THE EFFECT OF ACUTE EXERCISE ON COAGULATION FACTORS AND THE MECHANICAL PROPERTIES OF FIBRIN FIBERS

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DEDICATION

I would like to dedicate this thesis to my Wake Forest family, which includes both the Health & Exercise Science Department and the Wake Forest Athletic Department. Since arriving on campus in the Fall of 2014, the HES department has warmly welcomed, accepted, and encouraged me, even as an “untraditional” student. I have grown enormously through the classes I’ve taken, the professors I’ve worked with, and the classmates who have become some of my closest friends. Secondly, coming in and playing for the Wake Forest Field Hockey team my first year was an absolute blessing. Since, I have found a passion for coaching and developed meaningful relationships throughout the athletic community. I would not trade these past two years for the world and I owe that all to these two realms of people who make Wake Forest such a wonderful place.
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LIST OF ABBREVIATIONS

AFM: Atomic force microscopy
aPTT: Activated partial thromboplastin time
BMI: Body mass index
CAD: Coronary artery disease
CVD: Cardiovascular disease
DIC: Disseminated intravascular coagulation profile
HMWK: High molecular weight kininogen
KALL: Kallikrein
NOAC: Novel oral anticoagulant
OD: Older subject group with cardiovascular disease
PAI-1: Plasminogen activator inhibitor
PK: Prekallikren
PT: Prothrombin time
TAFI: Thrombin-activated fibrinolysis inhibitor
TF: Tissue factor
TFPI: Tissue factor pathway inhibitor
tPA: Tissue plasminogen activator
uPA: Urokinase
VKA: Vitamin K antagonist
YH: Younger, healthy subject group
II: Factor II
V: Factor V
VII: Factor VII
VIII: Factor VIII
IX: Factor IX
X: Factor X
XII: Factor XII
XI: Factor XI
ABSTRACT

PURPOSE: The objective of this study was to investigate the effect of acute exercise on coagulation factors and fibrin fiber properties in both younger, healthy subjects and older, subjects with cardiovascular disease (CVD). In addition, it was of interest to examine relationships between coagulation factors and fibrin fiber properties during acute exercise.

METHODS: 5 male subjects were recruited to the younger, healthy (YH) group and 5 male subjects were recruited to the older group with CVD (OD). Each participant performed a single session of an acute exercise protocol, having blood drawn pre-exercise and post-exercise. LabCorp performed disseminated intravascular coagulation (DIC) profiling on all blood samples, and fibrin fiber measurements were performed in the Wake Forest Olin Physics Laboratory using atomic force microscopy (AFM). Repeated measures ANOVA and Pearson’s correlations were used to analyze within and between group differences, and coagulation factor-fibrin fiber relationships, respectively. Effect sizes ($\eta^2$) were used to combat the small sample size and avoid type II errors.

RESULTS: 2-way repeated measures ANOVA revealed only one significant interaction: VIII ($p=.001, \eta^2=.778$). Paired sample t-tests showed the YH group had a significant increase in VIII from pre-exercise to post-exercise ($p=.002$) but the OD did not. Between group differences (YH vs. OD) were seen during exercise when measuring alpha-2-antiplasmin ($p=.018, \eta^2=.552$), antithrombin ($p=.026, \eta^2=.481$), and fibrin fiber extensibility ($p=.003, \eta^2=.691$). A significant increase in platelet levels was seen within groups from pre- to post-exercise ($p=.048, \eta^2=.507$). Pearson’s correlations revealed significant inverse correlations between VIII and fibrin fiber extensibility post-exercise
(r = -.804), and between platelets and fibrin fiber extensibility post-exercise (r = -.711).

Significant inverse relationships were also seen between pre-exercise alpha-2-antiplasmin and post-exercise fibrin extensibility (r = -.788), and between pre-exercise antithrombin and post-exercise fibrin extensibility (r = -.646).

**CONCLUSION:** Coagulation factors including VIII, alpha-2-antiplasmin, and antithrombin all appear to be affected by acute exercise. Furthermore, acute exercise appears to cause an increase in fibrin fiber extensibility in the OD group but not in the YH group. The four significant inverse correlations provide a basis for lowering fibrin fiber extensibility in response to exercise, by altering levels of pre-exercise VIII and platelets, and post-exercise alpha-2-antiplasmin and antithrombin.
REVIEW OF THE LITERATURE

Introduction

Cardiovascular disease (CVD) has and continues to be the number one cause of adult death in the United States (11). The high prevalence and mortality rates associated with CVD make its continued study of utmost importance. As of 2010, 83.6 million Americans over the age of 20 were living with at least one form of CVD (29). This prevalence represents 35.3% of the American adult population and the incidence is only expected to increase. Not only does CVD carry high prevalence rates, but its death toll is staggering. In 2010, CVD accounted for 31.9% of the 2,468,435 total deaths in the U.S. This means that CVD is responsible for almost 1 out of every 3 deaths in the U.S. (29).

Cardiovascular disease encompasses a wide range of diseases involving the heart and blood vessels, and can manifest in a multitude of ways. Early indications of CVD include angina pectoris, dyspnea, syncope, arrhythmias, and heart infections (i.e. myocarditis). The most common end points of CVD are myocardial infarction, stroke, and death (29). These outcomes can occur as a result of any type of CVD, including but not limited to coronary heart disease—including both coronary artery disease and peripheral artery disease—heart failure, cardiomyopathy, congenital heart defects and stroke. The most prevalent form of CVD is coronary artery disease (CAD), which involves the buildup of plaque or a blockage in one of the heart’s coronary arteries leading to myocardial ischemia or infarction (11).

The risk factors of CVD are fairly well documented and understood. Hypertension, smoking, poor diet, insufficient physical activity, obesity, high cholesterol, and abnormal blood glucose levels (diabetes) are all linked to an increased chance of
developing CVD (3). Many risk factors for CVD are modifiable and can be managed by lifestyle changes. Some risk factors however, cannot be modified. Age is a significant predictor of CVD risk. The older one gets, the greater their chance of developing CVD.

As of 2015, the American population of adults aged 60-79 years old had a 68.5% prevalence rate of CVD. In American adults above the age of 85, CVD remains the leading cause of death, exceeding cancer deaths and Alzheimer's disease deaths combined (58).

With such a high prevalence, CVD carries with it a very large economic burden. In 2010, the combined direct and indirect costs attributed to CVD were estimated to have reached $315.4 billion. For comparison, the total estimated a cost of cancer and benign neoplasms in 2008 was $201.5 billion (29). There is a staggering amount of money being spent to manage CVD, which serves as another reason why more research in this field is needed.

Pathophysiology of Coronary Artery Disease

Atherosclerosis is the underlying cause of most CVDs, including CAD. Atherosclerosis occurs when arteries become occluded due to the buildup of substances such as fats, cholesterol, and calcium. These obstructions are called plaques and can eventually reduce normal blood flow in the artery. It is important to note that atherosclerosis is considered an inflammatory disease and not simply a cholesterol storage issue, as was previously believed (46). Plaques normally form in regions of branching or geometric irregularity where blood flow goes through sudden changes in direction. The first sign of plaque deposition is the fatty streak lesion. Fatty streaks are composed of lipoproteins and start to emerge in the aorta and coronary arteries of most
people by 20 years of age. Fatty streaks can progress to a fibrous plaque as a result of smooth muscle cell (SMC) proliferation and continued lipid accumulation. Fibrous plaques usually take decades to form but occasionally can develop more quickly over the course of a few years. Once a plaque is present in a vessel it can change in size depending on blood flow and health conditions (6). The stability of the plaque determines the likelihood of plaque rupture and ensuing clotting activation.

Arterial plaque can undergo slow growth over time but can rupture at any point. When a plaque ruptures, the vessel in which it is located interprets the rupture as an injury to its lining. Thus the normal reaction is to activate the blood-clotting cascade, even though no external bleeding is occurring. Platelets are activated and eventually a clot is formed in the blood vessel, leading to a blockage of normal blood flow (30). The interaction that leads to the formation of a blood clot from a ruptured plaque is called the “coagulation cascade.”

The Coagulation Cascade

Thrombosis or “clot” formation is a major determinant of myocardial infarction and stroke (50). Thus it is important to examine the pathways that lead to both clot formation and clot degradation. Thrombosis and thrombolysis (clot breakdown) are complex processes that involve numerous pathways of factors, fibers, and proteins. The coagulation cascade is commonly thought to include three main pathways: the intrinsic system, extrinsic system, and common pathway. All three of these processes are illustrated in Figure 1 (2).
The intrinsic system is mobilized in reaction to a damaged vessel and activated by chemicals, collagen, exposed endothelium, and platelets (20). Activated Factor XII (XIIa) begins the clotting process by binding to the damaged endothelium. The ensuing activity of XIIa cleaves and thus activates its substrates Prekallikrein (PK) and XI. The activated forms of these substrates are Kallikrein (KALL) and Xla, respectively. High Molecular
Weight Kininogen (HMWK) is responsible for anchoring these substrates to the subendothelium. KALL then works to activate additional neighboring XII particles to build on this process. Once XI is activated, it diffuses into solution and activates IX. The cascade continues with activated IXa forming a complex with activated VIII. This multi-particle complex then binds to the damaged area, along with platelets, which then serves to activate X (69). This marks the end of the intrinsic system.

