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Fibrinolytic Adaptations to a Phase II Cardiac
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**FIBRINOLYTIC ADAPTATIONS TO A PHASE II CARDIAC
REHABILITATION PROGRAM**

By

Paul Robert Nagelkirk

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

FIBRINOLYTIC ADAPTATIONS TO A PHASE II CARDIAC REHABILITATION PROGRAM

By

Paul Robert Nagelkirk

Fibrinolysis, the process of dissolving a fibrin blood clot, plays a pivotal role in the development of vascular disease. Occlusive blood clots are responsible for most acute cardiovascular events, and patients with coronary artery disease (CAD) typically exhibit a blunted fibrinolytic capacity. Initiation of fibrinolysis involves the conversion of plasminogen to plasmin, which is primarily catalyzed by tissue plasminogen activator (tPA). Depressed tPA and elevations of its primary inhibitor, plasminogen activator inhibitor-1 (PAI-1) are associated with morbidity, mortality, and are independent risk factors for various cardiovascular outcomes. Exercise training promotes enhanced fibrinolytic potential in healthy individuals, and individuals with CAD who undergo 12 or more weeks of regular exercise as part of a cardiac rehabilitation program demonstrate improvements in tPA and PAI-1. Modern day third-party reimbursement practices often necessitate fewer than 12 weeks of exercise training in cardiac rehabilitation programs. It is unclear if training regimens shorter than 12 weeks will elicit fibrinolytic improvements. The purpose of the present study was to evaluate changes in plasma concentrations of tPA and PAI-1, as well as changes in expression of the tPA and PAI-1 genes in whole blood after three and six weeks of participation in a phase II cardiac rehabilitation program.

Fourteen CAD patients (12 male, 2 female) trained three days/week for six weeks. Exercise sessions adhered to American College of Sports Medicine (ACSM) guidelines

for intensity and duration. Blood samples were taken at baseline (BL), after three weeks (3W), and after six weeks of training (6W) in a cardiac rehabilitation program and analyzed for tPA activity and antigen, PAI-1 activity and antigen, and relative quantification of tPA and PAI-1 RNA. Linear regression revealed no confounding influences on any outcome variable. Data were then analyzed using repeated measures analysis of variance. Six weeks of training resulted in significant decreases in submaximal exercise heart rate and systolic blood pressure (SBP), and resting SBP ($p < 0.05$). No significant changes in plasma concentrations of tPA activity (BL = 0.69 ± 0.44 , 3W = 0.94 ± 0.62 , 6W = 0.77 ± 0.49 ng/ml, mean $\pm$ SD, $p = 0.391$) or antigen (BL = 13.1 ± 3.9 , 3W = 12.4 ± 3.7 , 6W = 11.8 ± 3.8 , mean $\pm$ SD, $p = 0.59$) were observed. No change was observed in plasma PAI-1 activity (BL = 17.0 ± 16.8 , 3W = 14.8 ± 22.5 , 6W = 17.9 ± 18.8 IU/ml, mean $\pm$ SD, $p = 0.29$) or antigen (BL = 28.3 ± 15.5 , 3W = 24.2 ± 20.2 , 6W = 22.4 ± 16.1 ng/ml, mean $\pm$ SD, $p = 0.15$). No change in tPA ($p = 0.45$) or PAI-1 ($p = 0.44$) gene expression was observed during six weeks of exercise training.

The six-week cardiac rehabilitation program yielded significant hemodynamic improvements, but did not alter fibrinolytic capacity. Based on the results of the present study and evidence in the literature, it is recommended that traditional cardiac rehabilitation programs that subscribe to ACSM guidelines include at least 12 weeks of regular exercise.

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER 1 - INTRODUCTION.....	1
CHAPTER 2 - REVIEW OF LITERATURE.....	5
Fibrinolysis mechanisms - overview	5
Cardiovascular disease and fibrinolysis.....	7
Exercise training and fibrinolysis	8
Exercise training and cardiovascular disease.....	12
Cardiac rehabilitation and fibrinolysis.....	15
Summary:.....	19
CHAPTER 3 - RESEARCH DESIGN / METHODS	21
Subjects:.....	21
Training:.....	22
Laboratory Measures:	22
Assays:	23
Statistical Analysis:.....	23
CHAPTER 4 - RESULTS.....	26
CHAPTER 5 - DISCUSSION	32
APPENDIX A - CONSENT FORM, MICHIGAN STATE UNIVERSITY	38
APPENDIX B - CONSENT FORM, WILLIAM BEAUMONT HOSPITAL	42
APPENDIX C - HUMAN SUBJECTS APPROVAL, MICHIGAN STATE UNIVERSITY	47
APPENDIX D - HUMAN SUBJECTS APPROVAL, WILLIAM BEAUMONT HOSPITAL.....	49
WORKS CITED	51

LIST OF TABLES

Table 1.	Subject Anthropometric Characteristics.....	28
Table 2.	Resting and Submaximal Exercise Hemodynamics.....	28

LIST OF FIGURES

Figure 1.	Individual values plus means $\pm$ SE for tPA activity and antigen at baseline (BL), after three weeks (3W) and after six weeks (6W) of exercise training.....	29
Figure 2.	Individual values plus means $\pm$ SE for PAI-1 activity and antigen at baseline (BL), after three weeks (3W) and after six weeks (6W) of exercise training.....	30
Figure 3.	tPA and PAI-1 mRNA expression at baseline (BL), after three weeks (3W), and after six weeks (6W) of exercise training. Data are expressed as means $\pm$ SE.....	31

Chapter 1 - INTRODUCTION

Hemostasis is defined as the cessation of bleeding. Following injury, blood loss is stemmed by the interaction of the severed blood vessel, platelets and soluble coagulation factors to form an insoluble fibrin clot. Fibrin clots are the result of a series of enzymatic reactions that ultimately cause the release of thrombin from prothrombin. A fibrin clot, or *thrombus*, may impair blood flow if not eliminated.

Fibrinolysis is the process by which an insoluble fibrin clot is degraded into fibrin dimer proteins, which are quickly cleared from circulation by the liver. *Plasmin*, the active form of the zymogen *plasminogen*, may cause proteolytic digestion of many coagulation proteins and is singularly responsible for fibrin dissolution. Thus, the key to stimulation of fibrinolysis is the conversion of plasminogen to plasmin. The most abundant and rapid plasminogen activator in the blood is *tissue plasminogen activator* (tPA). tPA is synthesized and released from the endothelium, and other tissues such as leukocytes (153) and sympathetic neurons (71, 72). The primary inhibitor of tPA is *plasminogen activator inhibitor-1* (PAI-1), which is produced by endothelial cells, smooth muscle cells, adipocytes, spleen cells, liver cells (4), and leukocytes (6, 16, 152). PAI-1 inhibits tPA by forming an inactive bimolecular complex. Plasma concentrations of tPA and PAI-1 are widely accepted markers of fibrinolytic activity and correlate with fibrinolysis as assessed by euglobulin clot-lysis time.

Coagulation and fibrinolysis play pivotal roles in the development of vascular disease. Vascular injury may be initiated by atherosclerotic plaque, leading to clot formation in an intact arterial wall. Ischemic coronary syndromes such as myocardial infarction (MI), sudden death, and unstable angina share a common pathophysiological

course that includes thrombus formation in or around ruptured coronary plaque (26, 50). Atheromatous lesions contain an abundance of pro-thrombotic elements as well as PAI-1, fibrinogen, fibrin, and fibrin degradation products (112, 147). In addition to the plaque itself, atherogenesis is characterized by chronic inflammation (63, 79), which, in turn, induces a procoagulant state. Furthermore, there is evidence of altered fibrinolytic status among patients with advanced CVD (22). Both PAI-1 and tPA are considered independent risk factors for cardiovascular disease (CVD). More specifically, decreased tPA and increased PAI-1 are associated with CVD (27, 76, 123, 131, 146), coronary artery disease (104, 122), ischemic events (24, 25, 56, 60, 142), stroke (73, 85), and morbidity and mortality (69, 90, 98, 141). Furthermore, impaired fibrinolysis is associated with other CVD risk factors (7, 43, 68, 75, 85, 92, 131). Thus, fibrinolysis is of considerable clinical significance and represents a viable target for therapeutic intervention in CVD.

Enhanced fibrinolysis is one of many cardioprotective adaptations associated with endurance exercise training. Athletes and individuals who report high levels of physical activity exhibit lower PAI-1 activity, lower tPA antigen (which is indicative of less tPA bound with PAI-1) and reduced tPA/PAI-1 complex formation compared to matched sedentary controls (30, 32, 81, 134). Longitudinal data confirm that aerobic training decreases tPA antigen and PAI-1 activity (37, 145).

A clear mechanism for the observed training-related changes in fibrinolysis has not been posited. Changes in plasma concentrations of tPA and PAI-1 may be the result of altered release rates of the stored proteins or a change in hepatic clearance. However, plasma concentrations of tPA and PAI-1 are largely genetically determined. Classic twin

studies have produced heritability estimates ranging from 42%-71% (17, 67) for PAI-1 and 30-60% for tPA (29, 48, 133). Expression of the tPA and PAI-1 genes in response to exercise training has not been studied, but mRNA levels have been reported to be modulated by elements that are known to be influenced by exercise. Experimental models of augmented aerobic metabolism results in increased tPA mRNA and reduced PAI-1 mRNA (33). Additionally, TGF-beta, a cytokine known to rise in response to acute physical exertion, upregulates PAI-1 (154). This response is attenuated by vascular endothelial growth factor (154), which increases in response to short-term exercise training (52). Thus, gene expression may be a significant contributor to any training-related adaptations in fibrinolysis.

Exercise training is a hallmark of traditional cardiac rehabilitation. Aerobic exercise elicits positive alterations at molecular, systemic, and whole body levels. Recent evidence suggests that individuals with the poorest baseline fibrinolytic capacity may realize the greatest improvement through regular exercise (77). Thus, individuals with CVD, who typically exhibit blunted fibrinolysis, may be more likely to experience hemostatic improvements through training compared to healthy men and women.

