SHORT-TERM CHANGES IN HEALTH STATUS AFTER CIGARETTE SMOKING

AND MODERATE INTENSITY EXERCISE

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

_____

Ayddin Reisi

Certificate of Approval:

_____	_____
L. Bruce Gladden Professor Health and Human Performance	Peter W. Grandjean, Chair Associate Professor Health and Human performance
_____	_____
Daniel Blessing Associate Professor Health and Human Performance	Stephen L. McFarland Acting Dean Graduate School

SHORT-TERM CHANGES IN HEALTH STATUS AFTER CIGARETTE SMOKING AND MODERATE INTENSITY EXERCISE

Ayddin Reisi

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
December 16, 2005

SHORT-TERM CHANGES IN HEALTH STATUS AFTER CIGARETTE SMOKING

AND MODERATE INTENSITY EXERCISE

Ayddin Reisi

_____
Signature of Author

_____
Date of Graduation

VITA

Ayddin Reisi, daughter of Dr. Reisi and Soosan Mardashti, was born on April 18, 1975, in Shiraz, Iran. She graduated from Asiyeh High School, Shiraz, Iran, in 1992. She attended Shiraz University of Medical Sciences, Shiraz, Iran, in a Bachelor of Science program in Physical Therapy. After four years, in August 2000, she graduated as third-best student. This was followed by a two years of work as a physical therapist at Nemazee Hospital, Shiraz. Then she attended Auburn University to study a Master of Science Degree in Health Promotion starting in January 2003.

THESIS ABSTRACT

SHORT-TERM CHANGES IN HEALTH STATUS AFTER CIGARETTE SMOKING AND MODERATE INTENSITY EXERCISE

Ayddin Reisi

Master of Science, December 16, 2005
(B. S., Shiraz University of Medical Sciences, 2000)

86 Typed Pages

Directed by Dr. Peter W. Grandjean

The combined effects of cigarette smoking and moderate-intensity aerobic exercise on physiological, inflammatory and blood clotting factors were examined in 12 casual smokers. Apparently healthy males between the ages of 19 and 35 years who self-reported that they smoked no more than 7 cigarettes per day were recruited to undergo each of three experimental conditions in randomized order: 1) a single bout of moderate-intensity aerobic exercise, 2) cigarette smoking, and 3) cigarette smoking just prior to a single bout of exercise. Blood samples were drawn prior to, immediately after and 10, 20 and 40 minutes after completing each condition. One additional blood sample was obtained again 24-hours after completing each condition. Data were analyzed using 3 (condition) x time (depending on the variable of interest) ANOVA. Simple main effects

and Duncan New Multiple Range tests were used to follow-up significant ANOVA findings. Smoking prior to exercise increased heart rate during moderate-intensity exercise and in the immediate period of recovery after exercise to a greater level than exercising or smoking alone. Systolic blood pressure was higher with smoking compared to exercise and diastolic blood pressure was significantly elevated after smoking. Pulmonary function was not significantly altered in any of the experimental conditions. White blood cell (WBC) count increased in all three conditions with a greater magnitude and duration in the exercise and combined smoking and exercise conditions versus smoking alone. C-reactive protein (CRP) was not significantly altered by smoking, exercise or the combination of the two conditions. Blood fibrinogen concentrations were greater with smoking and fibrinogen concentrations decreased similarly after each experimental condition. Smoking prior to exercise prolonged the elevation in platelet counts that occur after smoking or exercise alone. Activated partial thromboplastin time (APTT) was significantly greater with smoking and decreased after each condition; yet, prothrombin time (PT) was elevated similarly after each condition. We conclude from these results that smoking increases cardiovascular stress at rest, during exercise and recovery from exercise and smoking prolongs elevations in platelet counts that occur after exercise. However, smoking prior to exercise does not transiently alter inflammatory markers or appear to influence changes in blood clotting characteristics that are observed after exercise.

ACKNOWLEDGMENTS

I am most happy to express my gratitude to all of those who have contributed either directly or indirectly to the creation of this thesis. First, I would like to express my deepest appreciation to my advisor, Dr. Peter Grandjean, for providing scientific support, guidance and stimulation over the past two years. Dr. Gladden and Dr. Blessing deserve my appreciation for their comments and suggestions on this research. I would like to thank the graduate students in the Exercise Technology Laboratory (Sang-ouk "Tommy" Wee, Eric Plaisance, Mike Mestek, and Felipe Araya) for their assistance in data collection. My thanks also go to Mr. Kyle Taylor and the personnel in the Columbus Regional Hospital with their help in blood analysis.

I wish to warmly thank my husband, Arash, for all his understanding, help, and support in the past busy years. My deepest appreciation also go to my parents and sister for their wholehearted support during these years. I am greatly indebted to my family for encouraging me to pursue this degree, and supporting me along every step of the way. My warm-hearted thanks also go to my in-laws, and to Ali, Sahar, Pooyan, and Kian for their continuous support and encouragement.

Style manual or journal used: Journal of Applied Physiology

Computer software used: MS Word XP

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Brief Review of the Related Literature

Cigarette smoking is considered to be a major risk factor for carotid atherosclerosis, coronary heart diseases (1-5) chronic obstructive pulmonary disease and cancers of the upper digestive tract and lung (6). Current cigarette smokers have a risk of coronary heart disease death 52% higher than nonsmokers, ex-smokers, or pipe and cigar smokers (7). Even very light cigarette smoking considerably increases the risk of coronary heart disease in middle-age men (8). Cigarette smoking is associated with a high degree of elevated risk of mortality from all causes (9-12) and current cigarette smokers have a death rate from all causes, which is twice that of those who have never smoked. (13). According to the report of U.S. Surgeon General, more than 12 million Americans have died from smoking since 1964 and another 25 million Americans alive today will most likely die of a smoking-related illness. Although smoking-related mortality rates are declining, current statistics indicate that smoking kills an estimated 440,000 Americans each year and on average, men who smoke cut their lives short by 13.2 years, and female smokers lose 14.5 years (14).

The deleterious physical effects of inhaled cigarette smoking are well characterized. For example, it has been shown that there is a strong association between chronic smoking and various markers of systemic inflammation (15, 16). Chronic inflammation, as indicated by elevated C - reactive protein (CRP) (17-22) and white

blood cell (WBC) count (21, 23-25), is observed with greater prevalence and to a greater degree in smokers and is thought to increase the risk of coronary artery disease in those who smoke cigarettes (15, 16, 26-28). Elevated blood coagulation that is observed with chronic smoking is also thought to contribute to smokers' greater risk for coronary artery disease as well as other diseases (29). An elevated fibrinogen level (21, 24, 30), which is an indicator of blood coagulation, may predict cardiovascular disease and play a role at the early stages of the atherosclerotic process - as well as thrombosis and acute coronary syndromes. Smokers have lower baseline blood fibrinolytic activity, which appears to be a leading determinant of ischemic heart disease, compared to non-smokers(31-35). The lower baseline blood fibrinolytic activity in smokers could be attributed to a lower baseline level in tissue-type plasminogen activator (t-PA) (32) or elevated levels in its main inhibitor plasminogen activator inhibitor 1 (PAI-1) which are released from the endothelial cells of the vessel wall (33-36). Respiratory health disorders can also be identified among smokers, who as a group tend to have lower lung function test values compared to age-matched non-smokers (37, 38).

Part of the short-term deleterious physical effects of smoking may be attributed to its acute effects on inflammatory markers (CRP, WBC count), and blood coagulation factors as measured by fibrinogen concentrations, fibrinolytic activity, activated partial thromboplastin time (APTT), prothrombin time (PT), and platelet count (16, 26, 39). Smoking two cigarettes in succession increases WBC count (40) and chronic smokers have significantly higher values for CRP (16, 26, 32, 41), but the acute effect of smoking on CRP is currently uncharacterized. Although cigarette smokers tend to have elevated levels of fibrinogen (16, 26) and platelet count compared to non-smokers (42, 43),

fibrinogen remains unchanged after acute smoking (39, 40, 44) and no changes in platelet count are observed (40). The results of acute smoking and fibrinolytic activity are conflicting; cigarette smoking may elicit an increase in fibrinolytic activity due to an increase in t-PA (45) or it may not stimulate any changes in the level of t-PA and PAI-1 (35, 39). The acute effect of smoking on APTT and PT which are indicators of blood coagulation is not known; however, PT and APTT are lower among current cigarette smokers compared to nonsmokers (29, 46).

Likewise, smoking may also have acute detrimental effects on measures of cardiorespiratory function such as heart rate, blood pressure and pulmonary function. Acute smoking increases resting heart rate and blood pressure (44, 47-50) which are both indicative of increased cardiovascular stress. Smoking influence on pulmonary function is less consistent as lung function measurements can decrease or remain unchanged following acute smoking (37, 38). Combined with increased inflammation and risk of coagulation, smoking-related elevation in heart rate and blood pressure may acutely increase risk of an adverse cardiovascular outcome such as ischemia, myocardial infarction or sudden death.

The role of regular physical activity in the prevention of coronary heart disease has been well documented (51). However, an acute bout of physical exertion may elevate the risk of adverse cardiovascular events during and shortly after the exercise bout (52-54). As with cigarette smoking, acute exercise can influence inflammatory, blood coagulation and physiological parameters (36, 52, 55-58). Acute exercise is associated with a significant increase in WBC count (58, 59) and following triathlon and marathon

races, CRP markedly increases (58, 60). However, the short term changes in CRP after moderate-intensity exercise of short duration have not been characterized.

There are conflicting results on the effect of acute exercise on fibrinogen as fibrinogen can increase during acute exercise in healthy individuals after a marathon race (60). On the other hand, fibrinogen has remained unchanged following submaximal and maximal exercise tests (52, 61) or it may decrease in healthy adults immediately after a marathon race (62). Acute dynamic exercise increases fibrinolytic activity (52-54, 61, 63) and decreases APTT (52-54, 64). During strenuous physical exercise, increases in coagulation (shortening of APTT) and fibrinolytic activity (increase in t-PA activity) proceed in parallel (52). However, during recovery, while there is a sustained increase in coagulation, fibrinolytic activity demonstrates a sharp fall, which suggests a more favorable situation for clot formation after exercise. This phenomenon could constitute an enhanced risk for coronary artery thrombosis, which may contribute to exercise-related cardiovascular events (52-54). No significant changes in PT are observed after 60 minutes of aerobic exercise (65), but following a maximal exercise test, a decrease in PT has been observed (64). Both acute maximal and submaximal exercises increase platelet count significantly (64-67).

Blood pressure is typically lower than pre-exercise values following an acute bout of exercise (55, 56, 68, 69) and heart rate can remain above rest during the first 20 or 30 minutes of recovery (55, 56). In general a decrease in pulmonary function can be seen after maximal and submaximal exercise (57, 70) as forced vital capacity (FVC) is significantly reduced at 5 and 10 min post-exercise(57, 71, 72). However, there are conflicting results for forced expiratory volume in one second (FEV_1) which can decrease

significantly (71), or remain unchanged following maximal and submaximal exercise (57, 72).

It is estimated that 21.6 % of U.S. adults currently smoke cigarettes (73) and many of these individuals smoke occasionally (in social settings and/or periodically on any given week). In some instances smoking and manual labor, modest physical exertion and/or recreational physical activity occur simultaneously. Because of the detrimental cardiovascular and biochemical effects of smoking and the heightened risk of untoward cardiovascular events during or shortly after moderate-intensity exercise, it stands to reason that many people are regularly and unwittingly putting themselves at risk for an untoward cardiovascular event when they combine cigarette smoking and physical activity. The independent effects of cigarette smoking on the aforementioned physiologic and biochemical markers have been characterized - as have the effects of aerobic exercise. However, the combined effects of these activities have not been investigated. The purpose of this study is to determine the combined influences of smoking and moderate-intensity aerobic exercise on physiological, inflammatory, and blood coagulation responses in young healthy casual smokers.

Hypotheses

$H_{0:}$ Smoking prior to a single session of exercise will result in no difference in inflammatory, coagulation and physiological parameters compared to either the smoking or exercise conditions.

Ha: Smoking prior to exercise will increase markers of inflammation and coagulation, elevate cardiovascular stress as indicated by heart rate and blood pressure, and reduce lung function versus smoking or exercise alone.

Acute smoking increases WBC count (40) and chronic smokers have significantly higher values for CRP versus non-smokers (16, 26, 32) but at present there is no study on the effect of acute smoking on CRP. Acute exercise is associated with a significant increase in WBC count (58, 59, 64) and following triathlon and marathon races, CRP markedly increases (58, 60). However, the short term changes in CRP after moderate-intensity exercise of short duration have not been characterized.

Following acute smoking fibrinogen (39, 40, 44), and platelet count (40) remain unchanged and the results of acute smoking on fibrinolytic activity are conflicting (35, 39, 45), but based on the strongest evidence, it will remain unchanged (35, 39). The acute effect of smoking on APTT and PT is not known; however, PT and APTT are lower among current cigarette smokers compared to nonsmokers (29, 46). Acute exercise increases fibrinolysis (52-54, 61, 63), platelet count (64-67) and decreases APTT (52-54, 64). There are conflicting results on the effect of acute exercise on fibrinogen (52, 60-62) and PT (64, 65), but based on the strongest evidence fibrinogen (52, 61) and PT (65) will not change.

Acute smoking increases heart rate and blood pressure (44, 47-50) and reduces the ability to exhale forcefully as indicated by a reduction in $FEV_{1,}$

however, there are conflicting results for FVC and peak expiratory flow rate (PEFR) as they can decrease or remain unchanged following acute smoking (37, 38). Following acute exercise, heart rate increases above rest during the first 30 minutes (55, 56), and blood pressure decreases (55, 56, 68, 69). FVC is significantly reduced at 5 and 10 min post-exercise (57, 71, 72). However, there are conflicting results for FEV_1 which can decrease significantly (71) or remain unchanged following maximal and submaximal exercise (57, 72).