The extrinsic system works similarly to the intrinsic pathway, but requires components extraneous to the blood for the cascade to work. Once the endothelium is injured, the protein Tissue Factor (TF), which is housed in the subendothelium, becomes exposed to blood in the vessel. For this reason, extrinsic system is synonymous with TF initiated coagulation (26). Circulating VII binds to the exposed TF, forming a complex that promotes the auto-activation of VII. When Calcium and other phospholipids are present, this TF-VIIa complex binds and activates X, which signifies the beginning of the common pathway (69).

The common pathway is the most studied and well-understood portion of the coagulation cascade. The common pathway is the focus of the present investigation. The intrinsic and extrinsic pathways come together with the activation of X, and this conversion marks the start of the common pathway. Xa attaches to the phospholipid rich surface of activated platelets. From there it forms a complex with its cofactor, activated V. This complex functions to convert prothrombin into thrombin. Thrombin is considered the master regulator of the entire coagulation cascade. Thrombin serves as the enzyme responsible for converting fibrinogen to fibrin monomers, and does this by cleaving activation peptides from fibrinogen. It is the individual fibrin monomers that polymerize
to form a clot at the site of injury. Thrombin also activates XIII that circulates in the blood. This catalyzes the formation of covalent crosslink bonds between fibrin molecules that constitute the meshed clot. Additionally, thrombin activates more platelets that further fill in the clot. Thrombin activates both V and VIII to contribute to a positive feedback loop that amplifies the stimulation of the entire clotting process (69). VIII is critical for clot formation, and elevated levels of VIII are associated with increased risk of deep venous thrombosis (70).

As with all physiological function, thrombosis works in a tightly regulated manner. While the coagulation cascade leads toward clot formation, natural anticoagulants attempt to prevent or at least slow the formation of the clot. A pro-coagulant is any agent that promotes the coagulation of blood, for example prothrombin and fibrinogen. Conversely, an anticoagulant is any agent that prevents the coagulation of blood. Examples of natural anticoagulants present in blood vessels are antithrombin, tissue factor pathway inhibitor (TFPI), and thrombomodulin (26). Not only do anticoagulants prevent the initiation of coagulation but they also block the amplification of fibrinogen and platelet actions once they have already commenced. During the coagulation process thrombomodulin binds to thrombin and blocks its clotting ability by obstructing the fibrinogen binding sites on the thrombin molecule. Antithrombin inhibits the action of thrombin and TFPI slows the initiation of the extrinsic pathway by inhibiting TF (26).

Anticoagulants are also manufactured for use as medications and are commonly prescribed to patients at risk for CVD in order to prevent the formation of unnecessary clots. Both oral anticoagulant vitamin K antagonists (VKA) and newer non-vitamin K
antagonist novel oral anticoagulants (NOAC) work to prevent clotting that leads to threatening cardiovascular events and thrombotic disorders (52). Coumadin, also known by its generic name Warfarin, has been the most commonly prescribed anticoagulant medication for decades. Coumadin works by inhibiting the activity of the vitamin K-dependent coagulation factors—including II, VII, IX, and X—as well as Protein C and S (43). This results in an overall decrease in pro-coagulant activity within the body. Three non-vitamin K antagonist oral anticoagulants (NOAC), Dabigatran, Rivaroxaban, and Apixaban, have recently been approved by the FDA and are becoming increasingly used to manage those at risk for thrombosis (43). Dabigatran works by directly inhibiting thrombin, whereas Rivaroxaban and Apixaban inhibit Xa and thus interfere with its interaction with Va in the clotting process. The main limitation of NOAC use is the lack of a specific reversal agent for the case of a bleeding diathesis (43). However, the first of these reversal agents has been discovered for Dabigatran. Idarucizumab, an antibody fragment, has been found to completely reverse the anticoagulant effect of Dabigatran within minutes (60).

Once a clot is formed it must eventually be degraded or it will completely occlude the blood vessel. This function is achieved through a process called fibrinolysis (the breakdown of fibrin). The dissolution of a fibrin clot is dominated by the protease plasmin. Plasminogen is a circulating plasma zymogen that starts the fibrinolysis process by being converted to plasmin by both tissue plasminogen activator (tPA) and urokinase (uPA). Plasmin acts in a positive feedback manner to cleave both the tPA and uPA, resulting in active two-chain polypeptides. To promote its own degradation fibrin binds tPA and uPA to its surface, thus enhancing plasmin production. Once plasmin is formed
and activated, it cleaves fibrin resulting in blood-soluble degradation products. This complex process is modeled in Figure 2 (13). D-Dimer is a circulating fibrin degradation product and is the most commonly measured biomarker in this regard (19). Multiple molecules can slow fibrinolysis. The enzyme thrombin-activated fibrinolysis inhibitor (TAFI) is activated by thrombin during coagulation and reduces plasmin production. Plasminogen activator inhibitor-1 (PAI-1) and alpha-2-antiplasmin also regulate the clot dissolution process by slowing fibrinolysis (13).

There are many factors that impact hemostasis (i.e. blood clotting) including age, race, and gender. It has been hypothesized that blood rheology, defined as the biophysical and flow properties of blood (17), may be associated with risk factors for CVD. Blood passage time is a recently developed measurement made using a
microchannel array flow analyzer (MC-FAN) and has been found to be proportional to blood viscosity (39). A prolonged blood passage time signifies a reduction in normal blood flow. Seki et al., found that platelet count and fibrinogen levels were not significantly correlated with blood passage time (67). However, factors including age, body mass index, red blood cell count, white blood cell count, total cholesterol, low-density lipoprotein cholesterol, and triglyceride were all positively associated with blood passage time (67). This suggests that older age, obesity, dyslipidemia, and altered blood cell counts may be related to hemorheological disorders.

Age has also been shown to increase the propensity to develop venous thrombosis by more than 50-fold (26). Increased age is thought to increase the risk of thrombosis due to increases in circulating pro-coagulant proteins and a decrease in anticoagulants. In a study performed on young and old mice, inflammation was induced and the reaction of thrombomodulin (an anticoagulant) was examined. In the young mice, thrombomodulin expression initially decreased but eventually recovered to normal levels. But in the older mice, thrombomodulin decreased as well but then remained suppressed for prolonged periods of time. The older mice were also found to have increased fibrin formation and an increased mortality rate (69). This information makes the investigation of coagulation factors in older populations of particular interest.

Gender is another factor that may influence the blood coagulation process due to the presence of different sex hormones. Progestational and estrogenic hormones have been shown to alter both the coagulation and fibrinolysis systems, but the exact changes are inconsistent (8). Rat studies have shown that females have higher prothrombin levels, females are more sensitive to thrombin, and that males have twice the amount of
circulating fibrinogen (24, 41). Hormone replacement therapy using synthetic estrogen has also been shown to increase the risk for blood clots. Oral estrogens increase the production of thrombin and cause resistance to activated coagulation factor Protein C (65). Until the effects of sex hormones are better understood and to avoid gender interaction, most studies have exclusively studied male subjects.

**Fibrin Fiber Properties**

The ultimate objective of the coagulation process is to create a fibrin mesh in which the clot can form around. The properties of individual fibrin fibers have been difficult to study due to their extremely small size. Fibrin fibers are composed of 20-30% protein and the remaining 70-80% is water or solvent. This makes the fibrin fibers quite porous. The diameters of fibrin fibers are variable and cause the mass-length ratio of the fibers to vary by a factor of at least 100 (10). Diameter size is dependent on plasma concentrations of fibrinogen and thrombin. Increased levels of fibrinogen lead to larger fibrin fiber diameters (4). The opposite trend is seen with increased levels of thrombin in the blood as fibrin fibers decrease in diameter (63). At first this seems counterintuitive, given the coagulation cascade process, but despite thinner fibrin fibers, there is actually a greater amount of branching by these fibers. Current research suggests the force required to rupture a fiber increases with increasing fibrin fiber diameter (32).

Fibrin monomers polymerize in the blood to establish a network of branched fibers that result in the formation of a blood clot (47). Two mechanical properties that help to explain the physical properties of fibrin fibers are stiffness and extensibility. Stiffness is measured using Young’s modulus, which defines stiffness as stress divided by strain—the stiffer the fiber, the higher the Young’s modulus. The extensibility of a
fiber is the maximum strain that a fiber can endure without breaking. A related property known as the elastic limit is defined as the maximum strain a fiber can endure while being able to return to its original length. Therefore, all levels of strain below this limit can occur without any permanent, structural damage. Generally, a stiff fiber is not very extensible. However, Guthold and colleagues have shown that fibrin is a relatively stiff, yet very extensible type of fiber (32).