Cardiac rehabilitation programs typically include three days per week of supervised exercise lasting 20-60 minutes per session. Programs usually continue for 6-12 weeks. Intensity of exercise is titrated to optimize training benefits while minimizing patient risk. To date, six longitudinal studies have been published exploring the influence of cardiac rehabilitation on fibrinolytic parameters. Detailed in the following chapter, most studies utilized training regimens that are atypical of traditional cardiac rehabilitation programs. Many investigators have trained patients for 24-26 weeks (40,

77, 116, 121), and others utilized training intensities that were either far greater (138) or much less demanding (121) than recommended by the American College of Sports Medicine (3). Since studies of healthy individuals indicate that fibrinolytic improvements are highly dependent upon the specific exercise prescription (12, 37), methodological differences make it unclear if a traditional exercise-based cardiac rehabilitation program that adheres to international guidelines will elicit fibrinolytic improvements. This question is particularly important as insurance companies are now reimbursing hospitals and clinics for fewer rehabilitation sessions than in previous years, often compensating for no more than 18 sessions in six weeks (personal communication, Adam deJong, 7/26/2005). Understanding the time course of fibrinolytic adaptations during exercise training may provide insight as to the effectiveness of traditional cardiac rehabilitation programs.

The purpose of the present study was two-fold: (1) to assess fibrinolytic adaptations after three and six weeks of participation in an exercise-based cardiac rehabilitation program; and (2) to evaluate changes in expression of the tPA and PAI-1 genes after three and six weeks of participation in an exercise-based cardiac rehabilitation program. It was hypothesized that that no modifications would be observed in any measured variable after three weeks of cardiac rehabilitation, and that significant changes would be observed in plasma concentrations and gene expression of tPA and PAI-1 after six weeks of training.

Chapter 2 - REVIEW OF LITERATURE

Fibrinolysis mechanisms - overview

Hemostasis is defined as the cessation of bleeding. Occurring in distinct phases, hemostasis is initiated with platelets interacting with injured blood vessels and other platelets. This phase, known as *primary hemostasis*, ends with the formation of a clump of platelets. This *primary hemostatic plug* serves to temporarily arrest bleeding, but is fragile and easily dislodged from the vessel wall. *Secondary hemostasis* involves the interaction of soluble plasma proteins, or coagulation factors, in a series of complex enzymatic reactions that conclude with the thrombin catalyzed conversion of fibrinogen to insoluble fibrin. Deposition of fibrin strands on the platelet plug stabilizes the clot and allows healing to occur without further loss of blood.

If a clot remained intact after the damaged tissue healed, the vascular bed might become obstructed. Fibrinolysis, sometimes referred to as *tertiary hemostasis*, regulates the process of dissolving a fibrin clot. Fibrinolysis is activated in response to the initiation of the coagulation cascade. Activation of the fibrinolytic system produces plasmin, a proteolytic enzyme that is the active form of the zymogen plasminogen, which is able to digest fibrin or fibrinogen. The key factors in fibrinolysis are: (1) plasminogen; (2) plasmin; (3) plasminogen activators; and (4) plasminogen activator inhibitors. The latter two elements are the focal points of the present investigation.

Tissue plasminogen activator (tPA), a serine protease, is a rapid activator of plasminogen. Derived primarily from the endothelium, tPA can be found in other tissues such as leukocytes (153), sympathetic neurons (71, 72), the heart, kidneys and other organs (89). tPA has an affinity for fibrin with which it forms a bimolecular complex.

The catalytic efficiency of tPA for the activation of plasminogen is increased 1,000-fold in the presence of fibrin. Non-bound tPA has a low affinity for plasminogen and is thus not efficient in producing plasmin. Synthesis and release of tPA are stimulated by coagulation factor Xa, thrombin, bradykinin, and protein C. Furthermore, plasma concentrations of tPA may be elevated in response to hypotensive shock, pharmacologic stimulators, venous stasis, and physical exertion. tPA is inhibited by α 2-macroglobulin, α 1-antitrypsin, antithrombin III, α 2-antiplasmin, and a family of plasminogen activator inhibitors.

Plasminogen activator inhibitor-1 (PAI-1), part of the serine protease inhibitor (serpin) superfamily, provides rapid, specific inhibition of tPA and is the primary inhibitor of tPA in blood. PAI-1 can occur in an active inhibitory form, which inhibits tPA by forming a 1:1 complex with it. This form is unstable and will spontaneously convert to an inactive latent form that does not react with tPA (64). In humans, PAI-1 is produced by endothelial cells, smooth muscle cells, adipocytes, spleen cells, liver cells (4), and blood leukocytes (6, 16, 152). PAI-1 gene expression is induced by endotoxin, inflammatory cytokines (57, 127), lipoprotein (57), angiotensin II (42), transforming growth factor- β (TGF- β) (124), tumor necrosis factor- α (TNF- α) (58) and hypoxia (143). The main reservoir of PAI-1 in the blood exists in platelets (100-200 ng/ml in non-pathological conditions) but only 10% of this PAI-1 pool occurs in the active conformation (13, 31, 80). Plasma PAI-1 represents a much smaller source (5-20ng/ml) but is the most active pool in the blood (89). Blood concentrations of PAI-1 increase exponentially when platelets are activated due to injury or pathology.

Cardiovascular disease and fibrinolysis

Atherosclerosis is a complex, multifactorial process. However, ischemic coronary syndromes such as myocardial infarction (MI), sudden death, and unstable angina share common a pathophysiological course that includes thrombus formation in or around ruptured coronary plaque (26, 50). Autopsy studies that observed ruptured or cracked coronary atherosclerotic lesions in individuals without evidence of myocardial infarction (93) suggest plaque rupture must occur in combination with prothrombotic conditions in order for an ischemic event to occur. In vitro studies have shown that fibrin complexes formed in plasma from patients with a previous MI have tighter, more substantial network structures than gels formed in plasma from healthy subjects (10).

There is substantial evidence that hypercoagulability and depressed fibrinolytic capacity promote the formation and maintenance of thromboses both systemically and locally at the exposed surface of damaged plaque (86). Prospective studies have demonstrated that plasma concentrations of prothrombotic markers are predictors of subsequent cardiovascular events in healthy subjects (87, 91, 115, 118, 132), individuals with cardiovascular risk factors (74, 115) and evident coronary disease (142).

PAI-1 and tPA, the two critical fibrinolytic proteins that are the focus of this study, are considered independent risk factors for cardiovascular disease (CVD). Decreased tPA and increased PAI-1 are associated with CVD (27, 76, 123, 131, 146), coronary artery disease (104, 122), ischemic events (24, 25, 56, 60, 142), stroke (73, 85), morbidity and mortality (69, 90, 98, 141) in both men and women. Increased PAI-1 levels have been found in atherosclerotic lesions within the vessel wall (117, 128) and it is now understood that PAI-1 contributes to atherosclerotic progression in addition to its

role in fibrinolysis, including promotion of neointimal formation after vascular injury (106, 108). The magnitude of impairment in fibrinolytic potential may correspond to the extent of CVD, as tPA activity is higher in PAD patients with mild claudication versus patients with severe claudication (76). Furthermore, impaired fibrinolysis is independently associated with other CVD risk factors, including body composition (43, 75, 137), hyperlipidemia (7, 68, 85, 92, 131), diabetes, BMI, and low-density lipoprotein concentration (75). These data indicate a potential mechanistic link to the risk factors for CVD as well as the disease itself.

Exercise training and fibrinolysis

Exercise training elicits numerous physiological adaptations that relate to improved cardiovascular health. Among these cardioprotective adaptations is enhanced fibrinolytic capacity. Resting tPA and PAI-1 are correlated with maximal oxygen consumption (32), the gold standard indicator of aerobic fitness, and cross-sectional data indicate individuals who are regularly active demonstrate greater fibrinolytic potential than sedentary individuals. Speiser et al. (134) observed lower resting PAI-1 activity in younger athletes compared to sedentary controls. Lower tPA antigen, indicative of less tPA bound with PAI-1, has been observed in athletes compared to matched sedentary controls (81, 134). Furthermore, Depaz, et al. (30) observed reduced tPA/PAI-1 complex formation in trained versus untrained individuals. These cross-sectional observations have been supported by longitudinal data. Van den burg and associates (145) documented decreased tPA antigen, PAI-1 activity and PAI-1 antigen in a group of young healthy males that participated in twelve weeks of aerobic exercise training.

Furthermore, these investigators observed an increase in the tPA activity/tPA antigen ratio.

Exercise training may also influence the fibrinolytic response to acute bouts of exercise. Ferguson et al. (44) reported significantly higher elevations in global fibrinolysis, assessed by clot-lysis time, in trained versus untrained individuals following a maximal exercise test. Speiser et al. (134) observed higher elevations in tPA activity following maximal cycle ergometry in trained compared to untrained subjects. However, it was suggested that this was due to lower resting PAI-1 in the trained subjects, as tPA release was similar for both groups during exercise. Szymanski and Pate (139) also observed that active men experienced greater increases in tPA activity than sedentary men during moderate intensity exercise. Similar to the observations of Speiser's group, the inactive subjects also had higher resting PAI-1 activity. Furthermore, van den Burg, et al. (145) reported the relative increase in tPA from a maximal exercise test was not affected by training status. However, these authors did observe a significantly elevated tPA activity to antigen ratio post-exercise in the aerobically trained group. The relative change in this ratio from resting values was not significant between the trained and sedentary subjects. These data suggest baseline fibrinolytic profile may be the biggest influence of post-exercise values rather than differences in the magnitude of response.

Fibrinolytic adaptations to regular exercise may depend on training methods. Yarnell, et al. (155) reported that neither leisure-time nor work-related physical activity is associated with resting tPA or PAI-1. Thus, physical activity related to leisure or work may not be of sufficient intensity to modulate fibrinolytic activity. De Geus and associates (28) randomized sedentary men into groups that trained for either four or eight

months. A trend for decreased PAI-1 activity was observed, but these changes failed to reach the assigned level of significance due to large variation within groups. The training regimen included self-selected exercise frequency. Furthermore, the duration of the exercise sessions in the De Geus study differed among subjects by several hours per week, suggesting the variability of the results may have been impacted by inconsistent training regimens. Length of the training phase is also likely to influence magnitude of fibrinolytic adaptations. Bodary, et al. (12) demonstrated that very short-term training had no effect on resting measures of fibrinolysis. Sixteen apparently healthy men and women engaged in 50 minutes of moderate intensity treadmill exercise for 10 consecutive days. No change in resting tPA or PAI-1 was observed.

