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The effects of acute garlic supplementation on fibrinolytic potential in young, healthy, trained males

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The Effects of Acute Garlic Supplementation on Fibrinolytic Potential in Young, Healthy, Trained Males

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ABSTRACT

Purpose: The purpose of this project was to examine the effects of acute garlic supplementation on fibrinolytic potential and the fibrinolytic response to exercise in young healthy trained males. **Methods**: 18 healthy trained males (Age = 20.9 ± 2.2 years, Height = 178 ± 7.7 cm, Weight = 75.5 ± 9.6 kg, VO_2max = 59.8 ± 6.7 ml kg^{-1} min^{-1}) performed a graded treadmill test to volitional exhaustion. Blood samples were taken at rest, within two minutes post-exercise, and one hour post-exercise. Participants were randomly assigned to ingest either 900 mg of powdered garlic or a placebo three hours before the exercise session. The supplement was distributed in a double-blind, crossover fashion. Participants repeated the protocol with the other treatment after a 14-day washout period. Paired t-tests were used to compare height, weight, resting hematocrit, VO_2max, respiratory exchange ratio, and treadmill time between the two trials. A two-factor (treatment and time) repeated measures analysis of variance (ANOVA) was used to assess changes in tPA activity, tPA antigen, and PAI-1 activity. A priori statistical significance was set at $P < 0.05$. **Results:** A significant difference was found between the two treatment conditions for absolute and relative VO_2max. No significant differences were observed between the two treatment conditions for treadmill time or respiratory exchange ratio at VO_2max. There was no main effect for treatment and no treatment x time interaction for any of the fibrinolytic variables examined. **Conclusion:** Acute garlic supplementation does not alter fibrinolytic potential or the fibrinolytic response to exercise in young healthy trained males. Acute garlic supplementation does, however, cause a small but statistically significant increase in VO_2max. It remains unclear if this increase in VO_2max is of functional importance.

Chapter One

Introduction

The relative risk of myocardial infarction is 5.9 fold greater in the hour after vigorous exercise (Mittleman, et al. 1993). In the general population, the mechanism triggering the majority of cardiovascular events is the disruption of a vulnerable atherosclerotic plaque resulting in an occlusive thrombus (Giri et al., 1999) and studies examining individuals with exertion-related myocardial infarctions suggest a similar pathological process (Ciampricotti et al., 1990). Consequently, the post-exercise period may be a hazardous time for those at risk of coronary events. One of the major factors which determines if an atheroscleoritc plaque will become occlusive is the hemostatic balance in the blood (Nagelkirk, 2010). Since 4% to 15% of myocardial infarctions are exertion-related (Tofler, 1990), methods which reduce prothrombotic or increase antithrombotic hemostatic forces during exercise may be clinically significant.

Fibrinolysis, defined as the capacity to lyse insoluble fibrin clots, is primarily regulated by tissue plasminogen activator (tPA) (Collen & Lijnen, 1986) and the main circulating inhibitor of tPA; plasminogen activator inhibitor-1 (PAI-1) (Schleef & Loskutoff, 1988). tPA is a serine protease that activates the fibrinolytic system by converting the pro-enzyme plasminogen to the active enzyme plasmin, which then subsequently dissolves fibrin into fibrin degradation products (Gebbink, 2011). tPA is stored in small vesicles within vascular endothelial cells (Emeis et al., 1997) and is released in the presence of a variety of stimuli, especially increased levels of epinephrine (Chandler et al., 1992). PAI-1 is a serine protease inhibitor that inhibits the fibrinolytic system by acting as a substrate to tPA. The resulting cleavage of PAI-1 by tPA results in covalent binding of PAI-1 to tPA in a stable inactive 1:1: stochiometric complex (Gebbink, 2011).