It is difficult to analyze fibrin fibers because of their small size and because isolating them from a fibrin network is challenging. Atomic force-fluorescence microscopy has recently been developed to isolate and analyze the mechanical properties of fibrin monomers (48). The tip of the microscope is able to stretch individual fibers to incremental strain and a fluorescent microscope is used to image this process. Using this technology, Guthold and colleagues found that fibrin fibers are able to recover from very large strain and are very extensible (31). Quantitatively, uncross-linked fibrin fibers have an average extensibility of 226 +/- 52% and an elastic limit of 120%, meaning they could be stretched 2.2 times their original length and still recover. A partially cross-linked fibrin fiber was shown to have an average extensibility of 332 ± 71% (31). These findings indicate that fibrin fibers are both strong and extensible.

Physiologically, individual fibrin fibers polymerize and form networks of fiber. It is this mesh configuration that solidifies a blood clot, so these branched networks must also be studied in addition to the individual fibers. Whole fibrin film networks have extensibilities of 100 to 200% (28). These numbers are smaller than those found for individual fibers. This suggests that clot ruptures are not due to individual fiber ruptures but rather a disruption to the network of fibers (48).
In a recent unpublished study, the mechanical properties of fibrin fibers were examined in both young and healthy adults as well as older at risk adults. It was found that the fibrin fibers of the older at risk adults, who were taking aspirin, were more extensible than the young and healthy subjects. Potentially, if fibrin fibers are more extensible, they may be able to aggregate more platelets without breaking, thus forming a larger clot. The less extensible fibers of the young healthy adults, suggest that these fibers may break more easily and potentially prevent clot formation (45).

**Coagulation Factors and Fibrin in Cardiovascular Disease**

Both coagulation factors and fibrin fibers are affected by the presence of cardiovascular disease or its related risk factors. There is a clear association between high levels of fibrinogen and the risk of cardiovascular disease (51). As an acute phase protein, fibrinogen concentrations increase in response to most inflammatory conditions (7). Factor V and VIII have both been shown to have a significant association with coronary heart disease. D-dimer has additionally been associated with coronary heart disease risk (49). CVD is also associated with alterations in fibrin clot structure, including a more dense clot, with smaller pores, and more resistance to lysis (7). In unpublished findings, Guthold and colleagues have shown that fibrin fibers are 30% more extensible and 50% more elastic in older men with CVD taking aspirin, than in middle aged and older healthy men. This suggests that age and the presence of CVD may be influencing the structure and function of fibrin fibers. Despite these findings, it still remains unclear if there is a relationship between fibrin fiber properties and blood coagulation factors.
Effect of Acute Exercise

Cardiac Events

It is well established that regular exercise is a beneficial health behavior and can potentially reduce the risk of CVD (59). However exercise-induced thrombotic events such as myocardial infarction, ischemic stroke, and venous thromboembolism, remain a concern (61). Studies by Mittleman and colleagues have demonstrated that physically inactive people have the highest risk for cardiac events during acute exercise. Subjects who exercised less than one time per week were found to have a relative risk of myocardial infarction in the hour following heavy physical exertion of 107, when compared to less strenuous physical exertion or none. Yet in subjects who exercised at least five times per week, the relative risk of myocardial infarction following strenuous physical exertion was only 2.4 (55). Epidemiological studies like such have indicated that exercise may trigger cardiac events but the mechanism responsible for this is unclear.

Coagulation Factors

These exercise induced cardiac events have long been thought to occur because acute bouts of exercise create a hypercoagulable state in the blood. However, this view is no longer widely accepted and the effect of acute exercise on coagulation is not well understood (40). It appears that the blood of those with atherosclerosis and CVD may become hypercoagulable with exercise due to their predisposition to inflammation (14). When the intravascular hemostatic balance between coagulation and fibrinolysis becomes disturbed, “thrombotic” cardiac events are much more likely to occur.

During exercise the adrenal glands and certain neurons of the brain release the neurohormones epinephrine and norepinephrine (64). It has been found that
norepinephrine, but not epinephrine, causes increases in platelet activity and a platelet-mediated enhanced coagulation (38). Hyperactivity of platelets leads to enhanced platelet adhesiveness and aggregation. This in turn leads to more fibrin binding and larger, more dense blood clots. These factors may increase thrombotic risk, especially for the CVD population. Additionally in healthy, sedentary men, arterial platelet thrombus formation on collagen increases by 20% after 30 minutes of moderate intensity exercise at 70% of maximal oxygen uptake (9).

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the two most common ways to analyze a person’s coagulation status and measure clotting time (15). A study by Handa et al. demonstrated that submaximal exercise (below the lactate threshold) causes an insignificant 2% increase in PT, but a significant 5% decrease in aPTT (33). Similar findings were published in a more recent study performed on 25 healthy, untrained participants (53). After performing moderate exercise, defined as 80% of individual anaerobic threshold, PT showed an insignificant increase by 2%. In contrast, aPTT significantly decreased by 8%. The significant decrease in aPTT suggests that there is an increased activation of the coagulation system during exercise. At an exercise intensity of 100%, versus 80%, an even greater level of coagulation activity was observed (53). These decreases in aPTT reported by Handa et al. and Menzel & Hilberg are well documented, but the changes in PT have not been consistently observed (23). PT in response to exercise has been reported to both significantly decrease (27) and show no significant change (56).

The effect of acute exercise on other pro-coagulant factors, including Factor V and VIII, has also been studied. For the most part, V has been shown to be unaffected by
acute exercise. When measuring V activity of men who performed treadmill exercise until exhaustion, no significant differences were found between pre- and post-exercise levels of V activity (37). However, levels of VIII after exercise generally show large increases, suggesting a hypercoagulable state of the blood. Hedge et al. found that a treadmill run at 70-75% of VO\textsubscript{2} max significantly increases VIII activity levels by 38%. This exercise induced VIII activity remained elevated during a one-hour recovery period (35). Another study showed that moderate intensity exercise (80% individual anaerobic threshold) resulted in a 34% increase in VIII. Prior to exercise, VIII was measured as 85 ± 33 % of normal activity while immediately afterward it had increased to 114 ± 30% of normal activity (53).

The effects of acute exercise on fibrinogen levels has not been well studied, but a few studies on the topic have concluded that acute exercise tends to increase levels of circulating fibrinogen (34). In a study of male British Army recruits, increased levels of fibrinogen were observed after intensive exercise training. There was a consistent “acute-phase” response to strenuous exercise where the increase in fibrinogen level was seen after each of the five days of training (57).

D-Dimer forms as a result of fibrinolysis and thus is considered a fibrinolytic degradation product. D-Dimer has been shown to increase as a result of aerobic exercise bouts. Molz et al. have shown that an exercise session combining 30 minutes of aerobic cycling followed by maximal anaerobic training causes a significant increase in D-Dimer concentration immediately following the bout as well as 30 minutes afterwards (56). These changes have been observed to a greater extent when the exercise bout is prolonged. Following a marathon lasting anywhere from 2.3-2.6 hours, male subjects had
an average increase of 215% in plasma D-Dimer levels (62). This data strongly suggests that acute exercise is likely to increase fibrinolytic activity.

In a study done on 11 healthy, young subjects, the plasma concentration of tissue-plasminogen activator (tPA) was measured before and after a short maximal bicycle exercise test. Compared to resting levels, tPA was significantly elevated immediately post-exercise. These results indicate that in conjunction with an activation of pro-coagulant factors, there is also an increased response by the fibrinolytic system during acute exercise. The increased levels of tPA helps to maintain a stable condition of the blood by activating more plasmin for potential clot breakdown (22). The release of tPA during fibrinolysis appears to be the most important mechanism counteracting clotting and preventing thrombosis. Therefore the significant increases in tPA following exercise may be responsible minimizing the risk of thrombosis during or after exercise (21).

Plasminogen activator inhibitor (PAI-1) is the main inhibitor of tPA and thus acts as the major inhibitor of the fibrinolysis system (5). Following a maximal treadmill exercise test, PAI-1 decreases in healthy males and remains lowered for at least 10 minutes following the completion of exercise (18). It has also been found that people with metabolic syndrome and at risk for coronary heart disease, have a decrease in PAI-1 levels as a result of moderate intensity exercise (25). Similarly patients with peripheral artery disease have been shown to have a decrease in PAI-1 activity levels by as much as 40% during submaximal treadmill walking (72).

