
O'Donoghue Rehabilitation Institute
1122 N.E. 13th, ORI 1400
Oklahoma City, OK 73117-1039

RE: IRB No. 11841: The Age-Related Influences on Inflammatory Markers: A Pilot Study

Dear Dr. Gardner:

On behalf of the Institutional Review Board (IRB), I have reviewed your protocol modification form. It is my judgement that this modification allows for the rights and welfare of the research subjects to be respected. Further, it has been determined that the study will continue to be conducted in a manner consistent with the requirements of 45 CFR 46 or 21CFR 50 56 as amended; and that the potential benefits to subjects and others warrant the risks subjects may choose to incur.

This letter documents approval to conduct the research as described in:

Amend Form Dated: October 26, 2006
Protocol Version : 4 Dated: October 26, 2006
Consent form - Subject Version : 4 Dated: October 26, 2006
Recruitment flyer Dated: October 26, 2006 Cardiovascular Study

Amendment Summary:
1. Addition of new media flyer.
2. Revised exclusion criteria to define metabolic syndrome as having 3 or more characteristics of metabolic syndrome.
3. Revise protocol to move blood draw for the inflammatory markers and physician examination to the second visit.
4. Revised consent to update the order of the tests listed in the outline of each visit.

This letter covers only the approval of the above referenced modification. All other conditions, including the original expiration date, from the approval granted June 26, 2006 are still effective.

Any proposed change in approved research including the protocol, consent document, or other recruitment materials cannot be initiated without IRB approval except when necessary to eliminate immediate hazards to participants. Changes in approved research initiated without IRB approval to eliminate immediate hazards to the participant must be promptly reported to the IRB. Completion of approved research must be reported to the IRB.

If consent form revisions are a part of this modification, you will be provided with a new stamped copy of your consent form. Please use this stamped copy for all future consent documentation. Please discontinue use of all outdated versions of this consent form.

If you have any questions about these procedures or need additional assistance, please do not hesitate to call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu.

Sincerely yours,

Vicki Lampley, M.D., M.P.H.
Vice Chair, Institutional Review Board

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73190 • (405) 271-2045 • FAX: (405) 271-1677

CR#3

The University of Oklahoma
Health Sciences Center
INSTITUTIONAL REVIEW BOARD

IRB Number: 11841
Approval Date: April 11, 2007

April 13, 2007

Andrew Gardner, Ph.D.
O'Donoghue Rehabilitation Institute
1122 N.E. 13th, ORI 1400
Oklahoma City, OK 73117-1039

RE: The Age-Related Influences on Inflammatory Markers: A Pilot Study

Dear Dr. Gardner:

Thank you for completing and returning the IRB Application for Continuing Review (Progress Report) for the above-referenced study. You have indicated that the study is still active. At the meeting held March 26, 2007 the Institutional Review Board (IRB) reviewed and approved the Progress Report and determined that this study was appropriate for continuation.

This letter documents approval to conduct the research as described in:
Cont Review Form Dated: March 14, 2007
Protocol Version : 4 Dated: October 26, 2006
Consent form - Subject Version : 4 Dated: October 26, 2006
Priv - Research Auth 1 Dated: January 06, 2005

Please remember that any change in the protocol, consent document or other recruitment materials (advertisements, etc.) must be approved by the IRB prior to its incorporation into the study procedures. Submit a completed Protocol Modification form to the IRB office. Any serious, unanticipated adverse events involving participants enrolled in this study at OUHSC must be reported within four working days on the IRB Adverse Event Report form. Any event which involves the death of a participant must be reported no later than the next working day. All other adverse events (from outside sites) must be forwarded to the IRB office within 14 working days of receipt.

Approximately three months prior to the expiration date of this approval, you will be contacted by the IRB staff about procedures necessary to maintain this approval in an active status. Although every attempt will be made to notify you when a study is due for review, it is the responsibility of the investigator to assure that their studies receive review prior to expiration.

The approval of this study expires on February 29, 2008 and must be reviewed by the convened IRB prior to this time if you wish to remain in an active status. Federal regulations do not allow for extensions to be given on the expiration date.

If we can be of further assistance, please call the IRB office at (405) 271-2045 or send an email to irb@ouhsc.edu.

Sincerely yours,

Vicki Lampley, M.D., M.P.H.
Vice Chair, Institutional Review Board

Ltr_Prog_Appv_Active

Post Office Box 26901 • 1000 S.L. Young Blvd., Room 176
Oklahoma City, Oklahoma 73190 • (405) 271-2045 • FAX: (405) 271-1677

APPENDIX D

General Clinical Research Center Advisory Committee
Approval Letter

The University of Oklahoma
Health Sciences Center
GENERAL CLINICAL RESEARCH CENTER

FILE COPY

December 13, 2004

Dr. Andrew Gardner
Health & Sports Sciences
HHC 110

Re: The Age-Related Influences on Inflammatory Markers: A Pilot Study

Dear Dr. Gardner:

The above referenced protocol was reviewed and approved at the December GCRC Advisory Committee meeting. It is imperative that the GCRC is notified of any modifications in this protocol.

All correspondence to the IRB regarding this protocol must be copied and sent to the GCRC. Before any subjects can be seen for this protocol, it is your responsibility as the Principal Investigator to:

- Ensure the GCRC has copies of current consent forms, HIPAA authorization forms and IRB approval letters related to the protocol.
- Contact Melissa Hackney, Patient Care Manager, to schedule an in-service for the GCRC clinical staff.
- Ensure orders have been written for the protocol and signed off by the protocol M.D. and the GCRC Patient Care Manager.
- Contact John Eckmann to discuss data management issues.

In order to secure continuing funding of our GCRC, it is necessary to apprise reviewing bodies of our research accomplishments. Accordingly, it is essential that all manuscripts submitted for publication contain the following acknowledgment and a copy of the manuscript forwarded to the GCRC:

> "This work supported in part by the University of Oklahoma Health Sciences Center General Clinical Research Center grant M01-RR14467, National Center for Research Resources, National Institutes of Health."

If you have any questions or need assistance, please don't hesitate to contact me at 271-4272 or email philip-comp@ouhsc.edu.

Sincerely,

Philip C. Comp, MD
GAC Chairman

Mailing Address: P. O. Box 26901, ORI-W1400, Oklahoma City, OK 73190
Physical Address: 1122 N.E. 13th, Oklahoma City, OK 73117, Phone: (405) 271-4272 Fax: (405) 271-4273

APPENDIX E

Telephone Screening Form

SUBJECT DATA / MEDICAL HISTORY

Name:_____ Date:_____

Address:_____ Telephone:_____

_____ Email:_____

Age: _____ Race: 1 = Caucasian 4 = Asian
2 = African American 5 = American Indian
Sex: _____ 3 = Hispanic 6 = Other

Approx. Height:_____ Weight:_____ (Approx.) BMI:_____
Approx. Waist Circum. _____

Smoking History (Circle One):
1. Never
2. Past:
   # of yrs_____
   # of packs/day_____
   # of yrs quit_____
3. Current:

Medications_____
_____

Has the subject had any of the following medical conditions?

	YES	NO
Myocardial Infarct (Heart Attack):	____	____
Angina (Chest Pain):	____	____
Cerebral Vascular Accident (Stroke):	____	____
Aortic Aneurysm (thoracic or abdom.):	____	____
Hyperlipidemia (High cholesterol):	____	____
Pulmonary Disease (Lung disease):	____	____
Dyspnea (Shortness of breath):	____	____
Diabetes:	____	____
Hypo/Hyperthyroidism:	____	____
Active Cancer:	____	____
Renal Failure (Kidney disease):	____	____
Liver Disease:	____	____
Gastro-intestinal Disease:	____	____
(i.e.,Ulcers, GERD, Chronn's, Colitis)		
Arthritis:	____	____
Lupus:	____	____
Hypertension (High blood pressure):	____	____
Approximate B.P. = _____		
Congestive Heart Failure (CHF):	____	____
Lower Extremity Revascularization		
(leg operation for circulation):	____	____
Claudication (Calf pain while walking):	____	____
Living Independently at home:	____	____
Surgery within the past 3 months:	____	____
Regular/Recent Alcohol use:	____	____
(within past 10 days)		
Recent vaccination (influenze, pneumovax, etc.):	____	____
Colds or Infections with past 14 days:	____	____

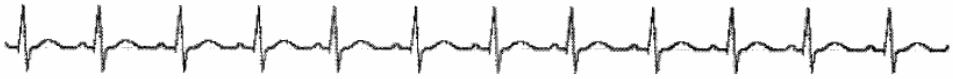
**If subject qualifies, set up appt. for 1st visit with Dr. Firdaus on a Tuesday morning. This is a fasting visit and all subjects shall refrain from stremuous activity/exercise 48 hours prior to each visit (specifically visit 2).*

APPENDIX F

Recruitment Flyer

Cardiovascular Study

The University of Oklahoma, Health and Exercise Sciences Department, is performing a study to investigate the effects of age, physical function, blood vessel function, and body fat on inflammatory substances in the blood stream in healthy adults.



Healthy men and women volunteers, between the ages of 20 and 90 years, are needed to complete this study. You will receive information regarding your cardiovascular health and percent body fat.

If interested, please contact: Luke Acree
(405)325-6839
email: lacree@ou.edu

APPROVAL
JUL 2 0 2005
OUHSC IRB

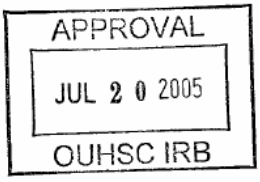
Luke Acree
(405) 325-6839
lacree@ou.edu

APPENDIX G

Recruitment Email

TO: All OUHSC Faculty and Staff
Subject Line: Cardiovascular Study

Volunteers who are 20 to 90 years of age and healthy are needed for a research study being conducted at the General Clinical Research Center. The purpose of the study is to examine the effects of aging on naturally occurring markers of inflammation in the blood stream. You will receive a report that includes information including basic cardiovascular measurements as well as a percent body fat assessment, after your participation is complete. If interested, contact Luke Acree at (405) 325-6839 or lacree@ou.edu for more information.

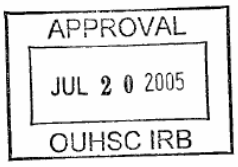


APPENDIX H

Recruitment Newspaper Advertisement

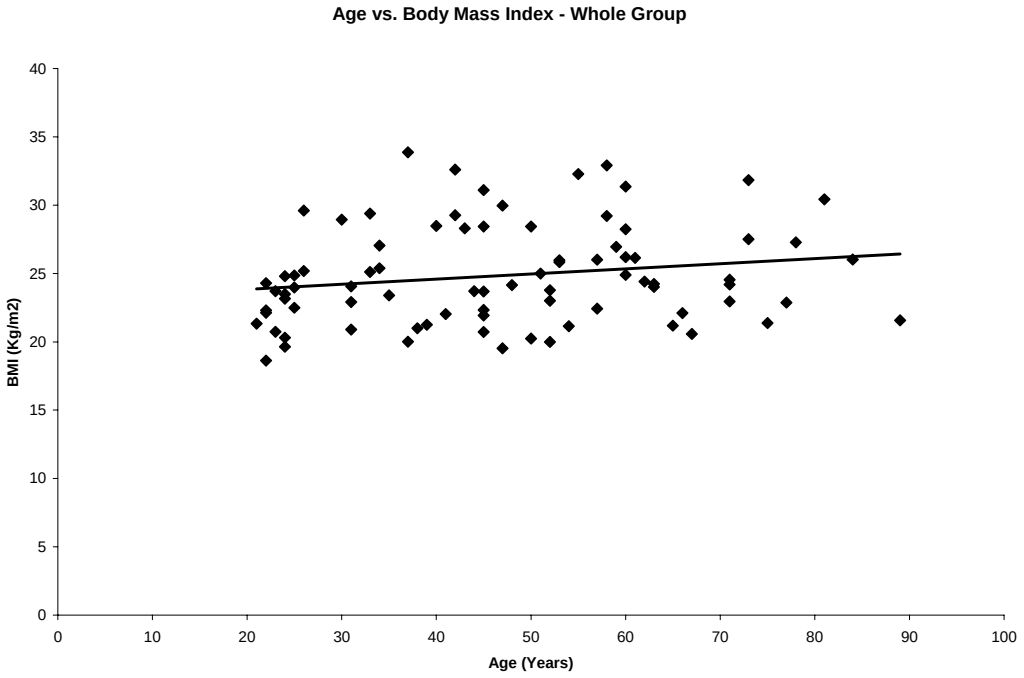
NEWSPAPER ADVERTISEMENTS

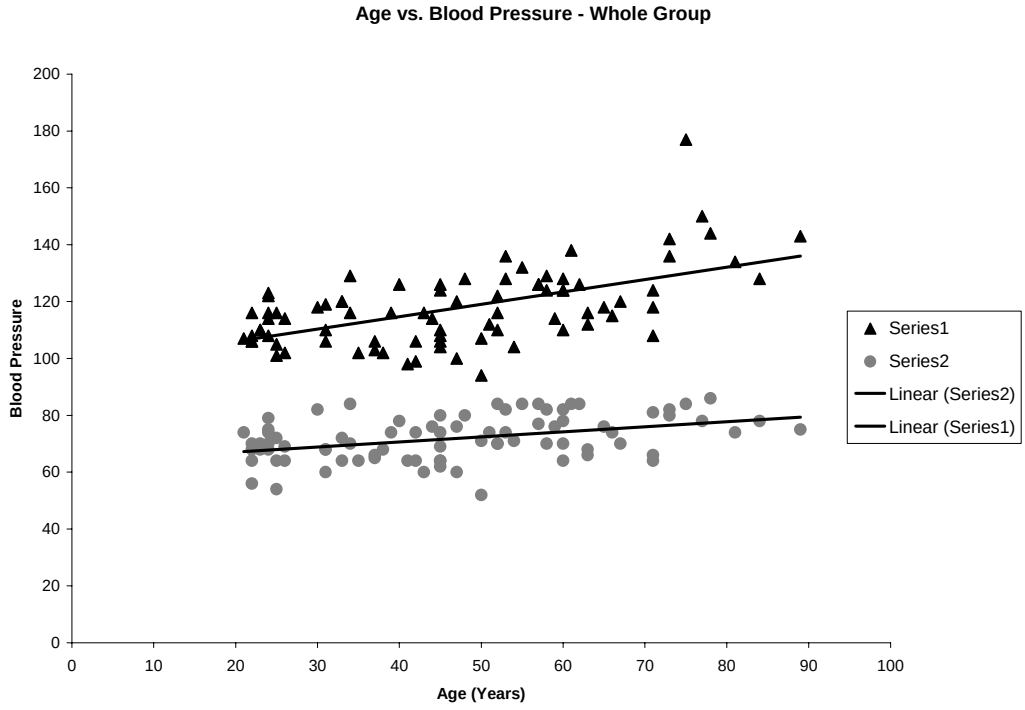
Participate in a research study at the OU Health Sciences Center, and receive a
CARDIOVASCULAR HEALTH ASSESSMENT
at no cost.
Volunteers must be between 20 and 90 years of age and healthy. Please call (405) 325-6839 for information. The University of Oklahoma is an equal opportunity institution.