The fibrinolytic improvements related to training may also be age-dependent. Aging is associated with a thrombophilic state that may contribute to cardiovascular complications (2, 32), and baseline fibrinolytic profile appears to exert a strong influence on training adaptations (134, 139). Stratton et al. (136) observed significant changes in resting fibrinolytic profile with training in older (60-82 years), but not younger (24-30 years) subjects. Similar training-related modifications are described elsewhere (14) while other investigators failed to demonstrate such changes (144). Methodological differences specifically related to the exercise regimen (*e.g.* intensity, duration, frequency, and weeks of training) in these studies make it difficult to draw decisive conclusions regarding the effect of age on hemostatic training adaptations. There is clearly a need for further research to elucidate the evaluate relationships between age, exercise intensity, duration, and modality and fibrinolytic adaptations to training.

The potential molecular mechanisms of fibrinolytic adaptations to regular exercise are poorly understood. Catecholamines are associated with release of tPA from endothelial cells (18, 113), and β -adrenergic blockade attenuates the normal fibrinolytic response to acute exercise (39). However, it is unlikely that catecholamines influence the fibrinolytic response, because tPA release occurs before an increase in epinephrine during and acute bout of exercise (38). Training-induced reductions in plasma catecholamines may be related to enhanced fibrinolytic capacity, but this relationship is unclear.

Increased blood flow due to repetitive bouts of physical exertion may exert multiple effects on the fibrinolytic system (110). Vascular shear stress may cause damage to the arterial intimal layer, particularly in regions disturbed by atherosclerotic plaque. Platelets and coagulation factors, notably thrombin, become activated at the site of injury, leading to increased release of both tPA and PAI-1 (110, 120). Elevated blood flow and vascular shear stress also enhance the basal formation of nitric oxide (NO) (126). NO inhibits platelet adhesion and aggregation and facilitates the dissolution of small platelet granules. Furthermore, NO regulates the release of both tPA and PAI-1 (126). It has been suggested that atherosclerosis-related impairment in NO synthesis and release may be mediated by the renin-angiotensin system, which is also involved in the regulation of fibrinolysis. In this system, angiotensinogen is converted to angiotensin I by the renal protease renin. Cleavage of angiotensin I by the angiotensin converting enzyme (ACE) yields angiotensin II, which is a potent vasoconstrictor and primary tool in the regulation of blood pressure. ACE may either promote increased levels of angiotensin I or bradykinin, which in turn induce the expression of PAI-1 and t-PA, respectively (15, 114). Studies of in vivo infusion of angiotensin II demonstrate a direct effect of

angiotensin on fibrinolysis (114). Training effects on the renin-angiotensin system may provide a link between regular exercise and fibrinolysis, but this remains speculative.

Recent findings related to atherosclerosis indicate a key role of inflammation in the disease process, and coronary disease is often characterized by high levels of circulating pro-inflammatory cytokines. Two such cytokines, TNF- α and IL-1 increase PAI-1 synthesis and/or release from endothelial cells and also decrease tPA synthesis (82). Regular exercise suppresses the activity of pro-inflammatory cytokines (107), though the extent to which this translates to fibrinolytic adaptations is unclear.

Exercise training and cardiovascular disease

Cardiac rehabilitation is a coordinated collection of interventions designed to improve physical, psychological, and social conditions so that patients with cardiovascular disease may preserve or resume optimal functioning and slow or reverse the progression of disease. This complex intervention may involve any of a variety of therapies including risk factor education, psycho-social counseling, drug therapy, nutritional and smoking cessation input. Nonetheless, the central element of cardiac rehabilitation is exercise therapy (45, 135, 149). A large body of evidence overwhelmingly suggests exercise-based interventions produce significant overall benefits compared to usual medical care, including increased physical performance, improved angina threshold and myocardial perfusion (36, 129). Various meta-analyses of the effects of exercise training among patients with CVD demonstrate numerous improvements in modifiable risk factors such as hypertension, lipid profile, smoking habit, body composition, and glucose tolerance, as well as improvements in health-related

quality of life as assessed by a range of outcome measures (140). Moreover, estimated reductions in total and cardiac mortality range from 20-32% (11, 100, 101, 140).

A primary goal of exercise training of an individual with cardiovascular disease is improved cardiac health, which necessitates improved myocardial perfusion. Exercise training attenuates ST-segment depression during exercise (36) and decreases perfusion defects on thallium scanning (129), indicating an increase in myocardial perfusion. Regional myocardial hypoperfusion may result from vascular stenosis, microvascular dysfunction (105), and microrheology (53). Each of these basic pathogenic components may be affected by exercise training, and several mechanisms may contribute to the exercise training-related improvements in cardiovascular health.

First, exercise training may arrest the progression of atherosclerosis, or even result in a net regression of coronary stenosis. A study of lifestyle changes including stress management, dietary modifications, and 3 hours of exercise training per week resulted in a significant regression of coronary stenoses compared to a non-exercising control group (102). This was associated with a 2.5-fold risk reduction in cardiac events after a 5-yr follow-up period (103). The Stanford Coronary Risk Intervention Project (62) did not observe atherosclerotic plaque regression in a group of patients undergoing exercise therapy, but the rate of change in minimal diameter per patient in this risk-reduction group was 47% less than for a non-exercising usual-care group. Similarly, the Heidelberg Regression Study used exercise and a low-fat diet to halt the progression of atherosclerosis, evidenced by an unchanged luminal diameter while the non-exercising control group experienced decreased luminal diameter (96).

Second, exercise training may improve myocardial circulation through the creation of collateral blood vessels. Animal studies suggest that long-term, intensive training increases coronary collateralization (20, 94, 125). Human data are more equivocal. Belardinelli observed significant increases in collateralization (9) while Niebauer did not (95). The lack of agreement between these studies is perplexing considering the exercise regimens employed were similar in intensity and frequency, the subject pools were comparable, and Niebauer's study trained its patients for a longer period of time than the Belardinelli project (12 months versus 8 weeks). One explanation for the negative findings reported by Niebauer is that the protracted training regimen resulted in net regression of atherosclerotic plaque, thus reducing the need for additional blood supply distal to the stenosis. It has also been suggested that angiography, the typical method for assessment of collateralization in humans, may not be sensitive enough to detect the formation of blood vessels smaller than 100 μm , especially in patients without previous MI (53, 95).

Finally, regular exercise may improve myocardial blood flow through enhanced dilation of microvasculature. Atherosclerosis is associated with progressive impairment of coronary endothelial function, which decreases nitric oxide (NO) release from endothelial cells. Since endothelium-derived nitric oxide is thought to be necessary to maintain an adequate vascular response to increased blood-flow demands during exertion, correction of endothelial dysfunction has become a rehabilitation goal of paramount importance. A recent study of 54 men and women with a recent MI documented significant improvement in endothelium-dependent vasodilation following three months of aerobic training, theorized to involve a chronic increase in NO production (148). NO

concentrations are influenced by various factors, each of which is susceptible to modifications through exercise.

L-arginine is the precursor to NO, and must be present at the active site of endothelial NO synthase (eNOS) for NO production. During exercise, augmented vascular shear stress increases the velocity of the endothelial high affinity/low-capacity transport system for L-arginine (109), which ensures substrate availability for eNOS. Furthermore, eNOS activity and expression are both enhanced in response to elevated shear stress both in vitro (23, 34, 49, 99) and in vivo (130, 151).

Reactive oxygen species (ROS) accelerate the degradation of NO, and are associated with atherosclerosis and endothelial dysfunction (111). Exercise training increases total oxygen uptake as well as production of ROS (70). However, regular exercise also improves endothelial function (55, 59). These seemingly contradictory facts may be explained by the fact that exercise training increases both eNOS and extracellular superoxide dismutase, a potent antioxidant. Through this mechanism, exercise training may attenuate the deleterious effects of ROS on NO.

Exercise training further affects endothelium-mediated vasomotion of coronary arteries by attenuating the paradoxical vasoconstrictive response to acetylcholine in patients with CVD (55, 59), thus improving peak flow velocity in larger conduit arteries, and increasing sensitivity and responsiveness to adenosine in smaller resistance vessels (59).

Cardiac rehabilitation and fibrinolysis

As described above, there is evidence that regular aerobic exercise elicits enhanced fibrinolytic capacity in healthy individuals. Exercise training is also effective

in improving rheological variables among individuals with confirmed diagnosis of or risk factors for CVD. Although post-menopausal women typically have an impaired fibrinolytic profile, DeSouza et al. (32) observed that trained post-menopausal women exhibit fibrinolytic profiles similar to pre-menopausal active women. Additionally, Lindahl et al. (84) observed significant decreases in tPA antigen and PAI-1 activity in patients with non-insulin dependent diabetes mellitus (NIDDM) in response to chronic aerobic exercise training combined with a nutritional intervention. Gardner (51) reported that patients with peripheral arterial disease (PAD) who expended fewer than 175 calories through physical activity were particularly susceptible to experiencing a prothrombotic state. In this study of 106 PAD patients subjects in the low physical activity group, as determined by monitoring with an accelerometer, exhibited lower tPA activity and higher PAI-1 activity than the moderate and high physical activity groups ($p < 0.05$). It has been hypothesized that training-related fibrinolytic improvements in a CVD population may be related to changes in body mass (144), suggesting the link between exercise and fibrinolysis may be circuitous with body composition mediating the association. However, Lindahl et al. (83) observed that a 5-6 kg reduction in body weight in obese individuals failed to have a significant effect on PAI-1, supporting a more direct relationship between exercise and hemostasis.