Alpha-2-antiplasmin is the main inhibitor of the fibrinolysis system. It works by forming plasmin-antiplasmin complexes on fibrin clots and thus inhibiting the function of plasmin (1). The most common way to measure alpha-2-antiplasmin levels is to measure
the plasmin-alpha-2-antiplasmin complex. During short maximal exercise, plasmin-alpha-2-antiplasmin was shown to increase by 75% after only 15 seconds, 172% after 45 seconds, and 261% after 90 seconds of exercise (36). Similar effects are seen with prolonged aerobic exercise. Thirty minutes after the completion of a downhill marathon run, plasmin-alpha-2-antiplasmin complexes were significantly increased as compared to resting pre-exercise values (71).

In review, there are specific coagulations factors that appear to be significantly affected by an acute bout of exercise. Factors indicating that there is increased pro-coagulation during acute exercise include: decreased aPTT, decreased whole-blood clotting time, and increased VIII (23). Concurrently, there are changes that indicate an increase in fibrinolysis during acute exercise as well, including: increased tPA, increased plasminogen, and increased D-Dimer (23). Thus, the current literature suggests that an acute bout of exercise likely leads to a hypercoagulable state of the blood, but that this is carefully balanced by an increase in fibrinolytic activity to prevent coagulation. These findings have been observed mainly in young healthy men, but have not been studied in older men at risk for CVD.

**Fibrin Fiber Properties**

Limited research has been conducted on the effect of acute exercise on fibrin fibers. In a study performed by Cadroy et al., subjects underwent a 30-minute bout of exercise on a bike ergometer at 50-70% of maximal oxygen uptake. Blood was drawn prior to and immediately following exercise, and was used to induce thrombus formation ex vivo. From the thrombus, fibrin deposition was quantified by immunologic determination of fibrin degradation products. It was determined that there was no
difference in fibrin concentration before versus after acute exercise (9). These findings suggest that overall concentration of fibrin fibers is not altered with exercise. Thus it is of interest to investigate whether mechanical properties of fibrin fibers, for example extensibility, are changing during acute exercise.

Limitations in the Literature

This subject of exercise-induced changes of blood coagulation factors and fibrin fiber properties has not been well studied. The studies that have been published have relatively small sample sizes, are uncontrolled, and mainly involve young, healthy individuals. Thus, the effects of acute exercise on coagulation factors and mechanical properties of fibrin fibers have not been well studied in older adults with CVD, who are at greatest risk for thrombotic cardiac events.

Study Aims

The aim of this study is to investigate the effect of acute exercise on coagulation factors and fibrin fiber properties in both young, healthy subjects as well as older subjects with CVD. Data generated from this investigation will help to identify the potential mechanisms associated with clotting related cardiac events occurring during exercise. The secondary aim of this study is to determine if there are relationships between coagulation factors and fibrin fiber mechanical properties in these two groups of male subjects. It is hypothesized that acute exercise will result in an increase in pro-coagulant measures in both groups, but that exercise will cause a greater increase in anticoagulant measures in the younger, healthy group (YH), compared to the older group with CVD (OD). However, it is hypothesized that acute exercise will cause a decrease in fibrin fiber extensibility in the YH group but not in the OD group. Furthermore it is hypothesized
that changes in pro-coagulation factors will be inversely correlated to changes in fibrin extensibility during acute exercise.
METHODS

Participants

Two groups of subjects were recruited for this study. The younger, healthy group (YH) consisted of men aged 20-23 years old with no risk factors for CVD. Five total men were recruited to this group from the Wake Forest University student population. The older group with CVD (OD) group consisted of men at least 60 years of age with known CVD or with at least two major risk factors for CVD. Five total men were recruited from new participants enrolling in either the Wake Forest Healthy Exercise and Lifestyle Programs (HELPS) or Therapeutic Lifestyle Change (TLC) programs. None of the participants of either group exercised regularly, defined as consistently exercising more than twice a week, prior to the study. Participants were not considered if they were taking medications that affect blood coagulation. Aspirin was allowable for the OD participants as it affects platelet aggregation and not blood coagulation. Women were excluded from the study as female sex hormones—specifically estrogen—alter blood coagulation.

Participant Safety

The Wake Forest University Institutional Review Board approved all procedures in this study. All subjects signed consent forms (Appendix A) and completed a medical history questionnaire prior to participation. In addition, each subject underwent an exercise stress test prior to participation. A symptom-limited RAMP treadmill test was used and was monitored by a Medical Doctor. Heart rate, ECG, and blood pressure were monitored throughout and recorded every two minutes during the test as well as in recovery. The exercise test was used to identify contraindications to exercise training and to determine maximal heart rate, which was used to calculate the appropriate exercise
intensity for each participant. CPR-certified staff and AED devices were also present for all tests.

**Acute Exercise Protocol**

After the initial screening, each participant performed a single study session of 40 minutes of aerobic exercise consisting of walking or jogging on a treadmill, or stationary biking. The mode of exercise was the choice of the participants as long as they maintained 70-85% of their maximal heart rate for the 40 minute bout. Rating of perceived exertion was also maintained between 13-15 during the session.

Immediately following the aerobic exercise, participants engaged in approximately 20 minutes of strength training exercises using free weights and weight machines. Each participant performed the leg press, leg extension, lat pulldown, and shoulder press exercises. Each participant used a moderate weight that they could perform two sets of 10-15 repetitions for each exercise. The principal investigator monitored all exercise procedures.

**Blood Collection and Analysis**

Participants had blood drawn by a registered nurse immediately prior to and following the acute exercise bout. Eight tubes were filled with 5 mL of venous blood from the cephalic vein in the antecubital area of the arm or the dorsal side of the hand both before and again immediately after the exercise bout. Six of the tubes contained 3.2% sodium citrate buffer and two tubes contained 7.2 mg K2 EDTA. The EDTA tubes and one sodium citrate tube were filled and stored at room temperature for the Disseminated Intravascular Coagulation (DIC) profile, performed by LabCorp (Winston-Salem). The DIC profile provides measures of the following coagulation factors: alpha-2-
antiplasmin, antithrombin activity, D-dimer, Factor V activity, Factor VIII activity, fibrinogen, plasminogen, platelets, prolonged activated partial thromboplastin time (aPTT), and prothrombin time (PT).

The remaining five tubes containing sodium citrate were filled and centrifuged in a Beckman model Tj-6 centrifuge for 10 minutes at 3700 RPM. The separated plasma was pipetted into fresh tubes (1mL plasma in each) and stored on ice for further testing. The plasma samples were transported to the Olin Physics Laboratory at Wake Forest University for atomic force microscopy to determine fibrin fiber properties. The remaining plasma was stored in plastic tubes and transported to LabCorp as part of the DIC profile.

**Fibrin Fiber Manipulation**

The plasma samples transferred to the Olin Physics Laboratory were stored at -80 degrees F until testing. Eventually the samples were aliquoted into 28µl samples and further stored at -80°C before analysis. Guthold et al. (31) have described the process by which blood clots are formed *in vitro* and individual fibrin fibers are isolated for testing. Prior to clot formation *in vitro*, individual 28 µl samples were thawed at room temperature for 5 minutes. Next, 8 µl of 100 mM CaCl₂ was added to the plasma sample to reach a final CaCl₂ concentration of 20 mM. Then 18 µl of this mixture was added to a previously prepared striated substrate followed by 2µl of human alpha thrombin (final concentration 0.1 NIH units/ml, Enzyme Research Laboratories, South Bend, IN). The ensuing reactions were allowed to proceed for approximately 60 minutes. A pipet tip was then used to carefully remove the top layer of the clot and the slide was rinsed with Fibrin Buffer-1 (pH 7.4, 10mM Hepes, 140mM NaCl). Following the removal of the clot’s top
layer, 24 nm fluorescent beads (Invitrogen, Fluospheres, Carlsbad, CA) diluted 1/100 with Fibrin Buffer-1 were added to the slide and the whole sample was allowed to incubate for 10 minutes. Samples were then rinsed and stored in Fibrin Buffer-2 (pH 7.4, 10 mM Hepes, 140 mM NaCl, 5 mM CaCl).

Fibrin fiber mechanical properties were found using a combined atomic force microscopy (AFM)/fluorescence microscopy technique. Fiber samples were prepared as described above and stored in a buffer solution. For analyses, the samples were placed on an inverted optical microscope (Zeiss Axiovert 200, Göttingen, Germany) and the AFM (Topometrix Explorer, Veeco Instruments, Woodbury, NY) was positioned on top of the microscope with the sample between the two. This set-up allows for manipulations to be viewed with the optical microscope while fibers are manipulated with the AFM, as can be seen in Figure 3.