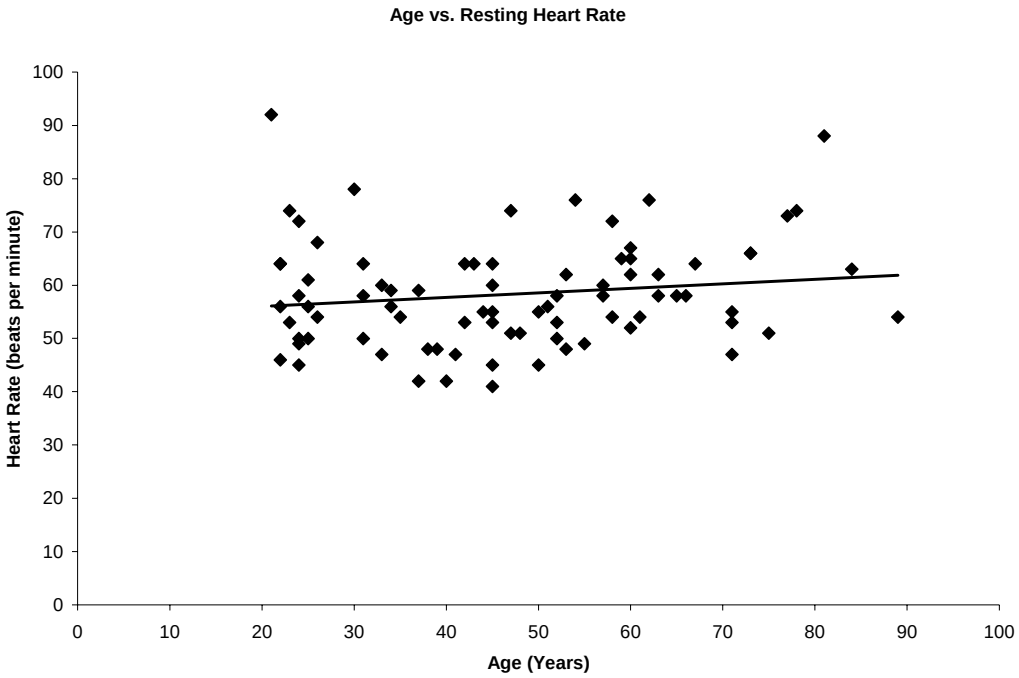


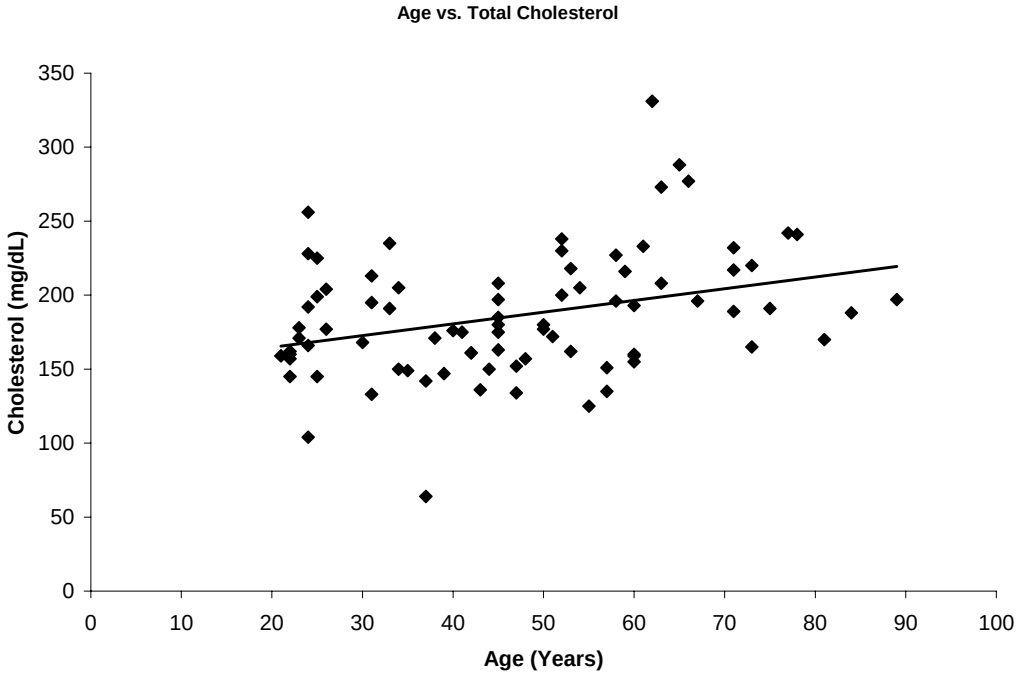
APPENDIX I

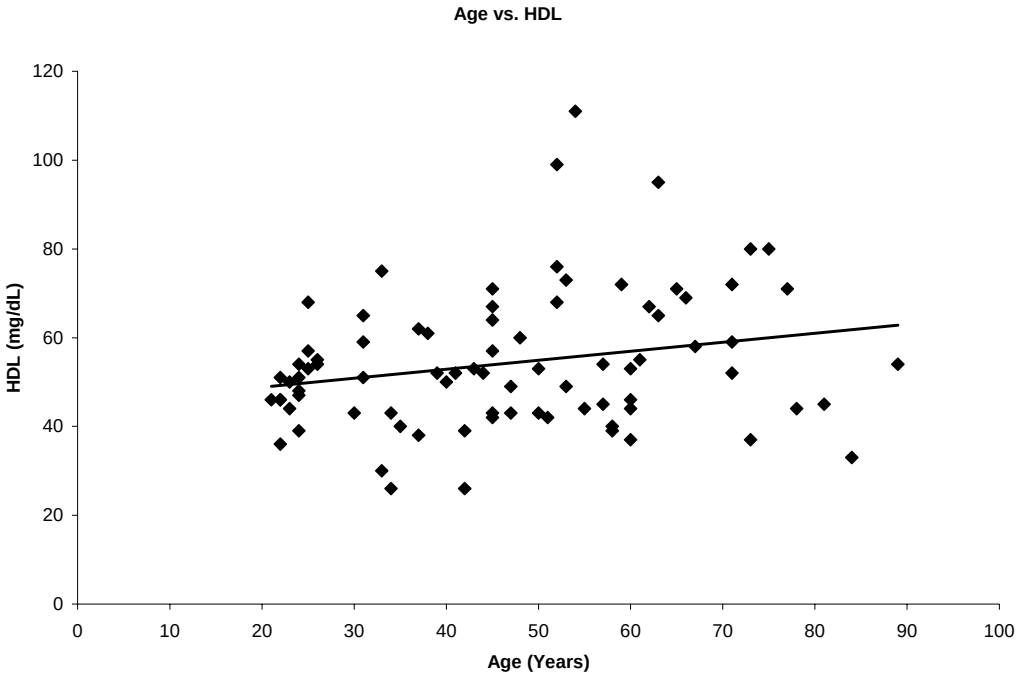
Scatter Plot
Age vs. Physical Characteristics

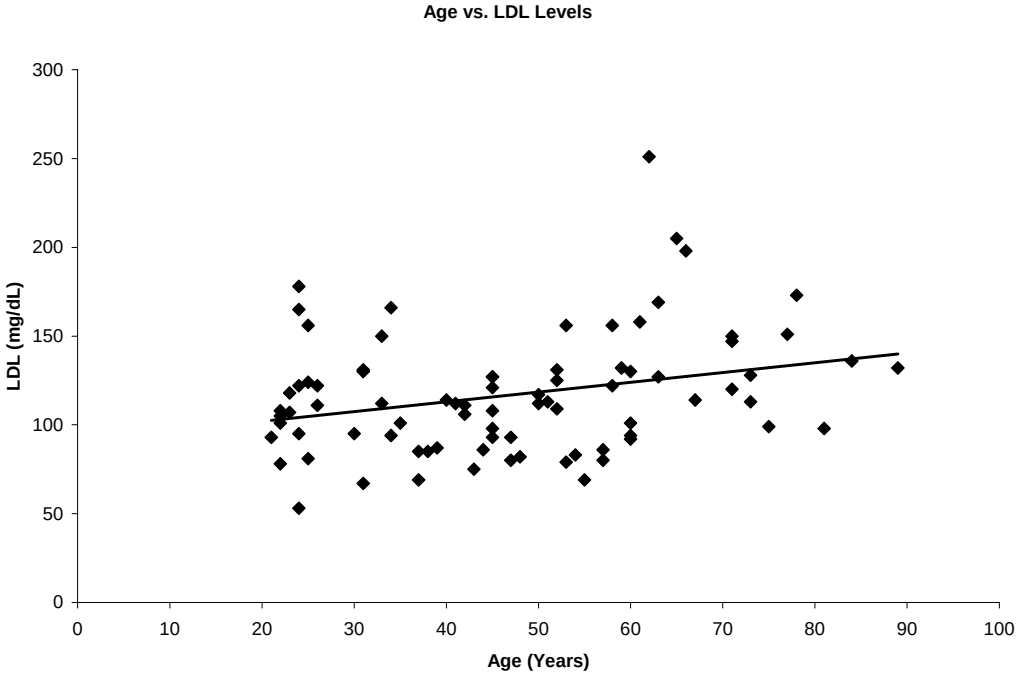

Age vs. Body Mass Index - Whole Group
BMI (Kg/m2)
Age (Years)
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
70
80
90
100

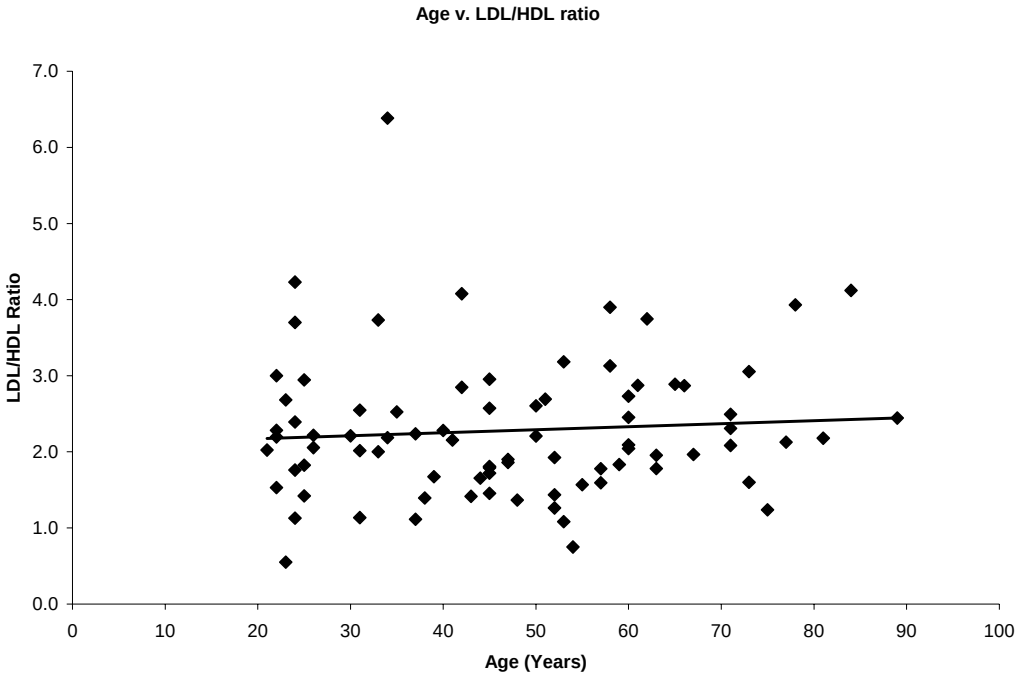

Age vs. Blood Pressure - Whole Group
Blood Pressure
Age (Years)
0
20
40
60
80
100
120
140
160
180
200
0
10
20
30
40
50
60
70
80
90
100
Series1
Series2
Linear (Series2)
Linear (Series1)

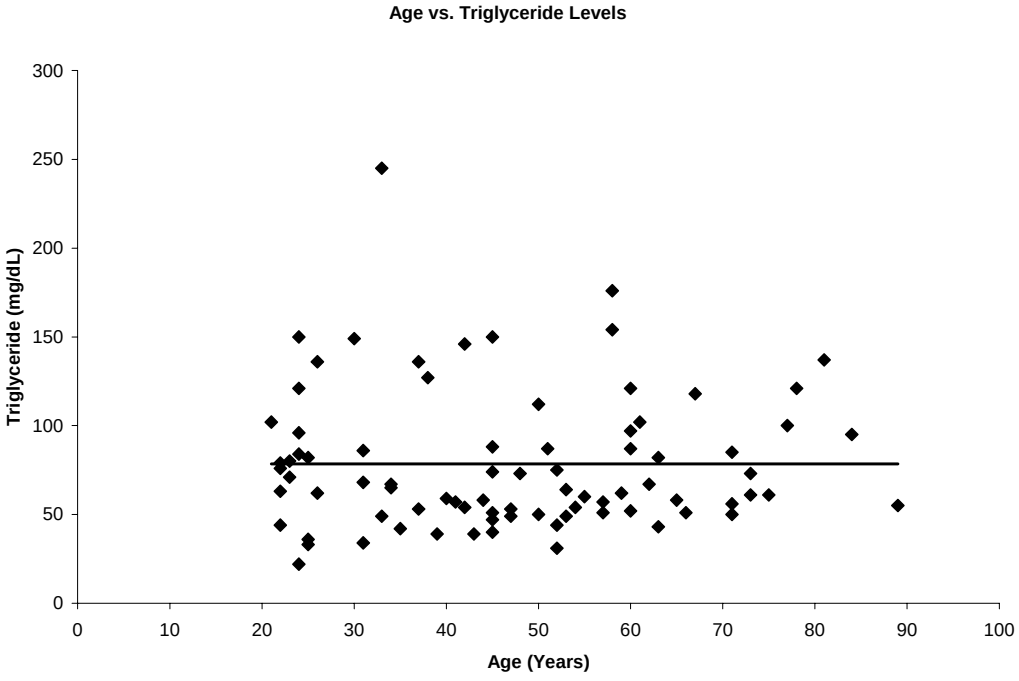

Age vs. Resting Heart Rate
Heart Rate (beats per minute)
Age (Years)
0
10
20
30
40
50
60
70
80
90
100