Limitations

The limitations to this study are as following:

1. Female participants will not be recruited in order to eliminate the potentially confounding effects related to gender on the inflammatory and blood coagulation markers (differences in body fat, body fat distribution, menstrual cycle fluctuations and potential influence of various birth control drugs).

2. Daily physical activity and dietary record and pre-blood draw questionnaire will be quantified, but not controlled. Participants will fast for at least 6 hour, abstain from smoking for 12 hour and from physical activity for 72 hour prior to the blood draw.

3. Only a single session of exercise will be studied.

Delimitations

The delimitations of this study are listed below:

1. The subjects will be recruited from Auburn University and the surrounding communities in Auburn-Opelika, Alabama.

2. Participants will consist of a minimum of twelve healthy male casual smokers (smoking no more than 7 cigarettes per day) between the ages of 19 and 35, and with a body mass index (BMI) less than 30 Kg/m^2.

3. Each participant will undergo three protocols: a single bout of moderate-intensity aerobic exercise, smoking two cigarettes, and smoking two cigarettes just prior to a single bout of exercise.

4. Participants will smoke 2 cigarettes of the same brand (Marlboro Light 100's containing 0.8 mg of nicotine each) within a 1/2-hour time period.

5. Experimental blood draws for each condition will be taken at baseline, immediately after, 10, 20, 40 minute and 24 hour after the end of the protocol.

Significance of the Study

The independent effects of cigarette smoking on the aforementioned physiologic and biochemical markers have been characterized - as have the effects of aerobic exercise; however, this is the first study that is being done on the combined influences of smoking prior to exercise in casual smokers. It is anticipated that this study will provide unique information on the combined influences of smoking and exercise on blood markers of clotting and inflammation and physiologic function (pulmonary and cardiovascular stress). The results may be used to reinforce what is known about the deleterious effects of smoking and to characterize the added physical risks, if any, that are taken when moderate physical exertion occurs with cigarette smoking. Also the acute changes in important health parameters consequent to cigarette smoking and physical exertion will be reported to scientific and clinical information outlets. These results may provide

additional rationale for smoking cessation programs - especially when physical activity is being recommended for improving health and positively changing lifestyles.

REVIEW OF LITERATURE

Introduction

This review of literature will begin with a discussion of atherosclerosis. The deleterious effects of chronic cigarette smoking will then be discussed. The next section will cover the acute effects of smoking on blood markers and physiological variables. The last section of this review will be about the effects of an acute bout of aerobic exercise on blood markers and physiological variables.

Overview of Atherosclerosis

Coronary atherosclerosis is the leading cause of death in all industrialized countries (74). Coronary atherosclerosis may result in the clinical syndromes of ischemic heart disease, angina pectoris, myocardial infarction, sudden cardiac death, and chronic heart failure. In the United States, each year approximately 1.5 million persons have myocardial infarction and 500,000 deaths occur (74). Each year more than 600,000 coronary artery bypass surgeries and more than 500,000 coronary angioplasties are performed (74).

Numerous studies on the pathophysiology of atherosclerosis in humans and animals led to the formulation of the response-to-injury hypothesis**,** which proposed endothelial dysfunction as the first step in atherosclerosis (75). According to the "response-to-injury" model, hemodynamic forces and chemical agents induce alterations

in the endothelium causing "injury" to the tissue layer (75, 76). Next monocytes attach to injured endothelium, migrate to intima (layer of connective tissue beneath endothelium) and take up cholesterol. Following that, platelets adhere to the endothelium and release growth factors. The adhesiveness of the endothelium with the monocytes, lipids and platelets results in the eventual formation of plaques (77). Endothelium dysfunction and injury induces the endothelium to have coagulant properties instead of anticoagulant (77). Platelet adhesion to injured endothelium and small blood clots (thrombi) may result in the initiation and generation of atherosclerosis (76). Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. When activated, platelets release growth factors that may contribute to the migration and proliferation of smooth muscle cells and monocytes. Activated platelets can accumulate on the walls of arteries and recruit additional platelets into an expanding thrombus (75). Plaque rupture and thrombosis are notable complications of advanced lesions that lead to unstable coronary syndromes or myocardial infarction (75). Possible causes of endothelial dysfunction leading to atherosclerosis include chemical agents introduced into the blood stream by cigarette smoking, hypertension, elevated and modified low-density lipoproteins, diabetes mellitus, infectious microorganisms and combinations of these or other factors (75).

The vascular inflammation that results from endothelial injury and plaque formation plays a key role in atherosclerosis and coronary artery disease (74, 75, 78). Inflammatory markers predict cardiovascular disease in the general population (78). Results from several epidemiologic studies provide compelling evidence to support associations between inflammatory markers, such as CRP and WBC count, with coronary heart disease (21). Several prospective studies have demonstrated a direct association

between circulating CRP levels and the risks of developing cardiovascular disease (17-22). Baseline levels of CRP predict the risk of future myocardial infarction, stroke, and peripheral atherosclerosis among apparently healthy middle-aged men (79) and women (19), even after adjustment for other known cardiovascular risk factors. WBC count, which rises during infections and inflammatory illnesses, also predicts coronary heart disease morbidity and mortality independent of traditional cardiovascular risk factors (21, 23-25).

Clotting factors are also implicated in the development of atherosclerosis (77). An elevated fibrinogen level is a predictor of cardiovascular disease and plays a role at the early stages of the atherosclerosis process (21, 24, 30). Kannel et al. (30) measured fibrinogen level in 1315 participants who were free of cardiovascular disease. During 12 years, cardiovascular disease developed in 165 men and 147 women. For both sexes, the risk of cardiovascular disease was correlated positively to higher fibrinogen levels.

Fibrinolytic activity, a process which breaks down clots, is related to the atherosclerosis process (31). Low fibrinolytic activity due to increased plasma levels of plasminogen activator inhibitor 1 (PAI-1) or reduced tissue plasminogen activator (t-PA) which are released from the endothelial cells of the vessel wall, plays a role at the early stages of the atherosclerotic process (80-82). Hamsten et al. (80) compared fibrinolytic activity in 71 patients who had survived a myocardial infarction with 50 healthy subjects of similar age, three years after the infarction. The reduced fibrinolytic activity in patients compared to controls could explain the role of fibrinolysis in atherosclerosis. The reduced fibrinolytic activity in patients was due to low tPA and mostly high levels of PAI-1.

Chronic Cigarette Smoking

Cigarette smoking increases the risk of stroke, peripheral vascular disease, chronic obstructive pulmonary disease (COPD), lower bone density, reproductive problems and many types of cancer, including cancers of the lip, oral cavity, and pharynx, esophagus, pancreas, larynx, lung, uterine cervix, urinary bladder, and kidney (83). More deaths are caused each year by tobacco use than by all deaths from human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides, and murders combined (83). Current cigarette smokers have a death rate from all causes which is twice that of those who have never smoked (13). The adverse health effects from cigarette smoking account for 440,000 deaths, or nearly 1 of every 5 deaths, each year in the United States. Although smoking-related mortality rates are declining, current statistics indicate that smoking kills an estimated 440,000 Americans each year and on average, men who smoke cut their lives short by 13.2 years, and female smokers lose 14.5 years (14). More specifically, cigarette smoking is considered to be an independent factor for carotid atherosclerosis, and coronary heart diseases (1-5). Smokers' risk of developing coronary heart disease is 2 to 4 times that of nonsmokers (84).

Chronic Smoking and Cardiovascular Disease

Following twenty-three years of follow up on 1,603 male and female smokers, 73 cases of coronary heart disease developed. In addition, coronary heart disease occurred more frequently in those who smoked > 10 cigarettes per day than in those who never smoked (4). Likewise, following a six-year follow-up on 119,404 female nurses who

were 30 to 55 years of age, 65 of the women died of fatal coronary heart disease and 242 had a nonfatal myocardial infarction (3). Results from this epidemiologic study showed that the number of cigarettes smoked per day was strongly associated with the risk of fatal coronary heart disease, nonfatal myocardial infarction, and angina pectoris. Even smoking 1 to 4 or 5 to 14 cigarettes per day was associated with a twofold to threefold increase in the risk of fatal coronary heart disease or nonfatal infarction (3).

The deleterious physical effects of inhaled cigarette smoking are well characterized, but the mechanisms by which cigarette smoking may initiate or accelerate atherosclerosis are not fully understood. Smoking affects various markers of systemic inflammation (15, 16). Therefore, smoking-related cardiovascular events may be due to elevated inflammatory markers in chronic cigarette smokers. Chronic inflammation, as indicated by elevated CRP and WBC count, is observed with greater prevalence and to a greater degree in smokers and is thought to increase the risk of coronary artery disease in those who smoke cigarettes (16, 26-28). The total WBC count increases in cigarette smokers (15) and decreases after smoking cessation (85). In 2003, Frohlich (16) measured CRP and WBC count in 2,305 men and 2,211 women, age 25-74 years. In men, current smokers showed significantly higher values for CRP and WBC count, compared to individuals who reported never smoking, with intermediate, but only slightly increased values for ex-smokers and for occasional smokers. Pack-years of smoking was positively associated with markers of inflammation. Conversely, duration of abstinence from smoking was inversely related to these markers. No such associations were found for CRP in women, but WBC count was increased in female cigarette smokers (16). Likewise, in a study comparing current smokers, former smokers and individuals who

had never smoked, CRP was higher among current smokers versus the other groups. In addition, a significant dose-response relationship was observed between cigarette smoking (cigarettes per day, pack-years) and CRP concentration (26).

Chronic exposure to cigarette smoke has been repeatedly demonstrated to cause alterations in blood coagulation factors, fibrinogen, platelet function and fibrinolytic activity, which may increase the risk of a cardiovascular event (86). Cigarette smokers tend to have elevated levels of fibrinogen compared to non-smokers (26, 39, 87) which may increase the risk of a cardiovascular event (21, 24, 30). Bazzano et al. (26) examined the relationship between cigarette smoking and fibrinogen in 4,187 current smokers, 4,791 former smokers, and 8,375 never-smokers 18 years of age and older. Cigarette smoking was related to elevated levels of fibrinogen and there was a positive and significant dose-response relationship between cigarettes per day and pack-years with levels of fibrinogen.

Faster blood coagulation observed with chronic smoking is thought to contribute to smokers' greater risk for coronary artery disease (29). Activated partial thromboplastin time (APTT) and prothrombin time (PT), which are measures of blood clotting time, are lower in chronic cigarette smokers, increasing the blood coagulation (29, 46). Yarnell and coworkers (29) reported a consistent decrease in APTT among smokers. The relation of PT with smoking has been evaluated in 3,604 white and 514 black males, aged 31-45 years. The mean prothrombin time was significantly shorter (0.2 seconds) among current cigarette smokers than among men who had never smoked (46).

Cigarette smokers tend to have elevated platelet counts compared to non-smokers (42, 43). Aghaji and coworkers (43) observed an elevated level of platelet count in male smokers versus non-smokers.

Not only are smokers more likely to form clot as indicated by elevated fibrinogen levels and lower APTT and PT, but also they have a lower tendency to break down clot (32-35, 39). The lower blood fibrinolytic activity in smokers could be attributed to a lower baseline level in t-PA (32, 39) or elevated levels of its main inhibitor, PAI-1 (33-36). In 1997, Simpson and coworkers (33) reported fibrinolytic activity in healthy individuals divided by smoking habit into current smokers, former smokers and non-smokers. PAI-1 antigen was significantly higher in smokers than in non-smokers with intermediate levels in former smokers, indicating an impaired fibrinolysis in smokers. In addition PAI -1 antigen and activity were correlated with pack-years of smoking.

Chronic Smoking and Pulmonary Disease

Ninety percent of all lung cancer deaths and approximately 80 to 90 percent of COPD (emphysema and chronic bronchitis) mortality rates are related to smoking (88). Even among smokers who have quit chronic lung disease accounts for 50 percent of smoking-related pulmonary conditions (88). In 1999, Iribarren and colleagues (6) followed 1546 men who smoked cigars and 16,228 who did not smoke over a 24 hour period, from 1971 through the end of 1995 for a first hospitalization or death from COPD, and through the end of 1996 for a diagnostic cancer. Cigar smokers, as compared with nonsmokers, were at higher risk for COPD, and cancers of the upper digestive tract and lung, with evidence of dose-response effects (cigars smoked per day).

Cigarette smoking during adolescence appears to reduce the rate of lung development and the level of maximum lung function that can be achieved. A consistent trend toward reduced pulmonary function was observed among chronic smokers versus nonsmokers (37). De (38) examined pulmonary function tests on 89 smokers. Pulmonary function test parameters including FVC, FEV_1, FEV_1/FVC and PEFR were reduced in chronic smokers in comparison to those of age-matched non-smokers. Further, it was noted that there is a dose-response relationship between the number of cigarettes smoked per day and these measures of lung function.

Summary

Cigarette smoking elevates inflammatory markers (CRP and WBC count), and enhances blood coaguability, which both could increase the risk of coronary heart disease in cigarette smokers. In addition cigarette smoking reduces lung function, which may increase the risk of COPD and cancer.

Acute Cigarette Smoking

Part of the deleterious physical effects of smoking may be attributed to its acute effects on inflammatory markers, blood coagulation factors, and physiological variables.