PAI-1 is produced by endothelial cells in an active form and is then secreted into the blood where it rapidly forms an inactive complex with tPA or it is released into the subendothelium where it binds to the extracellular matrix (Schleef & Loskutoff, 1988). PAI-1 is also produced by adipocytes in response to habitual elevations in tumor necrosis factor, insulin, and transforming growth factor beta (Alessi & Juhan-Vague, 2006). Regulation of PAI-1 occurs through a variety of mechanisms including endotoxin, thrombin, transforming growth factor β, and inflammatory cytokines (Schleef & Loskutoff, 1988). Coagulation, defined as the capacity to form insoluble fibrin clots, is achieved through a sequential process by which multiple inactive coagulation factors are converted to their active enzymatic form (Smith, 2003), ultimately resulting in conversion of prothrombin to thrombin. During exercise, markers of coagulation rise and remain elevated for hours into the post-exercise period (Hansen et al, 1990; Van Den Burg et al., 1995; Vanden Burg et al. 1997; Weiss et al. 1998; Hedge et al. 2000; Hillberg et al, 2003; Hillberg et al., 2003; Paton et al. 2004; Acil et al., 2007). Fibrinolysis also increases during exercise and thus maintains a balanced hemostatic environment (Chandler et al., 1992; Van Den Burg et al., 1995; Symanski et al., 1996; Van Den Burg et al., 1997; Fernhall et al. 1998; Hedge et al., 2000, Womack et al., 2001, Womack et al, 2003). However, at the cessation of exercise, fibrinolysis decreases rapidly (Hansen et al. 1990; Van Den Burg et al. 1995; Van Den Burg et al., 1997; Weiss et al. 1998; Cooper et al., 2004) and one hour post-exercise there is no longer any enhancement of the fibrinolytic system (Hilberg et al. 2003; Paton et al., 2004; Womack et al., 2006). This subsequently results in a hemostatic imbalance during the post-exercise period that favors clot formation.

For thousands of years the benefits of Garlic, *Allium sativum,* have been recognized and included in clinical treatment. In the codex of Ebers, an ancient Egyptian medical writing, garlic is written as an essential ingredient in 22 therapeutic formulas (Block, 1985). Hippocrates observed garlic to be an effective treatment for neoplasms as well as an effective diuretic (Singh & Singh, 2008). The Roman physician Dios-corides prescribed garlic as vermifuge to the Roman

army (Block, 1985). The prophet Mohammad recommended garlic as a treatment for scorpion stings (Singh & Singh, 2008). In modern times, Garlic still has the reputation of a highly recognized medicinal plant. In the two world wars, garlic was used as an antiseptic agent to treat wounds and prevent gangrene (Essman, 1984; Block, 1985). In 1944 Cavallito & colleagues identified this antiseptic component of garlic and it subsequently became the subject of two US patents (Block, 1985; Amagase et al., 2001). Albert Schweitzer reported to have used garlic as a medical treatment for amoebic dysentery (Block, 1985).

There are >3,000 scholarly publications which have examined and confirmed numerous therapeutic properties garlic (Amagase et al., 2001). One benefit is garlic's ability to improve fibrinolysis. Studies from the late 1970s to early 1990s reported substantial increases in fibrinolytic activity after acute and chronic supplementation (Bordia et al., 1975; Bordia et al., 1977, Bordia et al., 1978, Arora et al., 1981, Chutani & Bordia, 1981; Bordia et al., 1982; Gadkari et al. 1991). These studies also found garlic to be effective at enhancing fibrinolytic activity across a variety of forms including: raw garlic (Chutani & Bordia, 1981; Gadkari et al., 1991), fried garlic (Chutani & Bordia, 1981), and the essential oil of garlic (Bordia et al., 1975; Bordia et al., 1978; Arora et al., 1981; Bordia et al., 1982). Although the results of these studies are encouraging, they are limited because they fail to identify the specific parts of the fibrinolytic system that are affected by garlic. This limitation is inherently derived from the use of the euglobulin clot lysis method of measuring fibrinolytic activity (Kowalski et al., 1959) instead of directly measuring changes in tPA and PAI-I levels. There are also no prior studies which have combined exercise with garlic supplementation to evaluate the fibrinolytic effects of garlic during the post-exercise period.