Single fibers suspended over the ridges of the striated substrate were laterally pulled by the torquing of the AFM cantilever. NanoManipulator software (3rd Tech, Chapel Hill, NC) provides precise control of the AFM tip and collects force and position data during fiber manipulations. Fiber diameters were measured on the ridges of the striated substrate using tapping mode. From these data the actual force on the fiber was calculated; stress is defined as $S = F/A$ and extensibility $= (L' - L_{ori})/ L_{ori}$, where $A$ is the fiber cross-sectional area, $L_{ori}$ is the original length of the fiber, and $L'$ is the length of fiber just prior to rupture. Thus, extensibility was defined as the extension at which a fiber ruptures during stretching. Therefore if a fiber were stretched to two times its original length, it would have 100% extensibility. For each sample, 20-25 fibers were stretched and measured, and average values were determined.
Figure 3. Schematic of Single Fiber AFM/Fluorescence Microscopy Technique

(A) Fibrin fiber sample cover slides with wells and ridges are sandwiched between an AFM (top) and an inverted microscope (bottom). This allows for single fibers spanning the well to be mechanically manipulated by the AFM cantilever tip from above. (B) Top down view of a single fiber manipulation. (C) Snapshots of a single fiber manipulation during an extensibility test. Scale bar is 10 µm.

Data Analysis

All data was entered into SPSS version 22 and analyzed for normality.

Descriptive statistics were performed in order to determine means, standard deviations, and ranges. Abnormally skewed data were transformed to a normal distribution using a square root transformation, and if still abnormal then a natural log transformation.

Between and within group differences were analyzed using 2-way repeated measures ANOVA. Post-Hoc testing was performed using paired samples t-tests. Due to the small sample size, effect sizes were used to complement statistical hypothesis testing and to rule out Type II error. Effect sizes allow for an estimate of effect regardless of sample size, and provide a basis for how strongly two variables are related (44). SPSS reports
effect size using partial eta squared. Eta squared ($\eta^2$) is also known as the correlation ratio ($R^2$) and is defined as “the sums of squares (SS) for the effect of interest divided by the total sums of squares” (44). Partial eta squared follows the equation:

$$\eta^2 = \frac{SS_{between}}{SS_{between} + SS_{error}}$$ (44)

Effect sizes can be interpreted as small, medium, or large effects, corresponding to values of 0.2 for small, 0.5 for medium, and 0.8 for large (16). Bivariate Pearson correlations were performed to analyze associations between specific coagulation factors and fibrin fiber extensibility. A p-value of <0.05 was considered to be statistically significant for all analyses.
RESULTS

Demographics

A total of 10 subjects, 5 younger, healthy (YH) and 5 older subjects with CVD (OD), participated in this study. Descriptive statistics of demographic data for all subjects are presented in Table I.

All participants were male and white, non-Hispanic. The YH group ranged in age from 20-23 years, while the OD group ranged in age from 63-79 years. Body mass index (BMI) indicated the YH group was in the normal weight category, whereas the OD group was in the obese category. The extent of CVD varied among the 5 participants in the OD group. Type II diabetes, cardiomyopathy, and hypertension were conditions seen amongst the group. Two of the subjects had sustained a prior myocardial infarction. Three subjects had previously undergone CVD-related procedures including an acute aortic dissection repair, percutaneous transluminal coronary angioplasty, and coronary artery bypass graft surgery. Three of the five subjects were taking 81 mg of aspirin as well as other “cardiovascular” medication at the time of the study.

All participants of the study were analyzed for changes in coagulation factors and fibrin fiber extensibility pre- and post- acute exercise.

Table I: Demographics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>YH Group (n=5)</th>
<th>OD Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21 ± 1</td>
<td>73 ± 6</td>
</tr>
<tr>
<td>Age Range (yr)</td>
<td>20 - 23</td>
<td>63 - 79</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 ± 3.1</td>
<td>32.2 ± 5.7</td>
</tr>
</tbody>
</table>
Study Measures

All measurements were tested for normal distribution using the Kolmogorov-Smirnov test. All tests with significance values above 0.05 indicated that the data was normally distributed. All coagulation and fibrin fiber extensibility measurements were found to be normally distributed except for Factor VIII post-exercise (p= .031), and D-Dimer pre-exercise (p=.008) and D-Dimer post-exercise (p=.032). In order to allow for parametric testing, these measurements were transformed to obtain normal distributions. Factor VIII was normalized using a square root transformation. D-Dimer pre- and post-exercise was normalized using a natural log transformation, after the square root transformation did not result in normal distributions.

Means and standard deviations for each coagulation factor were determined for both the YH group and the OD group. These results can be seen in Tables II and III. Normal ranges of coagulation factor levels are presented in Appendix B. Means and standard deviations for fibrin fiber extensibility were also determined pre and post-exercise for both groups and can be found in Table IV.
### Table II: Mean Values (± SD) for Coagulation Factors Pre- and Post- Exercise, Younger Healthy Group

<table>
<thead>
<tr>
<th>Coagulation Factor</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10E3/uL)</td>
<td>330.7 ± 92.4</td>
<td>422.0 ± 162.1</td>
<td>+ 26.5</td>
</tr>
<tr>
<td>Prothrombin Time (s)</td>
<td>11.3 ± 1.0</td>
<td>11.4 ± 0.9</td>
<td>+ 0.3</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>29.2 ± 1.9</td>
<td>21.0 ± 11.8</td>
<td>- 28.1</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>248.0 ± 73.3</td>
<td>243.8 ± 74.7</td>
<td>- 1.7</td>
</tr>
<tr>
<td>Factor V Activity (%)</td>
<td>94.0 ± 7.6</td>
<td>94.0 ± 24.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Factor VIII Activity (%)</td>
<td>121.4 ± 55.9</td>
<td>380.8 ± 108.1</td>
<td>+ 197.2</td>
</tr>
<tr>
<td>Alpha-2-Antiplasmin (%)</td>
<td>107.4 ± 7.0</td>
<td>103.0 ± 11.1</td>
<td>- 4.1</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>111.0 ± 12.3</td>
<td>112.0 ± 9.8</td>
<td>+ 0.9</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>105.2 ± 10.8</td>
<td>111.2 ± 13.3</td>
<td>+ 5.7</td>
</tr>
<tr>
<td>D-Dimer (ug/FEU/mL)</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table III: Mean Values (± SD) for Coagulation Factors Pre- and Post- Exercise, Older CVD Group

<table>
<thead>
<tr>
<th>Coagulation Factor</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10E3/uL)</td>
<td>246.8 ± 74.9</td>
<td>248.6 ± 49.2</td>
<td>+ 0.7</td>
</tr>
<tr>
<td>Prothrombin Time (s)</td>
<td>11.3 ± 0.5</td>
<td>11.2 ± 0.5</td>
<td>- 0.9</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>27.8 ± 1.0</td>
<td>27.8 ± 1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>283.6 ± 70.3</td>
<td>298.8 ± 60.0</td>
<td>+ 5.4</td>
</tr>
<tr>
<td>Factor V Activity (%)</td>
<td>104.2 ± 18.5</td>
<td>115.6 ± 22.0</td>
<td>+ 10.9</td>
</tr>
<tr>
<td>Factor VIII Activity (%)</td>
<td>157.2 ± 34.7</td>
<td>178.4 ± 17.3</td>
<td>+ 13.5</td>
</tr>
<tr>
<td>Alpha-2-Antiplasmin (%)</td>
<td>84.8 ± 14.5</td>
<td>85.0 ± 14.8</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>90.2 ± 8.8</td>
<td>75.8 ± 42.3</td>
<td>- 16.0</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>97.2 ± 12.9</td>
<td>92.2 ± 17.4</td>
<td>- 5.1</td>
</tr>
<tr>
<td>D-Dimer (ug/FEU/mL)</td>
<td>0.6 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table IV: Mean Values (± SD) for Fibrin Fiber Extensibility Pre- and Post- Exercise

<table>
<thead>
<tr>
<th></th>
<th>YH Group</th>
<th></th>
<th>OD Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Ex</td>
<td>Post-Ex</td>
<td>Pre-Ex</td>
<td>Post-Ex</td>
</tr>
<tr>
<td>Extensibility (%)</td>
<td>176.0 ± 8.4</td>
<td>150.7 ± 10.5</td>
<td>189.4 ± 26.2</td>
<td>198.9 ± 19.3</td>
</tr>
<tr>
<td>Change (%)</td>
<td>- 14.4</td>
<td></td>
<td>+ 5.0</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance

Differences between the two subject groups and between the two time points (within groups) were assessed using 2-way repeated measures ANOVA for all coagulation measurements and fibrin fiber extensibility.

Platelet levels did not show a significant interaction between the group and time factors. There was a significant increase (p= .048) in platelet levels from pre- to post-exercise. As can be seen in Figure 4, most of this significant increase appears to be due to a change in the YH group even though the interaction was not significant. There was no significant difference in platelet levels between the subject groups.