Inflammatory Markers

Chronic smokers have significantly higher values for CRP (16, 26, 32, 41). The acute effects of smoking on CRP are currently uncharacterized, however the free radical oxidative stress which could arise from the gas or tar phase of cigarette smoke, can

initiate endothelial dysfunction and increase inflammatory responses on the vessel wall and contribute to the development of atherosclerosis (86). Blann et al. (40) observed a transient rise in WBC count following smoking two cigarettes in sequence, and in some individual smokers, the WBC count level peaked at 10 minutes or 30 minutes after smoking.

Blood Coagulation Markers

Although cigarette smokers tend to have elevated levels of fibrinogen compared to non-smokers (16, 26, 39), fibrinogen appears to remain unchanged after acute smoking (39, 40, 44). In a study reported by Blann and coworkers (40), immediately after, and at 10 and 30 min after smoking two cigarettes in sequence, there were no changes in fibrinogen concentration. Belch and coworkers (39), reported no changes in fibrinogen 10 minutes after smoking 3 cigarettes. Although cigarette smokers tend to have elevated levels of platelet count compared to non-smokers (42, 43), immediately, 10 and 30 minutes after smoking two cigarettes in sequence, there were no changes in platelet count (40). The acute effect of smoking on APTT and PT is not known; however, smoking is associated with an accelerated blood coagulation mechanism as manifested by a shortening of PT and APTT compared to nonsmokers (29, 46). APTT measures clotting time in the extrinsic pathway and PT is used to measure the clotting time in the intrinsic pathway. The decrease of both APTT and PT in chronic smokers shows that chronic smoking could affect both intrinsic and extrinsic pathways.

Although chronic smokers have lower baseline blood fibrinolytic activity, the results of acute smoking and fibrinolytic activity are conflicting (32-35, 39, 44). Kimura

et al. (44) reported changes in fibrinolysis after smoking two cigarettes in a 10-minute period. Blood samples were taken immediately and 15 minutes after smoking. Fibrinolytic activity was markedly increased after smoking as indicated by an increase in tPA antigen and activity, and remained higher than baseline even 15 minutes after smoking. No significant changes were observed in PAI-1 antigen.

Belch and coworkers (39) observed that 10 minuets after smoking three cigarettes, no changes were observed in PAI-1. Haire et al. (35) reported that smoking two cigarettes did not alter fibrinolysis, as it neither stimulated t-PA nor PAI, in healthy male cigarette smokers.

Physiological Variables

Different studies have shown an increase in resting heart rate and blood pressure following smoking one or two cigarettes (44, 47-50). Kool et al. (47) reported that after smoking one cigarette containing 1.3 mg of nicotine, systolic and diastolic blood pressure and heart rate increased significantly. Failla and coworkers (48) evaluated changes in heart rate and blood pressure over a 5-minute period immediately after smoking. Smoking caused increases in systolic and diastolic blood pressure and heart rate, which occurred soon after the beginning of smoking and persisted throughout the smoking and for 5-minutes after smoking. On average, smoking increased systolic blood pressure by 14%, diastolic blood pressure by 10% and heart rate by 27%.

Chronic smokers tend to have lower pulmonary function values compared to age-matched non-smokers (37, 38); however, changes in pulmonary function with acute smoking are less consistent, as lung function measurements can decrease or remain

unchanged following acute smoking (37, 38). Prokhorov and coworkers (37) indicated a reduction in FEV_1, and PEFR, but no significant changes were observed in FVC, and FEV_1/FVC. De et al. (38) evaluated pulmonary function in 89 smokers, 30 minutes after the smokers smoked two cigarettes. FEV_1 and FVC were reduced after smoking, but no changes were observed in PEFR.

Summary

Acute smoking increases WBC count. The changes in blood coaguability after acute smoking are different, as platelet count and fibrinogen concentration do not appear to change significantly and fibrinolytic activity may remain unchanged or increase. Acute smoking increases cardiovascular stress as indicated by an elevation in heart rate and blood pressure and reduces FEV_1.

Acute Moderate Aerobic Exercise

A single bout of physical exertion can elevate the risk of an adverse cardiovascular event during and shortly after exercise by modulating physiological, inflammatory and blood coagulation parameters.

Inflammatory Markers

Acute exercise increases WBC count (58, 59) and CRP (58, 60). Nieman and colleagues (59) reported that walking on a treadmill at 60% $\dot{V}O_{2max}$ for 45 minutes is associated with a significant, but moderate, increase in WBC count immediately after and up to 3-hours post exercise.

Taylor et al. (58) examined the inflammatory markers on 18 athletes before and after a 160-km triathlon involving canoeing, cycling, and running. WBC count increased immediately after the event and remained elevated for 30 minutes after the race and CRP increased by nearly 300% at the 24 hour post-exercise measurement.

In 1991, Weight and colleagues (60) examined the effect of a 42-km marathon race on CRP concentration in male and female distance athletes. Blood samples were drawn before, and up to 6 days after the race. CRP was markedly increased both 24 and 48 hours after the marathon and returned back to baseline at day 6.

Blood Coagulation Markers

There are conflicting results regarding the effect of acute exercise on fibrinogen. Fibrinogen levels were increased following a 42-km marathon race and remained elevated for up to 6 days after the race (60).

On the other hand, Rankinen et al. (61) reported no changes in fibrinogen concentrations after a maximal exercise test, a 30-minute submaximal exercise test at 50% $\dot{V}O_{2max}$ (aerobic threshold) or a 30-minute submaximal exercise test at 78% $\dot{V}O_{2max}$ (anaerobic threshold). Likewise, in a study by Van den Burg and colleagues (52), twenty-nine sedentary males underwent a maximal cycle ergometer test. Fibrinogen concentration remained unchanged throughout the exercise (0, 10, 15, 20, and 25 minutes) and during a 25-minute recovery period.

But on the other hand, after a marathon race, well-trained long-distance male runners displayed a significant decrease of fibrinogen which was still decreased 24 hours later, although 48 hours after the competition fibrinogen was back to baseline (62).

Both acute maximal and submaximal exercise increase platelet count significantly (64-67). Prothrombin time appears to decrease (64) or not change (65) after aerobic exercise. Ferguson and coworkers (64) reported increases in platelet count and decreases in PT immediately, and one hour after a standardized maximum exercise test on a treadmill. PT was shortened immediately after the exercise test, but was not different from baseline measurements within one hour of recovery. Likewise, platelet counts increased significantly after exercise, but returned back to normal in one hour of recovery.

Drygas (65) examined PT and platelet count changes following various models of exercise including a submaximal 18-minute, a prolonged submaximal 60-minute and a maximum stepwise bicycle ergometer test. None of the models caused a significant change in PT. Following the 18-minute exercise, a slight but significant increase in platelet count was observed, but the prolonged 60-minute exercise and the maximum exercise test caused a significant increase in platelet count.

Different studies have shown a decrease in APTT (54, 64) and an increase in fibrinolytic activity (54, 63) following an acute bout of exercise.

Van den Burg et al. (52) reported changes in fibrinolytic activity and APTT after a standardized cycle ergometer test. Blood samples were obtained at two exercise levels, 70% $\dot{V}O_{2max}$ (submaximal), 100% $\dot{V}O_{2max}$ (maximal) and during 25 minutes of recovery. Both during submaximal and maximal performance, fibrinolytic activity increased as tPA antigen and activity were increased. But during the 25 minutes of recovery as tPA activity decreased, fibrinolytic activity demonstrated a rapid decline. During submaximal and maximal exercise a decrease in APTT was observed. This decrease was sustained

during the recovery and at the end of recovery; it was shorter than observed during exercise.

Hegde and coworkers (53) examined fibrinolytic activity and APTT changes following a run at 70-75% $\dot{V}O_{2max}$ or walk at 1.2 mph for 30 minutes in a random order. Blood was obtained at rest, immediately after, and every 20 minutes during a one hour recovery. Walking did not alter the activity of either tPA or APTT as compared with rest. But immediately after the run, tPA was significantly increased; however it demonstrated a negative slope during the one hour recovery time. APTT was significantly decreased after the run and remained at this level during the one hour recovery.

Rankinen et al. (61) reported fibrinolytic activity after a maximal exercise test, 30 minutes of submaximal exercise at 50% $\dot{V}O_{2max}$ (aerobic threshold) and 30 minutes of submaximal exercise at 78% $\dot{V}O_{2max}$ (anaerobic threshold). Blood samples were drawn before, immediately, and 24 hour after each test. TPA activity increased immediately following all three tests. PAI-1 activity decreased during maximal and anaerobic threshold test, but not during the aerobic threshold test. The 24 hour post-exercise fibrinolytic activity values were all back to normal.

During strenuous physical exercise, increases in coagulation (shortening of APTT) and fibrinolytic activity (increase in t-PA activity) proceed in parallel (52). However, during recovery, while there is a sustained increase in coagulation, fibrinolytic activity demonstrates a sharp fall, which suggests a more favorable situation for clot formation after exercise. This phenomenon could constitute an enhanced risk for coronary artery thrombosis, which may contribute to exercise-related cardiovascular events (52-54).

Physiological Variables

Following an acute bout of aerobic exercise, heart rate can remain above rest during the first 20 or 30 minutes of recovery (55, 56) and different studies have reported that blood pressure is typically lower than pre-exercise values with a decrease in systolic blood pressure and no change in diastolic blood pressure (55, 56, 68). One study found that following a 30 minute bout of cycle ergometer exercise at 50 or 75 % of $\dot{V}O_{2max}$, diastolic blood pressure was lower during 45 minutes of recovery compared with before exercise (69).

Brown et al. (55) measured heart rate and blood pressure changes in 12 normotensive males. Subjects completed a 45 minute cycle exercise at 50% $\dot{V}O_{2max}$ and heart rate and blood pressure were measured in the last 1 minute of exercise and at 5, 15, 20, 40, and 60 minutes of recovery. Systolic blood pressure after exercise was significantly lower than rest at most measurement intervals except for a nonsignificant increase and decrease at 5 and 60 minutes of recovery, respectively. Systolic blood pressure was lowest from 15 to 40 minutes of recovery where it was 5-8 mmHg below the resting value. Diastolic blood pressure did not change during and after exercise. Heart rate during recovery was above rest and the differences were significant only from 5 to 20 minutes of recovery.

In general a decrease in pulmonary function can be seen after maximal and submaximal exercise (57, 70-72). Coast and coworkers (57) examined FVC and FEV_1 immediately after, and 5, 10 and 15 minutes following a maximal cycle ergometer test. FVC was decreased immediately following exercise, but it returned to near original levels within 5 minutes post exercise and FEV_1 was not altered following exercise.

O'Kroy et al. (71) have investigated changes in FVC and FEV_1 following three different intensities and durations of treadmill running on active runners. The experiment included a graded maximal test to exhaustion, a 7 minute test at 90% of $\dot{V}O_{2max}$, and a 30 minute test at 60% of $\dot{V}O_{2max}$. FVC and FEV_1 were measured pretest, 5, 10, and 30 minutes post exercise. FVC was different between times but not between intensities. FVC was decreased at 5 and 10 minutes post-test compared with pre and 30 minutes. FEV_1 was significantly reduced at 5 and 10 minutes post-test, with no differences found between intensities. FEV_1 had returned to near baseline values by 30 minutes post-test in the 60% intensity while the maximal and 90% intensities remained lower than pretest values. FVC recovery trends were similar to FEV_1 in that the higher intensity decrements remained depressed from pretest values while the lower intensities returned to near pretest values.

Cordain and colleagues (72) performed two bouts of exercise on separate days; one to maximal heart rate and one to 85% of maximal heart rate for 20 minutes. FVC and FEV_1 were measured prior to, and at 5, 15, 30, 60 and 120 minutes post-exercise. FVC decreased significantly at 5, 15, 30, and 60 minutes following both maximal and submaximal exercise, however the changes between the two exercise bouts were not significantly different from one another. FEV_1 generally remained unchanged and showed no difference between the two exercise bouts.

Summary

A single bout of aerobic exercise is associated with a significant increase in WBC count. Although following a triathlon and marathon race, CRP markedly increases, the

short-term changes in CRP after moderate-intensity exercise of short duration have not been characterized. Acute exercise increases blood coaguability, as platelet count increases and APTT decreases. There are conflicting results on the effect of acute exercise on fibrinogen and PT, with differences in the exercise protocols, training status and the analytical methods used for the blood analysis probably being responsible for the reported inconsistencies. Fibrinolytic activity increases during and immediately after exercise, but it demonstrates a sharp fall within the recovery period. Heart rate remains above rest during the first 30 minutes of exercise recovery, systolic blood pressure decreases and a reduction in FVC is observed immediately post-exercise.

Conclusion

The combined risk of coagulation, increased inflammation and cardiovascular stress following smoking and exercise, may acutely increase risk of an adverse cardiovascular outcome such as ischemia, myocardial infarction or sudden death, and will reduce lung function values. Therefore, it was the purpose of this study to determine the combined influences of smoking and moderate-intensity aerobic exercise on physiological, inflammatory and blood coagulation responses in young healthy casual smokers.

METHODS

Overview

Apparently healthy young male casual smokers will undergo each of three conditions in a randomized order: 1) a single bout of moderate-intensity aerobic exercise, 2) cigarette smoking, and 3) cigarette smoking just prior to a single bout of exercise. Blood samples will be drawn prior to, immediately after, 10, 20, 40 minutes and 24 hours after completing each condition. A pre- and post-pulmonary function test will be performed, and heart rate and blood pressure will be monitored continuously throughout each condition. The protocol will span approximately eight visits (~10 hours) and consists of three 5-day dietary and physical activity interventions. A general experimental timeline is presented in Figure 1.