Similar to studies involving apparently healthy subjects, fibrinolytic improvements related to exercise training of a CVD population may depend on duration and/or intensity of training. Estelles, et al. (40) studied a group of post-MI patients, that entered an exercise training program and another group of patients that did not. The non-exercise control group experienced a significant decline in fibrinolytic potential over 6

months, as evidenced by decreased tPA activity and increased PAI-1 activity. Fibrinolytic capacity did not specifically improve in response to training (i.e. nonsignificant increase in tPA activity) but the exercise group did not experience this decrement in fibrinolysis.

Six months of aerobic training produced significant fibrinolytic changes in a recent study by Killewich, et al. (77). Twenty-one men with intermittent claudication underwent 6 months of treadmill exercise training and were compared to a group with intermittent claudication who did not train. Significant increases in tPA activity and decreases in PAI-1 activity were observed in the exercise group, while no fibrinolytic changes were observed in the non-exercising group. It was noted that patients with the highest baseline PAI-1 experienced the greatest decline, suggesting that those with the greatest fibrinolytic impairment may benefit most from regular exercise.

Paramo, et al. (104) studied 30 survivors of a first MI and 30 healthy controls who underwent 9 months of cardiac rehabilitation training. Patients had higher tPA antigen and PAI-1 activity and antigen at baseline than healthy control subjects. Three months of training elicited a significant decrease in PAI-1 activity ($p < 0.01$), and 9 months of exercise produced a decrease in PAI-1 antigen ($p < 0.05$).

Not all published studies showed a fibrinolytic benefit of participating in at least 3 months of exercise-based cardiac rehabilitation. Rigla, et al. (116) evaluated 27 diabetic patients and 11 healthy controls before and after a 3 month training program. The primary finding of this study was a decrease in thrombomodulin after training, indicating improved endothelial integrity. Regarding fibrinolysis, type I diabetics experienced a significant increase in PAI-1 activity ($p < 0.05$). Type II diabetics demonstrated a similar,

though not statistically significant, response to training. No change was observed in tPA activity. Since healthy controls exhibited similar results, the authors disregard this unusual finding as biologically unimportant. It is not clear from the description of the methods used why such a finding was observed. One explanation is that 10 of the enrolled subjects were current smokers, which has substantial effects on fibrinolytic parameters.

A more recent investigation (121) randomized 29 male patients with congestive heart failure (CHF) to training or control groups. The training program included 26 weeks of combined strength and endurance exercise 4 times per week, 2 supervised and 2 “at home,” unsupervised, sessions. Home training sessions lasted 11 minutes and included 4 exercises for relaxation, flexibility and strength, and one endurance exercise. Supervised exercise sessions included these same 5 “home” exercises plus an interval workout on a cycle ergometer designed for CHF patients. The cycle ergometer workout alternated 30-second work phases with 60-second recovery phases a total of 10 times. Work phases were at an intensity associated with 50% of the individual’s maximum exercise capacity. Training improved exercise tolerance but none of the endothelial-derived variables under examination, including tPA and PAI-1. It is probable that the exercise stimulus, which included approximately 5-10 minutes of moderate-intensity aerobic activity, may not have been adequate to elicit the molecular adaptations necessary to produce significant hemostatic improvements.

Only one published investigation of the fibrinolytic adaptations during cardiac rehabilitation used an exercise regimen of less than 3 months. Suzuki and associates (138) studied 56 post-MI patients before and after one month of exercise training.

Compared to a non-exercising control group of MI patients, coagulation activity was suppressed following training as evidenced by numerous markers including FVIII activity, vWF antigen, VII activity, and thrombin-antithrombin. In a subset of 20 patients who underwent physical training, tPA antigen and PAI-1 activity decreased ($p < 0.05$). This was an atypical cardiac rehabilitation program in that it involved two 40-minute sessions per day of treadmill walking and cycle ergometry for six days per week. The extreme duration and frequency of exercise explains the rapid fibrinolytic improvements that were not apparent in other studies until 3-9 months of training.

Historically, phase II cardiac rehabilitation programs included 3 days per week of supervised exercise lasting 20-60 minutes for 3-6 months. In recent years, insurance companies reduced the number of exercise sessions for which they would reimburse hospitals and clinics. Today, most programs are 6-12 weeks in duration. The literature suggests three months of training is sufficient to elicit positive fibrinolytic changes, but it is unclear if fewer than 12 weeks of traditional cardiac rehabilitation is beneficial with regard to hemostasis.

Summary:

In summary, fibrinolysis is clinically significant due to its regulation of clot dissolution, particularly in regard to thrombosis in or around atherosclerotic plaque. Two of the primary elements of the fibrinolytic process, tPA and PAI-1, are strong predictors of many outcomes related to CVD. Low plasma concentrations of tPA and high concentrations of PAI-1 are considered independent risk factors for CVD. Exercise training produces numerous cardiovascular benefits, which may include enhanced fibrinolytic capacity and, thus, reduced risk of acute cardiovascular events. Individuals

with CVD often exhibit reduced fibrinolytic capability and may experience profound hemostatic improvements following a regular training regimen. Exercise training is the cornerstone of modern cardiac rehabilitation, and previous studies have demonstrated that fibrinolytic improvements may be realized through typical rehabilitation programs lasting three to nine months. Exercise regimens of more than three months that adhered to exercise guidelines provided by the American College of Sports Medicine (3) were effective in producing fibrinolytic improvements (40, 77), and one study demonstrated that as few as three months of training improved fibrinolytic potential (104). Only one study examined fibrinolysis in patients with CVD after fewer than three months of training, but the exercise regimen involved very high-intensity and high-frequency activity that would not be part of a traditional cardiac rehabilitation program (138). The present study is the first to assess fibrinolytic adaptations during a cardiac rehabilitation program of fewer than 12 weeks that utilized an exercise prescription as recommended by the American College of Sports Medicine.

Chapter 3 - RESEARCH DESIGN / METHODS

All methods described herein have been approved by Michigan State University's Committee on Research Involving Human Subjects and William Beaumont Hospital's Human Investigation Committee. Each participant had the study explained in full, and provided written informed consent prior to enrollment in the study.

Subjects:

Individuals referred for Phase II (monitored) cardiac rehabilitation at William Beaumont Hospital in Royal Oak, MI were recruited to participate in this study. This cardiac rehabilitation program enrolls approximately 300 patients in its Phase II program annually, and has been shown to produce numerous beneficial physiological adaptations through six to eight weeks of physical training (47). To determine effect sizes for training adaptations in fibrinolysis, data were collected from patients with coronary artery disease preparing to begin Phase II cardiac rehabilitation ($n = 4$) and those who recently completed six weeks of cardiac rehabilitation ($n = 3$). It was estimated that 10 subjects would be sufficient to detect statistically significant training-induced increases in plasma tPA and decreases in PAI-1 at an alpha level of $P < 0.05$ with power of 0.8. Tobacco users and those with diagnosed liver disease were excluded from the study due to the possible effects on the variables under examination. Subjects were encouraged not to modify their course of pharmacological treatments during this study unless otherwise directed by their personal physicians. Individuals who discontinued or began taking a medication likely to influence fibrinolytic variables (i.e. fibrates, statins, β -blockers, ACE inhibitors) during participation in the study were excluded from data analysis. Twenty-two volunteers qualified and agreed to participate in the study. Three subjects withdrew from the study

due to prolonged illness, four provided unusable baseline blood samples, and one was excluded for discontinuing a significant medication. Thus, 14 patients (12 male, 2 female) completed the study and were included in final analyses.

Training:

Subjects participated in 18 sessions (3 sessions/week) of structured exercise therapy as part of the Phase II cardiac rehab program at Beaumont Hospital. Supervised exercise sessions consisting of bouts of treadmill walking, cycling, or combined arm/leg ergometry were conducted three days per week for approximately one hour per session. Exercise intensity corresponded to 50-70% of heart rate reserve. Subjects were monitored continuously during exercise via ECG by hospital personnel. Data from subjects who participated in fewer than 80% of the scheduled exercise sessions or who required more than eight weeks to complete 18 sessions were not included in the final analyses.

Laboratory Measures:

Each participant's height, weight, hip circumference, waist circumference, resting systolic and diastolic blood pressure was measured at program entry and exit by Beaumont Hospital personnel. To determine changes in submaximal exercise responses as a result of the rehabilitation program, 13 participants completed constant-load, treadmill exercise tests during their 2nd and 18th exercise sessions. Intensity for these tests was set at the workload prescribed at the beginning of the phase II program. Steady-state heart rate and blood pressure were collected during these exercise tests.

Blood samples were taken at three time points: prior to enrollment in Phase II cardiac rehabilitation, after nine sessions (approximately three weeks), and after 18

sessions (approximately six weeks). Following a 12 hour overnight fast, subjects reported to the Beaumont rehabilitation center where blood was drawn from an antecubital vein. All blood samples were acquired between 6 and 10 AM to control for diurnal variations in fibrinolysis (5, 66). Blood samples were collected in tubes containing 1:10 0.45M sodium citrate, pH 4.3 (Biopool Stabilyte™) and platelet-poor plasma was isolated by centrifugation at 11,200 g for 20 min at 4°C. Plasma aliquots were frozen and stored at -80°C until assayed. Blood to be used for assessment of mRNA was collected in commercially available tubes containing a lysing and a stabilizing buffer (Qiagen, Inc., Valencia CA) and stored at -20°C until assayed.

Assays:

Plasma concentrations of tPA and PAI-1 were measured using enzyme-linked immunosorbancy assays (ELISA). All blood assays were performed in Michigan State University's Fibrinolysis Research and Genetics Laboratory in the Department of Kinesiology. Samples were measured in duplicate, and blood assays were batched so that all data points for a given subject were run using the same kit. Intra-assay coefficients of variation in the Fibrinolysis Research and Genetics Laboratory are consistently < 5% for these measurements when this protocol is used (personal communication, Christopher Womack, Ph.D.).