Aims and Hypothesis

Aim 1- To determine if acute supplementation with garlic influences plasma tPA levels at rest, within 2-minutes following a cardiorespiratory fitness test, and 1-hour after completion of a cardiorespiratory fitness test as compared to a placebo.

Hypothesis 1- Acute supplementation with garlic will increase plasma tPA activity and decrease plasma tPA antigen at rest and will elevate tPA activity and tPA antigen levels within 2-minutes following a cardiorespiratory fitness test and 1-hour after completion of a cardiorespiratory fitness test as compared to a placebo.

Aim 2- To determine if acute supplementation with garlic influences plasma PAI-1 levels at rest, within 2-minutes following a cardiorespiratory fitness test, and 1-hour after completion of a cardiorespiratory fitness test as compared to a placebo.

Hypothesis 2- Acute supplementation with garlic will decrease plasma PAI-1 levels at rest, within 2-minutes following a cardiorespiratory fitness test, and 1-hour after completion of a cardiorespiratory fitness test as compared to a placebo.

Significance of this Study

To date only three studies have examined the effect of acute garlic supplementation on tPA levels in humans (Kiesewettier et al., 1990; Jung et al., 1991; Legnani et al., 1993). Only two studies have evaluated the effect of acute garlic supplementation on PAI-1 activity (Jung et al., 1991; Legnani et al., 1993). Yet, there is no overall consensus between them regarding the ability of acute supplementation to enhance fibrinolytic potential. Kiesewettier & colleagues observed 300 mg and 600 mg of powdered garlic to increase tPA activity 56% and 51%, respectively, after 5 hours. Moreover, the 600 mg of powdered garlic was shown to improve tPA activity in all participants. Jung & colleagues in a similar study observed 900 mg of dried garlic to enhance tPA

activity by 86% after 5 hours. In contrast to these two studies, Legnani & colleagues administered 900 mg of dried garlic powder and observed no significant change in tPA activity or antigen after 2, 4, and 6 hours.

It should also be realized that all of these studies had female participants (Kiesewettier et al., 1990; Jung et al., 1991; Legnani et al., 1993) and two of the studies had participants who were active tobacco smokers (Jung et al., 1991; Legnani et al., 1993). Cederblad et al. (1977) and Giardina et al., (2004) have observed fibrinolytic activity to be variable across the menstrual cycle. None of the three studies indicated if they standardized their female participants' treatment trials to menstrual cycle status. Research has also shown smoking tobacco alters fibrinolysis by decreasing tPA activity and antigen levels (Newby et al., 1999; Pretorius et al., 2002) and also by decreasing the tPA/PAI-1 molar ratio (Barua et al., 2002). These two factors were controlled for in the present study.

An additional strength of the current study is that it uniquely maximizes the potential to detect a difference between trials. Basal tPA and PAI-1 activity levels are low (Rijken et al., 1983; Declerck et al., 1988). This necessitates either a large effect size or a large number of participants to detect differences in these variables at rest. Large changes in fibrinolysis occur when stress is applied to the cardiovascular system. Research has shown exercise to be a sufficient stress to observe differences in fibrinolytic potential between groups that are not readily available at rest (Hamouradtidis et al., 1988). Thus, exercise may increase the potential to observe differences in the fibrinolytic profile between the garlic and placebo trials. Lastly, the effects of garlic on the exercise induced alterations in fibrinolysis are not known.

Chapter Two

Review of the Literature

Objective

The objective of this chapter is to provide an overview of: 1) the effects of garlic supplementation on fibrinolytic potential, 2) the effects of acute exercise on fibrinolytic potential, 3) the effects of acute exercise on coagulation potential, and 4) the methodological considerations for research involving hemostasis.