Participants

Twelve male volunteers between the ages of 19 and 35 years, who self-reported that they smoked no more than 7 cigarettes per day were recruited from Auburn University, Auburn-Opelika, AL, and surrounding communities through flyers and verbal invitations. All participants had a BMI less than 30 kg/m^2 and were apparently healthy. Apparently healthy is defined as no known cardiovascular, pulmonary or metabolic disease, no blood disorders, no signs or symptoms suggestive of underlying cardiovascular disease and currently free of any physical condition that may elevate

markers of immune system function (colds, allergies, musculoskeletal problems, infections, skin abrasions, etc.) and not currently taking medications known to influence the dependent variables of interest in this study.

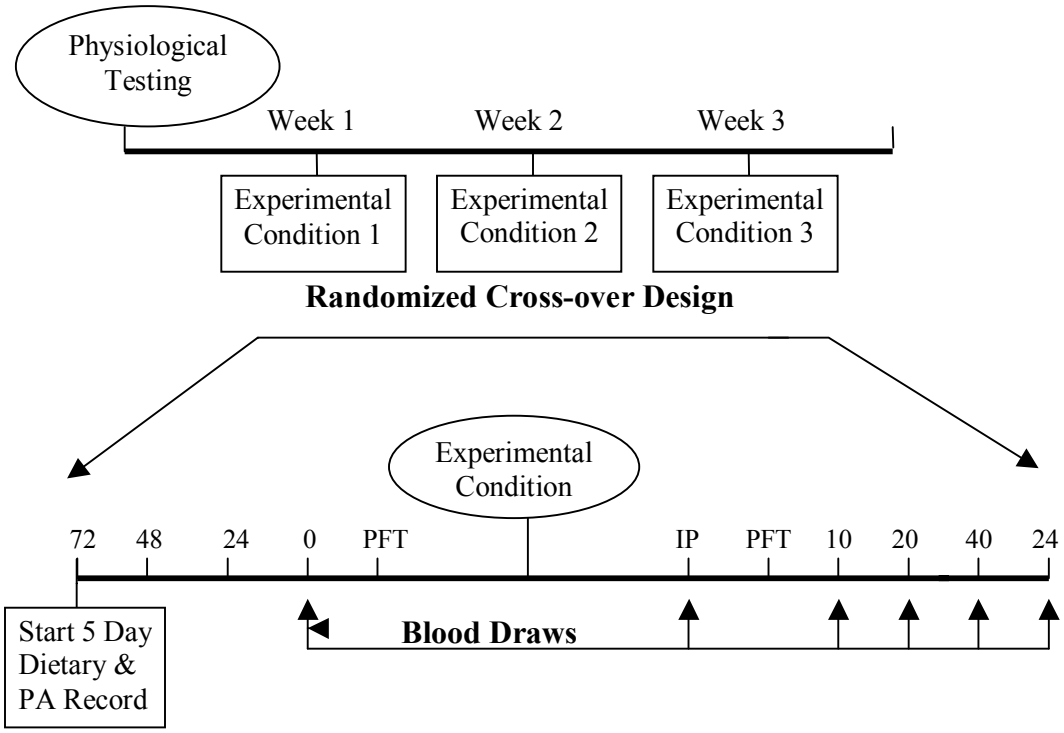

Figure 1. General Experimental Timeline. Physiological measurements consist of anthropometric measurements, pulmonary function test, physician exam and a graded exercise test. Participants will undergo 3 conditions in a randomized order: 1) a single bout of moderate-intensity aerobic exercise, 2) cigarette smoking, and 3) cigarette smoking just prior to a single bout of exercise. A 5-day physical activity (PA) and dietary record will be kept for each condition 3 days prior to, the day of and the day following the experimental condition. Blood samples will be drawn prior to, immediately post (IP),

10, 20, 40 minutes and 24 hours after completing each condition and a pre- and post-pulmonary function test (PFT) will be performed.

Preliminary Screening

Volunteers were asked to come to the Auburn University Exercise Technology Lab and were fully informed about the study. They read and signed an IRB-approved informed consent document. Next, they completed Health History and Physical Activity Questionnaires. All volunteers who met entry criteria for the study were evaluated for preliminary physiological assessment and further screening which included: 1) a physician exam and review of self-reported health history to determine each volunteer's appropriateness and safety for participation in the graded exercise test, 2) height, weight, BMI, waist and hip circumferences and relative body fat, 3) lung volumes and pulmonary function measures, 4) a maximal graded treadmill test (stress test), with heart rate, blood pressure and 12-lead electrocardiographic tracings. A physician was present and available during all of the physiological assessments.

Relative body fat was measured by using Lange® Skinfold Calipers to measure skin folds at seven different sites (1, 89). Pulmonary function was tested via an automated open-loop spirometer (Breeze 6.1 software, Medical Graphics Corp., St Paul, MN). The maximal graded exercise test was performed on a motor driven treadmill using the Bruce Protocol to determine each participant's maximal oxygen consumption ($\dot{V}O_{2max}$). Participants' respiratory gases [oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$)] were measured using an automated breath-by-breath system (Medical Graphics CardiO_2 Integrated Metabolic System, Med Graphics Corp., St. Paul,

MN). Standard gases were used to calibrate both analyzers before each test. Heart rate and blood pressure were taken following five minutes of supine rest, during the final 60 seconds of each stage, and at one, three, and five minutes during active recovery using a Polar® Heart Rate Monitor and their blood pressure was taken manually using a mercury sphygmomanometer. Rate of perceived exertion (RPE) was obtained during the final 30 seconds of each stage. The graded exercise test was considered a maximal effort if the participant met any two of the following criteria: the respiratory exchange ratio (RER) was ≥1.1, the subject's maximum heart rate was no more than 10 beats below his age-predicted maximum, or the participant experienced volitional fatigue.

Following completion of all the screening tests, the physician reviewed the exercise test results in order to determine the appropriateness and safety for participation in this study. All eligible participants were given 5-day dietary and physical activity record forms, and instructed on how to appropriately complete these forms.

Experimental Procedures

Participants underwent each of three experimental conditions in a randomized order: 1) a single bout of moderate-intensity aerobic exercise, 2) cigarette smoking, and 3) cigarette smoking just prior to a single bout of exercise. Each experimental condition was separated by at least one week to eliminate the influence of the previous condition on the dependent variables of interest. All data were collected in a standard format for all three conditions. Participants reported to the Exercise Technology Lab after a 6 to 8 hour fast (limited to water intake only), after abstaining from alcohol or tobacco for the previous 12 hours and moderate-to-vigorous-intensity physical activity for the previous

72 hour period. Participants were weighed, fitted with a heart rate monitor and then sat upright and quietly for 5 minutes in a phlebotomy chair. After 5 minutes of seated rest, blood pressure and heart rate were measured. Next an indwelling venous catheter was inserted into an antecubital vein and a baseline blood sample was drawn. Then participants underwent a baseline pulmonary function assessment (a forced vital capacity maneuver - FVC) until two maneuvers met American Thoracic Society standards and were within 5% of each other.

The exercise condition included treadmill walking / jogging at 60 to 70% of maximal exertion until 500 kcals were expended (~40 to 60 minutes). The cigarette smoking condition consisted of smoking 2 cigarettes of the same brand (Marlboro Light 100's containing 0.8 mg of nicotine each) within a 1/2-hour time period. All cigarette smoking occurred in a designated outdoor smoking area while the participant was seated. Investigators monitored smoking from a distance sufficient to avoid second-hand smoke inhalation. The combined condition included cigarette smoking immediately followed by treadmill walking/jogging.

While exercising on the treadmill, participant's $\dot{V}O_2$ were measured at different time points by using the automated breath-by-breath system to calculate caloric expenditure. We multiplied participants $\dot{V}O2$ (L/min) by five to calculate the total kcals expended during different intervals of the exercise. To calculate the time remaining we subtracted the total kcals expended from our goal of 500 kcal and then divided it by the current kcals/min rate.

Blood samples were drawn immediately after and 10, 20 and 40 minutes after completing each condition. The indwelling catheter was removed after the 40 minute

blood sample. A post-condition pulmonary function test was performed between the immediately post and 10-minute post blood samples. Heart rate was monitored continuously and blood pressure obtained at regular intervals after each condition. Participants were requested to remain in the laboratory until their heart rate returned to 60 to 100 beats per minute, blood pressures approximated baseline values and the participant indicated being capable of leaving safely.

One additional blood sample was obtained 24-hours after completing each condition. Participants were asked to fast for 6 to 8 hours, and abstain from smoking, alcohol consumption and outside moderate-intensity physical activity until after their 24-hour post-condition blood sample was obtained. Participants submitted their dietary and physical activity logs for each week after completing the 24-hour condition. All experimental data collection occurred during the weekday and during early afternoon-evening hours (between 3 and 7 pm).

Blood Sampling

An indwelling venous catheter (20-gauge x 1 1/2" teflon-coated) was inserted into an antecubital vein, capped with a multi-sample injection port and secured with medical tape. For the baseline blood draw, we obtained ~ 21.5 mL of blood (14 mL into two red-top tubes, 4.5 mL into a blue-top tube and 3 mL into a purple-top tube). After this and subsequent blood samples (except the final sample), we introduced ~1.5 mL of heparin-saline flush in order to maintain catheter patency. For the immediately after and 10, 20 and 40 minutes blood draws, each time we obtained ~ 7.5 mL of blood (4.5 mL into a blue-top tube and 3 mL into a purple-top tube). One additional 17 mL blood sample (14

mL into two red-top tubes and 3 mL into a purple-top tube) was obtained by needle 24-hours after completing each condition. The total volume of blood drawn for each condition was ~68.5 mL (~51.5 mL on the first day and ~17 mL 24 hours later). CRP was analyzed from the samples in the red-top tubes. Blood clotting variables (fibrinogen, PAI-1, APTT and PT) were measured in the samples in the blue-top tubes and purple top tube samples were used for the analysis of platelet count, WBC count, hemoglobin and hematocrit.

Biochemical Analysis

High sensitivity CRP was analyzed using an enzyme-linked immunosorbent assay (ELISA) according to the MP Biomedicals instructions (High sensitivity C-reactive protein enzyme immunoassay kit). WBC count, platelet count, hemoglobin and hematocrit were analyzed in duplicate by using the CELL-DYN 1700 autoanalyzer (Abbott Diagnostics, Abbott Park, IL). WBC count analysis was determined by using the electrical impedance method, which passes through an aperture of the Von Behrens WBC Transducer (90). This transducer has a known size and the cells are counted by changes in electrical resistance as they pass through the transducer. The Von Behrens WBC Transducer also determines the size of the WBCs (neutrophils, lymphocytes, basophils, and eosinophils). The amplitude of the impulse is proportional to the size of the cell that passes through the transducer. Platelet count was measured by using the electrical impedance method (90). The whole blood is analyzed after a 1:12801 dilution is made. The diluted specimen enters the Von Behrens RBC (red blood cell)/Platelet transducer through an aperture by means of a vacuum. The red blood cells and platelets are

measured by electrical impedance. The amplitude of the impulse is proportional to the size of the cell that passes through the transducer. The hematocrit (HCT) is determined by using the ratio of RBC to plasma and it is expressed as a percentage of the whole blood. The HCT is calculated from the red blood cell count and the mean corpuscular volume (MCV). The following equation is used: HCT = (RBC x MCV)/10. The hemoglobin measurement is obtained by using the Modified Cyanomethemoglobin Method. This colorimetric assay is read by the instrument using a filtered photodetector with a wavelength of 540 nanometers. Relative plasma volume shifts at each blood sampling time were calculated from hemoglobin and hematocrit.

Fibrinogen, APTT and PT were analyzed using the STA Compact (Diagnostica Stago, Parsippany, N.J.) coagulation instrument according to the manufacturer's instructions (STA-R Operators Manual). All of the results were run in duplicate within four hours. The instrument uses an electromagnetic mechanical clot detection system. The cuvette contains a steel ball and when the patient specimen and reagents are added, a clot will form and the steel ball will stop moving and the time will be recorded in seconds. The PT was measured by adding 50 µL of patient sample in sodium citrate to 50 µL of STA-Neoplastin CI Plus 10. The specimen and reagent are mixed to allow the clot to form. The APTT was measured by adding 50 µL of patient sample to 50 µL of $CaCl_2$, then after a short incubation, 50 µL of PTT reagent is added so that the analysis can begin. The fibrinogen was measured by adding 100 µL of patient sample to fibrinogen reagent to make a 1:20 final dilution.

Dietary and Physical Activity Record

In order to reduce the possible confounding influence of dietary nutrients and outside physical activity on changes in the inflammatory markers and blood clotting factors, participants were asked to record their food intake 3 days prior to and throughout each experimental condition. In order to familiarize each participant with dietary record keeping and the dietary requirements of the study, a dietary record example was given to each participant. Caloric intake and the percentages of carbohydrate, fat and protein were assessed from individual dietary records. The dietary records were analyzed by using a commercially available software package (Food Processor v. 7.4, ESHA Research, Salem, OR).

Participants were also asked to avoid any strenuous exercise 3 days prior to and throughout each of the experimental conditions. This was verified verbally and by using a self-reported 5-day physical activity questionnaire for each of the experimental conditions (91).

Statistical Analysis

The dependent variables of interest were the inflammatory (CRP and WBC count), blood coagulation (fibrinogen, APTT, PT, PAI-1, platelet count), cardiovascular (heart rate and blood pressure), and the pulmonary variables (FVC, FEV_1, FEV_1/FVC, PEFR, $FEF_{25-75\%}$). The independent variables were the experimental conditions (a single bout of exercise, smoking and acute smoking prior to a single bout of exercise) and time (depending on the variable of interest).