RNA was isolated from whole blood using commercially available kits (Qiagen, Inc., Valencia CA) and amplified using real time PCR. As endogenous control to correct for potential variation in RNA loading and quantification, RNA Pol II was used. RNA was treated with DNA-Free (Ambion part no: 1906), according to manufacturer's protocol, to remove any genomic DNA contamination. 0.5ug of RNA was converted into

cDNA using Taqman Reverse Transcription reagents (Applied Biosystems no. N808-0234), according to ABI directions. Relative levels of expression for PAI-1, tPA and RNA Pol II were determined using Taqman Gene Expression Assays for RNA Pol II (no.Hs00172187_m1), tPA (no.Hs00263492_m1), and PAI-1(no. Hs00167155_m1) and Taqman mastermix (ABI no.4304437) according to manufacturer's standard protocol (10min 95C initial denaturation, followed by 40 cycles of 95C 15s and 60C 60s). Levels of PAI-1 and tPA expression were normalized to RNA pol II, and fold changes of gene expression are relative to baseline. Gene expression assays were done in Michigan State University's Genomics Technology Support Facility.

Statistical Analysis:

Statistical calculations included means, SD, and SE. Student's *t* test for paired samples was used to assess changes from baseline to program conclusion for the following variables: weight, waist circumference, hip circumference, waist/hip ratio, body mass index (BMI), resting blood pressure, heart rate, submaximal exercise heart rate and blood pressure. All paired comparisons were two-tailed. Linear regression was used to assess the relationship between the outcome variables (plasma concentrations of tPA activity, tPA antigen, PAI-1 activity, PAI-1 antigen, and tPA and PAI-1 RNA) and any potential confounders, including comorbid diagnoses, medication use, waist:hip ratio, body mass index (BMI), weight change, month of enrollment in the program, and elapsed time since coronary event. When no confounding influences were observed, change in plasma concentrations and gene expression of tPA and PAI-1 from baseline (BL) to three weeks (3W) and six weeks of training (6W) were assessed using repeated measures ANOVA. Tukey's post-hoc test was used to elucidate the differences in the event

statistical significance was observed. Statistical significance for all analyses was set at $\alpha = 0.05$. $P \leq 0.10$ was considered a nonsignificant trend. Unless otherwise stated, all values are displayed as means $\pm$ SD.

Chapter 4 - RESULTS

Subject anthropometric characteristics are displayed in Table 1. Twelve males and two females, aged 67.4 ± 10.5 yrs, completed the study. Time elapsed from the most recent cardiac event or surgery to enrollment in the cardiac rehabilitation program was 77.7 ± 14.5 days. No significant changes in weight, body mass index (BMI), waist circumference, hip circumference or waist:hip ratio were observed following the six-week training regimen ($P > 0.05$). As expected, baseline values for plasma tPA and PAI-1 indicated a blunted fibrinolytic profile. Unpublished data from our laboratory show that comparably aged, sedentary healthy individuals exhibit higher tPA activity (0.92 ± 0.35 vs. 0.65 ± 0.43 IU/ml), lower tPA antigen (5.8 ± 1.8 vs. 13.1 ± 3.9 ng/ml) and lower PAI-1 activity (8.1 ± 12.1 vs. 17.0 ± 16.8 IU/ml) than participants in the present study.

Resting and submaximal exercise HR, SBP and DBP are displayed in Table 2. Six weeks of participation in the cardiac rehabilitation program produced significant decreases in resting HR, as well as resting and exercise SBP ($P < 0.05$). A non-significant trend was observed for decreased resting DBP ($P = 0.075$) and increased resting HR from baseline to 6 weeks.

Linear regression was used to assess potential confounding influences on the outcome variables in question. Prevalence of comorbid diagnoses that may influence fibrinolytic parameters are as follows: 21% diabetes mellitus, 67% hypertension, and 56% hypercholesterolemia. None of these conditions were predictive of the change in tPA activity, tPA antigen, PAI-1 activity or PAI-1 antigen ($p > 0.05$) during the six weeks of training. Medications such as ACE inhibitors, anticoagulants, aspirin, nitrates, platelet inhibitors, calcium channel blockers, and diuretics were likewise unrelated to tPA

and PAI-1 changes ($p>0.05$). All participants were taking beta-blockers and statins, so these classes of medication were not included in regression analyses. Waist:hip ratio and weight change during the six-week cardiac rehabilitation program also demonstrated no relationship to changes in tPA and PAI-1 ($p>0.05$).

Repeated measures ANOVA showed no significant changes in tPA or PAI-1 following six weeks of training. Plasma concentrations of tPA activity (BL= 0.69 ± 0.44 , 3W= 0.94 ± 0.62 , 6W= 0.77 ± 0.49 ng/ml, mean $\pm$ SD, $p=0.391$) and antigen (BL= 13.1 ± 3.9 , 3W= 12.4 ± 3.7 , 6W= 11.8 ± 3.8 , mean $\pm$ SD, $p=0.59$) are shown in figure 1. Figure 2 illustrates the changes in PAI-1 activity (BL= 17.0 ± 16.8 , 3W= 14.8 ± 22.5 , 6W= 17.9 ± 18.8 IU/ml, mean $\pm$ SD, $p=0.29$) and antigen BL= 28.3 ± 15.5 , 3W= 24.2 ± 20.2 , 6W= 22.4 ± 16.1 ng/ml, mean $\pm$ SD, $p=0.15$).

Gene expression responses are displayed in Figure 3. RNA data were normalized to the housekeeping gene RNA Pol II and displayed as fold changes relative to baseline. Repeated measures ANOVA showed no changes in tPA ($p=0.45$) or PAI-1 ($p=0.44$) gene expression during six weeks of exercise training.

Table 1: Subject Anthropometric Characteristics (N=14)

	Baseline	6 Weeks
Height (cm)	174.9 ± 7.0	-
Weight (kg)	92.2 ± 14.7	92.4 ± 13.1
Waist circumference (cm)	106.5 ± 12.4	102.9 ± 12.4
Hip circumference (cm)	110.7 ± 12.0	110.0 ± 11.3
Waist/Hip Ratio	0.96 ± 0.07	0.94 ± 0.10
BMI	30.3 ± 5.2	30.3 ± 5.3

Table 2: Resting and Submaximal Exercise Hemodynamics (N=13)

	Baseline	6 Weeks
Resting HR (bpm)	61.4 ± 8.8	68.7 ± 12.2 †
Resting SBP (mmHg)	129.7 ± 20.7	120.0 ± 17.9 *
Resting DBP (mmHg)	73.8 ± 10.5	70.5 ± 7.4 †
Submaximal HR (bpm)	94.1 ± 16.8	87.9 ± 17.0 *
Submaximal SBP (mmHg)	137.5 ± 16.2	126.2 ± 18.5 *
Submaximal DBP (mmHg)	67.8 ± 13.0	66.5 ± 9.7

* P<0.05 compared to baseline

† P≤0.10 compared to baseline

Figure 1.

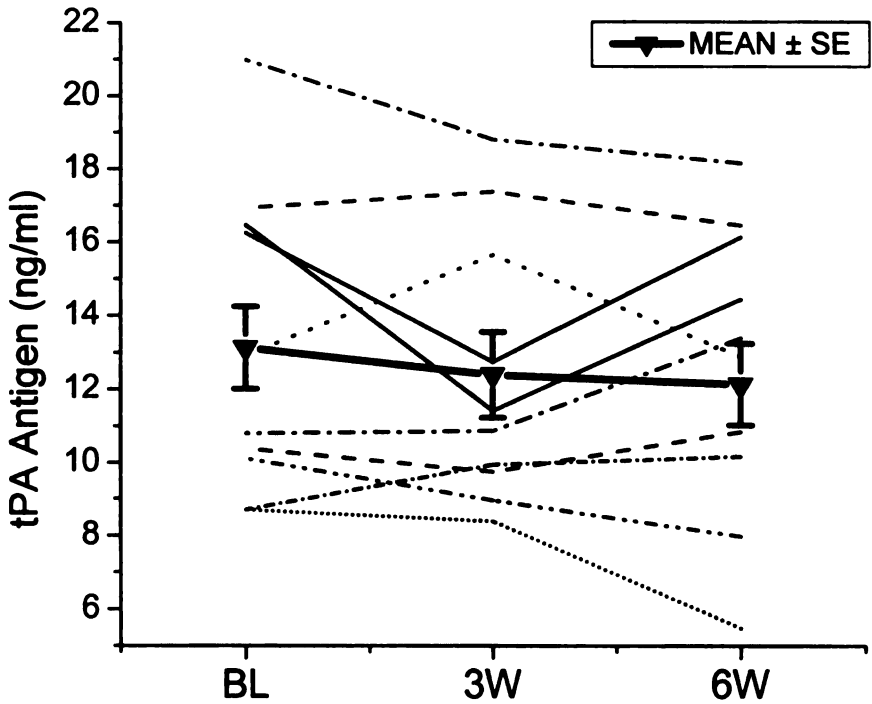
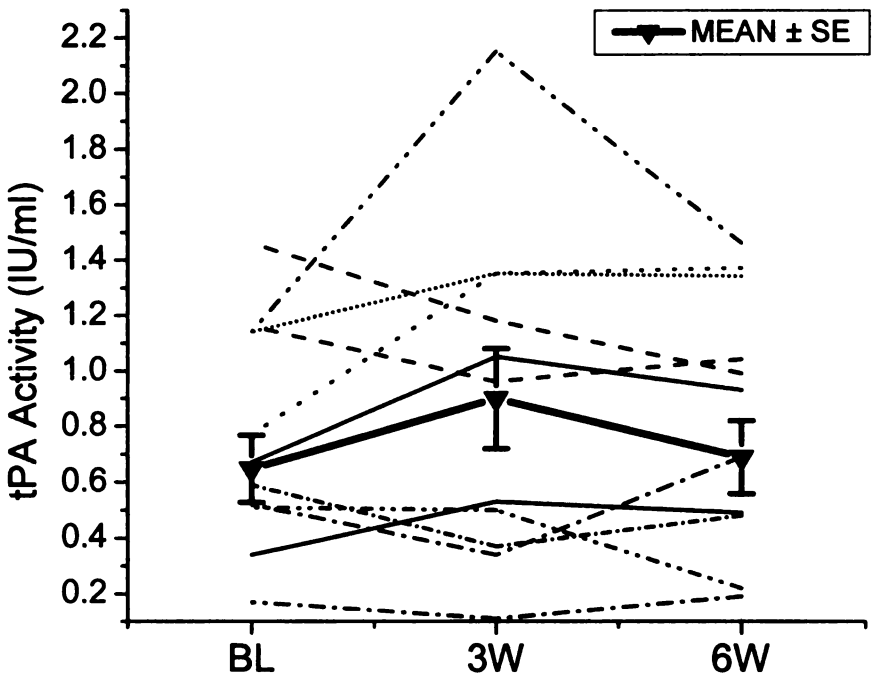


Figure 2.