Regulation of Fibrinolysis

Fibrinolysis, defined as the capacity to lyse insoluble fibrin clots, is primarily regulated by tissue plasminogen activator (tPA) (Collen & Lijnen, 1986) and the main circulating inhibitor of tPA; plasminogen activator inhibitor-1 (PAI-1) (Schleef & Loskutoff, 1988). tPA is a protease which activates the fibrinolytic system through the conversion of the pro-enzyme plasminogen to the active enzyme plasmin (Gebbink, 2011). This subsequently results in the dissolution of fibrin into fibrin degradation products (Gebbink, 2011). tPA is stored within vascular endothelial cells (Emeis et al., 1997) and is released from the endothelium in response to a variety of stimuli. However, in regards to exercise, approximately half of the tPA release is due to increased circulation of epinephrine (Chandler et al., 1992).

PAI-1 is a protease inhibitor, which inhibits the fibrinolytic system by acting as a substrate to tPA. Upon cleavage of PAI-1 by tPA, a covalent bond forms between the two producing an inactive complex (Gebbink, 2011). PAI-1 is also produced by endothelial cells in an active form and is then secreted into the blood where it rapidly forms an inactive complex with tPA (Schleef & Loskutoff, 1988). PAI-1 is also produced by adipocytes in response to habitual elevations in tumor necrosis factor, insulin, and transforming growth factor beta (Alessi & Juhan-Vague 2006). Like tPA, the regulation of PAI-1 occurs through a variety of mechanisms

including endotoxin, thrombin, transforming growth factor β, and inflammatory cytokines (Schleef & Loskutoff, 1988).

Effects of Garlic on Fibrinolysis

Studies dating back to the late 1970s have demonstrated that garlic has the ability to positively affect fibrinolytic potential (Bordia et al., 1975; Bordia et al., 1977, Bordia et al., 1978, Arora et al., 1981, Chutani & Bordia, 1981; Bordia et al., 1982; Gadkari et al. 1991; Bordia et al., 1998). These studies observed a wide range (15-130%) of fibrinolytic enhancement (Bordia et al., 1975; Bordia et al., 1977; Bordia et al., 1978). This large range of observed values may be due to differences in dosage form. The essential oil of garlic (Bordia et al., 1975; Bordia et al, 1978; Arora et al., 1981) produced larger increases in fibrinolytic activity as compared to fried garlic (Chutani & Bordia, 1981;) and garlic with butter (Bordia et al., 1975; Bordia et al. 1982). This may be due to the fact that consuming a fatty diet produces hyperlipemia, which has been postulated to impair fibrinolytic potential (Bordia et al., 1975; Bordia et al., 1982). Luley & colleagues (1986) used dried garlic; however, no improvements in fibrinolytic potential were observed with its administration. The large range of observed values may also be due to differences in dosage amount. Bordia and colleagues (1977, 1978) observed a 130% in fibrinolytic activity, after three months of supplementing 1 gram of garlic oil per kilogram of bodyweight daily. Conversely, Gadkari and colleagues (1991) observed a smaller increase in fibrinolytic activity (21.8%) after 2 months of dosing with 10 grams of raw garlic daily. Similarly, Arora and colleagues (1981) used a smaller dose, 3.75 milligrams of garlic oil daily, which results in an observed 32.5% increase in fibrinolytic activity by 8 weeks.

Interestingly, the literature also shows that garlic can enhance fibrinolytic activity after acute doses. Bordia and colleagues (1975) administered 50 grams of garlic along with 100 grams of butter to 10 healthy subjects. After 3 hours there was a 15% increase in fibrinolytic activity. Within the same study on a separate trial, men were fed 100g of butter and over the same time course saw a 49% decline in fibrinolytic activity. This strongly suggests an acute ability of garlic

to enhance the fibrinolytic system. Similarly, Chutani & Bordia (1981) compared the effect of fried and raw garlic on fibrinolytic activity in 20 patients with ischemic heart disease. Garlic was administered in the morning in fried or raw doses amounting to 0.5 grams per kilogram of body weight. Fibrinolytic activity increased in the control group by 5% after 6 hours; however, this difference was not significant. In the experimental trials, fibrinolytic activity significantly increased by 72% and 63% six hours following the administration of raw and fried garlic, respectively. Moreover, these elevated levels of fibrinolytic activity were maintained up to 12 hours after administration.