Factor VIII activity was the only coagulation factor that had a significant interaction between the two independent variables (p= .001). Post-hoc paired samples t-tests and independent t-tests were used to further investigate this interaction and determine where significant differences existed. The t-tests indicated there was a significant difference in VIII from pre to post exercise in the YH group (p= .002) but not for the OD group (p= .304). As seen in Figure 5, VIII activity increased in the YH group from 121.4 ± 55.9% to 380.8 ± 108.1% from pre to post exercise, whereas VIII activity in the OD group only increased from 157.2 ± 34.7% to 178.4 ± 17.3%. Independent samples t-tests concluded that there was a significant difference between the YH group and the
OD group at post-exercise for levels of VIII (p= .001) but there was no between group difference in VIII pre-exercise.

Figure 4: Changes in Platelets Pre vs Post Exercise

Figure 5: Changes in Factor VIII Pre- vs. Post Exercise
Alpha-2-antiplasmin activity did not have a significant interaction between group and time factors. There was no significant difference in alpha-2-antiplasmin from pre- to post-exercise. There was a significant difference, however, between the two subject groups (p= .018). Figure 6 shows that the YH group had higher levels of alpha-2-antiplasmin activity at both pre- and post-exercise, (107.4 ± 7.0% to 103.0 ± 11.1%, respectively), compared to the OD group (84.8 ± 14.5% to 85.0 ± 14.8%, respectively).

Figure 6: Changes in Alpha-2-Antiplasmin Pre- vs. Post Exercise
Antithrombin activity did not have a significant interaction between group and time factors. There was no significant difference in antithrombin activity from pre- to post-exercise. However, there was a significant difference between groups (p = .026). Antithrombin activity levels were found to be significantly greater in the YH group as compared to the OD group. Figure 7 shows that the YH group had higher levels of antithrombin at pre- and post-exercise, (111.0 ± 12.3% to 112.0 ± 9.8%, respectively), compared to the OD group (90.2 ± 8.8% to 75.8 ± 42.3%, respectively). This difference appears to be mainly due to a decrease in the OD group, but again the interaction was not significant (p = .467).

Figure 7: Changes in Antithrombin Pre- vs. Post Exercise
For each of the other coagulation factors that were measured, including prothrombin time, aPTT, fibrinogen, V activity, plasminogen, and D-Dimer, no significant interaction existed between group and time factors. There were also no significant within group differences from pre- to post-exercise, nor were there significant between group differences.

The last analysis of variance was performed on fibrin fiber extensibility. Fibrin fiber extensibility did not show a significant interaction between and within groups. Fibrin extensibility also had no significant difference from pre- to post-exercise. Yet, there was a significant difference between groups (p=.003). As can be seen in Figure 8, the OD group had significantly greater fibrin fiber extensibility than the YH group. Despite the non-significant interaction (p=.073), the OD group showed an increase (+5%) in fibrin fiber extensibility during exercise, whereas the YH group showed a decrease (-14%).

Figure 8: Changes in Fibrin Fiber Extensibility Pre- vs. Post Exercise
**Effect Sizes**

In order to account for the small sample size, lack of power, and to avoid Type II errors, effects sizes were used to assess changes. Partial eta squared ($\eta^2$) was used to measure effect size. The significant interaction observed in VIII revealed a large effect size of $\eta^2 = 0.788$. The significant within group difference in platelet concentration revealed a moderate effect size of 0.507. Moderate effect sizes were seen for the other three significant between group differences; alpha-2-antiplasmin ($\eta^2 = 0.522$), antithrombin ($\eta^2 = 0.481$), and fibrin fiber extensibility ($\eta^2 = 0.691$).

**Correlations**

Finally, the associations between coagulation factors and fibrin fiber properties at both pre- and post- exercise were examined with Pearson’s correlations. Four significant ($p < .05$) relationships were observed and are presented in Table V and Figure 8. While cause and effect cannot be determined, these findings indicate that there are potential important relationships between blood coagulation measures and fibrin fiber properties during acute exercise.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets, post-exercise</td>
<td>Fibrin extensibility, post-exercise</td>
<td>-.711</td>
</tr>
<tr>
<td>VIII, post-exercise</td>
<td>Fibrin extensibility, post-exercise</td>
<td>-.804</td>
</tr>
<tr>
<td>Alpha-2-antiplasmin, pre-exercise</td>
<td>Fibrin extensibility, post-exercise</td>
<td>-.788</td>
</tr>
<tr>
<td>Antithrombin, pre-exercise</td>
<td>Fibrin extensibility, post-exercise</td>
<td>-.646</td>
</tr>
</tbody>
</table>
Conclusions

In conclusion, these results may identify potential mechanisms responsible for exercise-induced cardiac events that may occur. All four coagulation factors that had significant between or within group differences were also significantly, inversely correlated to fibrin fiber extensibility. For example, there was a significant difference between the YH group and the OD group in FVIII activity post-exercise, and that post-
exercise FVIII activity had a strong inverse correlation to post-exercise fibrin fiber extensibility. This may suggest that the increase in FVIII observed in the YH group during exercise may result in a decrease in fibrin fiber extensibility, and decrease the risk of developing a blood clot during exercise. Similar conclusions can be drawn from the significant inverse relationships between platelets, alpha-2-antiplasmin, and antithrombin, and fibrin fiber extensibility. This finding would need to be replicated in a larger, more diverse group of subjects before definitive conclusions can be drawn.
DISCUSSION

A number of studies have evaluated the effect of acute exercise on selected coagulation factors (33, 34, 35, 56). The majority of these studies have been conducted on young, healthy populations and none have examined the effect of acute exercise on the mechanical properties of fibrin fibers. Thus it was the aim of this investigation to evaluate the effect of acute exercise on coagulation factors and fibrin fiber properties in both young, healthy subjects as well as older subjects at risk for CVD. In addition, this study sought to identify possible relationships between individual coagulation factors and fibrin fiber properties during acute exercise.

Participant Demographics

The younger, healthy group (YH) consisted of healthy males aged 20-23 years, which is similar to the subjects included in other studies (9, 22, 53). In contrast, this study also included a group of males aged 60 years or older with a history of CVD or at least two risk factors for the disease (OD). This population has not previously been included in studies examining the effects of acute exercise on coagulation. The average BMI of the OD group classified them as obese, further increasing their risk for CVD. The subjects in the OD group had a varying array of CVD—several had prior myocardial infarction or invasive heart surgery, whereas several had multiple CVD risk factors. It is also important to note that three of the five OD subjects were regularly taking aspirin, a drug that affects platelet activity, at the time of the study. This may have had an impact on the findings of this investigation, particularly platelet measurements.
Pre-Exercise Coagulation Measures and Fibrin Fiber Properties

All averages for pre-exercise measures of coagulation factors in the YH group fell within normal ranges (Appendix B). The OD group had two pre-exercise coagulation measures fall outside of the normal range. Pre-exercise levels of Factor VIII activity in the OD group were higher than normal, which indicates elevated pro-coagulation activity. Secondly, pre-exercise D-Dimer levels were higher than normal in the OD group. This is likely due to extremely high levels measured in individual 6 and individual 9. Pre-exercise fibrin fiber extensibility measurements fell within normal ranges for both the YH and the OD group (48), although few studies have evaluated this measure in great detail so comparisons are limited.

Exercise Induced Changes in Coagulation Factors

Factor VIII was the one coagulation factor that had a significant interaction between groups and pre-post exercise. This significant interaction was supported by a large effect size. The YH group had a significant increase in VIII from pre- to post-exercise, but a non-significant increase was seen for the OD group. A significant increase in VIII during exercise has been previously reported (35, 53). However it was somewhat surprising to see a greater increase in the pro-coagulant VIII in the YH group than in the OD group, as this suggests the former to be at greater risk for developing blood clots during exercise. Although speculative, the greater change in VIII observed in the YH group compared to the OD group during exercise may be related to the higher absolute exercise intensity in the YH subjects. While both groups performed the acute bout of exercise at similar relative intensity levels (70-85% HR), the YH subjects were all vigorously running at that level whereas the OD subjects were walking at moderate pace.
The literature does suggest that pro-coagulants may be positively associated with exercise-intensity (23) and thrombus formation may be more likely with high exercise intensity (9).

Levels of alpha-2-antiplasmin were found to be significantly different between the two groups, with higher levels of activity in the YH group. Alpha-2-antiplasmin is an inhibitor of plasmin and therefore is responsible for slowing down the process of fibrinolysis. This finding suggests the YH group has more difficulty degrading fibrin and is potentially more likely to form a clot. This contrasts a prior publication that suggests CVD populations have higher resistance to lysis as their fibrin fibers are more dense, making them more difficult to degrade (7). Although speculative, the difference in alpha-2-antiplasmin levels between the two groups could be due to the need for less inhibition of fibrinolysis in the OD group due to their dense fibrin structure.

Another significant finding was higher levels of antithrombin activity in the YH group compared to the OD group. Antithrombin is responsible for inactivating enzymes of the coagulation cascade and thus is considered an anticoagulant. This finding suggests the higher levels of antithrombin protects the YH subjects against exercise-induced coagulation, which may be necessary given the observed increase in VIII in YH subjects during exercise. Changes in antithrombin during acute exercise have not been reported, and therefore this finding cannot be compared to other investigations.