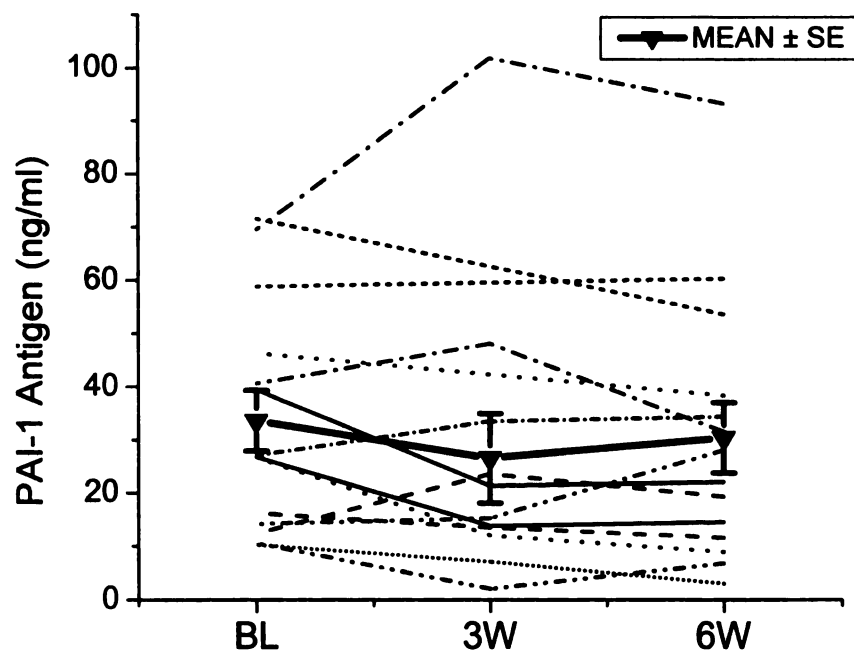
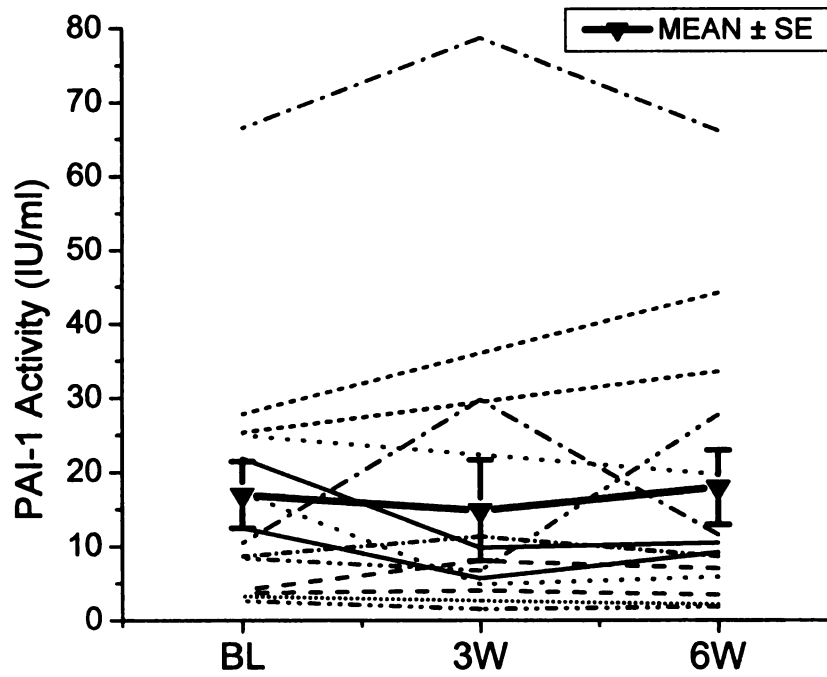
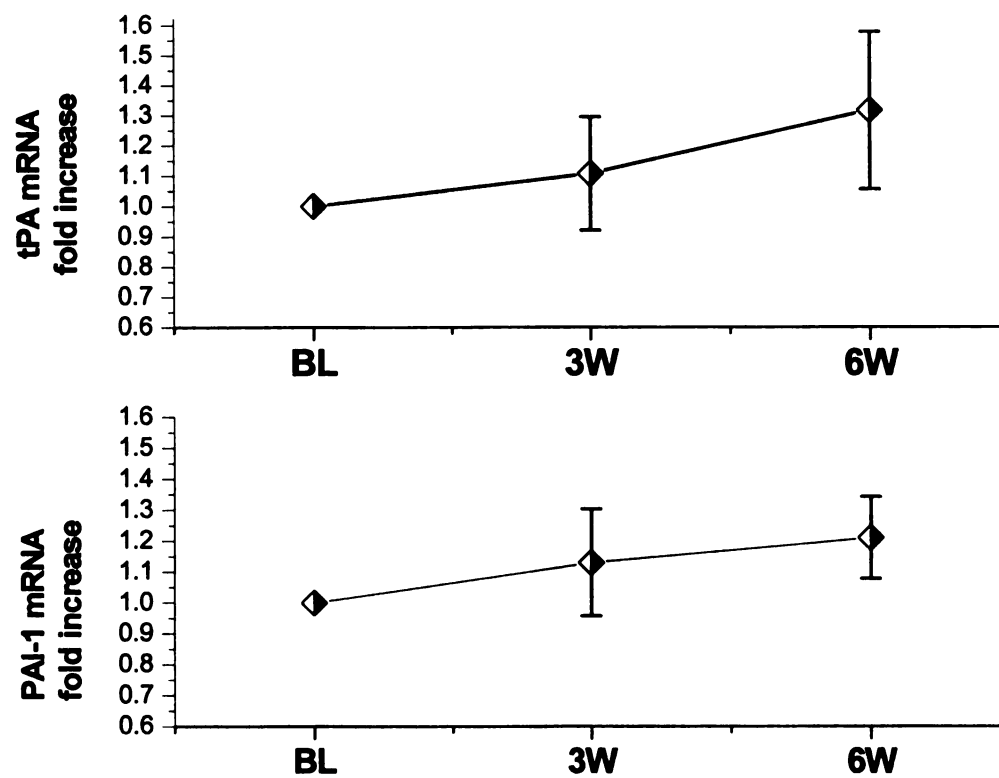


Figure 3.



Chapter 5 - DISCUSSION

Fibrinolytic capacity, as indicated by plasma concentrations of tPA and PAI-1, is directly related to coronary risk. Exercise training promotes numerous cardiovascular benefits, possibly including an enhanced ability to lyse an occlusive blood clot. The purpose of the present study was to assess the fibrinolytic adaptations during six weeks of participation in an exercise-based cardiac rehabilitation program. Earlier investigations of training-induced fibrinolytic adaptations in a population with cardiovascular disease have reported conflicting results. Variability in exercise intensity, duration, and length of training phase are likely explanations for the discordant observations.

Phase 2 cardiac rehabilitation historically included up to 12 weeks of supervised exercise. Modern-day third party reimbursement practices often necessitate fewer than 12 weeks of training. Hospital-based rehabilitation programs consisting of ten (54), eight (41), and six weeks of training (47, 97) produce significant improvement in various measures of physical and psychological functioning. Improvements in hemodynamic variables such as resting HR and BP tend to plateau with training regimens lasting longer than six weeks, so many insurance companies compensate for no more than 18 exercise sessions in six weeks (personal communication, A.T. de Jong). Participants in the present study realized significant hemodynamic improvements at rest and during submaximal exercise in response to six weeks of cardiac rehabilitation, as previously documented from this rehabilitation program (47).

With respect to fibrinolysis, well-controlled studies of 36 weeks of training induced significant increases in tPA (77) and decreases in PAI-1 (40, 77). Paramo, et al. reported decreased PAI-1 activity after 12 weeks of a nine-month training program (40,

104), suggesting that a rehabilitation program of this length is sufficient to affect positive fibrinolytic alterations. Paramo's study and the present investigation both utilized training regimens that agree with recommendations of the American College of Sports Medicine (1), including three days of activity per week for up to 40 minutes at an intensity associated with 40-85% of heart rate reserve. The Paramo study documented improvements in exercise capacity and HDL concentrations, and indicated the improved exercise tolerance was a relevant explanation for the observed fibrinolytic improvements. The present study similarly documented improved submaximal exercise tolerance without corresponding improvements in fibrinolytic capacity. Previous data from the Beaumont Hospital rehabilitation program also indicate the six week training regimen significantly improves total cholesterol, HDL and LDL concentrations (47). Thus, improvements in exercise performance and lipid profile do not appear to be directly related to fibrinolytic changes. Paramo also reported a significant decrease in plasma lipoprotein(a) (Lp(a)), which was not assessed in our study. Lp(a) is postulated to possess antifibrinolytic properties by competing with plasminogen for fibrin binding sites (8, 78). Exercise training studies of Lp(a) have produced equivocal results (35, 150), so it is unclear if this, and not duration of the training phase, explains the discordant fibrinolytic observations between Paramo's and the present study.