Data were analyzed using 3 (condition) x variable times (depending on the variable of interest) repeated measures analysis of variance (ANOVA). Among the blood samples, fibrinogen, APTT, and PT were analyzed by 3 x 5, platelet count and WBC count by 3 x 6, and finally CRP by 3 x 2 ANOVA. Heart rate and blood pressure were analyzed by 3 x 5 and pulmonary variables by 3 x 2 ANOVA. Simple main effects and Duncan New Multiple Range tests were used to follow up significant ANOVA findings. CRP was analyzed by change score CRP (CRPpost – CRPpre) using a Non-parametric Sarage Test, since CRP values were not normally distributed (92). The comparison wise error was set at $p < 0.05$ level.

RESULTS

The purpose of this study was to determine the combined influences of smoking and moderate-intensity aerobic exercise on physiological, inflammatory and blood coagulation responses in young healthy casual smokers. The results of this study are presented in the following order: 1) participant characteristics; 2) physiological responses to smoking and exercise; 3) pulmonary responses to smoking and exercise; 4) biochemical responses to smoking and exercise.

Participant Characteristics

Twenty participants agreed to participate in this study. Eight participants dropped out of the study due to physical issues, unrelated to our intervention, that precluded participation (e.g., developed heart dysrhythmia requiring medication, a liver condition requiring medication, and one had severe vasovagal response to venipuncture) and time conflicts. As a result, twelve participants met the criteria and finished the study. Of the twelve participants, seven were Asian-Indians, four were South Korean and one was Turkish. Participants' baseline physiological characteristics are summarized in Table 1.

Age (yrs)	25 ± 4	19 - 34
Height (inches)	69.5 ± 2	65.5 - 73
Weight (lbs)	167.0 ± 18.9	127.0 – 188.3
BMI (kg/m^2)	24.6 ± 2.7	20.1 – 28.3
Waist (inches)	32.5 ± 3.1	26.0 – 36.5
% Fat	20.0 ± 5	9 - 25
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	39.2 ± 5.7	31.9 – 48.1

Table 1. Baseline physiological characteristics. BMI= body mass index; % Fat = body fat expressed as percentage of body weight; $\dot{V}O_{2max}$= maximal oxygen consumption. All values are presented as group means ± 1 standard deviation with the group range.

Physiological Responses to Smoking and Exercise

Physiological Changes during Exercise

The exercise sessions were designed to expend 500 kcals of energy at 60-70% of each participant's $\dot{V}O_{2max}$. As expected, the average exercise $\dot{V}O_2$, RER, rate of caloric expenditure and RPE were similar for both exercise sessions ($p > 0.05$ for all). In both of the exercise trials, the participants reached the target energy expenditure in 39 to 60 minutes of treadmill walking/jogging. The average steady state exercise heart rate was significantly higher in the SE (smoke and exercise condition) versus the EX (exercise condition) condition ($p = 0.0342$, $F_{1, 11} = 5.84$). The average steady state physiological measurements during exercise are summarized in Table 2.

$\dot{V}O_2$	EX	1.99 ± 0.30	1.63 – 2.62
	SE	1.99 ± 0.34	1.66 – 2.62
RER	EX	0.96 ± 0.05	0.86 – 1.02
	SE	0.95 ± 0.03	0.92 – 0.99
HR	EX	153 ± 9	136 - 167
	SE	159 ± 11	149 – 185
RPE	EX	12.7 ± 1.1	11 – 14
	SE	12.4 ± 1.4	11 - 15
Tkcals	EX	506 ± 3	502 – 511
	SE	506 ± 14	492 - 549
Time	EX	51.9 ± 7.2	39 - 62
	SE	51.9 ± 7.3	39 - 60

Table 2. Steady state physiological measurements during exercise. EX = exercise condition; SE = smoke and exercise condition; $\dot{V}O_2$ = oxygen consumption ($L \cdot min^{-1}$); RER = respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$); HR = heart rate ($beats.min^{-1}$); RPE = Borg rating of perceived exertion-classical scale (6-20); Tkcals = total kcals expended with exercise; Time = duration of exercise (minutes). All data are presented as means ± 1 standard deviation with the range value.

Changes in Heart Rate and Blood Pressure with Smoking and Exercise

Post-condition heart rates at 10, 20, and 40 minutes were significantly different after smoking and exercise (SE) than with smoking (SM) or exercise (EX) alone.

Post-condition heart rates remained higher with SE as compared with EX alone and heart rates after EX were significantly greater versus SM ($p < 0.0001$, $F_{8, 88} = 15.29$). Post-condition heart rate responses are presented in Table 3.

HR	Baseline	IPS	IPE	10-min	20-min	40-min
SM	73 ± 7^{a}	82 ± 11^{b}	.	72 ± 7^{a}	71 ± 7^{a}	70 ± 7^{a}
EX	73 ± 9^{a}	.	133 ± 10^{b}	$93 \pm 7^{c*}$	$88 \pm 9^{c*}$	$81 \pm 8^{d*}$
SE	72 ± 8^{a}	87 ± 7^{b}	132 ± 20^{c}	$100 \pm 11^{d\dagger}$	$94 \pm 7d^{\dagger}$	$89 \pm 8^{b\dagger}$

Table 3. Post-condition heart rate (HR) responses (beats.min^{-1}). SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition; IPS = immediately post smoking; IPE = immediately post exercise; 10-min = 10 minutes post condition; 20-min = 20 minutes post condition; 40-min = 40 minutes post condition. All values are presented as means $\pm$ 1 standard deviation. Means with similar lower-case superscripts are similar; * = significantly different from smoking condition; † = significantly different from smoking condition and exercise condition.

The average systolic blood pressure in the smoking condition was significantly greater than exercise, but not significantly different from the average systolic blood pressure observed with the smoking and exercise combination ($p = 0.0144$, $F_{2, 22} = 5.17$). See Figure 2.

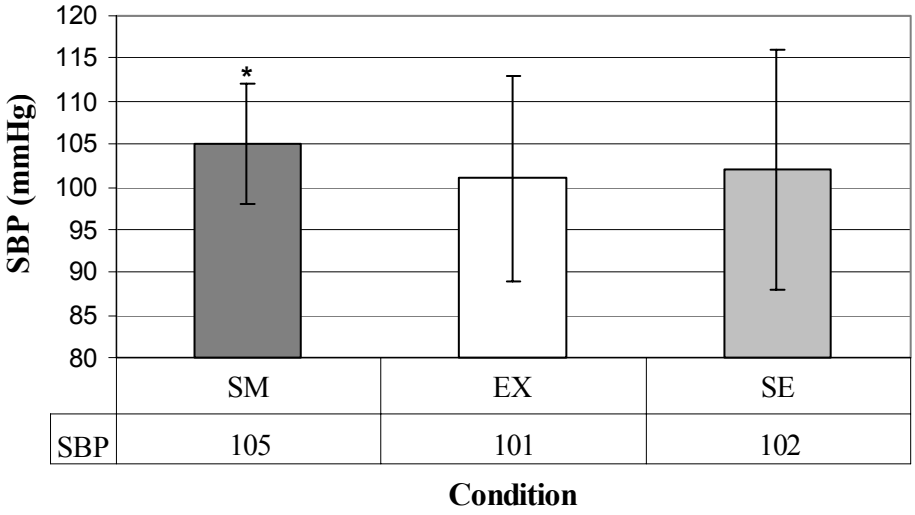

Figure 2. Systolic blood pressure (SBP) changes across conditions. SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition. All values are condition main effects (average for each condition across time) $\pm$ 1 standard deviation. * = significantly different from exercise condition.

Systolic blood pressure increased similarly immediately after smoking (SM), exercise (EX), and the combined (SE) conditions and returned to baseline values within 10 minutes of recovery ($p < 0.0001$, $F_{5, 55} = 14.82$). See Figure 3.

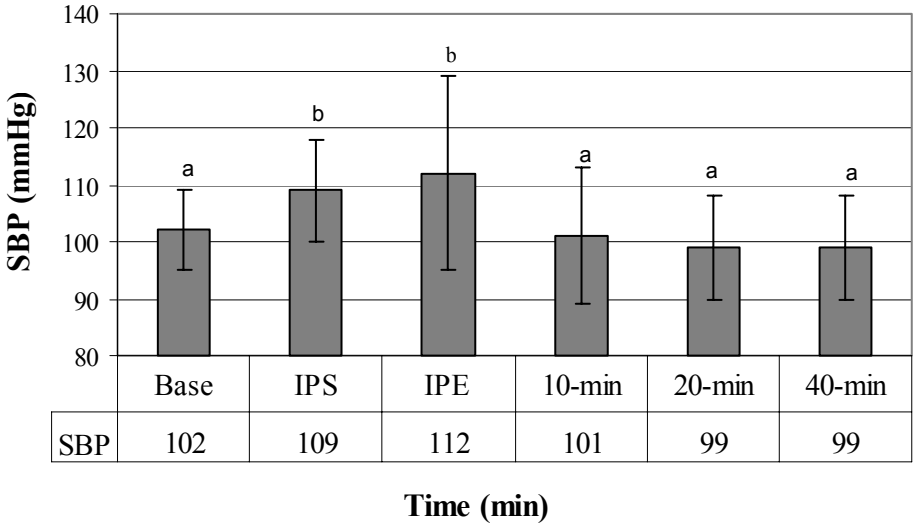

Figure 3. Systolic blood pressure (SBP) changes across time. Base = baseline; IPS = immediately post-smoking; IPE = immediately post-exercise; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post-condition; means with similar lower-case superscripts are similar. All values are time main effect means (average at each time across conditions) $\pm$ 1 standard deviation.

The average diastolic blood pressures were not different between conditions ($p > 0.05$). However, diastolic blood pressure significantly increased immediately after smoking when compared to baseline, and post-exercise recovery values ($p = 0.0071$, $F_{5, 55} = 3.58$). See Figure 4.

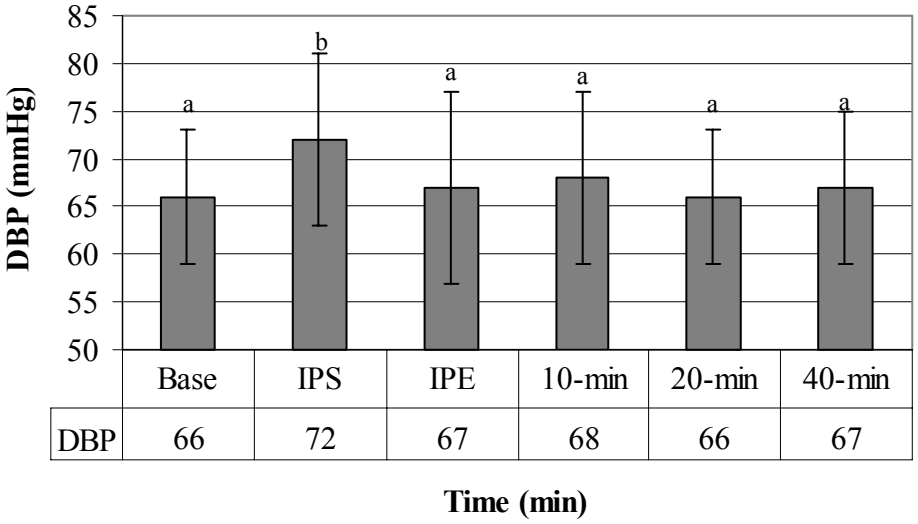

Figure 4. Diastolic blood pressure (DBP) changes across time. Base = baseline; IPS = immediately post-smoking; IPE = immediately post-exercise; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post-condition; means with similar lower-case superscripts are similar. All values are time main effects (average at each time across conditions) $\pm$ 1 standard deviation.

Pulmonary Responses to Smoking and Exercise

Measures of pulmonary function were not different between conditions and did not change from baseline values in any condition ($p > 0.05$ for all). Measures of pulmonary function are summarized in Table 4.

Variable		Pre-Condition	Post-Condition
FVC	SM	4.51 ± 0.60	4.58 ± 0.54
	EX	4.50 ± 0.63	4.54 ± 0.60
	SE	4.55 ± 0.59	4.59 ± 0.60
FEV_1	SM	3.80 ± 0.49	3.86 ± 0.50
	EX	3.81 ± 0.52	3.83 ± 0.50
	SE	3.82 ± 0.50	3.85 ± 0.47
FEV_1/FVC	SM	84 ± 6	84 ± 6
	EX	85 ± 5	85 ± 6
	SE	84 ± 5	84 ± 6
PEFR	SM	9.36 ± 1.78	9.04 ± 1.98
	EX	9.34 ± 1.69	9.08 ± 1.86
	SE	9.51 ± 2.06	9.23 ± 1.89
$FEF_{25-75\%}$	SM	4.09 ± 0.87	4.08 ± 0.92
	EX	4.07 ± 0.80	3.97 ± 0.77
	SE	4.04 ± 0.86	4.11 ± 0.10

Table 4. Pulmonary changes following smoking and exercise. FVC = forced vital capacity (L); FEV_1 = forced expiratory volume in one second (L); FEV_1 / FVC = the ratio of FVE_1 /FVC (%); PEFR = peak expiratory flow rate (L/min); $FEF_{25-75\%}$ = forced expiratory flow rate during the middle 50% of exhalation (L/sec); SM = smoking condition; EX = exercise condition; SE = smoking and exercise condition. All values are presented as means ± 1 standard deviation.

Biochemical Responses to Smoking and Exercise

Changes in Inflammatory Markers Following Smoking and Exercise

WBC increased immediately post-intervention in all three conditions. However, the WBC increase was of greater magnitude and duration with EX and SE versus smoking alone ($p < 0.0001$, $F_{10, 108} = 4.31$). The WBC remained elevated up to 40 minutes after the intervention with EX and SE but not with smoking. WBC returned to baseline levels in 24 hours in all three conditions. Changes in WBC following smoking and exercise are summarized in Table 5.