Age vs. Total Cholesterol
Cholesterol (mg/dL)
Age (Years)
0
50
100
150
200
250
300
350
10
20
30
40
60
70
80
90


Age vs. HDL
HDL (mg/dL)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

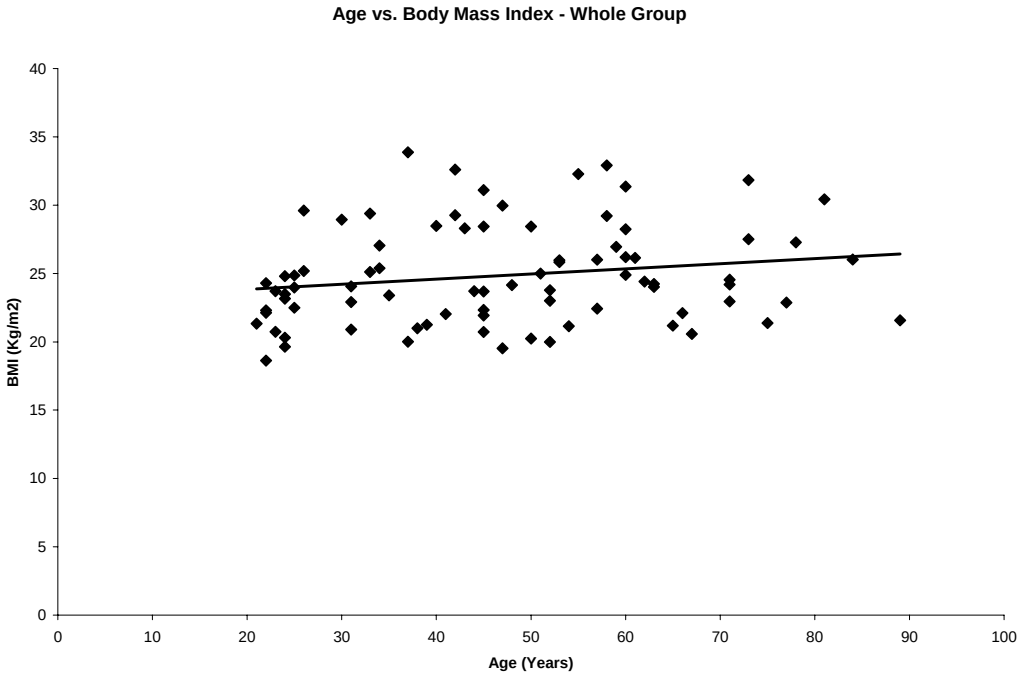

Age vs. LDL Levels
LDL (mg/dL)
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

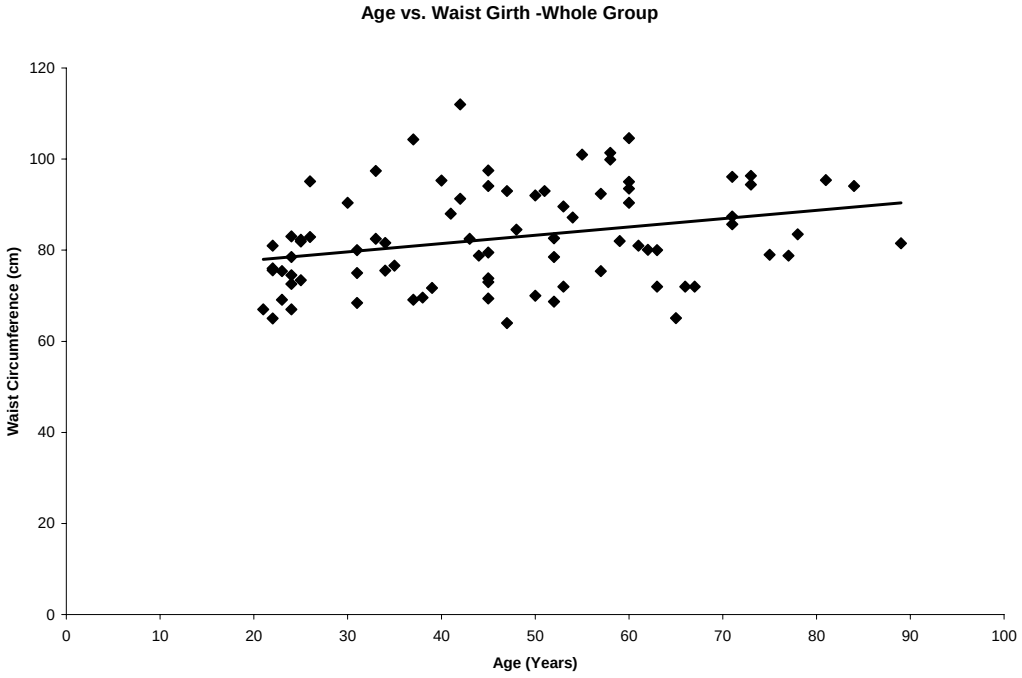

Age v. LDL/HDL ratio
LDL/HDL Ratio
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

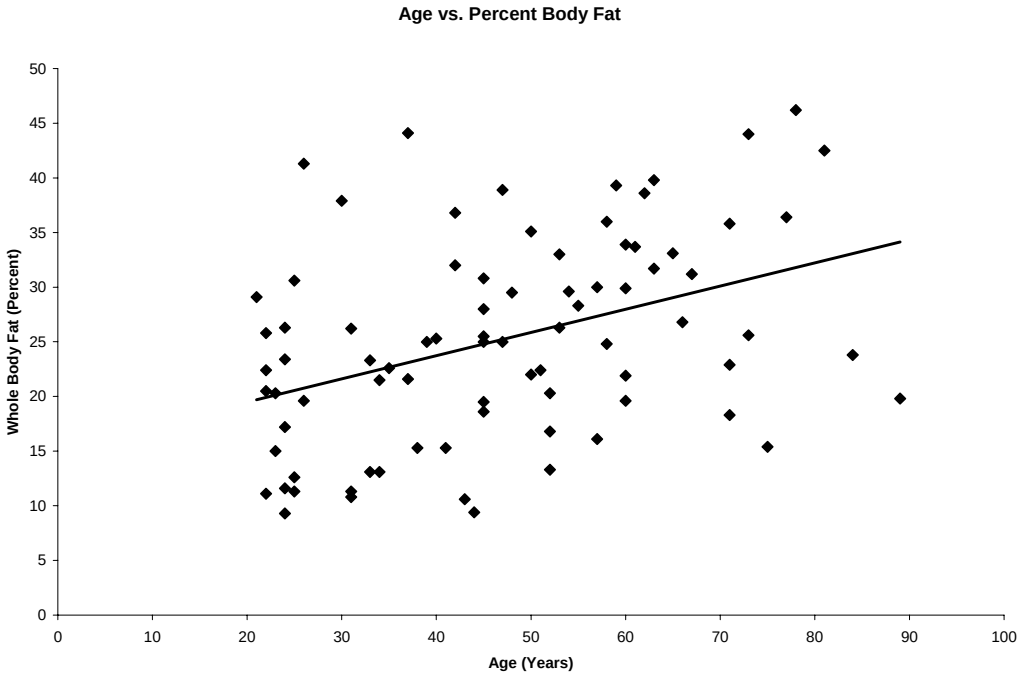

Age vs. Triglyceride Levels
Triglyceride (mg/dL)
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

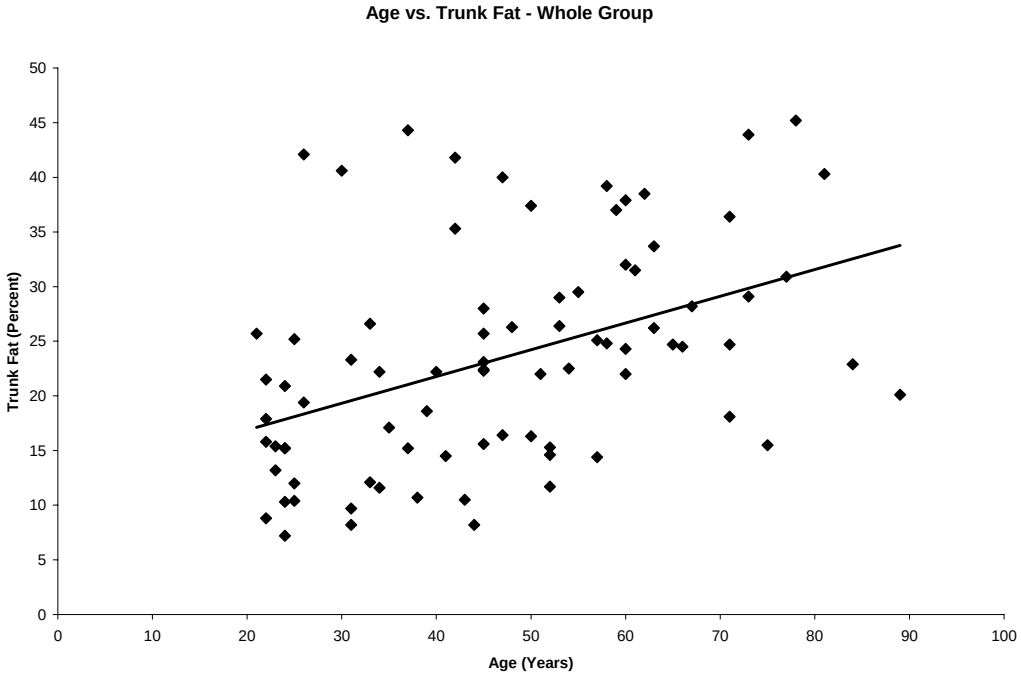
APPENDIX J

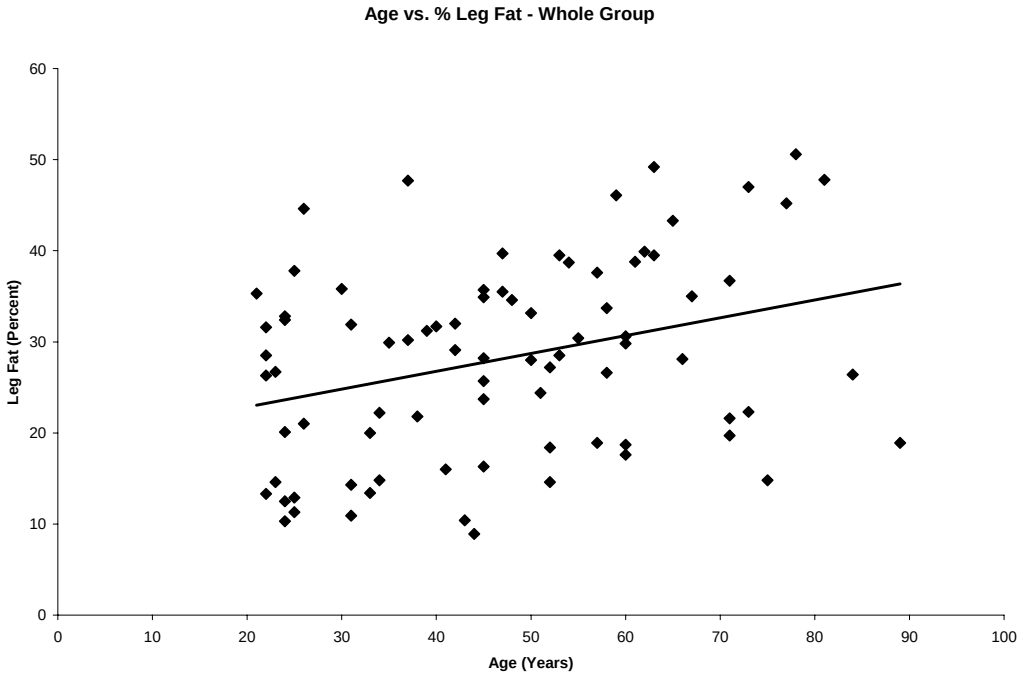
Scatter Plots
Age vs. Body Composition Measurements

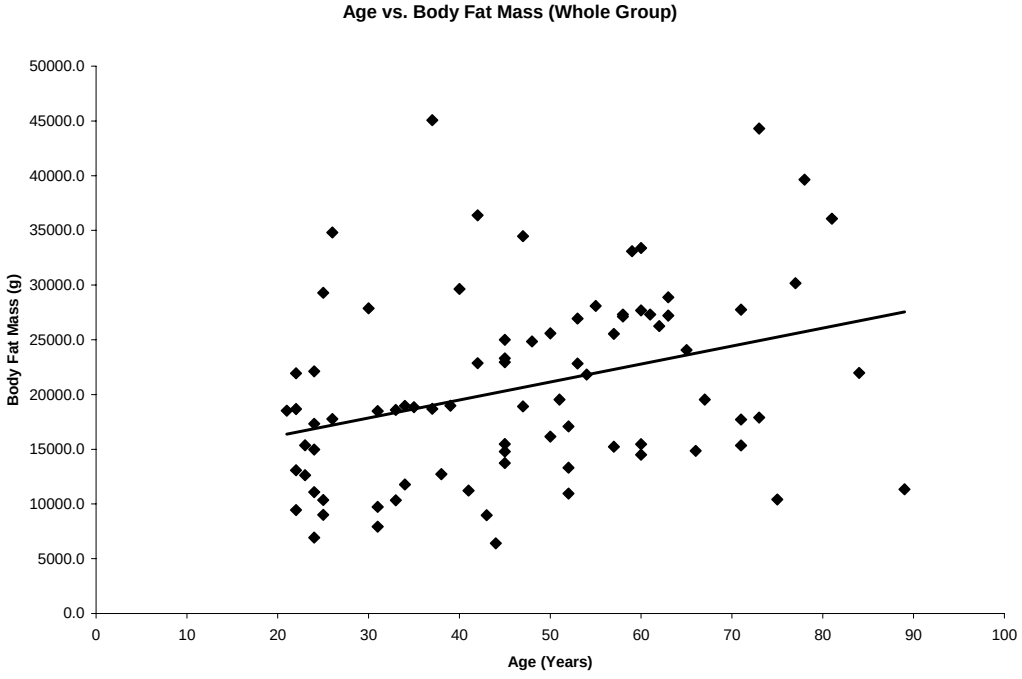

Age vs. Body Mass Index - Whole Group
BMI (Kg/m2)
Age (Years)
0
5
10
15
20
25
30
35
40
0
10
20
30
40
50
60
70
80
90
100


Age vs. Waist Girth -Whole Group
Waist Circumference (cm)
Age (Years)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100


Age vs. Percent Body Fat
Whole Body Fat (Percent)
Age (Years)
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100