Only one previously published study examined the fibrinolytic adaptations to fewer than 12 weeks of cardiac rehabilitation. Suzuki, et al. observed significant decreases in PAI-1 activity after one month of training (138). These investigators also reported significant increases in plasma concentrations of norepinephrine after training, possibly facilitating the documented improvements in fibrinolysis. However, one would

expect elevated catecholamines to provoke greater release of active tPA (18, 113), which was not observed. Suzuki failed to show improvements in exercise tolerance as the exercise and sedentary control groups increased submaximal exercise time equally, and exercise SBP was greater post-training than at baseline. The only explanation offered by the authors for the improved fibrinolytic potential was a significant decrease in hematocrit following training. It was suggested that the reduced hematocrit reflected increased plasma volume and decreased blood viscosity. This hypothesis, though tenuous, cannot be disregarded based on our results, as hematocrits were not collected in the present study. The primary difference between Suzuki's and the present study is the prescribed exercise frequency and duration. Patients in the Suzuki study exercised 80 minutes per day, six days per week -- far greater than is recommended by the ACSM. Thus, the collective evidence in published literature suggests that 12 weeks of participation in an exercise-based cardiac rehabilitation program that adheres to established guidelines for exercise prescription is effective in promoting enhanced fibrinolysis, and that six weeks is not. It is unclear if fibrinolytic adaptations to training may be realized in 7-11 weeks of cardiac rehabilitation participation.

Adipose tissue is a known site of PAI-1 expression (4). Previous investigations indicate that plasma levels of PAI-1 correlate closely to BMI and visceral fat accumulation (4), and some have speculated that training-induced changes in PAI-1 are actually the result of fat loss (19). In the present study, no statistical relationship was observed between waist-to-hip ratio or waist circumference and the change in PAI-1 during the six weeks of training. It should also be noted that baseline and 6-week BMI were not associated with PAI-1 activity or antigen at the same time point. Recent

evidence suggests that both tPA and PAI-1 are synthesized in skeletal muscle (65).

Fibrinolytic alterations from training that correlate to changes in BMI may be related to increased muscle mass as opposed to, or in addition to reductions in fat. The exercise stimulus in the present study did not appear to influence body composition as indicated by BMI and waist/hip ratio, which may explain the lack of fibrinolytic changes.

Strategies for weight loss in a coronary disease population are not well-defined (61), and only modest improvements in body composition through phase II cardiac rehabilitation are described in the literature (88). The relationship between training-induced modifications in fibrinolysis and body fatness must be studied more carefully in patients with CVD.

In the present study subjects were enrolled over the course of a 10 month period (August – June), introducing the possibility of seasonal fluctuations altering the variables under examination (28). Recent evidence suggests, however, that exercise training may reverse the seasonal changes in tPA and PAI-1 (145). Our results do not indicate any influence of season on fibrinolytic training adaptations, as month of enrollment in the phase II program was not related to the change in any outcome variable from baseline to six weeks.

Recent findings describe the expression of tPA and PAI-1 genes in human peripheral blood cells (119, 152). Basal leukocyte expression of other genes is amplified following a course of resistance training (46) and an acute bout of aerobic exercise (21). To date, there are no published investigations of leukocyte expression of tPA and PAI-1 genes during a course of aerobic exercise training. Our results indicate that the six week exercise program did not elicit changes in expression of the tPA and PAI-1 genes. This

may partially explain the lack of change in plasma concentration of the two fibrinolytic proteins or may simply reflect no influence of training on tPA and PAI-1 gene expression in nucleated blood cells, implying no relationship exists between leukocyte RNA and plasma concentrations of tPA and PAI-1. Further investigation is required to clarify this issue.

Limitations of the present study include the lack of a non-exercising control group. The repeated measures design of the project allows each subject serve as his/her own control, but we are unable to control for the effect of time that might also influence the fibrinolytic variables under examination. The subject pool was a convenience sample that included citizens of Royal Oak, MI and its surrounding areas. The observations reported herein may not apply to individuals from other demographic areas. Another limitation involves the gene expression data, which were derived from whole blood. Due to methodological considerations, we were unable to assess RNA expressed from other cells, particularly muscle and endothelial cells, which release a significant proportion of tPA and PAI-1. Gene expression of blood leukocytes may not reflect transcription in other tissues. Finally, subject safety precluded the discontinuation of medications that may impact tPA and PAI-1. Although statistical analyses did not indicate a pharmacological influence on our data, we cannot exclude the possibility. Likewise, comorbid diagnoses may have affected our outcome variables, although no statistical relationship was observed.

In conclusion, the results of the present study demonstrate that a six week, exercise-based cardiac rehabilitation program does not provide an adequate training stimulus to elicit positive fibrinolytic changes. Depressed fibrinolytic activity is

associated with recurrence of cardiac events (50). As such, the lack of fibrinolytic improvement observed in this study may reflect a need to re-evaluate the recommended exercise prescription for individuals with cardiovascular disease, as well as insurance reimbursement policies for cardiac rehabilitation. The literature suggests rehabilitation programs that adhere to recommended exercise guidelines should continue for at least 12 weeks. Fibrinolytic improvements may also be achieved through shorter programs that utilize greater frequency, intensity, or duration of exercise. The benefit of a more aggressive rehabilitation program would be reduced cost for delivery of service. Significant fibrinolytic improvements could be realized in 24 sessions over four weeks, as opposed to 36 sessions in 12 weeks. However, the risk to the patient may vastly outweigh the benefits. Furthermore, retention rates would likely decline, further increasing risk of recurrent events and potentially negating the cost benefit. Given the clinical importance of hemostasis, future studies are needed to elaborate on the time course of fibrinolytic changes during cardiac rehabilitation. Other future studies might explore possible effects of gender, race, and genetic polymorphisms on the fibrinolytic adaptations to exercise among healthy people as well as those with CVD.

APPENDIX A

CONSENT FORM, MICHIGAN STATE UNIVERSITY

Consent Form

The influence of genetic polymorphisms on hemostatic adaptations to exercise in patients with cardiovascular disease.

Principal Investigators:

Christopher J. Womack, Ph.D.	Michigan State University	517-353-5222
Adam deJong	William Beaumont Hospital	248-655-5749
Barry Franklin, Ph.D.	William Beaumont Hospital	248-655-5749

Summary of the research protocol: You have been asked to participate in a study investigating how genetics affects blood clotting adaptations to exercise. We are trying to determine if certain genes influence changes in enzymes related to blood clotting that typically change after an individual participates in regular aerobic exercise. Specifically, we are interested in the enzymes tPA and PAI-1, which both influence how well you are able to break down blood clots that form in your body. We will obtain 10 cc (about 2 tablespoons) of blood from a vein in your arm. From this sample, we will be able to measure your levels of tPA and PAI-1 and will also extract some DNA to determine if your genetics affect your levels of these enzymes. This blood draw will occur in the morning and we ask that you do not eat or drink anything except water for 12 hours prior to the test. You may take your medications as usual except for those that you are required to take with food. You will also be asked to fill out a brief questionnaire that assesses your physical activity for the past seven days. The questionnaire and blood draw will be administered four separate times: prior to your first exercise training session; after 3 weeks of regular exercise; after 6 weeks of exercise; and 6 weeks after your last supervised exercise session.

Estimate of subject's time: Each test will take approximately 40 minutes. You will rest for 30 minutes prior to the blood draw and then we will obtain the blood sample.

Experimental procedures: Following a 12-hour fast, you will report to the William Beaumont Hospital Cardiac Rehabilitation Center. You will then assume a seated position for 30 minutes to stabilize your levels of tPA and PAI-1, during which time you will fill out the physical activity questionnaire. Ten ml (about 2 tablespoons) of blood will then be obtained from a vein in your arm. The blood sample will be analyzed for enzymes, which reflect your ability to break down blood clots (tPA and PAI-1). A portion of the blood will be used to analyze your DNA to see if your inherited genetics might be related to these enzymes. Your blood may be stored for future analysis of other genetic properties that investigators deem important at a later date.

Risks/Discomforts: Risks for blood drawing can include discomfort, bruising, and, in rare instances, infection, lightheadedness, and fainting. We will use sterile procedures and trained personnel to ensure that there is minimal discomfort with obtaining the blood samples.

Payment: There is no payment for participation in this study.

Voluntary participation: Your participation in this study is voluntary and dependent upon your consent. You may choose to withdraw from the study at any time. In addition, you may request at any time that your stored DNA be disposed of and made unavailable for further analysis. If you refuse participation or withdraw from the study, it will not affect the quality of care that you are currently receiving at William Beaumont Hospital. In addition, any results of the testing that you complete will be available to you.

Confidentiality and anonymity: Your privacy will be protected to the maximum extent allowable by law. Any publication or presentation of the results of this study will not identify you personally. Furthermore, your records and test results will only be available to the investigators of this study and will not be shared with others, except at your approval or request.

Contact person: Should you have any questions and/or concerns about your participation in the study, please contact:

Chris Womack, Ph.D.
3 IM Sports Circle
Michigan State University
East Lansing, MI 48824
517-353-5222

or

Barry Franklin, Ph.D.
Director, Cardiopulmonary Rehabilitation Services
William Beaumont Hospital
Royal Oak, MI
248-655-5750

If your questions pertain to participant's rights as human subjects of research, please contact:

Dr. Peter Vasilenko, Ph.D., Chair
University Committee on Research Involving Human Subjects
202 Olds Hall
Michigan State University
E. Lansing, MI 48823
517-355-2180

Injury: If you are injured as a result of your participation in this research project, William Beaumont Hospital will provide emergency medical care if necessary. If the injury is not caused by the negligence of the investigators, you are personally responsible

for the expense of this emergency care and any other medical expenses incurred as a result of this injury.

If you agree to join this study, please sign your name below. By completing and returning this form, you indicate your voluntary agreement to participate.

Signature of subject

Signature of investigator

Date

APPENDIX B

CONSENT FORM, WILLIAM BEAUMONT HOSPITAL

HIC # 03-115
07/24/03

Consent Form and Authorization for Disclosure of Protected Health Information

Dr. Barry Franklin and the Cardiac Rehabilitation Program at William Beaumont Hospital are engaged in research investigating how genetics affect blood clotting adaptations to exercise. This investigational study is known as:

THE INFLUENCE OF GENETIC POLYMORPHISMS ON HEMOSTATIC ADAPTATIONS TO EXERCISE IN PATIENTS WITH CARDIOVASCULAR DISEASE.

You may not participate in this study if you are currently enrolled in another related research study.

• INTRODUCTION

We are trying to determine if certain known genes influence changes certain enzymes related to blood clotting that typically change after an individual participates in regular aerobic exercise. You have the right to know about the procedures that will be performed during the study. This information is not meant to frighten or alarm you. It is to inform you of potential risks/benefits so you can decide with confidence whether or not to participate in this study. Please read this information carefully and ask as many questions as you like before deciding whether you want to take part.