WBC	Baseline	IPC	10-min	20-min	40-min	24-hr
SM	6.1 ± 0.6^{a}	6.9 ± 0.9^{b}	6.2 ± 0.5^{a}	6.1 ± 0.7^{a}	6.3 ± 0.7^{a}	6.3 ± 0.8^{a}
EX	6.1 ± 0.9^{a}	$7.7 \pm 1.5^{bc*}$	$7.0 \pm 1.5^{b*}$	$7.1 \pm 1.5^{b*}$	$8.3 \pm 2.3^{c*}$	6.0 ± 0.7^{a}
SE	6.2 ± 1.2^{a}	$7.9 \pm 1.1^{b*}$	$7.1 \pm 1.1^{c*}$	$7.2 \pm 1.3^{c*}$	$7.9 \pm 1.6^{b*}$	6.3 ± 0.9^{a}

Table 5. Changes in WBC (white blood cell) count (x 10^9/L) following smoking and exercise. SM smoking condition; EX = exercise condition; SE = smoke and exercise condition; IPC = immediately post-condition; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post condition; 24-hr = 24 hours post-condition; means with similar lower-case superscripts are similar; * = significantly different from smoking condition. All values are presented as means $\pm$ 1 standard deviation.

For CRP, median and range values are presented since CRP concentration was not normally distributed. A Non-parametric Savage Test on change values indicated changes in CRP between conditions was not significant ($p > 0.05$). The changes in CRP have been summarized in Table 6.

D_CRP	Median	Range
SM	-0.013	-0.667 – 1.726
EX	-0.001	-1.144 - 2.063
SE	0.074	- 0.185 – 0.802

Table 6. Changes in C-reactive protein (CRP mg/L) following smoking and exercise. D_CRP = CRP measured 24 hours post-intervention – CRP measured at baseline; SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition.

Changes in Clotting Factors Following Smoking and Exercise

The average fibrinogen concentration in the smoking condition was significantly greater than exercise alone and smoking and exercise combined ($p = 0.0073$, $F_{2, 22} = 6.20$). See Figure 5.

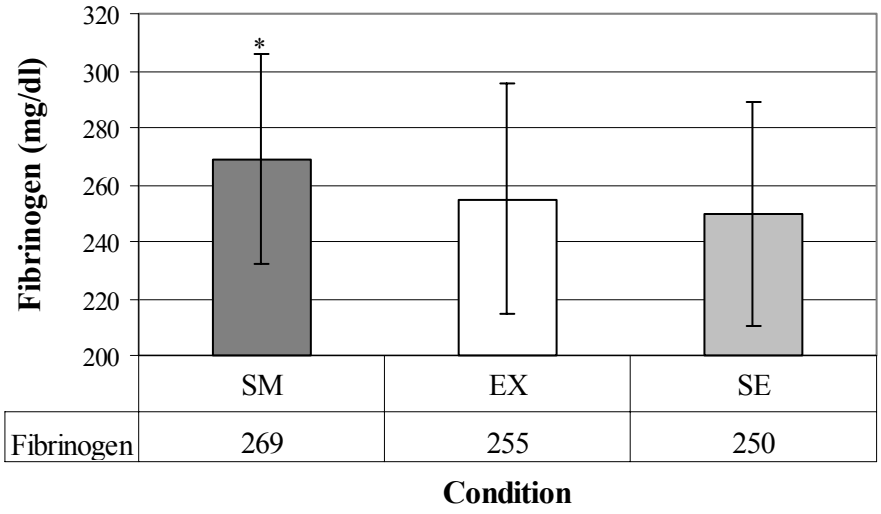

Figure 5. Fibrinogen changes across conditions. SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition. All values are condition main effects (average for each condition across time) $\pm$ 1 standard deviation. * = significantly different from EX and SE condition.

The acute fibrinogen response across time was similar in all three conditions. Fibrinogen was lower 10 minutes post-condition and continued to decrease up to 40 minutes after each of the interventions ($p < 0.0001$, $F_{4, 44} = 12.36$). See Figure 6.

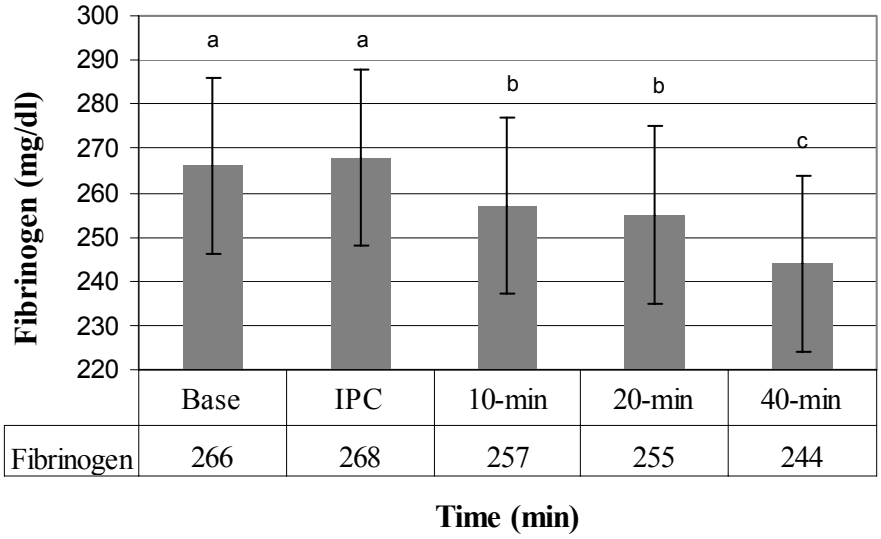

Figure 6. Fibrinogen changes across time. Base = baseline; IPS = immediately post-smoking; IPE = immediately post-exercise; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min= 40 minutes post-condition; means with similar lower-case superscripts are similar. All values are time main effects (average at each time across conditions) $\pm$ 1 standard deviation.

Platelet counts increased significantly immediately post-intervention in all three conditions. With smoking, platelets returned to baseline levels within 10 minutes after smoking the last cigarette, decreased below baseline levels at 20 and 40 minutes post-intervention and returned to baseline counts in 24 hours. The same pattern was evident after EX; however, the increase in platelet count immediately post-exercise was of greater magnitude than after SM. The platelet count response after SE was similar to that of EX and SM except that platelet counts remained elevated up to 10 minutes

post-intervention ($p = 0.0045$, $F_{10, 108} = 2.77$). Changes in platelet count are summarized in Table 7.

Platelet	Baseline	IPC	10-min	20-min	40-min	24-hr
SM	256 ± 52^{a}	260 ± 52^{b}	235 ± 49^{a}	229 ± 50^{ac}	217 ± 58^{c}	248 ± 46^{a}
EX	253 ± 48^{a}	$284 \pm 50^{b*}$	247 ± 50^{ac}	230 ± 57^{cd}	221 ± 62^{d}	248 ± 54^{ac}
SE	249 ± 57^{a}	$295 \pm 57^{b*}$	$256 \pm 49^{a*}$	234 ± 46^{c}	225 ± 38^{c}	249 ± 55^{a}

Table 7. Changes in platelet count (x 10^9/L) following smoking and exercise. SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition; IPC = immediately post-condition; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post-condition; 24-hr = 24 hours post-condition; means with similar lower-case superscripts are similar; *= significantly different from smoking condition. All values are presented as means $\pm$ 1 standard deviation.

The average APTT in the smoking condition was significantly greater than EX and SE conditions ($p = 0.0141$, $F_{2, 22} = 5.21$). See Figure 7.

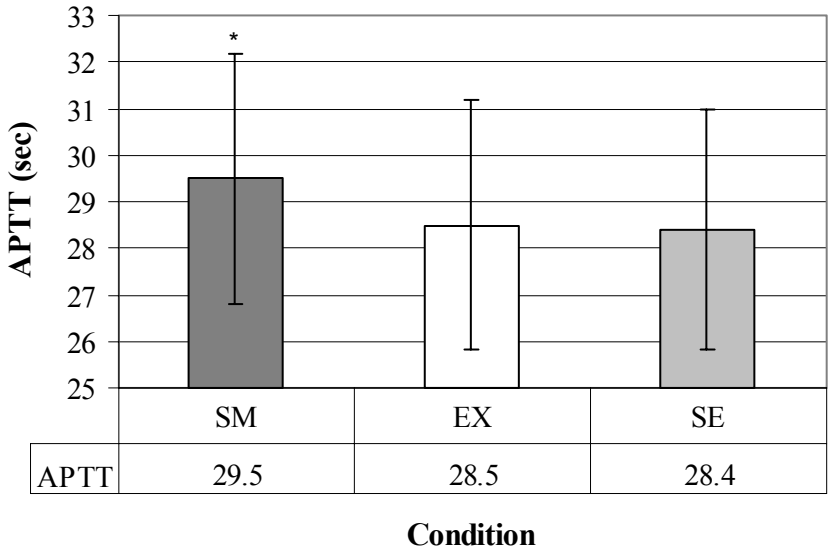

Figure 7. APTT (activated partial thromboplastin time) changes across conditions. SM = smoking condition; EX = exercise condition; SE = smoke and exercise condition. All values are condition main effects (average for each condition across time) $\pm$ 1 standard deviation. * = significantly different from EX and SE condition.

Post-condition APTT decreased similarly across time in all three conditions. APTT was shorter immediately after each condition and in 40-minutes, decreased up to 5 seconds from baseline measurements ($p < 0.0001$, $F_{4, 44} = 77.47$). See Figure 8.

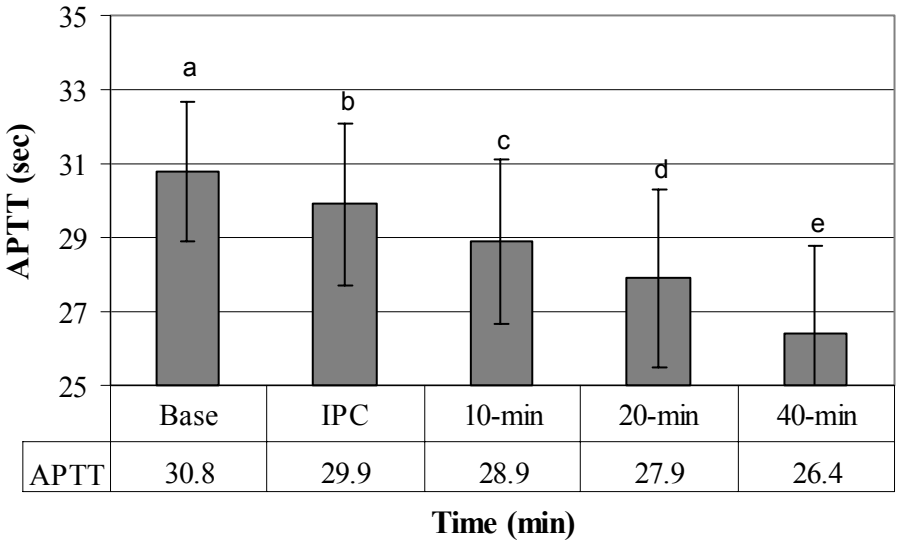

Figure 8. APTT (activated partial thromboplastin time) changes across time. Base = baseline; IPS = immediately post-smoking; IPE = immediately post-exercise; 10-min = 10-minutes post condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post-condition; means with similar lower-case superscripts are similar. All values are time main effects (average at each time across conditions) $\pm$ 1 standard deviation.

PT was not different between conditions ($p > 0.05$). However, the PT in all three conditions responded similarly by increasing significantly at 10 minutes post-intervention and returning back to baseline values after 40 minutes of recovery ($p = 0.0024$, $F_{4, 44} = 4.89$). See Figure 9.

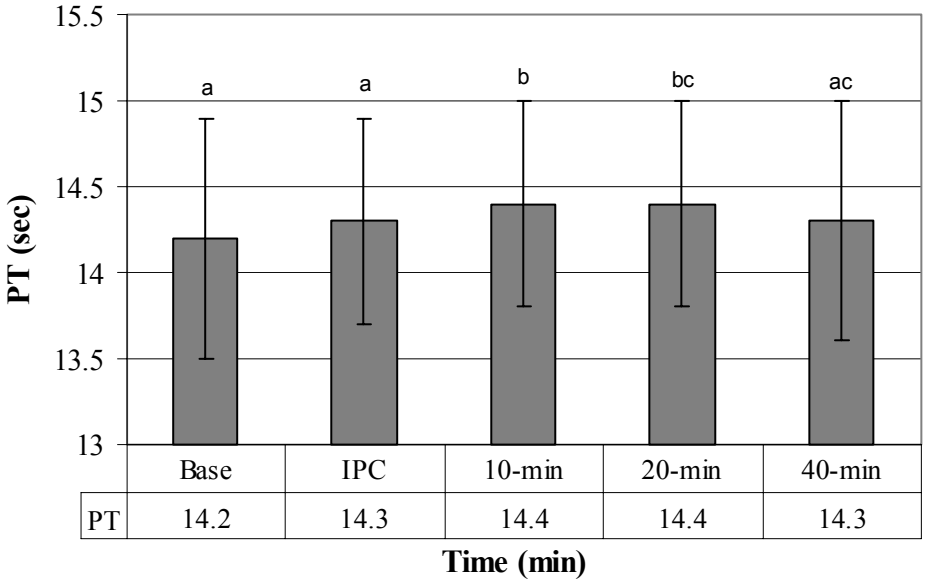

Figure 9. PT (partial thromboplastin time) changes across time. Base = baseline; IPS = immediately post-smoking; IPE = immediately post-exercise; 10-min = 10 minutes post-condition; 20-min = 20 minutes post-condition; 40-min = 40 minutes post-condition; means with similar lower-case superscripts are similar. All values are time main effects (average at each time across conditions) $\pm$ 1 standard deviation.