Platelets were the one measurement that had a statistically significant difference within groups from pre to post exercise. It appears that the change in the YH group was driving this finding, as the YH group had a 26.5% increase in platelets post-exercise whereas the OD group only increased 0.7%. The increase in platelets observed in the YH
group during exercise could be related to exercise intensity, as discussed earlier. In addition, three of the OD subjects were taking aspirin during the time of the study, which has known inhibitory effects on platelet activity (66). Although platelet activity is not technically part of the coagulation cascade, higher platelet levels in the YH group would appear to result in a greater potential for thrombus formation during exercise.

In addition to the statistically significant differences between and within the two experimental groups, there were several non-significant findings of interest. Both fibrinogen and Factor V were higher in the OD group than the YH group. This finding is consistent with observation that circulating fibrinogen is a characteristic of CVD (51). The OD group also showed non-significant increases in both fibrinogen and V from pre to post-exercise, which indicates a slightly greater pro-coagulant potential. However the OD group had lower levels of VIII when compared to the YH group (Figure 4), which is somewhat contradictive as VIII functions prior to both V and fibrinogen in the coagulation cascade. This finding suggests that there must be an event happening after VIII that is causing an increase in the pro-coagulant activity in OD subjects.

Levels of the anticoagulant plasminogen were seen to be slightly lower in the OD group than the YH group. The OD group also showed a trend for plasminogen levels to decrease post-exercise, whereas the YH group tended to increase in plasminogen levels post-exercise. These observations suggest that the CVD patient may have a more difficult time both regulating the forward progress of the clotting cascade as well as the degradation of a fibrin clot once it’s been formed. But again, strong conclusions should not be made from these non-significant observations.
There were two coagulation measurements in the present study that did not respond as expected, based on previous reports in the literature. First, D-Dimer has been documented to increase during acute exercise in healthy subjects (56, 62). Findings from this study indicate that there was no change in D-Dimer levels from pre- to post-exercise in either subject group. These findings therefore suggest that the acute exercise being performed is having no effect on the breakdown on a fibrin clot. It is possible that the abnormally high resting levels of D-Dimer in the OD group did not allow for a post-exercise increase to occur. Given our observations that acute exercise results in an increase in pro-coagulation and inhibition of anticoagulation, there is a greater threat of blood clot formation if fibrinolysis activity is not simultaneously increasing.

The literature also suggests that there is normally a decrease in aPTT activity during acute exercise (33, 53). aPTT is a measure of clotting time and tests the efficiency of the intrinsic and common pathways of the coagulation cascade. The YH group in the present study showed a slight decrease in aPTT post-exercise but there was no change in the OD group. It is unclear why aPTT did not decrease during exercise as has been reported.

**Exercise Induced Changes in Fibrin Fiber Extensibility**

In the present study, the OD group demonstrated an increase (+5%) in fibrin fiber extensibility during exercise, whereas the YH group had a decrease (-14%) in fibrin fiber extensibility. This difference in activity between the two groups may be very important, as greater extensibility of fibrin fibers has been shown to increase the risk of clot formation (45). An increase in fibrin fiber extensibility can create a more occlusive blood clot, as it can expand as more cells are entrapped. In contrast, a decrease in fibrin
extensibility may result in fibrin fibers that are more easily broken or dissolved. These fiber findings suggest that acute exercise may increase the extensibility of fibrin fibers and thus increase the risk of thrombosis in the OD group.

**Relationship Between Coagulation Factors and Fibrin Extensibility**

Bivariate Pearson’s correlations were run to analyze relationships between coagulation factors and fibrin fiber extensibility, both pre and post-exercise. Although significant correlations cannot determine cause and effect, they can provide insight into important relationships between coagulation factors and fibrin fiber properties during acute exercise. To our knowledge, no studies have examined the relationship between coagulation factors and fibrin fiber properties.

We observed a significant inverse correlation between platelets and fibrin fiber extensibility post-exercise. This indicates that as platelet levels increase, there is a decrease in fibrin fiber extensibility. Although speculative, it could be beneficial to decrease fibrin extensibility as platelet levels increase in order to prevent expansion of a clot.

The second significant, inverse correlation was between VIII activity and fibrin fiber extensibility post-exercise. This relationship indicates that as levels of VIII in the blood increase during exercise, there is a decrease in fibrin fiber extensibility. This may also be protecting against clot expansion during acute exercise.

Pre-exercise levels of alpha-2-antiplasmin were significantly, inversely correlated with post-exercise fibrin fiber extensibility. Thus, low levels of alpha-2-antiplasmin prior to exercise are associated with greater fibrin extensibility following an acute bout of exercise. Alpha-2-antiplasmin’s main responsibility in the clotting process is to inhibit
fibrinolysis or slow the breakdown of a fibrin clot. Therefore it appears that individuals who possess a greater ability to degrade a fibrin clot (less circulating alpha-2-antiplasmin) prior to exercise, are more likely to have greater fibrin fiber extensibility following a bout of exercise. Should this result be confirmed in a large study, measuring alpha-2-antiplasmin may be a way to identify those with greater fibrin fiber extensibility and subsequent thrombotic risk during exercise.

The final significant inverse correlation was observed between pre-exercise antithrombin and post-exercise fibrin extensibility. Antithrombin is a natural anticoagulant that inhibits many factors of the coagulation cascade, most importantly thrombin. Therefore antithrombin is primarily responsible for inactivating the thrombin-led conversion of fibrinogen to fibrin. Thus it appears that low antithrombin activity at rest is related to greater fibrin extensibility post-exercise. This is an alarming relationship, as low antithrombin activity alone is a risk for blood clotting, but when combined with highly extensible fibrin fibers the risk may be further increased.

**Study Limitations and Future Directions**

The main limitation of this study is the small sample size, and resulting lack of power. Although effect size was used to counter this issue, a larger group of participants should be recruited to confirm these findings. It was difficult to have a larger sample size, as the measurements used in this study are very expensive and time consuming to perform. The DIC profile, which was performed pre- and post-exercise in each subject, costs $300, for a total of $6000 in this small study. Also, measuring fibrin fiber properties is expensive, requires sophisticated equipment, and involves many hours to perform measurements on each fiber sample.
Another limitation was the inclusion of just male subjects. The results of this study are not generalizable to women as female sex hormones may effect coagulation. Thus future studies will have to examine females and eventually compare the two genders. The men in the OD group were recruited based on their history of CVD events and CVD risk factors. Because of differences in their medical history, it’s difficult to place them all in one “group” without recognizing that there are many potential differences among these subjects that may have affected our results. Lastly, men in the OD group were allowed to participate even if they were regularly taking aspirin. This may have effected some coagulation measurements, particularly platelets. In order to avoid this limitation in the future, groups of younger, healthy men taking aspirin or older men with CVD not taking aspirin should be studied.

An additional area for future study would be to assess other known coagulation factors. We were limited to certain coagulation factors in the DIC profile test available through LabCorp, however it is possible to measure other coagulation factors from blood samples. For example, a number of studies have examined Factor VII, X, XIII, tPA, and PAI (18, 21, 22, 25), allowing for more thorough investigation of both pro-coagulant and anticoagulant activity during exercise. It would also be important to perform further correlations between coagulation measurements and additional fibrin fiber properties, such as stiffness and diameter.

Another important direction of this line of research is to examine the effect of chronic exercise (and possibly diet manipulation) on these coagulation factors and the mechanical properties of fibrin fibers. While it is important to understand the acute effects of exercise on coagulation factors and fibrin fibers, as this will provide important
insight into the mechanism of exercise induced thrombotic events, it is also important to
determine if these responses can be modified by chronic exercise training or diet change.

Conclusions

Despite its limitations, this study provides further insight toward our
understanding of the effect of acute exercise on coagulation factors and fibrin fiber
properties. Coagulation factors including VIII, alpha-2-antiplasmin, antithrombin all
appear to be affected by acute exercise and may be related to differences in age and/or the
presence of CVD. Further, acute exercise appears to cause an increase in fibrin fiber
extensibility in the OD group but not in the YH group. Gaining a better understanding of
these coagulation factors and fibrin fiber properties may lead to greater understanding
and possible prevention of thrombotic vascular events during exercise. Further study is
warranted to confirm and expand on the findings observed in this pilot study.
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35. Hegde SS, Goldfarb AH, Hegde S. Clotting and fibrinolytic activity change during 

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37. Iatridis SG, Ferguson JH. Effect of physical exercise on blood clotting and 


Appendix A

INFORMED CONSENT
THE EFFECTS OF LIFESTYLE CHANGE ON BLOOD COAGULATION AND FIBRIN FIBER PROPERTIES

You are invited to be in a research study. The purpose of this research study is to determine if lifestyle modifications (exercise, diet, weight loss) alters the blood clotting factors and/or the mechanical properties of the blood clot components – specifically the fibrin fibers. You are being asked to take part in this study because you have participated in a prior study of exercise on blood coagulation/fibrin properties and you have recently participated in the WFU Therapeutic Lifestyle Change (TLC) Program. This study will require you to return to the laboratories of Wake Forest University once for approximately one hour. Your participation is voluntary. Please take your time in making your decision as to whether or not you wish to participate. Ask the study staff to explain any words or information contained in this informed consent document that you do not understand. You may also discuss the study with your friends and family.