Age vs. Trunk Fat - Whole Group
Trunk Fat (Percent)
Age (Years)
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100

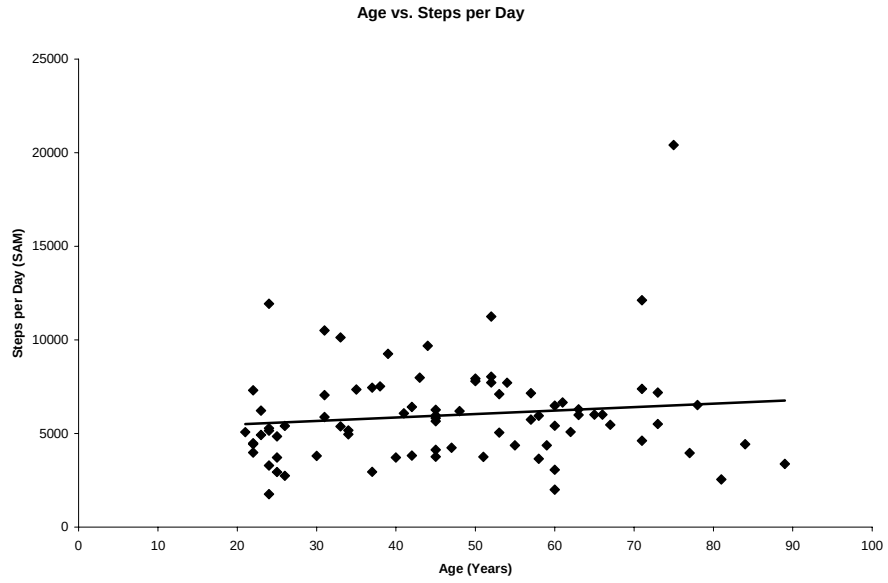

Age vs. % Leg Fat - Whole Group
Leg Fat (Percent)
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

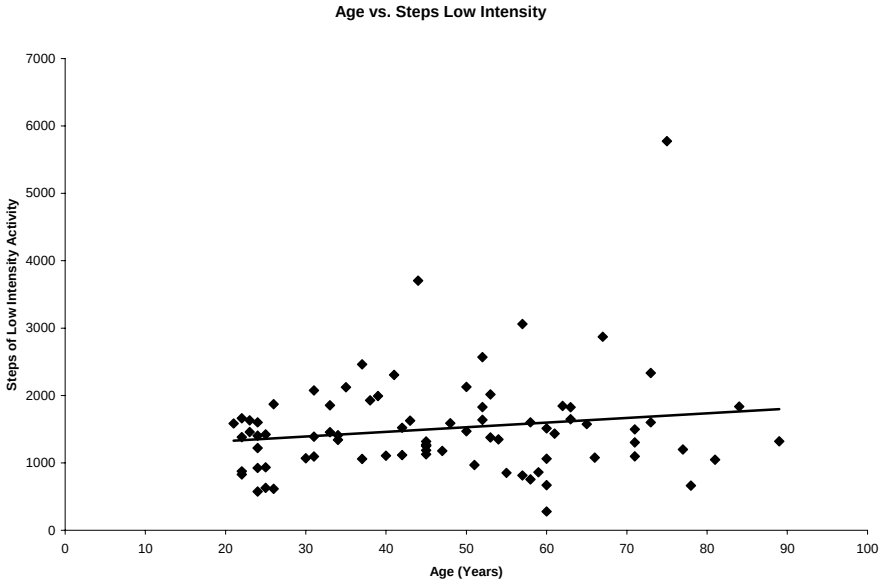

Age vs. Body Fat Mass (Whole Group)
Body Fat Mass (g)
0.0
5000.0
10000.0
15000.0
20000.0
25000.0
30000.0
35000.0
40000.0
45000.0
50000.0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

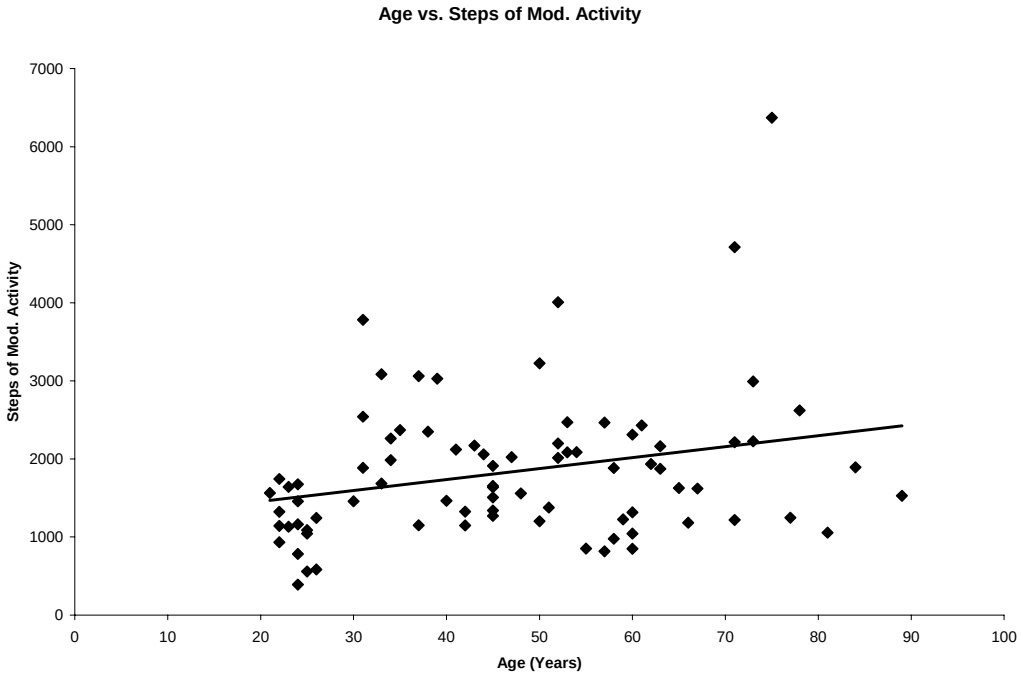

Age vs. Trunk Fat Mass (Whole Group)
Trunk Fat Mass (g)
30000.0
25000.0
20000.0
15000.0
10000.0
5000.0
0.0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

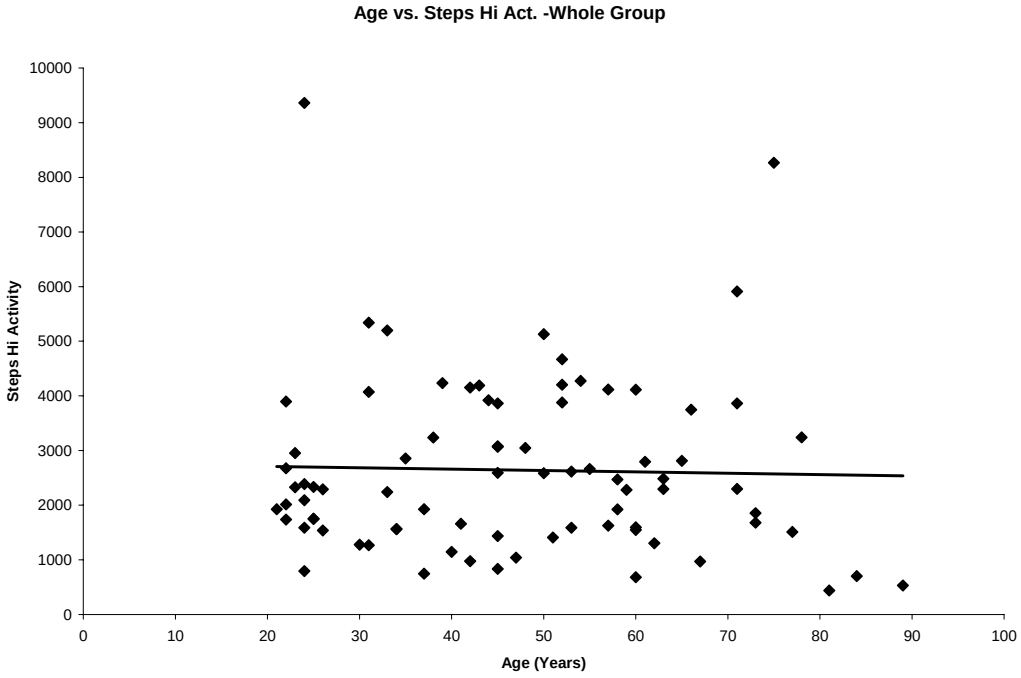
APPENDIX K

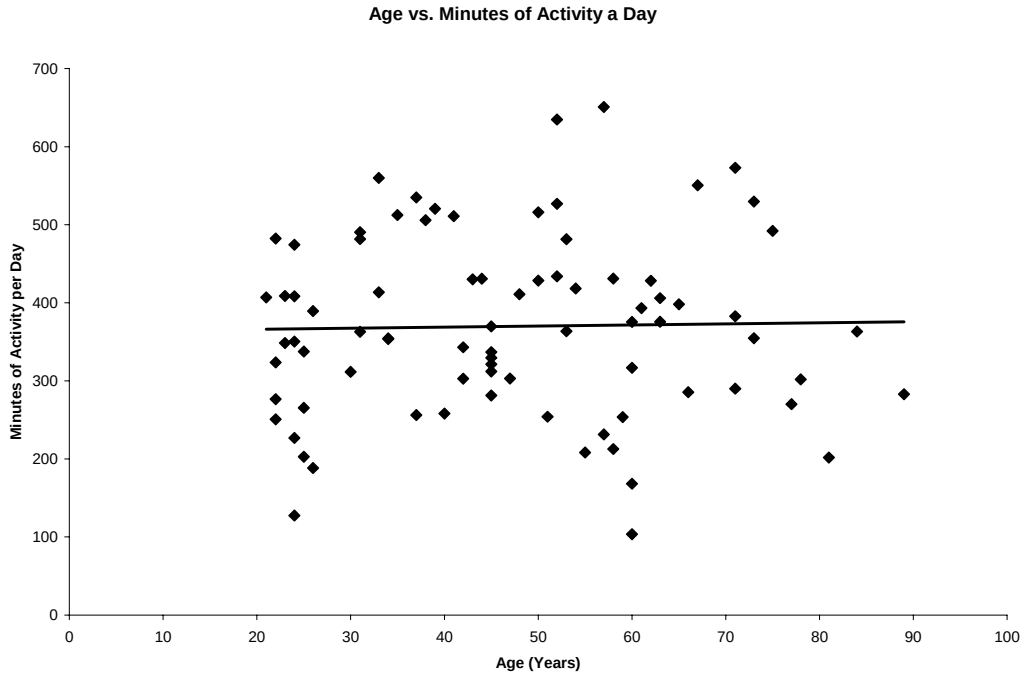
Scatter Plots
Age vs. Physical Activity Measurements

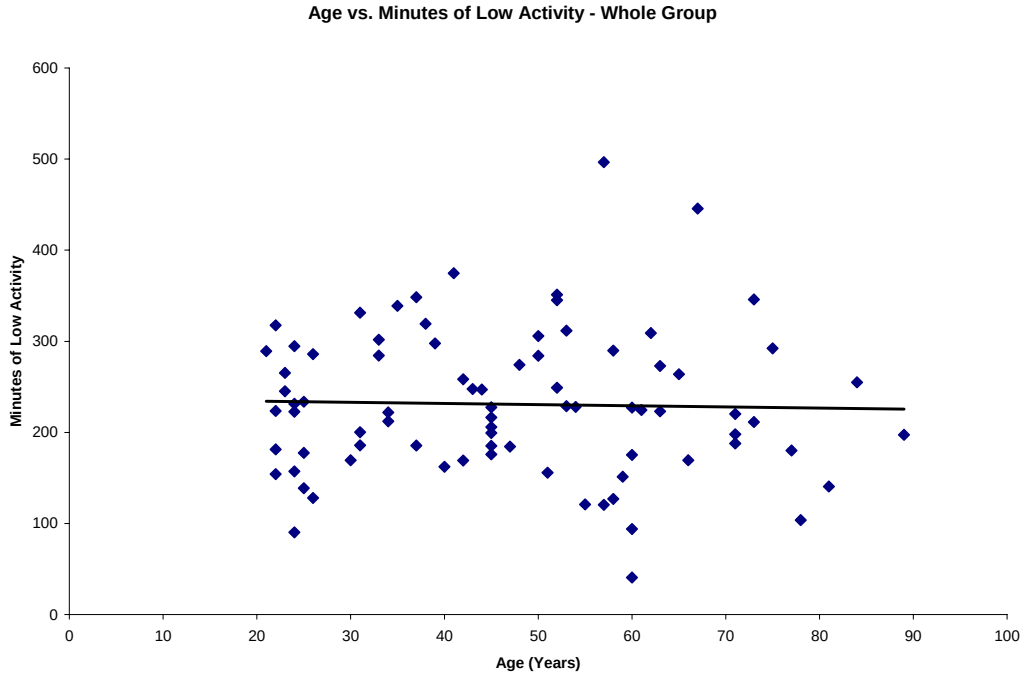

Age vs. Steps per Day
Steps per Day (SAM)
25000
20000
15000
10000
5000
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

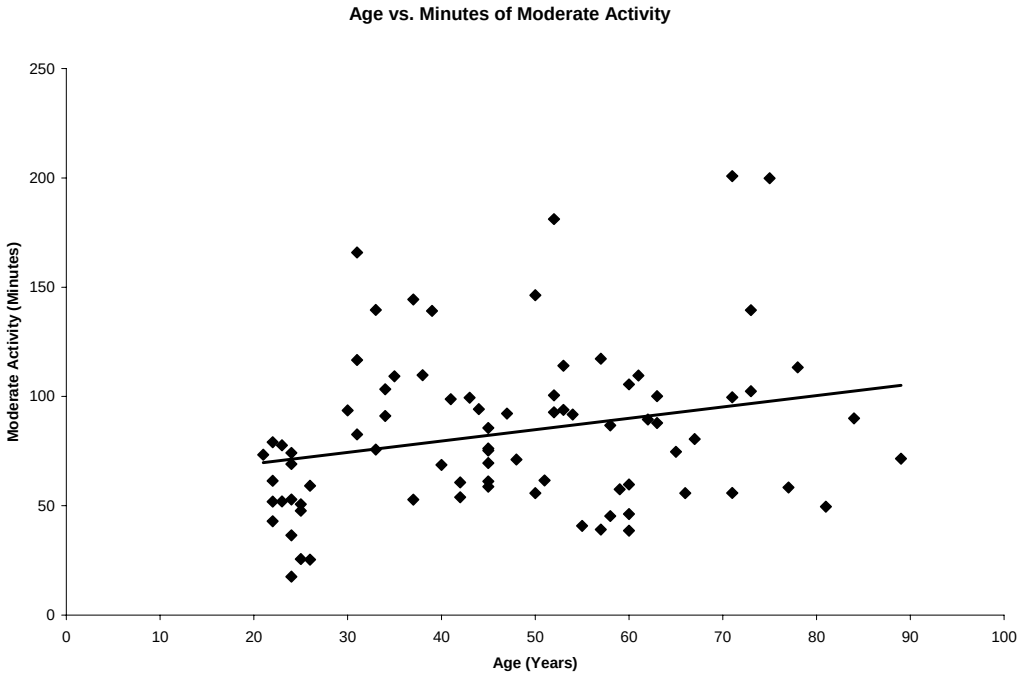

Age vs. Steps Low Intensity
Steps of Low Intensity Activity
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