DISCUSSION

This is the first study to examine the combined influences of smoking and moderate-intensity aerobic exercise on physiological, inflammatory, and blood coagulation responses in young healthy casual smokers. Combination of smoking and exercise appears to increase cardiovascular stress during moderate-intensity exercise and in the immediate period of recovery after exercise. In addition, systolic and diastolic blood pressures were both elevated after smoking. Pulmonary function was not significantly altered by smoking, exercise or the combination of the two conditions. Blood fibrinogen concentrations are also elevated with smoking, but smoking does not appear to alter the fibrinogen or WBC responses to exercise. Smoking and exercise together prolongs the elevation in platelet counts that occur after smoking or exercise alone. However, changes in these clotting factors after smoking did not have a significantly negative impact on clotting times as measured by activated partial thromboplastin time (APTT) or prothrombin time (PT) after exercise. In addition, CRP was not significantly altered by smoking, exercise or the combination of the two conditions. We conclude from these results that smoking increases cardiovascular stress at rest, during exercise and recovery from exercise. Greater fibrinogen concentrations occur with smoking and smoking prolongs elevations in platelet counts that occur after exercise. However, smoking prior to exercise does not transiently alter inflammatory

markers or appear to influence changes in blood clotting characteristics that are observed after exercise.

The participants in our study were all casual smokers who self reported that they smoked less than seven cigarettes per day. The participants in this study were not involved in regular exercise activities. They kept a moderate lifestyle activity with most of their physical activity limited to walking within the campus. The participants' average BMI categorized them in the 40th percentile of the same age and gender in the population. The mean body fat percentage puts the participants in the 30th percentile (below average) of the population based on age and gender (Institute of Aerobics Research, Dallas, TX, 1994). The average $\dot{V}O_{2max}$ for the participants was below average which ranks their cardiorespiratory fitness among the 30^{th} percentile of the same age and gender in the population (Institute for Aerobics Research in Dallas, TX, 1994).

The WBC count, CRP, fibrinogen, platelet count, APTT and PT were all within normal range in our participants. However, chronic smokers averaging 12-20 cigarettes per day tend to have elevated levels of WBC count, CRP (16), fibrinogen (26), and platelet count (43), and lower level of APTT (29) and PT (46) compared to non-smokers. The young age of our participants and the fact that they were casual smokers, could explain the normality of their baseline inflammatory and clotting variables.

The higher heart rate during exercise in the SE condition versus the EX condition is supported by the previous studies. Heart rate increases after smoking (45, 48-51) which supports the finding of having higher levels of heart rates during exercise in the SE condition versus exercising alone.

The observed increase in heart rate after smoking is consistent with the results reported in previous studies in which smoking one to two cigarettes increases heart rate (45, 48-51). The significantly higher heart rate levels within the recovery period in the SE condition, versus the EX condition, is supported by previous studies. Following smoking, heart rate increases (45, 48-51), which causes the SE condition to elicit higher heart rates within the recovery period compared to the EX condition alone.

The elevation in systolic blood pressure after smoking is supported by previous studies indicating an increase in systolic blood pressure immediately after smoking one or two cigarettes (45, 48-51). Since the systolic blood pressure is lower in the recovery period for the SE condition compared to the SM, exercise seems to attenuate the increase in systolic blood pressure that is observed with smoking alone.

The observed increase in diastolic blood pressure immediately after smoking is consistent with other studies reporting an elevation in diastolic blood pressure immediately following smoking (48, 49). Diastolic blood pressure remained unchanged after exercise which is supported by studies indicating no change in diastolic blood pressure following an acute bout of aerobic exercise (55, 56, 68). However the average diastolic blood pressures were not different between the three conditions.

In this study, exercise and smoking did not alter the pulmonary function values. However, other researchers have reported a reduction in some of the pulmonary function values following smoking or exercising alone (38, 39, 58, 72, 73). The decrease observed in FEV_1 after smoking one or two cigarettes in other studies was mostly measured in heavy smokers (38, 39); however, our participants were all casual smokers. The studies that showed a reduction in FVC following an acute bout of exercise were on either active

runners (72) or were measured after a maximal bout of exercise (58) which are different from our study that included inactive individuals undergoing a submaximal bout of aerobic exercise. It does not appear that smoking one or two cigarettes or moderate-intensity-exercise affects acute changes in pulmonary function in healthy young casual smokers.

The increase observed in WBC count immediately post-intervention in all of the conditions is supported by other studies. Following an acute bout of exercise (59, 60) and immediately after smoking two cigarettes (41), an increase in WBC count is observed. Since the increase in WBC count is greater in the SE and EX condition compared to the SM condition, smoking does not appear to influence the WBC count responses to exercise.

Measures of CRP were not different between conditions and did not change from baseline values after smoking and exercise. There is limited evidence for the effect of acute smoking and exercise on CRP. Following a triathlon and marathon race, CRP markedly increases (59, 61); however, the short term changes in CRP after a short duration aerobic exercise and following acute smoking have not been reported before.

The increase observed in fibrinogen level in the smoking condition versus the EX and the SE condition, indicates that smoking affects fibrinogen but does not affect fibrinogen responses to exercise. The decrease observed in fibrinogen responses across time in all of the three conditions is highly variable with other studies. Following smoking two to three cigarettes, fibrinogen remains unchanged (40, 41) and the results of acute exercise on fibrinogen are not consistent which may increase following a marathon

race (61), remain unchanged after a maximal and submaximal exercise test (53, 62), or decrease following a marathon race (62).

The increase observed in platelet count immediately post-intervention in all of the conditions is observed in other studies after acute exercise, but not following smoking. Both acute maximal and submaximal exercise increase platelet count (65, 66), but after smoking two cigarettes in sequence, no changes in platelet count were observed (41). The greater increase in platelet count in the SE condition versus the smoking indicates that smoking prolongs elevations in platelet counts that occur after exercise.

The lower APTT in the EX and SE condition compared to the smoking condition, indicates that acute smoking does not negatively affect APTT. The decrease observed in APTT across time is consistent with studies indicating a decrease in APTT within and during recovery of a single bout of aerobic exercise (53, 54). APTT response with smoking and exercise (SE) is similar to what was observed with exercise and no additional influence is observed.

The increase in PT at 10 minutes post-intervention in all the conditions is not consistent with other studies. Following a maximal exercise test PT decreases and there are no changes observed in PT after a submaximal exercise test (65, 66). Up to now no study has been reported on the effect of acute smoking on PT, but chronic smokers have lower levels of PT (47). At present, there is no clear explanation for this observed increase in PT across time.

APTT measures clotting time in the extrinsic pathway and PT is used to measure the clotting time in the intrinsic pathway. The decrease of APTT in the extrinsic and

increase of PT within the intrinsic pathway will even each other, indicating no significant changes in clotting time following smoking and exercise in all of the three conditions.

In summary, smoking prior to exercise increases cardiovascular stress as indicated by an elevation in heart rate during and after exercise, however it does not affect pulmonary function measures. Smoking prior to exercise does not appear to influence changes in inflammatory markers and blood clotting characteristics that are observed after exercise. Young casual smokers could start an exercise program without increasing their risk of an untoward cardiovascular event with exercise.

REFERENCES

1. **American College of Sports Medicine.** ACSM's Guidelines for Exercise Testing and Prescription (6th ed.). Baltimore, MD: Lippincott Williams & Wilkins, 2000.

2. **Djousse L, Myers, R. H., Province, M. A., Hunt, S. C., Eckfeldt, J. H., Evans, G., & etl.** Influence of apolipoprotein E, smoking, and alcohol intake on carotid atherosclerosis: National Heart, Lung, and Blood Institute Family Heart Study. *Stroke* 33: 1357-1366, 2002.

3. **Willett WC, Green, A., Stampfer, M. J., Speizer, F. E., Colditz, G. A., Rosner, B. & et al.** Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. *The New England Journal of Medicine* 317: 1303-1309, 1987.

4. **Kiyohara Y, Ueda, K., & Fujishima, M.** Smoking and cardiovascular disease in the general population in Japan. *Journal of Hypertension - Supplement* 8: S9-S15, 1990.

5. **Diez-Roux AV, Nieto, F. J., Comstock, G. W., Howard, G., & Szklo, M.** The relationship of active and passive smoking to carotid atherosclerosis 12-14 years later. *Preventive Medicine* 24: 48-55, 1995.

6. **Iribarren C, Tekawa, I. S., Sidney, S., & Friedman, G. D.** Effect of cigar smoking on the risk of cardiovascular disease, chronic obstructive pulmonary disease, and cancer in men. *New England Journal of Medicine* 340: 1773-1178, 1999.

7. **Jajich CL, Ostfeld, A. M., & Freeman, Jr, D. H.** Smoking and coronary heart disease mortality in the elderly. *Journal of the American Medical Association* 252: 2831-2834, 1984.

8. **Rosengren L, Wilhelmsen., & Wedel, H.** Coronary heart disease, cancer and mortality in male middle-aged light smokers. *Journal of Internal Medicine* 231: 357-362, 1992.

9. **Doll R, Peto, R., Boreham, J., & Sutherland, I.** Mortality in relation to smoking: 50 years' observations on male British doctors. *British Medical Journal* 328: 1519-1528, 2004.

10. **Hammond EC, & Horn, D.** Smoking and death rates: Report on forty-four months of follow-up of 187,783 men. 2. Death rates by cause. *Journal of the American Medical Association* 166: 1294-1308., 1958.

11. **Jacobs EJ, Thun, M. J., & Apicella, L. F.** Cigar smoking and death from coronary heart disease in a prospective study of US men. *Archives of Internal Medicine* 159: 2413-2418, 1999.

12. **Jacobs DR, Adachi, Jr. H., Mulder, I., Kromhout, D., Menotti, A., Nissinen, A., & et al.** Cigarette smoking and mortality risk: Twenty-five-year follow-up of the Seven Countries Study. *Archives of Internal Medicine* 159: 733-740, 1999.

13. **Hawthorne VM, & Fry, J. S.** Smoking and health: The association between smoking behavior, total mortality, and cardiorespiratory disease in West Central Scotland. *Journal of Epidemiology & Community Health* 32: 260-266, 1978.

14. **United States Department of Health & Human Services.** New Surgeon General's Report Expands List of Diseases Caused by Smoking (online). http://www.hhs.gov/news/press/2004pres/20040527a.html (27 May, 2004).

15. **Smith MR, Kinmonth, A. L., Luben, R. N., Bingham, S., Day, N. E., Wareham, N. J. & et al.** Smoking status and differential white cell count in men and women in the EPIC-Norfolk population. *Atherosclerosis* 169: 331-337, 2003.

16. **Frohlich M, Sund, M., Lowel, H., Imhof, A., Hoffmeister, A., Koenig, W.** Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). *European Heart Journal* 24: 1365-1372, 2003.

17. **Danesh J, Wheeler, J. G., Hirschfield, G. M., Eda, S., Eiriksdottir, G., Rumley, A. & et al.** C-reactive protein and other circulating markers of inflammation in the

prediction of coronary heart disease. *New England Journal of Medicine* 350: 1387-1397, 2004.

18. **Ridker PMC, M., Stampfer, M. J., Tracy, R. P., & Hennekens, C. H.** Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine* 336: 973-979, 1997.

19. **Ridker PM, Buring, J. E., Shih, J., Matias, M. & Hennekens, C. H.** Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 98: 731-733, 1998.

20. **Strandberg TE, & Tilvis, R. S.** C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. *Arteriosclerosis, Thrombosis & Vascular Biology* 20: 1057-1060, 2000.

21. **Danesh J, Collins, R., Appleby, P., & Peto, R.** Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *Journal of the American Medical Association* 279: 1477-1482, 1998.

22. **Rohde LE, Hennekens, C. H., & Ridker, P. M.** Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *American Journal of Cardiology* 84: 1018-1022, 1999.

23. **Weijenberg MP, Feskens, E. J., & Kromhout, D. L.** White blood cell count and the risk of coronary heart disease and all-cause mortality in elderly men. *Arteriosclerosis, Thrombosis & Vascular Biology* 16: 499-503, 1987.

24. **Yarnell JW, Baker, I. A., Sweetnam, P. M., Bainton, D., O'Brien, J. R., Whitehead, P. J., & et al.** Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 83: 836-844, 1991.

25. **Haim M, Boyko, V., Goldbourt, U., Battler, A., & Behar, S.** Predictive value of elevated white blood cell count in patients with preexisting coronary heart disease: the Bezafibrate Infarction Prevention Study. *Archives of Internal Medicine* 164: 433-439, 2004.

26. **Bazzano LA, He, J., Muntner, P., Vupputuri, S., & Whelton, P. K.** Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. *Annals of Internal Medicine* 138: 891-897, 2003.

27. **Bermudez EA, Rifai, N., Buring, J. E., Manson, J. E., & Ridker, P. M.** Relation between markers of systemic vascular inflammation and smoking in women. *American Journal of Cardiology* 89: 1117-1119, 2002.

28. **De Maat MP, & Kluft, C.** The association between inflammation markers, coronary artery disease and smoking. *Vascular Pharmacology* 39: 137-139, 2002.

29. **Yarnell JW, Sweetnam, P. M., Rumley, A., & Lowe, G. D.** Lifestyle factors and coagulation activation markers: the Caerphilly Study. *Blood Coagulation & Fibrinolysis* 12: 721-728, 2001.