Procedures

If you choose to participate, the visit (4-6 months after previous study visit) will consist of you exercising in the Reynolds Gym under the direct supervision of Dr. Brubaker and students from Wake Forest University. These individuals are certified to provide basic life support (CPR) and utilize an automated external defibrillator in the event of a medical emergency as well as to activate the Emergency Medical Response of Wake Forest University and W-S Forsyth County EMS by calling 9911 if needed. Moreover, the exercise bout will be terminated at any time at your request or if abnormal signs and/or symptoms are observed by the study staff. As with all exercise studies, we “err on the side of caution” and will discontinue the session if there are any medical concerns.

The exercise session is identical to the one you performed in the prior study and will take approximately 1 hour. This session includes a single “bout” of aerobic-type exercise. The exercise bout will begin with ~5 minutes of warm-up exercise (light walking, gentle calisthenics, etc.). You will then begin to walk on a treadmill and gradually increase the intensity of your exercise (i.e., walk at a faster speed) until you achieve a heart rate that is equivalent to 70-85% of the level you obtained on the maximal exercise test. Your heart rate will be verified and regulated with a Polar Heart Monitor worn on your chest and wrist during the exercise bout. The goal is to have you exercise at this intensity continuously for 30 minutes, but if you cannot, you will be given the opportunity to stop and rest as much as needed. Following the “aerobic” exercise component, you will do 15 minutes of “cool-down” exercise that would include stretching and light weight training (10 exercises) with light hand-held weights. We will make sure your heart rate levels return to pre-exercise levels and that you will be free of any abnormal symptoms before discharging you.

Just prior to the aforementioned exercise “bout”, an experienced phlebotomist will draw 5ml of blood (1 teaspoon) from a vein in your arm into each of 6 tubes. This procedure (same amount of blood) would be repeated immediately following the bout of exercise. Consequently, a total of 60 ml of blood (equal to approximately 4 tablespoons of blood) will be drawn from you during this visit. Your blood sample will be stored until analyzed with a label that contains a unique identifier and will not include any identifiable information about you such as your name, address, telephone number, social security number, medical record number or any of the identifiers outlined in the HIPAA Privacy Rule. The
unique identifier will be a randomly assigned number and only the principal investigator will have access to the code that links the unique identifier to you.

**Risks and Benefits**

The research that may be performed with your blood sample is not designed to help you specifically. It might help people who have vascular disease at some point in the future, but it is not known if this will happen. The results of the research performed with your blood will not be given to you or your doctor. The results will not be put in your medical record. You will be in the study only for the one session described above and there is no follow-up testing or intervention of any type. You can stop participating at any time and there is no harm done to you by withdrawing from the study.

Being in this study does involve some risk to you from the blood draw and the exercise. You may experience discomfort, bruising and/or bleeding where the needle is inserted. Occasionally some people become dizzy lightheaded or feel faint. Infection may occur on rare occasions. These risks are minimized by using sterile procedures and having an experienced phlebotomist draw your blood. Frequent donation of blood can result in low iron in your blood (iron deficient anemia) but you are only giving a very small amount in this study. If concerned, you should discuss the risk of being in this study with the study staff.

The risks associated with exercise are rare, especially under the supervision of trained professionals, but do include potential for heart attack, irregular heartbeats, blood pressure and/or heart rate abnormalities, but these are minimized in this study by performing a preliminary exercise “stress test” to identify potential contraindications to exercise. Furthermore, your exercise session is individually prescribed based on the initial exercise test and carefully monitored by trained and experienced staff that can provide basic life support if needed and/or activate EMS if advanced life support is required. The risk of harm or discomfort that may happen as a result of this study is not expected to be more than in daily life or from routine medical tests.

Even if you do not finish the visit and/or elect to withdraw from the study, you will be given a $25.00 gift card to compensate you for study related expenses (i.e. gas, parking, tolls) Overall, this study will provide us with a better understanding of the role of fibrin fibers (and other blood clotting measures) in the formation of blood clots and determine if lifestyle changes have any impact on these measures. Participation in this research study may involve providing information that you consider confidential or private. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to have access to research records, will be made to keep your information safe and confidential. Laboratory test results generated as a result of your participation in the research study will be stored on a password protected personal computer of the Principle Investigator that is provided by Wake Forest University. These results will be kept secure, with access to this information limited to individuals with proper authority and to those who are directly involved with this research study. The data generated in this study will be retained as long as scientifically useful and will be destroyed properly thereafter.

Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. You can notify the Principal or Student Investigators, at any time, if you wish to decline participation or withdraw from the study. The investigators also have the right to stop your participation in the study at any time. This could be because it is in your best medical interest, or you do not qualify for a study group of interest.

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**Wake Forest University**
Institutional Review Board
IRB Number: IRB00022087
Date Approved 5/25/2015
Version Valid Until: 5/24/2016
For questions about the study or in the event of a research-related injury, contact the study investigator, Dr. Peter Brubaker at (336) 758-4683 (office) or (336) 416-9686 (cell phone). The Institutional Review Board (IRB) is a group of people who review the research to protect your rights. If you have questions about your rights as a research participant, you should contact the Office of Research and Sponsored Programs at (336) 758-5888. You will be given a copy of this signed consent form.

By signing below, you indicate that you are willing to participate in this research project

________________________________ _______________________________________
Subject Name (Printed)

________________________________ _______________________________________
Subject Signature Date

________________________________ _______________________________________
Person Obtaining Consent Date
Appendix B

Normal Blood Coagulation Values as defined by LabCorp, Disseminated Intravascular Coagulation (DIC) Profile, Comprehensive Plus

<table>
<thead>
<tr>
<th>Measure</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10E3/uL)</td>
<td>140 - 415</td>
</tr>
<tr>
<td>Prothrombin Time (s)</td>
<td>9.1 - 12.0</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>24 - 33</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>193 - 423</td>
</tr>
<tr>
<td>Factor V Activity (%)</td>
<td>60 - 140</td>
</tr>
<tr>
<td>Factor VIII Activity (%)</td>
<td>50 - 150</td>
</tr>
<tr>
<td>Alpha-2-Antiplasmin (%)</td>
<td>83 - 123</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>75 - 135</td>
</tr>
<tr>
<td>Plasminogen (%)</td>
<td>78 - 130</td>
</tr>
<tr>
<td>D-Dimer (ug/FEU/mL)</td>
<td>0.0 - 0.4</td>
</tr>
</tbody>
</table>
Georgia Holland

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Education

*Wake Forest University*, Winston Salem, NC, May 2016
Master of Science, Health and Exercise Science
Faculty Advisor: Dr. Peter Brubaker, PhD
GPA: 3.8

*Yale University*, New Haven, CT, May 2014
Bachelor of Science, Molecular Cellular and Developmental Biology
Thesis: “Characterization of endometrial cell proliferation in a hyperinsulinemic, euglycemic mouse model”
Faculty Advisor: Dr. Clare Flannery, MD
GPA: 3.5

Research Positions

Graduate Student Researcher

*Wake Forest University Department of Health & Exercise Science, Winston Salem, NC*
- Assisted in the administering of acute aerobic treadmill and anaerobic weight circuit testing
- Centrifuged participant blood samples using a Beckman model Tj-6 for later coagulation factor and fibrin fiber profiling
- Transformed and statistically tested data using SPSS version 22

Undergraduate Student Researcher

*Yale University Department of Molecular, Cellular and Developmental Biology, New Haven, CT*
- Assisted in the dissection of the endometrium and ovaries of mutated MKR mice
- Performed 2-day immunohistochemistry using p27 and cyclin D antibodies on prepared slides of uterus tissue from 5 wild type and 9 MKR mice
- Analyzed H&E, Ki67, and IHC stained slides looking for abnormalities in the cells of the uteri
Publications and Presentations


“Characterization of endometrial cell proliferation in a hyperinsulinemic, euglycemic mouse model” Poster presented at MCDB Student Research Symposium, Yale University, April 2014

Awards and Honors

Yale University Commencement Student Marshal, 2014
Francis Gordon Brown Prize, 2013

Laboratory and Computer Skills

Immunohistochemistry; microinjection; spectrophotometry
Microsoft Word, Excel, PowerPoint; SPSS

Professional Memberships

American College of Sports Medicine, Fall 2014 - Present

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