The aforementioned studies strongly support the hypothesis that garlic supplementation can enhance the fibrinolytic system. However, the specific targets of improvement in the fibrinolytic system are unknown due to the use of euglobulin clot lysis time in these studies. The euglobulin clot lysis method is a technique that calls for the generation of a standard clot followed by recording the lysis time of said clot (Kowalski et al., 1958). This provides an adequate assessment of overall fibrinolytic activity; however, it does not allow researchers to specifically identify the aspects of the fibrinolytic system that are being altered by garlic supplementation. More recent studies have attempted to bypass this limitation by directly measuring tPA and PAI-1 levels before and after garlic administration. Kiesewttier & colleagues (1990) administered single doses of 100 mg, 300 mg, and 600 mg of powdered garlic or placebo orally. Five hours following administration they observed a 56.3% and 51% increase in tPA activity in the 300 mg and 600 mg trials, respectively. Despite the similarities in overall percent increase, the 600 mg trial was the only dose that led to increased activity in all participants. Jung & colleagues (1991) similarly administered single doses of 900 mg of powdered garlic or placebo. Five hours after administration, tPA activity rose 86.1%. Legnani & colleagues (1993) administered a single dose of 900 mg of powdered garlic as well. tPA activity levels increased 300% after 4 hours and 406% after 6 hours. PAI-1 was investigated by both Jung et al (1991) and Legnani et al (1993), however, neither research group reported any significant changes in this variable.

Increased tPA activity could explain the shortening of the euglobulin clot lysis time observed with garlic supplementation. However, it should also be realized that fibrinolytic potential has pronounced circadian variations (Angleton et al., 1988). PAI-1 activity and tPA antigen are significantly higher in the morning as compared to evening values (Angleton et al., 1988). In contrast, tPA activity is significantly lower in the morning and inversely correlated (r=-0.57) with PAI-1 activity (Angleton et al., 1988). Therefore, a portion of the impressive increases in tPA activity observed by these studies was due to normal diurnal variations rather than garlic supplementation. In support of this, tPA activity increased 35% and 31.4% in the placebo trials, in the Kiesewettier and colleagues (1991) and Jung and colleagues (1991) studies.

It should also be noted that these studies had female participants (Kiesewetter et al., 1990; Jung et al., 1991; Legnani et al., 1993) and participants who were active tobacco smokers (Jung et al., 1991; Legnani et al., 1993). Cederblad et al. (1977) and Giardina et al. (2004) have observed fibrinolytic activity to be variable across the menstrual cycle. None of the three studies indicated if they standardized their female participants' treatment trials to menstrual cycle status. Research has also shown smoking tobacco alters fibrinolysis by decreasing tPA activity and antigen levels (Newby et al., 1999; Pretorius et al., 2002) and also by decreasing the tPA/PAI-1 molar ratio (Barua et al., 2002). These two factors were controlled for in the present study.