30. **Kannel WB, Wolf, P. A., Castelli, W. P., & D'Agostino, R. B.** Fibrinogen and risk of cardiovascular disease. The Framingham Study. *Journal of the American Medical Association* 258: 1183-1186, 1987.

31. **Meade TW, Ruddock, V., Stirling, Y., Chakrabarti, R., & Miller, G. J.** Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. *Lancet* 342: 19076-19107, 1993.

32. **Allen RA, Kluft, C., & Brommer, E. J.** Effect of chronic smoking on fibrinolysis. *Arteriosclerosis* 5: 443-450, 1985.

33. **Simpson AJ, Gray, R. S., Moore, N. R., & Booth, N. A.** The effects of chronic smoking on the fibrinolytic potential of plasma and platelets. *British Journal of Haematology* 97: 208-213, 1997.

34. **Dotevall A, Rangemark, C., Eriksson, E., Kutti, J., Wadenvik, H., & Wennmalm, A.** Cigarette smoking increases thromboxane A2 formation without affecting platelet survival in young healthy females. *Thrombosis & Haemostasis* 68: 583-588, 1992.

35. **Haire WD, Goldsmith, J. C., & Rasmussen, J.** Abnormal fibrinolysis in healthy male cigarette smokers: role of plasminogen activator inhibitors. *American Journal of Hematology* 31: 36-40, 1989.

36. **El-Sayed MS**. Effects of exercise on blood coagulation, fibrinolysis and platelet aggregation. *Sports Medicine* 22: 282-298, 1996.

37. **Prokhorov AV, Emmons, K. M., Pallonen, U. E., & Tsoh, J. Y.** Respiratory response to cigarette smoking among adolescent smokers: A pilot study. *Preventive Medicine* 25: 633-640, 1996.

38. **De A. K. T, M. M., Matah, S. C., & Roy, D. C.** Importance of smoking index for assessing lung damage by lung function tests. *Indian Journal of Chest Diseases & Allied Sciences* 31: 165-169, 1989.

39. **Belch JJ, McArdle, B. M., Burns, P., Lowe, G. D. & Forbes, C. D**. The effects of acute smoking on platelet behavior, fibrinolysis and haemorheology in habitual smokers. *Thrombosis & Haemostasis* 51: 6-8, 1984.

40. **Blann AD, Kirkpatrick, U., Devine, C., Naser, S., & McCollum, C. N.** The influence of acute smoking on leucocytes, platelets and the endothelium. *Atherosclerosis* 141: 133-139, 1998.

41. **Das I**. Raised C-reactive protein levels in serum from smokers. *Clinica Chimica Acta* 153: 9-13, 1985.

42. **Tell GS, Grimm, R. H. Jr., Vellar, O. D. & Theodorsen, L.** The relationship of white cell count, platelet count, and hematocrit to cigarette smoking in adolescents: the Oslo Youth Study. *Circulation* 72: 971-974, 1985.

43. **Aghaji M, Nnabuko, R., Uzuegbunam, C., & Oyeka, I. C.** The relationship of white blood cell and platelet counts to cigarette smoking in adult Nigerians. *Central African Journal of Medicine* 36: 273-278, 1990.

44. **Kimura S, Nishinaga, M., Ozawa, T., & Shimada, K.** Thrombin generation as an acute effect of cigarette smoking. *American Heart Journal* 128: 7-11, 1994.

45. **Allen RA, Kluft, C., & Brommer, E. J.** Acute effect of smoking on fibrinolysis: increase in the activity level of circulating extrinsic (tissue-type) plasminogen activator. *European Journal of Clinical Investigation* 14: 354-361, 1984.

46. **Freedman DS, Byers, T., Barboriak, J. J., Flanders, W. D., Duncan, A., Yip, R., & et al.** The relation of prothrombin times to coronary heart disease risk factors among men aged 31-45 years. *American Journal of Epidemiology* 136: 513-524, 1992.

47. **Kool MJ, Hoeks, A. P., Struijker Boudier, H. A., Reneman, R. S., & Van Bortel, L. M.** Short- and long-term effects of smoking on arterial wall properties in habitual smokers. *Journal of the American College of Cardiology* 22: 1881-1886, 1993.

48. **Failla M, Grappiolo, A., Carugo, S., Calchera, I., Giannattasio, C., & Mancia, G.** Effects of cigarette smoking on carotid and radial artery distensibility. *Journal of Hypertension* 15: 1659-1664, 1997.

49. **Behr MJ, Leong, K. H., & Jones, R. H.** Acute effects of cigarette smoking on left ventricular function at rest and exercise. *Medicine & Science in Sports & Exercise* 13: 9-12, 1981.

50. **Rotstein A, Sagiv, M., Yaniv-Tamir, A., Fisher, N., & Dotan, R.** Smoking effect on exercise response kinetics of oxygen uptake and related variables. *International Journal of Sports Medicine* 12: 281-284, 1991.

51. **Haennel RG, & Lemire, F.** Physical activity to prevent cardiovascular disease. How much is enough? *Canadian Family Physician* 48: 65-71, 2002.

52. **Van den Burg PJ, Hospers, J. E., van Vliet, M., Mosterd, W. L., & Huisveld, I. A.** Unbalanced haemostatic changes following strenuous physical exercise. A study in young sedentary males. *European Heart Journal* 16: 1995-2001, 1995.

53. **Hegde SS, Goldfarb, A. H., & Hegde, S.** Clotting and fibrinolytic activity change during the 1 h after a submaximal run. *Medicine & Science in Sports & Exercise* 33: 887-892, 2001.

54. **Lin X, El-Sayed, M. S., Waterhouse, J., Reilly, T.** Activation and disturbance of blood haemostasis following strenuous physical exercise. *International Journal of Sports Medicine* 20: 149-153, 1999.

55. **Brown SP, Li, H., Chitwood, L. F., Anderson, E. R., & Boatwright, D.** Blood pressure, hemodynamic, and thermal responses after cycling exercise. *Journal of Applied Physiology* 75: 240-245, 1993.

56. **Hagberg JM, Montain, S. J., & Martin, W. H. 3rd.** Blood pressure and hemodynamic responses after exercise in older hypertensives. *Journal of Applied Physiology* 63: 270-276, 1987.

57. **Coast JR, Haverkamp, H. C., Finkbone, C. M., Anderson, K. L., George, S. O., & Herb, R. A.** Alterations in pulmonary function following exercise are not caused by the work of breathing alone. *International Journal of Sports Medicine* 20: 470-475, 1999.

58. **Taylor C, Rogers, G., Goodman, C., Baynes, R. D., Bothwell, T. H., Bezwoda, W. R. & et al.** Hematologic, iron-related, and acute-phase protein responses to sustained strenuous exercise. *Journal of Applied Physiology* 62: 464-469, 1987.

59. **Nieman DC, Nehlsen-Cannarella, S. L., Donohue, K. M., Chritton, D. B., Haddock, B. L., Stout, R. W. & et al.** The effects of acute moderate exercise on leukocyte and lymphocyte subpopulations. *Medicine & Science in Sports & Exercise* 23: 578-585, 1991.

60. **Weight LM, Alexander, D. & Jacobs, P.** Strenuous exercise: Analogous to the acute-phase response? *Clinical Science* 81: 677-683, 1991.

61. **Rankinen T, Vaisanen, S., Penttila, I., & Rauramaa, R.** Acute dynamic exercise increases fibrinolytic activity. *Thrombosis & Haemostasis* 73: 281-286, 1995.

62. **Prisco D, Paniccia, R., Bandinelli, B., Fedi, S., Cellai, A. P., Liotta, A. A., & et al.** Evaluation of clotting and fibrinolytic activation after protracted physical exercise. *Thrombosis Research* 89: 73-78, 1998.

63. **Dufaux B, Order, U., & Liesen, H.** Effect of a short maximal physical exercise on coagulation, fibrinolysis, and complement system. *International Journal of Sports Medicine* 12: 38-42, 1991.

64. **Ferguson EW, Bernier, L. L., Banta, G. R., Yu-Yahiro, J., & Schoomaker, E. B.** Effects of exercise and conditioning on clotting and fibrinolytic activity in men. *Journal of Applied Physiology* 62: 1416-1421, 1987.

65. **Drygas WK**. Changes in blood platelet function, coagulation, and fibrinolytic activity in response to moderate, exhaustive, and prolonged exercise. *International Journal of Sports Medicine* 9: 67-72, 1988.

66. **Ersoz G, Zergeroglu, A. M., & Yakaryilmaz, A.** The effect of submaximal exercise on platelet aggregation during late follicular and midluteal phases in women. *Thrombosis Research* 108: 147-150, 2002.

67. **Chicharro JL, Sanchez, O., Bandres, F., Guantes, Y., Yges, A., Lucia. A., & et al.** Platelet aggregability in relation to the anaerobic threshold. *Thrombosis Research* 75: 251-257, 1994.

68. **Boone JBJ, Probst, M. M., Rogers, M. W., & Berger, R.** Postexercise hypotension reduces cardiovascular responses to stress. *Journal of Hypertension* 11: 449-453, 1993.

69. **MacDonald J, MacDougall, J., & Hogben, C.** The effects of exercise intensity on post exercise hypotension. *Journal of Human Hypertension* 13: 527-531, 1999.

70. **Haverkamp HC, Metelits, M., Hartnett, J., Olsson, K., & Coast, J. R.** Pulmonary function subsequent to expiratory muscle fatigue in healthy humans. *International Journal of Sports Medicine* 22: 498-503, 2001.

71. **O'Kroy JA, Loy, R. A., & Coast, J. R.** Pulmonary function changes following exercise. *Medicine & Science in Sports & Exercise* 24: 1359-1364, 1992.

72. **Cordain L, Rode, E. J., Gotshall, R. W., & Tucker, A.** Residual lung volume and ventilatory muscle strength changes following maximal and submaximal exercise. *International Journal of Sports Medicine* 15: 158-161, 1994.

73. **Center for Disease control and Prevention**. Cigarette smoking among adults-United States, 2003. *Morbidity and Mortality Weekly Report* 54: 509-513, 2005.

74. **American College of Sports Medicine.** ACSM's Recourse Manual for Guidelines for Exercise Testing and Prescription (4th ed.). Baltimore, MD: Lippincott Williams & Wilkins, 2001.

75. **Ross R**. Atherosclerosis: An inflammatory disease. *New England Journal of Medicine* 340: 115-126, 1999.

76. **Ross R**. The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 362: 801-809, 1993.

77. **Glagov S, Weisenberg, E., Zarins, C.K., Stankunavicius, R., Kolettis, G.J.** Compensatory enlargement of human atherosclerotic coronary arteries. *New England Journal of Medicine* 316: 1371-1375, 1987.

78. **Kuller LH, Tracy, R.P., Shaten, J., Meilahn, E.N.** Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *American Journal of Epidemiology* 144: 537-547, 1996.

79. **Ridker PMC, M. Stampfer, M.J. Tracy, R.P. Hennekens, C.H**. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 97: 425-428, 1998.

80. **Hamsten A, Wiman, B., de Faire, U., Blomback, M.** Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *New England Journal of Medicine* 313: 1557-1563, 1985.

81. **Hamsten A, de Faire, U., Walldius, G., Dahlen, G., Szamosi, A., Landou, C., Blomback, M., Wiman, B.** Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 2: 3-9, 1987.

82. **Salomaa V, Stinson J.D. V., Kark, J.D., Folsom, A.R., Davis, C. E., and Wu K. K.** Association of fibrinolytic parameters with early atherosclerosis: The Aric study. *Circulation* 91: 284-290, 1995.

83. **Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion**. Health effects of cigarette smoking (online). http://www.cdc.gov/tobacco/factsheets/HealthEffectsofCigaretteSmoking_Factsheet.htm (Feb. 2004).

84. **American Heart Association**. Risk factors and coronary heart disease (online). http://www.americanheart.org/presenter.jhtml?identifier=4726 (8 Oct. 2005).

85. **Jensen EJ, Pedersen, B., Frederiksen, R., Dahl, R.** Prospective study on the effect of smoking and nicotine substitution on leucocyte blood counts and relation between blood leucocytes and lung function. *Thorax* 53: 784-789, 1998.

86. **Ambrose JA, Barua, R.S.** The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology* 43: 1731-1737, 2004.

87. **Muscari A, Bastagli, L., Poggiopollini, G., Tomassetti, V., Massarelli, G., Cappelletti, O., Plate, L., Boni, P., Puddu, P.** Different associations of C-reactive protein, fibrinogen and C3 with traditional risk factors in middle-aged men. *International Journal of Cardiology* 83: 63-71, 2002.

88. **American Lung Association**. Smoking 101 fact sheet (online). http://www.lungusa.org/site/pp.asp?c=dvLUK9O0E&b=39853 (Nov. 2004).

89. **Jackson AS, Pollock, M. L., & Ward, A.** Generalized equations for predicting body density of women. *Medicine & Science in Sports & Exercise* 12: 175-181, 1980.

90. **Kendall R, Benoit, E., Bogiages, J., Bordenkircher, R., Caple, K., Chen, L. L., & et. al.** Performance evaluation of the Abbott Cell-Dyn 1800 automated hematology analyzer. *Laboratory Hematology* 9: 143-152, 2003.

91. **Blair SN, Haskell, W. L., Ho, P., Paffenbarger, R. S. Jr., Vranizan, K. M., Farquhar, J. W. & Wood, P. D.** Assessment of habitual physical activity by a seven-day recall in a community survey and controlled experiments. *American Journal of Epidemiology* 122: 794-804, 1985.

92. **Hajek J.** A course in Nonparametric Statistics. San Francisco: Holden-Day, 1969, p. 83.