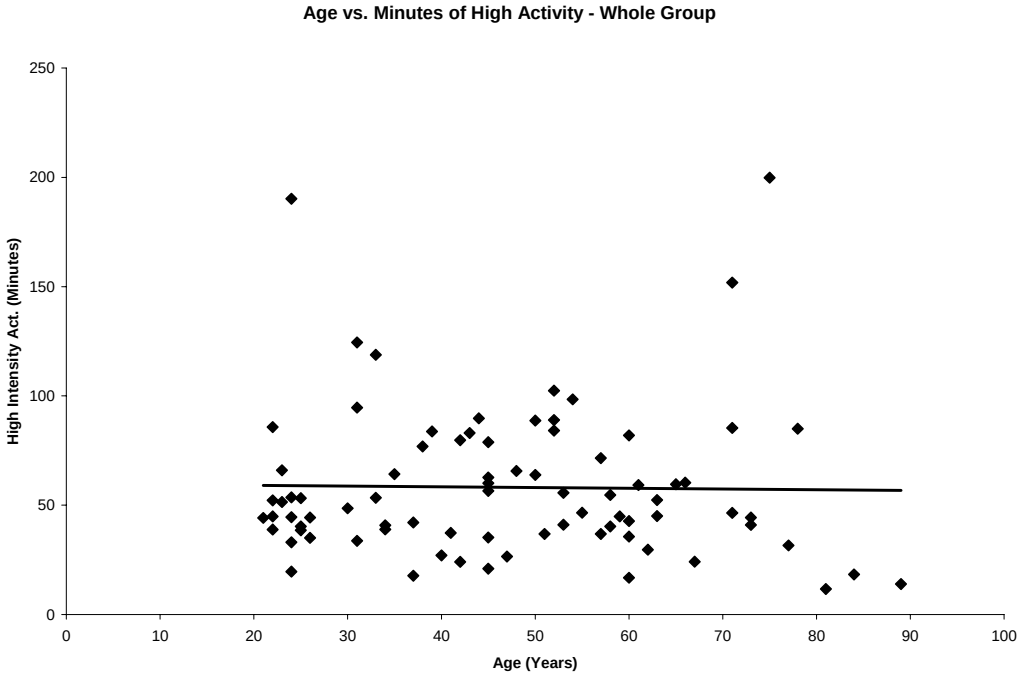

Age vs. Steps of Mod. Activity
Steps of Mod. Activity
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

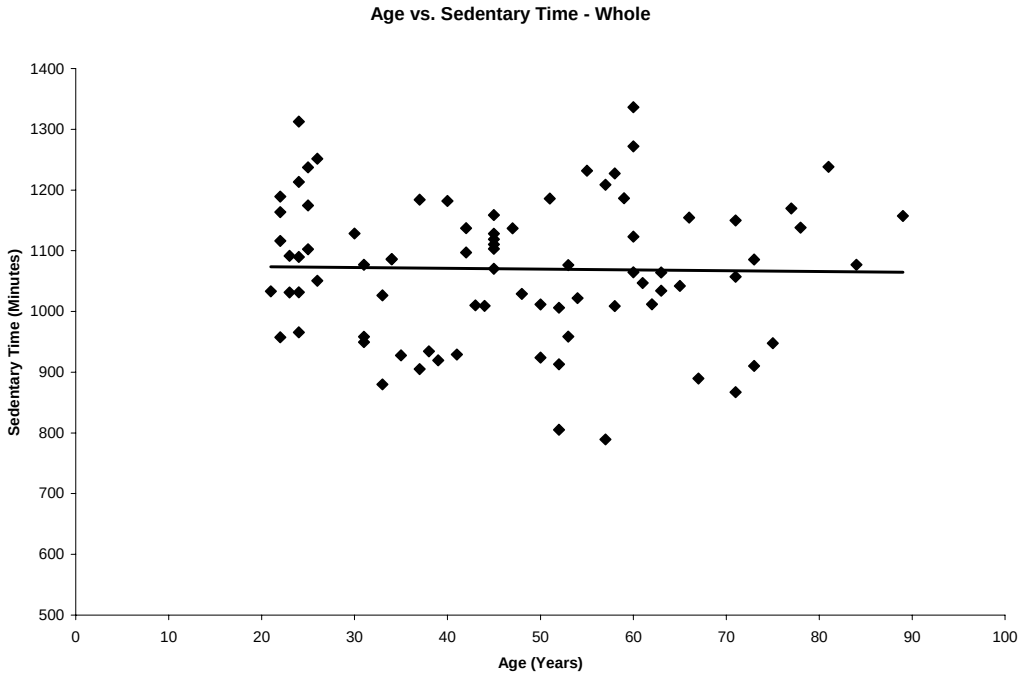

Age vs. Steps Hi Act. -Whole Group
Steps Hi Activity
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)


Age vs. Minutes of Activity a Day
Minutes of Activity per Day
Age (Years)
0
100
200
300
400
500
600
700
0
10
20
30
40
50
60
70
80
90
100


Age vs. Minutes of Low Activity - Whole Group
Minutes of Low Activity
Age (Years)
0
100
200
300
400
500
600
0
10
20
30
40
50
60
70
80
90
100


Age vs. Minutes of Moderate Activity
Moderate Activity (Minutes)
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)


Age vs. Minutes of High Activity - Whole Group
High Intensity Act. (Minutes)
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
90
100
Age (Years)


Age vs. Sedentary Time - Whole
Sedentary Time (Minutes)
1400
1300
1200
1100
1000
900
800
700
600
500
0
10
20
30
40
50
60
70
80
90
100
Age (Years)

APPENDIX L

General Linear Model Results
Sum of Squares
for
C-Reactive Protein

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.541[a]	2	1.271	.448	.641
Intercept	28.103	1	28.103	9.905	.002
age	1.271	1	1.271	.448	.505
sex	1.358	1	1.358	.479	.491
Error	221.297	78	2.837		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .011 (Adjusted R Squared = -.014)

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21.523[a]	3	7.174	2.731	.050
Intercept	1.013	1	1.013	.386	.536
age	8.978	1	8.978	3.417	.068
perbfat	18.982	1	18.982	7.224	.009
sex	4.366	1	4.366	1.662	.201
Error	202.315	77	2.627		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .096 (Adjusted R Squared = .061)

Tests of Between-Subjects Effects

Dependent Variable: CRP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.938[a]	3	5.646	2.101	.107
Intercept	13.638	1	13.638	5.075	.027
AGE	3.881	1	3.881	1.444	.233
TRNKFAT	14.397	1	14.397	5.358	.023
SEX	.921	1	.921	.343	.560
Error	206.901	77	2.687		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .076 (Adjusted R Squared = .040)

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.896[a]	3	9.632	3.804	.013
Intercept	6.962	1	6.962	2.750	.101
age	3.802	1	3.802	1.502	.224
bmi	26.354	1	26.354	10.410	.002
sex	5.000	1	5.000	1.975	.164
Error	194.943	77	2.532		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .129 (Adjusted R Squared = .095)

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.344[a]	4	7.336	2.867	.029
Intercept	4.018	1	4.018	1.570	.214
age	4.073	1	4.073	1.592	.211
perbfat	.448	1	.448	.175	.677
bmi	7.821	1	7.821	3.056	.084
sex	.346	1	.346	.135	.714
Error	194.494	76	2.559		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .131 (Adjusted R Squared = .085)

Tests of Between-Subjects Effects

Dependent Variable: CRP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.926[a]	4	7.231	2.820	.031
Intercept	4.079	1	4.079	1.591	.211
AGE	3.809	1	3.809	1.485	.227
TRNKFAT	.030	1	.030	.012	.914
BMI	11.988	1	11.988	4.674	.034
SEX	4.394	1	4.394	1.713	.194
Error	194.913	76	2.565		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .129 (Adjusted R Squared = .083)

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.830[a]	3	6.610	2.495	.066
Intercept	44.282	1	44.282	16.713	.000
age	.001	1	.001	.001	.981
hdl	17.289	1	17.289	6.525	.013
sex	5.275	1	5.275	1.991	.162
Error	204.009	77	2.649		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .089 (Adjusted R Squared = .053)

Tests of Between-Subjects Effects

Dependent Variable: crp

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33.767[a]	4	8.442	3.375	.013
Intercept	.252	1	.252	.101	.752
age	1.183	1	1.183	.473	.494
bmi	13.937	1	13.937	5.573	.021
hdl	4.871	1	4.871	1.948	.167
sex	7.060	1	7.060	2.823	.097
Error	190.071	76	2.501		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .151 (Adjusted R Squared = .106)

Tests of Between-Subjects Effects

Dependent Variable: CRP

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30.216[a]	5	6.043	2.341	.050
Intercept	3.887	1	3.887	1.506	.224
SEX	3.337	1	3.337	1.293	.259
AGE	4.203	1	4.203	1.628	.206
TRNKFAT	.073	1	.073	.028	.867
BMI	11.764	1	11.764	4.557	.036
SEX * AGE	1.290	1	1.290	.500	.482
Error	193.623	75	2.582		
Total	372.950	81			
Corrected Total	223.839	80			

a. R Squared = .135 (Adjusted R Squared = .077)

APPENDIX M

General Linear Model Results
Sum of Squares
for
Fibrinogen

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	117.145[a]	58	2.020	1.209	.319
Intercept	1154.043	1	1154.043	690.579	.000
AGE	101.029	44	2.296	1.374	.213
SEX	.138	1	.138	.082	.777
AGE * SEX	15.521	13	1.194	.714	.731
Error	36.765	22	1.671		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .761 (Adjusted R Squared = .131)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	125.907[a]	59	2.134	1.600	.117
Intercept	114	1	.114	.085	.773
PULSE	8.762	1	8.762	6.571	.018
AGE	96.156	44	2.185	1.639	.111
SEX	1.140	1	1.140	.855	.366
AGE * SEX	15.252	13	1.173	.880	.584
Error	28.003	21	1.333		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .818 (Adjusted R Squared = .307)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	122.585[a]	59	2.078	1.393	.202
Intercept	6.022	1	6.022	4.037	.058
PERBFAT	5.441	1	5.441	3.647	.070
AGE	91.567	44	2.081	1.395	.207
SEX	3.888	1	3.888	2.606	.121
AGE * SEX	14.560	13	1.120	.751	.698
Error	31.324	21	1.492		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .796 (Adjusted R Squared = .225)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	122.794[a]	59	2.081	1.405	.196
Intercept	18.289	1	18.289	12.343	.002
PERFTRK	5.649	1	5.649	3.813	.064
AGE	91.148	44	2.072	1.398	.205
SEX	2.536	1	2.536	1.712	.205
AGE * SEX	13.976	13	1.075	.726	.721
Error	31.116	21	1.482		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .798 (Adjusted R Squared = .230)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	117.947[a]	59	1.999	1.112	.411
Intercept	170.928	1	170.928	95.072	.000
STPHIDAY	.619	1	.619	.344	.564
AGE	96.852	44	2.201	1.224	.319
SEX	.217	1	.217	.120	.732
AGE * SEX	13.405	13	1.031	.574	.847
Error	35.958	20	1.798		
Total	1507.863	80			
Corrected Total	153.905	79			

a. R Squared = .766 (Adjusted R Squared = .077)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	126.501[a]	60	2.108	1.538	.143
Intercept	.019	1	.019	.014	.907
PULSE	3.707	1	3.707	2.705	.116
PERFTRK	.594	1	.594	.434	.518
AGE	90.073	44	2.047	1.494	.167
SEX	1.721	1	1.721	1.256	.276
AGE * SEX	12.857	13	.989	.722	.723
Error	27.408	20	1.370		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .822 (Adjusted R Squared = .288)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	126.540[a]	61	2.074	1.440	.190
Intercept	.001	1	.001	.001	.982
PULSE	3.736	1	3.736	2.594	.124
PERFTRK	.175	1	.175	.121	.731
PERBFAT	.039	1	.039	.027	.871
AGE	90.044	44	2.046	1.421	.205
SEX	.246	1	.246	.171	.684
AGE * SEX	12.765	13	.982	.682	.757
Error	27.369	19	1.440		
Total	1524.217	81			
Corrected Total	153.909	80			

a. R Squared = .822 (Adjusted R Squared = .251)

Tests of Between-Subjects Effects

Dependent Variable: FIBRIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	128.624[a]	62	2.075	1.395	.226
Intercept	.018	1	.018	.012	.913
PULSE	4.991	1	4.991	3.356	.085
PERFTRK	1.204	1	1.204	.810	.381
PERBFAT	.733	1	.733	.493	.492
STPHIDAY	.061	1	.061	.041	.842
AGE	90.588	44	2.059	1.384	.236
SEX	.001	1	.001	.000	.985
AGE * SEX	11.548	13	.888	.597	.825
Error	25.281	17	1.487		
Total	1507.863	80			
Corrected Total	153.905	79			

a. R Squared = .836 (Adjusted R Squared = .237)

APPENDIX N

General Linear Model Results
Sum of Squares
for
Tissue Plasminogen Activator

Tests of Between-Subjects Effects

Dependent Variable: TPAACT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.379[a]	57	.112	.959	.567
Intercept	32.082	1	32.082	275.020	.000
AGE	4.698	44	.107	.915	.610
SEX	.054	1	.054	.466	.502
AGE * SEX	1.386	12	.116	.990	.488
Error	2.566	22	.117		
Total	45.469	80			
Corrected Total	8.945	79			

a. R Squared = .713 (Adjusted R Squared = -.030)

Tests of Between-Subjects Effects

Dependent Variable: TPAACT

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.380[a]	58	.110	.900	.636
Intercept	3.104	1	3.104	25.409	.000
TRIG	.001	1	.001	.008	.932
AGE	3.978	44	.090	.740	.803
SEX	.047	1	.047	.386	.541
AGE * SEX	1.385	12	.115	.945	.525
Error	2.565	21	.122		
Total	45.469	80			
Corrected Total	8.945	79			

a. R Squared = .713 (Adjusted R Squared = -.079)