Fukao & colleagues (2007) uniquely examined the effects of garlic administration on markers in tPA and PAI-1 in vitro by using cultured human umbilical vein endothelial cells (HUVECs), fibrin film, and chromogenic assays. They observed that garlic preparations at the concentrations of 0.4 $\mu g*mL^{-1}$, 100 $\mu g*mL^{-1}$, 500 $\mu g*mL^{-1}$, and 1 $mg*mL^{-1}$ did not influence tPA or PAI-1 secretions from the HUVECs. However, when tPA was incubated with garlic on a fibrin film, the lysis area of fibrin increased 1.8x as a result of increased degradation of fibrin by tPA mediated activation of plasminogen. The assay also demonstrated increased plasmin generation by tPA in the presence of garlic. Kinetic analysis showed that the maximum velocity achieved by the enzymatic system (V_{max}) did not change but that the substrate concentration required to reach

half of V_{max} decreased with garlic. The efficiency of the enzymatic reaction between tPA and plasminogen was also found to be enhanced which resulted in an 8.7x increase in total reactivity. Together these findings suggest garlic does not increase the secretion of tPA from the vascular endothelium but rather acts as a cofactor that increases the affinity between tPA and plasminogen.

Harenberg and colleagues (1988) also conducted a unique study of the effects of garlic supplementation on fibrinoylsis. The researchers measured concentrations of fibrinopeptide Bβ 1-42 in the blood following administration of 600 mg of dried garlic for 4 weeks in a group of 20 out-patients with hyperlipoproteinemia. Fibrinopeptide Bβ1-42 is a product of the lysis of fibrin by plasmin (Nossel et al., 1979). Thus, the concentration of fibrinopeptide Bβ 1-42 in the blood is an indicator of the magnitude of fibrinolytic activation. Harenberg et al., (1988) observed a 10% and 42% increase in fibrinopeptide Bβ 1-42 concentration over baseline after 2 and 4 weeks of garlic administration, respectively. These results suggest that fibrinolysis increases following garlic supplementation by enhancement of the activation of plasminogen. Since tPA is the main activator of plasminogen, this study indirectly supports the hypotheses that garlic either increases the amount of enzymatically active tPA or acts as a cofactor that increases the affinity between tPA and plasminogen.

Table 2.1. The Effects of Garlic Supplementation on Fibrinolysis .

Study	Problem	Participants	Design	Finding(s)
Bordia et al. (1975)	Effect of the essential oils of garlic on FA and fibrinogen	10 healthy volunteers	100 g of butter with 4 slices of bread plus 50 g of garlic oil or placebo.	↑ FA 15% 3 hr after garlic administration
Bordia et al. (1977)	Effect of essential oil of garlic on serum FA in patients with coronary artery disease	I) 10 healthy individuals II) 10 patients with old myocardial infarction III) 20 patients with acute myocardial infarction	Groups I and II administered 1 g of the essential oil extract of raw garlic per kg of body weight per day for 3 mo. Group III administered 1 g of the essential oil extract of raw garlic per kg of body weight per day for 20 days.	I) ↑FA 130% by 3rd mo II) ↑FA 83% by 3rd mo III) ↑ FA 63% above post-infarction value after 10 d ↑ FA 95.5% above post-infarction value after 20 d
Bordia et al. (1978)	The effect of essential oil of garlic on FA in patients of coronary artery disease.	I) 20 Healthy volunteers II) 20 Patients with coronary artery disease	The essential oil extract from 1 g of raw garlic, administered daily for 3 mo.	I) ↑ FA 130% by 3rd mo II) ↑FA 83% by 3rd mo
Nossel et al. (1979	Sequence of fibrinogen proteolysis	10 female patients receiving an abortion	Patients received intrauterine infusion of hypertonic saline	Plasmin cleaves fibrinopeptide Bβ1-42 from fibrin
Arora et al. (1981)	Effect of long-term garlic use on markers of ischemic heart disease	I) 30 individuals with ischemic heart disease II) 20 healthy volunteers	2 x 0.625 mg capsules, containing the essential oil of garlic, taken 3 times a day for 12 wk.	↑ FA 52.4% and 52.8% in both groups at 4th wk. ↑ FA 26.5% and 32.5% in both groups at 8th wk. ↔ in FA above resting levels at 12th wk.