• DESCRIPTION OF THE STUDY

This study involves collection of blood samples from 90 participants in the William Beaumont Hospital Cardiac Rehabilitation Program. Thirty of the participants will be untrained and just entering the program, 30 will be participants who have completed phase II of the program and 30 will be participants who have completed at least 12 weeks of phase III. Following a 12-hour fast, participants will report to the William Beaumont Hospital Cardiac Rehabilitation Center. They will then assume a seated position for 30 minutes. Ten ml (about 2 tablespoons) of blood will then be obtained from an arm vein. The blood sample will be analyzed for enzymes, which reflect the body's ability to break down blood clots (tPA and PAI-1). A portion of the blood will be used to analyze DNA to see if the genetics might be related to these enzymes.

• TISSUE BANKING:

In this study, your DNA and your blood plasma will be retained in case there are other potential genetic markers of these enzymes that are identified. Your samples will be coded and the code and access to the samples will only be accessible by the investigators of this study, specifically Dr. Barry Franklin, Dr. Chris Womack, Mr. Paul Nagelkirk, and Mr. Adam Coughlin. These are the only individuals that will perform the analyses of your samples. The results of your genetic analyses will be available to you, but will not be available to anyone else. The samples will be stored for a period of five years. However, you may request at any time that these samples be disposed of and made unavailable for further analysis.

HIC APPROVED **EXPIRES**
JUL 28 2003 JUL 28 2004

Pt. Initials Date

HIC # 03-115
07/24/03

• **RISKS, SIDE EFFECTS AND DISCOMFORTS:**

Side effects seen with the use of blood drawing include:

Bruising Infection

• **BENEFITS:** Exercise training typically improves enzymes that break down blood clots, but the mechanism for changes in these enzymes is unknown. Genetics have a large influence on the levels of these enzymes, but it is unknown if having a certain genetic profile will cause someone to have a larger or smaller change in these enzymes as a result of an exercise training program. By finding out whether there are certain genetic markers that influence an individual's change in these with exercise training, it will better enable clinicians to specifically target therapies for improving these enzymes. However, there is no guarantee that you will personally benefit from participation in this study.

• **ALTERNATIVE OPTIONS:**

You may elect to not participate in this study. This will have no affect on your level of care in the William Beaumont Hospital Cardiac Rehabilitation Program.

• **ECONOMIC CONSIDERATIONS:**

The cost of analysis for the blood samples and DNA are covered by the study and you are not financially responsible for these costs in any way. However, this study requires your current participation in the William Beaumont Hospital Cardiac Rehabilitation Program, which is considered routine care. If routine care costs normally covered by a third party payor are not covered by your insurance, the costs will be your responsibility.

• **COMPENSATION:**

Your participation in this study is voluntary. You should understand the possible effects or hazards that might occur during the course of the study as described in this consent form. Should inadvertent injury or damage result from your participation in this study, there are no designated funds provided for subsequent medical care or compensation by either the investigator or William Beaumont Hospital. However, you do not waive any legal rights by signing this consent form.

• **CONFIDENTIALITY, DISCLOSURE AND USE OF YOUR INFORMATION:**

Your results will remain confidential but may be used by the investigators. Your medical and billing records of the study will remain confidential but may be disclosed or used by the following or their representatives: the investigators, William Beaumont Hospital, the Food and Drug Administration, and other governmental agencies. Your healthcare insurer including Medicare and Medicaid also have access to your medical and billing records of the study. The purpose for this disclosure or use is, for example, to assure compliance with study protocol, to evaluate the effectiveness of the study, or to provide protection to you as a study subject. The disclosure and use of your information will continue after your participation in the study, there is no expiration date for the use of your medical and billing records from the study.

HIC APPROVED EXPIRES
JUL 28 2003 JUL 28 2004

Pt. Initials Date

HIC # 03-115
07/24/03

• **STOPPING STUDY PARTICIPATION**

Your participation is voluntary and you may choose not to participate or withdraw from the study at any time without penalty or loss of benefits to which you are otherwise entitled, or without jeopardizing your medical care by your physician at William Beaumont Hospital or by the Cardiac Rehabilitation Program. However, if you do not agree to sign this Consent and Authorization you will not be able to participate in this study.

If you withdraw from the study, you must provide a written statement to the investigators of your choice to withdraw. However, where the study has relied on your consent and authorization for the time you participated in the study, your consent and authorization cannot be withdrawn.

Based on his judgment, the investigator in charge of the study can decide to remove you from this study without your consent for any appropriate reason, which will be explained to you.

• **CONTACTS:**

You may contact the Principal Investigator, Dr. Barry Franklin of this study at (248) 655-5749 to answer any questions you might have about your study participation or in case you think you may have any research related injuries. If you have any questions regarding your rights as a human research subject, you may contact the Institutional Review Board (Human Investigation Committee) Chairman at (248) 551-0662.

• **STATEMENT OF VOLUNTARY PARTICIPATION:**

I have read the above, have asked questions and have received answers about this study to my satisfaction. I understand what I have read and willingly give my consent to participate in **THE INFLUENCE OF GENETIC POLYMORPHISMS ON HEMOSTATIC ADAPTATIONS TO EXERCISE IN PATIENTS WITH CARDIOVASCULAR DISEASE**. I understand that I will receive a copy of this document and will be promptly informed of any new findings regarding this study. I further authorize the use of disclosure of my health and personal information contained in records as described above.

		<u>Alternative Signature</u> <u>IF RESEARCH SUBJECT UNABLE TO SIGN</u>
Research Subject Name		
Hospital Number		Name (please print)
Research Subject Signature		Relationship to Subject
Date Time		As the personal/legal representative of the study subject, please list the basis for your authority to sign this Consent and Authorization:
		Court-appointed guardian ☐
		Next of Kin ☐

HIC APPROVED EXPIRES

Pt. Initials Date

07/24/2003 08/08/2004

HIC # 03-115
07/24/03

Durable Power of Attorney ☐

Signature _____
Date/Time _____

(Please check the appropriate box)
Witness to Signature Only ☐ OR
Witness to Consent Process and Signature ☐

Witness Name (Please Print)

Witness Signature Date Time

In accordance with Hospital Policy #304, all consent forms must be signed by a Beaumont employee who witnesses the subject sign the form. The witness must observe the entire consent process including the signature if: 1) the subject is unable to read or write, but otherwise understands English (see FDA Information Sheets), or; 2) if a short form consent is used (see 21CFR50.27(b)(2) or 45CFR117(b)(2)).

• **INVESTIGATOR/AUTHORIZED CONSENT PROVIDER STATEMENT:**

I have explained this study and have offered the study subject an opportunity for any further discussion or clarification.

Name (please print)

Phone Number

Signature

Date

Time

HIC APPROVED

JUL 28 2003

EXPIRES

JUL 28 2004

Pt. Initials Date

APPENDIX C

HUMAN SUBJECTS APPROVAL, MICHIGAN STATE UNIVERSITY

**MICHIGAN STATE
UNIVERSITY**

**Renewal
Application
Approval**

April 7, 2005

To: Christopher Womack
3 Im Sports Circle
Msu

Re: IRB # 03-289 Category: EXPEDITED 2-2
Renewal Approval Date: April 7, 2005
Project Expiration Date: April 6, 2006

Title: THE INFLUENCE OF GENETIC POLYMORPHISMS ON HEMOSTATIC ADAPTATIONS TO
EXERCISE IN PATIENTS WITH CARDIOVASCULAR DISEASE

The University Committee on Research Involving Human Subjects (UCRIHS) has completed their review of your project. I am pleased to advise you that the renewal has been approved

The review by the committee has found that your renewal is consistent with the continued protection of the rights and welfare of human subjects, and meets the requirements of MSU's Federal Wide Assurance and the Federal Guidelines (45 CFR 46 and 21 CFR Part 50). The protection of human subjects in research is a partnership between the IRB and the investigators. We look forward to working with you as we both fulfill our responsibilities.

Renewals: UCRIHS approval is valid until the expiration date listed above. If you are continuing your project, you must submit an *Application for Renewal* application at least one month before expiration. If the project is completed, please submit an *Application for Permanent Closure*

Revisions: UCRIHS must review any changes in the project, prior to initiation of the change. Please submit an *Application for Revision* to have your changes reviewed. If changes are made at the time of renewal, please include an *Application for Revision* with the renewal application.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects, notify UCRIHS promptly. Forms are available to report these issues.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with UCRIHS.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at UCRIHS@msu.edu. Thank you for your cooperation.

Sincerely,



Peter Vasilenko, Ph.D.
UCRIHS Chair

c: Paul R. Nagelkirk
3 IM Circle

APPENDIX D

HUMAN SUBJECTS APPROVAL, WILLIAM BEAUMONT HOSPITAL

Beaumont

William Beaumont Hospital
Royal Oak

Human Investigation Committee
Richard L. Lucarotti, Pharm.D.
Chairman

July 28, 2003

Barry Franklin, PhD
Division of Cardiac Rehabilitation
William Beaumont Hospital
Royal Oak, MI 48073

Dear Investigator:

RE: Project HIC-2003-115

**THE INFLUENCE OF GENETIC POLYMORPHISMS ON HEMOSTATIC
ADAPTATIONS TO EXERCISE IN PATIENTS WITH CARDIOVASCULAR DISEASE**

It is my belief that the above referenced project with consent form (dated July 24, 2003) involves no more than minimal risk to human subjects as detailed in Docket #87N-0032 of the Federal Register (6/18/91).

This project has been granted **FULL APPROVAL** under the Expedited Review policy [21 CFR 56.110] of the Human Investigation Committee. A progress report is requested by May 28, 2004. Submit with this report a computer diskette with an excel spreadsheet of subjects enrolled. See attached example.

NOTE:

The number assigned this project is HIC-2003-115. This number **MUST** be used on all correspondence with reference to this project.

Sincerely,



Richard L. Lucarotti, Pharm. D.
Chairman
Human Investigation Committee

/js
(expedrvw)

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