APPENDIX O

General Linear Model Results
Sum of Squares
for
Plasminogen Activator Inhibitor – 1

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3199.944[a]	59	54.236	.815	.736
Intercept	2269.741	1	2269.741	34.107	.000
AGE	2753.889	45	61.198	.920	.606
SEX	54.136	1	54.136	.813	.377
AGE * SEX	355.054	13	27.312	.410	.949
Error	1397.500	21	66.548		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .696 (Adjusted R Squared = -.158)

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3588.863[a]	60	59.814	1.186	.346
Intercept	281.311	1	281.311	5.578	.028
BRADBP	388.919	1	388.919	7.712	.012
AGE	2852.381	45	63.386	1.257	.295
SEX	97.647	1	97.647	1.936	.179
AGE * SEX	349.896	13	26.915	.534	.876
Error	1008.580	20	50.429		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .781 (Adjusted R Squared = .122)

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3215.963[a]	60	53.599	.776	.778
Intercept	110.166	1	110.166	1.595	.221
TRIG	16.019	1	16.019	.232	.635
AGE	2154.282	45	47.873	.693	.848
SEX	37.338	1	37.338	.541	.471
AGE * SEX	370.715	13	28.517	.413	.947
Error	1381.481	20	69.074		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .700 (Adjusted R Squared = -.202)

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3326.846[a]	60	55.447	.873	.668
Intercept	4.677	1	4.677	.074	.789
BDYFATMS	126.902	1	126.902	1.998	.173
AGE	2403.918	45	53.420	.841	.694
SEX	152.172	1	152.172	2.395	.137
AGE * SEX	421.738	13	32.441	.511	.892
Error	1270.598	20	63.530		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .724 (Adjusted R Squared = -.105)

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3447.053[a]	60	57.451	.999	.526
Intercept	40.994	1	40.994	.713	.409
PERFTRK	247.109	1	247.109	4.296	.051
AGE	2313.280	45	51.406	.894	.635
SEX	213.607	1	213.607	3.714	.068
AGE * SEX	437.154	13	33.627	.585	.839
Error	1150.390	20	57.520		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .750 (Adjusted R Squared = -.001)

Tests of Between-Subjects Effects

Dependent Variable: PAI1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3654.779[a]	61	59.914	1.208	.333
Intercept	246.613	1	246.613	4.971	.038
BRADBP	207.726	1	207.726	4.187	.055
PERFTRK	65.916	1	65.916	1.329	.263
AGE	2470.235	45	54.894	1.106	.419
SEX	160.997	1	160.997	3.245	.088
AGE * SEX	378.082	13	29.083	.586	.836
Error	942.665	19	49.614		
Total	7422.024	81			
Corrected Total	4597.444	80			

a. R Squared = .795 (Adjusted R Squared = .137)

APPENDIX P

Raw Data

ID Number	Age	Decade	Group	Height	Weight	BMI	Waist Girth	Gender	Medications	Rest Ankle SBP
1001	22	2	1	177.5	69.7	22.12	75.6	1	0	118
1002	57	5	2	174	67.9	22.43	75.4	2	0	150
1003	25	2	1	180.4	80.9	24.86	81.9	1	0	134
1004	59	5	2	163	71.6	26.95	82	2	0	124
1005	58	5	2	185.4	100.4	29.21	99.9	1	0	150
1006	40	4	2	180.2	92.5	28.49	95.3	1	0	154
1007	44	4	2	175.8	73.3	23.72	78.8	1	0	140
1008	52	5	2	177.4	72.4	23.01	78.5	1	0	140
1009	33	3	1	177.8	92.9	29.39	97.4	1	0	140
1010	51	5	2	180.7	81.6	24.99	93	1	0	130
1011	24	2	1	195.6	88.6	23.16	83	1	0	152
1012	50	5	2	162.5	75.1	28.44	92	2	0	124
1013	26	2	1	183.5	84.8	25.18	82.9	1	0	114
1014	54	5	2	162.56	55.86	21.14	87.2	2	0	130
1015	48	4	2	171.9	71.4	24.16	84.5	2	0	140
1016	45	4	2	166	57.1	20.72	73	1	0	122
1017	26	2	1	160.6	76.36	29.61	95.1	2	0	112
1018	55	5	2	170	93.3	32.28	101	1	0	160
1019	23	2	1	188	83.8	23.71	75.4	1	0	122
1020	24	2	1	181	66.5	20.30	72.6	1	0	138
1021	66	6	3	153.1	51.8	22.10	72	2	0	136
1022	61	6	3	164.2	70.5	26.15	81	2	0	152
1023	37	3	1	175.9	61.9	20.01	69.1	2	0	120
1024	38	3	1	164.2	56.6	20.99	69.6	2	0	130
1025	42	4	2	184.5	111	32.61	112	1	0	122
1026	50	5	2	168	57.1	20.23	70	2	0	120
1027	47	4	2	165.1	53.2	19.52	64	2	0	116
1028	45	4	2	178.4	90.5	28.44	94.1	1	0	122
1029	52	5	2	177.8	63.2	19.99	68.7	2	0	136
1030	39	3	1	167.6	59.7	21.25	71.7	2	0	136
1031	67	6	3	165	56	20.57	72	2	0	140
1032	53	5	2	163.9	69.4	25.83	72	2	0	134
1033	65	6	3	161.3	55.1	21.18	65.1	2	0	120
1034	63	6	3	160.02	61.5	24.02	72	2	0	120
1035	57	5	2	175.8	80.4	26.01	92.4	1	0	132
1036	31	3	1	173	72	24.06	80	1	0	136
1037	58	5	2	156.2	80.3	32.91	101.4	2	0	152
1038	35	3	1	164.2	63.1	23.40	76.6	2	0	122
1039	25	2	1	183.2	80.5	23.99	82.3	1	0	110
1040	24	2	1	174	75.1	24.81	78.5	1	0	120
1041	25	2	1	185.4	77.3	22.49	73.4	2	0	122
1042	22	2	1	178.5	77.4	24.29	81	1	0	130
1043	43	4	2	175.6	87.3	28.31	82.5	1	0	120
1044	41	4	2	179	70.6	22.03	88	1	0	118
1045	60	6	3	170.2	81.8	28.24	93.5	1	0	130
1046	22	2	1	165.1	60.8	22.31	76	2	0	130
1047	33	3	1	174.5	76.5	25.12	82.5	1	0	128
1048	34	3	1	175.3	78	25.38	75.5	1	0	132
1049	45	4	2	174	66.4	21.93	73.8	2	0	130
1050	63	6	3	168	68.4	24.23	80	2	0	138
1051	21	2	1	157.5	52.9	21.33	67	2	0	120
1052	60	6	3	183	83.4	24.90	95	1	0	146
1053	23	2	1	166.5	57.5	20.74	69.1	2	0	118
1054	24	2	1	168	66.3	23.49	74.5	2	0	136
1055	24	2	1	167.5	55.1	19.64	67	2	0	140
1056	71	7	3	183	82.2	24.55	96.1	1	0	142
1057	22	2	1	162.5	49.2	18.63	65	2	0	128
1058	45	4	2	164.5	64.1	23.69	79.5	2	0	130
1059	31	3	1	159.1	58	22.91	68.4	2	0	124
1060	34	3	1	177.8	85.5	27.05	81.6	1	0	140
1061	62	6	3	162.3	64.3	24.41	80.1	2	0	136
1062	77	7	3	170	66.1	22.87	78.8	2	0	180
1063	45	4	2	175.5	95.8	31.10	97.5	1	0	150
1064	52	5	2	178.3	75.6	23.78	82.6	1	0	142
1065	42	4	2	162	76.8	29.26	91.3	2	0	130
1066	45	4	2	157.5	55.4	22.33	69.4	2	0	134
1067	73	7	3	171.4	80.8	27.50	96.3	1	0	156
1068	89	7	3	166.6	59.9	21.58	81.5	1	0	148
1069	71	7	3	181.6	75.7	22.95	87.4	2	0	122
1070	31	3	1	176.1	64.8	20.90	75	1	0	126
1071	47	4	2	170	86.6	29.97	93	2	0	148
1072	84	7	3	177.6	82.1	26.03	94.1	1	0	130
1073	30	3	1	164.6	78.4	28.94	90.4	2	0	130
1074	78	7	3	168.1	77.1	27.28	83.5	2	0	156
1075	53	5	2	175.2	79.7	25.97	89.6	1	0	144
1076	60	6	3	185.5	107.9	31.36	104.6	1	0	144
1077	71	7	3	177.2	76	24.20	85.7	1	0	140
1078	60	6	3	188	92.6	26.20	90.4	1	0	140
1079	37	3	1	165.7	93	33.87	104.3	2	0	106
1080	73	7	3	171	93.1	31.84	94.4	2	0	150
1081	81	7	3	157.5	75.5	30.44	95.4	2	0	144
1082	75	7	3	182	70.8	21.37	79	1	0	192

ID Number	Rest Brachial SBP	Rest ABI	Rest Brachial DBP	Rest Pulse	PA Score	SAM Low Cadence Steps	Medium Cadence
1001	106	1.11	56	46	5	3239	3394
1002	126	1.19	84	60	5	5900	4752
1003	116	1.16	64	61	7	1881	2098
1004	114	1.09	76	65	5	1691	2405
1005	124	1.21	70	54	3	1946	2520
1006	126	1.22	78	42	2	2130	2816
1007	114	1.23	76	55	7	24894	13833
1008	116	1.21	70	58	7	5405	5948
1009	120	1.17	64	60	3	4305	4983
1010	112	1.16	74	56	7	2899	4111
1011	122	1.25	79	45	6	1115	758
1012	107	1.16	71	55	3	4321	3534
1013	114	1.00	64	68	6	1212	1153
1014	104	1.25	71	76	7	2601	4028
1015	128	1.09	80	51	5	3172	3112
1016	106	1.15	69	45	6	2248	2533
1017	102	1.10	69	54	3	3673	2440
1018	132	1.21	84	49	7	1627	1628
1019	110	1.11	68	53	7	2860	2221
1020	123	1.12	70	50	7	4983	4125
1021	115	1.18	74	58	6	2125	2334
1022	138	1.10	84	54	5	2787	4721
1023	103	1.17	65	42	7	12350	15356
1024	102	1.27	68	48	7	3811	4645
1025	99	1.23	64	64	3	3275	3368
1026	94	1.28	52	45	7	7686	11656
1027	100	1.16	60	51	3	3069	5266
1028	104	1.17	62	41	7	5110	6675
1029	110	1.24	70	53	7	10287	16037
1030	116	1.17	74	48	7	5829	8859
1031	120	1.17	70	64	3	2854	1611
1032	128	1.05	74	48	3	5938	7273
1033	118	1.02	76	58	7	6264	6474
1034	112	1.07	68	58	3	3176	4167
1035	126	1.05	77	58	6	1580	1586
1036	110	1.24	68	50	7	3275	5640
1037	129	1.18	82	72	7	11137	13096
1038	102	1.20	64	54	6	6178	6902
1039	105	1.05	54	50	7	2480	2206
1040	108	1.11	68	49	6	2369	3253
1041	101	1.21	72	56	6	2777	2125
1042	116	1.12	70	56	6	3173	4380
1043	116	1.03	60	64	7	11342	15148
1044	98	1.20	64	47	6	15985	14695
1045	110	1.18	64	67	7	8253	10457
1046	108	1.20	68	64	7	6077	6473
1047	120	1.07	72	47	7	12782	21275
1048	116	1.14	70	56	6	10649	15000
1049	110	1.18	64	60	7	3275	4262
1050	116	1.19	66	62	3	14490	14859
1051	107	1.12	74	92	6	9567	9445
1052	124	1.18	78	52	7	5087	19021
1053	110	1.07	70	74	7	6435	6467
1054	116	1.17	75	58	6	6329	5757
1055	114	1.23	74	72	5	5767	4883
1056	124	1.15	81	53	7	7749	13164
1057	106	1.21	64	64	3	10402	9965
1058	108	1.20	64	55	6	9209	13349
1059	106	1.17	60	58	7	9632	26245
1060	129	1.09	84	59	6	9415	15868
1061	126	1.08	84	76	2	12894	13520
1062	150	1.20	78	73	3	8500	8832
1063	124	1.21	80	64	6	8404	10654
1064	122	1.16	84	50	7	11248	15081
1065	106	1.23	74	53	7	10558	9196
1066	126	1.06	74	53	7	8770	9351
1067	142	1.10	80	66	6	11284	15688
1068	143	1.03	75	54	3	9194	10641
1069	108	1.13	66	55	3	7656	8496
1070	119	1.06	68	64	7	12352	15139
1071	120	1.23	76	74	3		
1072	128	1.02	78	63	3	15700	16173
1073	118	1.10	82	78	3	8617	11727
1074	144	1.08	86	74	1	2646	10449
1075	136	1.06	82	62	4	3992	6044
1076	124	1.16	70	62	3	7343	9103
1077	118	1.19	64	47	3	4554	14338
1078	128	1.09	82	65	1	10696	16341
1079	106	1.00	66	59	3	7334	7960
1080	136	1.10	82	66	3	16262	20837
1081	134	1.07	74	88	3	7272	7319
1082	177	1.08	84	51	6	9996	11029

ID Number	SAM High Cadence Steps	SAM Total Steps	SAM Sedentary Time	SAM Low Cadence Time
1001	7584	14217	1864	618
1002	3130	13782	1521	957
1003	3514	7493	2363	357
1004	4479	8575	2329	297
1005	4969	9435	3169	328
1006	2199	7145	2272	312
1007	26333	65060	6781	1659
1008	11464	22817	2698	1020
1009	6626	15914	3036	841
1010	4212	11222	3542	465
1011	1539	3412	2544	175
1012	15096	22951	2977	836
1013	3031	5396	2466	252
1014	8252	14881	1972	440
1015	6079	12363	2053	547
1016	7698	12479	2198	397
1017	4498	10611	2061	561
1018	5089	8344	2354	231
1019	4571	9652	2142	481
1020	33277	42385	3432	822
1021	7386	11845	2276	334
1022	5433	12941	2034	436
1023	9655	37361	4540	1747
1024	6402	14858	1847	631
1025	12180	18823	3336	496
1026	9341	28683	3340	1105
1027	2713	11048	2960	480
1028	12565	24350	4602	717
1029	18689	45013	3223	1406
1030	12388	27076	2690	871
1031	964	5429	884	443
1032	7706	20917	2824	918
1033	11185	23923	4144	1049
1034	4786	12129	2051	430
1035	7993	11159	2347	234
1036	12175	21090	3220	555
1037	17166	41399	7013	2014
1038	8310	21390	2700	986
1039	6925	11611	4882	547
1040	4630	10252	2115	432
1041	4561	9463	2153	456
1042	7730	15283	4562	591
1043	29208	55698	7041	1726
1044	11498	42178	6442	2598
1045	19067	37777	15654	1156
1046	18598	31148	8086	1260
1047	35824	69881	6067	2080
1048	11782	37431	8206	1676
1049	2152	9689	2986	477
1050	18205	47554	8201	2165
1051	11623	30635	6238	1746
1052	12426	36534	24362	739
1053	11640	24542	4064	1045
1054	8258	20344	4075	1164
1055	9913	20563	7571	981
1056	22964	43877	6284	1176
1057	13079	33446	8410	1683
1058	18102	40660	7475	1589
1059	37065	72942	6590	1389
1060	10984	36267	7617	1488
1061	9104	35518	7068	2158
1062	10711	28043	8294	1277
1063	10167	29225	7912	1530
1064	28859	55188	6908	1710
1065	6786	26540	7617	1794
1066	21418	39539	7754	1437
1067	11840	38812	7651	1489
1068	3684	23519	8055	1374
1069	16014	32166	8012	1308
1070	7557	35048	5708	1973
1071		0		
1072	5997	37870	9204	2178
1073	10273	30617	9086	1363
1074	12923	26018	4539	413
1075	4603	14639	3120	663
1076	28460	44906	7770	1212
1077	17982	36874	2638	670
1078	11277	38314	7527	1606
1079	5182	20476	8201	1285
1080	12930	50029	6338	2409
1081	3055	17646	8586	974
1082	14312	35337	7685	1641

ID Number	SAM Medium Cadence Time	SAM High Cadence Time	SAM Total Activity Time	Monitoring Time Period	Days
1001	154	167.00	939	2803	2
1002	226	71	1254	2775	2
1003	96	81	534	2897	2
1004	113	88	498	2827	2
1005	117	104	549	3718	3
1006	132	52	496	2768	2
1007	633	603	2895	9676	7
1008	274	263	1557	4255	3
1009	224	158	1223	4259	3
1010	184	110	759	4301	3
1011	34	38	247	2791	2
1012	164	261	1261	4238	3
1013	50	69	371	2837	2
1014	177	190	807	2779	2
1015	142	131	820	2873	2
1016	117	157	671	2869	2
1017	116	87	764	2825	2
1018	78	89	398	2752	2
1019	102	101	684	2826	2
1020	188	676	1686	5118	4
1021	110	119	563	2839	2
1022	213	115	764	2798	2
1023	724	211	2682	7222	5
1024	217	152	1000	2847	2
1025	158	234	888	4224	3
1026	529	231	1865	5205	4
1027	240	69	789	3749	3
1028	311	245	1273	5875	4
1029	725	410	2541	5764	4
1030	407	245	1523	4213	3
1031	80	24	547	1431	1
1032	336	164	1418	4242	3
1033	297	237	1583	5727	4
1034	193	101	724	2775	2
1035	76	139	449	2796	2
1036	247	283	1085	4305	3
1037	603	380	2997	10010	7
1038	318	187	1491	4191	3
1039	101	152	800	5682	4
1040	144	104	680	2795	2
1041	99	104	659	2812	2
1042	199	172	962	5524	4
1043	693	579	2998	10039	7
1044	685	259	3542	9984	7
1045	475	439	2070	17724	12
1046	298	363	1921	10007	7
1047	962	819	3861	9928	7
1048	688	308	2672	10878	8
1049	194	54	725	3711	3
1050	697	357	3219	11420	8
1051	443	267	2456	8694	6
1052	842	307	1888	26250	18
1053	306	260	1611	5675	4
1054	273	176	1613	5688	4
1055	228	206	1415	8986	6
1056	592	507	2275	8559	6
1057	463	293	2439	10849	8
1058	598	395	2582	10057	7
1059	1151	864	3404	9994	7
1060	725	273	2486	10103	7
1061	625	207	2990	10058	7
1062	414	224	1915	10209	7
1063	492	249	2271	10183	7
1064	690	578	2978	9886	7
1065	421	167	2382	9999	7
1066	427	438	2302	10056	7
1067	722	289	2500	10151	7
1068	498	97	1969	10024	7
1069	389	324	2021	10033	7
1070	695	201	2869	8577	6
1071					
1072	769	157	3104	12308	9
1073	754	391	2508	11594	8
1074	452	339	1204	5743	4
1075	272	119	1054	4174	3
1076	413	567	2192	9962	7
1077	611	462	1743	4381	3
1078	746	302	2654	10181	7
1079	366	123	1774	9975	7
1080	971	309	3689	10027	7
1081	344	81	1399	9985	7
1082	506	346	852	2493	2

ID Number	Steps/day	Min/day	stploday	stpmdday	stphiday	minloday	minmdday	minhiday	sedday
1001	7304	482	1664	1744	3896	317	79	86	958
1002	7152	651	3062	2466	1624	497	117	37	789
1003	3725	265	935	1043	1747	177	48	40	1175
1004	4368	254	861	1225	2281	151	58	45	1186
1005	3654	213	754	976	1925	127	45	40	1227
1006	3717	258	1108	1465	1144	162	69	27	1182
1007	9682	431	3705	2059	3919	247	94	90	1009
1008	7722	527	1829	2013	3880	345	93	89	913
1009	5381	414	1456	1685	2240	284	76	53	1026
1010	3757	254	971	1376	1410	156	62	37	1186
1011	1760	127	575	391	794	90	18	20	1313
1012	7798	428	1468	1201	5129	284	56	89	1012
1013	2739	188	615	585	1538	128	25	35	1252
1014	7711	418	1348	2087	4276	228	92	98	1022
1015	6197	411	1590	1560	3047	274	71	66	1029
1016	6263	337	1128	1271	3864	199	59	79	1103
1017	5409	389	1872	1244	2293	286	59	44	1051
1018	4366	208	851	852	2663	121	41	47	1232
1019	4918	349	1457	1132	2329	245	52	51	1091
1020	11925	474	1402	1161	9363	231	53	190	966
1021	6008	286	1078	1184	3746	169	56	60	1154
1022	6660	393	1434	2430	2796	224	110	59	1047
1023	7449	535	2462	3062	1925	348	144	42	905
1024	7515	506	1928	2349	3238	319	110	77	934
1025	6417	303	1116	1148	4152	169	54	80	1137
1026	7935	516	2126	3225	2584	306	146	64	924
1027	4244	303	1179	2023	1042	184	92	27	1137
1028	5968	312	1252	1636	3080	176	76	60	1128
1029	11245	635	2570	4006	4669	351	181	102	805
1030	9255	521	1992	3028	4234	298	139	84	919
1031	5463	550	2872	1621	970	446	81	24	890
1032	7101	481	2016	2469	2616	312	114	56	959
1033	6015	398	1575	1628	2812	264	75	60	1042
1034	6294	376	1648	2162	2484	223	100	52	1064
1035	5747	231	814	817	4117	121	39	72	1209
1036	7054	363	1095	1887	4072	186	83	95	1077
1037	5956	431	1602	1884	2469	290	87	55	1009
1038	7349	512	2123	2371	2855	339	109	64	928
1039	2943	203	629	559	1755	139	26	39	1237
1040	5282	350	1221	1676	2385	223	74	54	1090
1041	4846	337	1422	1088	2336	234	51	53	1103
1042	3984	251	827	1142	2015	154	52	45	1189
1043	7989	430	1627	2173	4190	248	99	83	1010
1044	6083	511	2306	2119	1658	375	99	37	929
1045	3069	168	671	850	1549	94	39	36	1272
1046	4482	276	874	931	2676	181	43	52	1164
1047	10136	560	1854	3086	5196	302	140	119	880
1048	4955	354	1410	1986	1560	222	91	41	1086
1049	3760	281	1271	1654	835	185	75	21	1159
1050	5996	406	1827	1874	2296	273	88	45	1034
1051	5074	407	1585	1564	1925	289	73	44	1033
1052	2004	104	279	1043	682	41	46	17	1336
1053	6227	409	1633	1641	2954	265	78	66	1031
1054	5150	408	1602	1457	2091	295	69	45	1032
1055	3295	227	924	782	1589	157	37	33	1213
1056	7382	383	1304	2215	3864	198	100	85	1057
1057	4439	324	1381	1323	1736	223	61	39	1116
1058	5822	370	1319	1911	2592	228	86	57	1070
1059	10510	490	1388	3782	5341	200	166	124	950
1060	5169	354	1342	2262	1566	212	103	39	1086
1061	5085	428	1846	1936	1303	309	89	30	1012
1062	3956	270	1199	1246	1511	180	58	32	1170
1063	4133	321	1188	1507	1438	216	70	35	1119
1064	8039	434	1638	2197	4204	249	101	84	1006
1065	3822	343	1521	1324	977	258	61	24	1097
1066	5662	330	1256	1339	3067	206	61	63	1110
1067	5506	355	1601	2225	1680	211	102	41	1085
1068	3379	283	1321	1529	529	197	72	14	1157
1069	4617	290	1099	1219	2298	188	56	47	1150
1070	5884	482	2074	2542	1269	331	117	34	958
1071									
1072	4431	363	1837	1892	702	255	90	18	1077
1073	3803	311	1070	1457	1276	169	94	49	1129
1074	6524	302	663	2620	3240	104	113	85	1138
1075	5050	364	1377	2085	1588	229	94	41	1076
1076	6491	317	1061	1316	4114	175	60	82	1123
1077	12120	573	1497	4713	5911	220	201	152	867
1078	5419	375	1513	2311	1595	227	106	43	1065
1079	2956	256	1059	1149	748	186	53	18	1184
1080	7185	530	2335	2992	1857	346	139	44	910
1081	2545	202	1049	1056	441	140	50	12	1238
1082	20411	492	5774	6371	8267	292	200	200	948

ID Number	BMD (g/cm2)	% Total Body Fat	% Fat - Leg	% Fat - Arms	% Fat - Trunk	T. Mass - Total Body	T. Mass - Leg
1001	1.161	11.1	13.3	9.5	8.8	70984.4	13574.1
1002	1.002	30	37.6	29.5	25.1	67943.5	15792.7
1003	1.272	12.6	12.9	10.1	12	80378.3	16751.2
1004	1.031	39.3	46.1	38.5	37	71799.4	25339.9
1005	1.198	24.8	26.6	21.4	24.8	101986.6	33461.3
1006	1.048	25.3	31.7	22.9	22.2	93526.4	20606.4
1007	1.381	9.4	8.9	9.8	8.2	72057.9	22445.7
1008	1.191	16.8	18.4	16.7	15.3	72404.3	24088.6
1009	1.185	23.3	20	17.9	26.6	92930.7	18666.3
1010	1.04	22.4	24.4	19.6	22	80115.3	15841.2
1011	1.396	11.6	12.5	10.6	10.3	88720.6	30204.6
1012	0.966	35.1	33.15	37.1	37.4	77185.1	23648.8
1013	1.189	19.6	21	15.3	19.4	84604.8	27715.7
1014	1	29.6	38.7	35.7	22.5	56406.5	20959
1015	1.149	29.5	34.6	34.2	26.3	71830.9	24487.8
1016	0.954	18.6	23.7	16.7	15.6	57974.6	18091.5
1017	0.978	41.3	44.6	36.3	42.1	78025.2	27944.3
1018	1.326	28.3	30.4	20.8	29.5	92408	28636.3
1019	1.248	15	14.6	11.9	15.4	86535.7	27279.8
1020	1.237	9.3	10.3	9.7	7.2	67215.1	20874.3
1021	0.964	26.8	28.1	38.6	24.5	52891	18072.7
1022	1.126	33.7	38.8	35.1	31.5	70393.3	14876.2
1023	1.207	21.6	30.2	24	15.2	61904.6	21761.6
1024	1.152	15.3	21.8	12.9	10.7	58419.9	19748.9
1025	1.145	36.8	32	32.1	41.8	113686.7	34171.5
1026	1.132	22	28	22.1	16.3	57727.1	23199.4
1027	1.041	25	35.5	26.9	16.4	53284.2	20521.2
1028	1.029	19.5	16.3	16.5	22.3	90687.9	28617.2
1029	1.118	20.3	27.2	24.8	14.6	62849.5	69762.4
1030	1.106	25	31.2	32.8	18.6	60819.4	22729.7
1031	1.027	31.2	35	39.8	28.2	55811.5	18848
1032	1.05	33	39.5	33	29	68195.8	27681.2
1033	0.97	33.1	43.3	42.8	24.7	55586	20899.5
1034	0.99	39.8	49.2	49	33.7	58699.7	21635.2
1035	1.347	16.1	18.9	12.5	14.4	80563.7	27706.4
1036	1.241	10.8	10.9	9.6	9.7	72750.9	24716.3
1037	0.946	36	33.7	35.4	39.2	81060.5	12306
1038	1.015	22.6	29.9	23	17.1	63058.6	23003
1039	1.234	11.3	11.3	10.7	10.4	79732.2	27467.8
1040	1.284	17.2	20.1	15.5	15.2	74506	25142.3
1041	1.179	30.6	37.8	29.9	25.2	77501.7	31691.2
1042	1.141	22.4	28.5	22.5	17.9	76956.7	27786.9
1043	1.251	10.6	10.4	8.5	10.5	86301.8	27268.3
1044	1.126	15.3	16	13.6	14.5	70181.4	23096.8
1045	1.353	19.6	17.6	15.2	22	82428.3	25399
1046	1.093	25.8	31.6	29.5	21.5	59113.8	20347.1
1047	1.068	13.1	13.4	12.1	12.1	77174.6	28177.3
1048	1.268	13.1	14.8	11	11.6	79609.7	26040.5
1049	1.235	28	34.9	34.5	22.4	66776.2	24927.2
1050	1.004	31.7	39.5	36.1	26.2	68890.3	24479.1
1051	1.144	29.1	35.3	29.7	25.7	52504.5	18980.7
1052	1.26	21.9	18.7	21.1	24.3	82716.3	26339.8
1053	1.09	20.3	26.7	26.1	13.2	57548.3	22356.1
1054	1.096	26.3	32.8	30.6	20.9	67462	24932
1055	1.161	23.4	32.4	28.3	15.2	53479.2	20689.2
1056	1.246	22.9	21.6	19.9	24.7	81996.3	25097.7
1057	0.968	20.5	26.3	21.6	15.8	49762	17869.8
1058	1.194	30.8	35.7	32.8	28	64301.8	15546.3
1059	1.15	26.2	31.9	24.5	23.3	57967.8	20788.5
1060	1.114	21.5	22.2	17.1	22.2	85453.9	28295.1
1061	1.041	38.6	39.9	45.5	38.5	65779.1	21891.5
1062	0.98	36.4	45.2	40	30.9	66762.4	24698.6
1063	1.208	25	25.7	22.3	25.7	97268.7	33336.8
1064	1.055	13.3	14.6	11	11.7	75071.3	25104.2
1065	1.098	32	29.1	30.2	35.3	78593.3	25002.3
1066	1.08	25.5	28.2	29	23.1	54909.4	20587.8
1067	1.17	25.6	22.3	23.1	29.1	80254.3	25313.9
1068	1.07	19.8	18.9	20.3	20.1	59972	18777
1069	1.242	35.8	36.7	39.8	36.4	75630.7	24175.6
1070	1.147	11.3	14.3	8.5	8.2	68062.6	23330.4
1071	1.104	38.9	39.7	41.3	40	86822.7	28417.6
1072	1.12	23.8	26.4	21.3	22.9	83222.5	28312.7
1073	1.002	37.9	35.8	39.8	40.6	77882.1	24912.3
1074	0.945	46.2	50.6	50.5	45.2	78336.9	27876
1075	1.18	26.3	28.5	23.5	26.4	80131.1	25748
1076	1.084	33.9	30.6	31	37.9	109100.5	36316.8
1077	1.118	18.3	19.7	13.2	18.1	77949.9	27472.4
1078	1.138	29.9	29.8	24.7	32	92886.2	27803
1079	1.089	44.1	47.7	41.4	44.3	94471.4	37030
1080	1.058	44	47	45.5	43.9	94261.4	34528.1
1081	0.949	42.5	47.8	45.2	40.3	75468.3	28697.1
1082	1.2	15.4	14.8	14.4	15.5	70408.4	23844.9

ID Number	T. Mass - Arms	T. Mass - Trunk	Body Fat Mass	Trunk Fat Mass	Triglyceride	Cholesterol (mg/dL)	HDL (mg/dL)
1001	8730.5	314267.1	9440.9	27655.5	79	145	51
1002	6872.4	30601.8	25546.8	7681.1	57	151	54
1003	10284.6	38034.5	10368.8	4564.1	33	199	68
1004	7572	34575	33099.5	12792.8	62	216	72
1005	11714.2	50976.5	27128.4	12642.2	176	196	39
1006	11490.3	16545	29647.9	3673.0	59	176	50
1007	4499.5	36286.9	6413.2	2975.5	58	150	52
1008	8914.4	34178.3	13322.4	5229.3	75	200	76
1009	10284.7	48274.7	18586.1	12841.1	245	191	30
1010	9302.5	40064.1	19548.1	8814.1	87	172	42
1011	10943	42538.2	11090.1	4381.4	84	166	54
1012	7880.7	41118.8	25586.9	15378.4	112	177	43
1013	10385.9	40667	17767.0	7889.4	136	204	55
1014	5470.5	25976.3	21829.3	5844.7	54	205	111
1015	7540.7	35009.8	24853.5	9207.6	73	157	60
1016	6410.9	29338.9	13740.0	4576.9	88	175	64
1017	6222.8	39486.4	34799.2	16623.8	62	177	54
1018	11036.4	47857.7	28092.0	14118.0	60	125	44
1019	11768	42650.1	12634.2	6568.1	80	178	44
1020	7377.9	34296.2	6923.2	2469.3	22	104	47
1021	5187.5	24441.8	14862.4	5988.2	51	277	69
1022	7378	33558.9	27312.6	10571.1	102	233	55
1023	6675.5	29365	18695.2	4463.5	53	142	62
1024	5876.3	28329	12735.5	3031.2	127	171	61
1025	12816.6	61820.5	36379.7	25841.0	146	161	26
1026	2991.2	24552.3	16163.6	4002.0	50	180	53
1027	5386.4	23386.4	18915.9	3835.4	53	134	43
1028	12394.4	44702	14782.1	9968.5	150	180	42
1029	6173.2	30664.6	17095.1	4477.0	31	230	99
1030	6839.6	27225.2	18975.7	5063.9	39	147	52
1031	6001.7	27109.3	19534.0	7644.8	118	196	58
1032	6400.3	29877.5	26937.3	8664.5	49	162	73
1033	5393.3	25443.8	24068.7	6284.6	58	288	71
1034	5524.2	27009.7	28880.3	9102.3	82	208	65
1035	9687.3	37717.9	15226.5	5431.4	51	135	45
1036	9290	34065.6	7929.8	3304.4	68	195	51
1037	9126.1	42401.3	27317.4	16621.3	154	227	40
1038	6655.4	29182	18854.5	4990.1	42	149	40
1039	10348.7	37131.9	9009.7	3861.7	82	225	53
1040	9387.1	34878	14975.7	5301.5	150	256	48
1041	7953.7	33712.5	29295.6	8495.6	36	145	57
1042	8497.5	35543.2	21932.7	6362.2	63	157	36
1043	13085.5	41724.3	8975.4	4381.1	39	136	53
1044	8224.1	33866.4	11229.0	4910.6	57	175	52
1045	11350.2	40462	14507.4	8901.6	97	159	46
1046	6554.6	28130.7	18680.0	6048.1	76	162	46
1047	8706.6	35721.9	10341.4	4322.3	49	235	75
1048	10301.5	38068.1	11782.2	4415.9	67	205	26
1049	6062.5	30870.7	23304.9	6915.0	47	197	67
1050	8002.9	31701.2	27211.7	8305.7	43	273	95
1051	5274.5	24369	18534.1	6262.8	102	159	46
1052	9822.3	41469.1	15467.9	10077.0	52	193	53
1053	6285.5	24663.9	15365.4	3255.6	71	171	50
1054	7245.1	30330.9	22127.5	6339.2	121	228	39
1055	4982.2	23823.1	17327.3	3621.1	96	192	51
1056	9374	42630.8	17711.2	10529.8	50	232	72
1057	4811.6	22828.4	13087.4	3606.9	44	160	46
1058	4209.3	21096.6	22955.7	5907.0	51	208	71
1059	5691.7	27250	18491.7	6349.3	86	213	65
1060	10494.5	41234	18970.8	9153.9	65	150	43
1061	6729.8	33344.6	26245.9	12837.7	67	331	67
1062	3404.4	31386.8	30176.6	9698.5	100	242	71
1063	11277.5	47573.2	24998.1	12226.3	74	185	43
1064	9737	34953.6	10960.4	4089.6	44	238	68
1065	9193.1	40270.7	22870.7	14215.6	54	161	39
1066	5845	24885.6	15484.5	5748.6	40	163	57
1067	9506.6	40075.4	17896.7	11661.9	73	165	37
1068	7089.4	29388.5	11334.7	5907.1	55	197	54
1069	7549.8	39019.5	27756.5	14203.1	85	189	52
1070	9343.3	29650.1	9733.0	2431.3	34	133	59
1071	9280.8	44231.5	34468.6	17692.6	49	152	49
1072	8935.9	40664	21970.7	9312.1	95	188	33
1073	8273.8	40513.6	27881.8	16448.5	149	168	43
1074	7199.4	39018.1	39638.5	17636.2	121	241	44
1075	9969.8	40048.4	22837.4	10572.8	64	218	49
1076	11876.6	55500.5	33384.8	21034.7	87	155	37
1077	8738.5	37030.1	15356.1	6702.4	56	217	59
1078	7781.8	49737	27680.1	15915.8	121	160	44
1079	8848.6	43756.5	45062.9	19384.1	136	64	38
1080	8611.5	46242.8	44302.9	20300.6	61	220	80
1081	7543.3	34991.6	36073.8	14101.6	137	170	45
1082	8003.2	33719.8	10420.4	5226.6	61	191	80

ID Number	LDL (mg/dL)	L/H Ratio	Glucose	[CRP] mg/L	[TPA]ant	[TPA]act	[Fibrinogen]	[PAI-1]act
1001	78	1.5	74.0	0.6	3.190	0.48	4.860	0.00
1002	86	1.6	88.0	0.8	4.900	0.66	4.433	0.00
1003	124	1.8	95.0	0.2	3.924	0.40	6.793	1.62
1004	132	1.8	94.0	0.6	6.199	0.45	9.724	7.59
1005	122	3.1	94.0	0.8	8.332	0.20	2.956	26.35
1006	114	2.3	79.0	0.2	7.660	0.48	13.350	7.99
1007	86	1.7	85.0	1.3	3.608	0.99	3.625	0.00
1008	109	1.4	92.0	3.4	4.719	0.50	2.806	3.42
1009	112	3.7	86.0	3.2	9.000	0.23	4.250	14.82
1010	113	2.7	100.0	0.4	5.111	1.16	2.978	0.21
1011	95	1.8	86.0	0.2	7.399	0.59	2.267	6.16
1012	112	2.6	70.0	0.4	8.379	0.18	3.991	25.15
1013	122	2.2	76.0	0.4	4.229	0.83	2.245	0.77
1014	83	0.7	60.0	0.3	7.791	0.84	2.827	0.00
1015	82	1.4	49.0	3.9	7.693	0.69	3.280	2.26
1016	93	1.5	70.0	0.2	9.752	0.14	2.913	40.03
1017	111	2.1	82.0	3.7	6.124	0.19	3.129	2.38
1018	69	1.6	82.0	0.9	7.268	0.52	2.892	9.94
1019	118	2.7	82.0	0.4	3.052	0.67	7.849	0.21
1020	53	1.1	67.0	2.5	8.706	0.26	2.676	15.89
1021	198	2.9	77.0	0.5	2.595	1.30	2.827	0.00
1022	158	2.9	73.0	1	13.248	0.38	4.077	24.61
1023	69	1.1	76.0	0.7	3.575	0.45	2.547	5.71
1024	85	1.4	69.0	0.2	8.412	0.50	3.150	2.83
1025	106	4.1	97.0	1.6	2.758	1.01	6.017	0.00
1026	117	2.2	86.0	1.9	4.327	0.86	2.806	0.00
1027	80	1.9	84.0	0.2	4.556	0.84	3.431	0.00
1028	108	2.6	86.0	1.1	8.379	0.36	2.655	8.84
1029	125	1.3	82.0	0.2	6.353	0.78	2.547	0.57
1030	87	1.7	74.0	0.7	4.196	0.72	2.892	1.40
1031	114	2.0	105.0	0.4	7.562	0.73	5.349	5.12
1032	79	1.1	80.0	0.9	5.797	0.35	2.784	8.36
1033	205	2.9	84.0	1.7	9.033	1.00	2.827	1.82
1034	127	2.0	85.0	0.8	41.974	1.09	4.142	0.00
1035	80	1.8	89.0	0.3	22.693	2.61	3.107	1.04
1036	130	2.5	79.0	0.2	4.000	0.70	2.741	2.68
1037	156	3.9	94.0	4.2	9.621	0.27	2.655	14.97
1038	101	2.5	69.0	6.1	2.529	0.62	3.000	0.00
1039	156	2.9	58.0	1.1	8.150	0.79	2.159	0.30
1040	178	3.7	86.0	0.2	5.667	0.81	2.978	3.33
1041	81	1.4	67.0	0.6	7.856	0.79	4.034	0.51
1042	108	3.0	86.0	4.2	7.922	0.19	2.806	24.94
1043	75	1.4	75.0	0.4	5.026	1.017	4.745	0.000
1044	112	2.2	78.0	0.2	6.181	1.263	4.797	0.000
1045	94	2.0	67.0	2	8.968	0.627	4.424	4.085
1046	101	2.2	70.0	2.1	6.006	0.389	5.257	10.770
1047	150	2.0	92.0	0.2	5.554	0.423	2.374	5.934
1048	166	6.4	72.0	0.2	8.265	0.828	2.292	0.042
1049	121	1.8	74.0	0.2	4.248	0.759	3.845	0.597
1050	169	1.8	79.0	0.8	5.503	0.683	5.093	1.918
1051	93	2.0	75.0	0.9	4.725	0.322	3.968	17.904
1052	130	2.5	80.0	1.8	11.454	0.297	4.197	19.066
1053	107	0.5	81.0	0.2	4.374	1.240	5.168	0.000
1054	165	4.2	71.0	0.6	22.777	0.562	4.443	1.786
1055	122	2.4	70.0	0.5	4.022	0.718	3.768	0.000
1056	150	2.1	93.0	1.1	8.340	0.639	4.967	1.072
1057	105	2.3	81.0	1.6	4.549	1.047	4.482	0.000
1058	127	1.8	70.0	1.6	6.583	1.287	5.462	0.359
1059	131	2.0	82.0	0.8	11.629	0.734	4.158	1.046
1060	94	2.2	74.0	0.3	8.014	0.501	3.394	9.290
1061	251	3.7	66.0	0.7	17.052	0.828	4.096	3.530
1062	151	2.1	90.0	1.9	9.028	2.430	4.706	0.000
1063	127	3.0	68.0	2.3	8.692	0.507	3.992	5.141
1064	131	1.9	83.0	0.2	20.417	0.511	4.158	10.241
1065	111	2.8	72.0	3.2	9.822	0.442	5.067	16.847
1066	98	1.7	86.0	0.5	4.399	1.224	3.735	0.121
1067	113	3.1	84.0	0.4	11.880	1.092	4.614	2.552
1068	132	2.4	86.0	0.4	9.028	1.195	6.571	2.288
1069	120	2.3	85.0	0.8	8.868	0.639	5.749	5.538
1070	67	1.1	72.0	6.8	7.512	0.970	5.740	0.000
1071	93	1.9	105.0	0.9	7.361	0.251	4.044	17.957
1072	136	4.1	92.0	3.1	8.667	1.327	5.492	0.000
1073	95	2.2	75.0	0.6	16.611	0.354	5.172	10.505
1074	173	3.9	76.0	1.7	8.592	0.724	5.479	5.802
1075	156	3.2	90.0	1.2	12.132	0.495	5.390	19.013
1076	101	2.7	78.0	2.5	10.000	1.636	4.027	0.000
1077	147	2.5	81.0	0.8	10.972	0.696	4.734	12.276
1078	92	2.1	86.0	1.7	21.346	0.275	7.164	17.877
1079	85	2.2	86.0	10.4	12.282	0.377	5.552	7.784
1080	128	1.6	87.0	0.9	8.893	1.285	4.081	1.786
1081	98	2.2	73.0	71	10.550	0.214	4.680	29.133
1082	99	1.2	77.0	0.2	11.680	0.659	4.057	4.270