FA= fibrinolytic activity (measured by euglobin clot lysis time), FPB β= Fibrinopeptide Bβ, tPA ACT= tissue plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1= plasminogen activator inhibitor 1, HUVEC=human umbilical vein endothelial cells

Table 2.1. The Effects of Garlic Supplementation on Fibrinolysis (Continued).

Study	Problem	Participants	Design	Finding(s)
Chutani & Bordia (1981)	Effect of fried versus raw garlic on FA in man	I) 20 patients with ischemic heart disease II) 30 patients with ischemic heart disease	I) A single dose of 0.5 g/kg body weight of raw or fried garlic. II) Raw or fried garlic pods administered in doses of 0.5 g/kg of body weight.	I) ↑FA 72% by 6 and 12 h with raw garlic ↑ FA 63% and 66% by 6 and 12 h respectively with fried garlic II) ↑ FA 53% at end of first wk with raw garlic ↑FA 84.8% by the end of 1 mo with raw garlic ↑ FA 40% by the end of first wk with fried garlic ↑ FA 72% by the end of 1 mo with fried garlic
Bordia et al. (1982)	The effect of garlic oil on FA and platelet adhesiveness in man	I) 20 healthy volunteers II) 20 healthy volunteers	I) 75 mg butter plus usual diet for 3 wk II) 75 mg of butter plus 0.25 mg/kg body weight of garlic oil for 3 wk.	↑ FA 36.3% with a fatty diet and garlic oil by 3 wk.
Luley et al. (1986)	Effect of dried garlic on FA and fibrinogen levels	51 hyperlipemic patients	3 x 425 mg tablets, containing either a placebo or dried garlic	↔ difference in FA observed
Harenberg et al. (1988)	Effect of dried garlic on blood coagulation, fibrinoylsis, and platelet aggregation in patients with hyperlipoproteinemia.	20 out-patients with hyperlipoproteinemia.	600 mg of dried garlic pills, 3 x 200 mg daily, for 4 wk.	↑ FpB β 1-24 10% and 42% after 2nd and 4th wk, respectively

FA= fibrinolytic activity (measured by euglobin clot lysis time), FPB β= Fibrinopeptide Bβ, tPA ACT= tissue plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1= plasminogen activator inhibitor 1, HUVEC=human umbilical vein endothelial cells

Table 2.1. The Effects of Garlic Supplementation on Fibrinolysis (Continued).

Study	Problem	Participants	Design	Finding(s)
Kiesewettier et al. (1990)	Effect of garlic on blood fluidity and FA	10 healthy volunteers	100 mg, 300 mg, or 600 mg of powdered garlic or placebo	↑tPA ACT 56.3% in 300 mg trial after 5 h ↑ tPA ACT 51% in 600 mg trial after 5 h
Gadkari et al. (1991)	Effect of raw garlic on FA and clotting time.	I) 30 healthy medical students II) 20 healthy medical students	10 g of raw garlic for 2 mo.	↑FA 21.8% in group I by 2 mo.
Jung et al. (1991)	Effect of garlic powder on cutaneous microcirculation	10 healthy volunteers	3 x 300 mg of pure garlic powder capsules or 3 x 300 mg of placebo	↑ tPA ACT 86.1% after 5 h
Legnani et al. (1993)	Effect of dried garlic on fibrinolysis and platelet aggregation in healthy subjects.	10 healthy volunteers	900 mg of dried garlic powder	↔ difference in tPA ACT or tPA AG after 2, 4, or 6 h
Bordia et al. (1998)	Effect of Darlic on Fibrinolytic and Fibrinogen in patients with CAD	I)30 patients with CAD II)30 patients with CAD	I) 2x2 capsules containing 1 g of raw garlic II) 2x2 capsules containing placebo	I) ↑ FA 39.4% and 55.1% by 1.5 and 3 mo, respectively ↔ in fibrinogen levels II) ↔ in all measures
Fukao et al. (2007)	Antithrombotic effects of odorless garlic powder in vitro	24-well multiplates with seeded human umbilical vein endothelial cells Fibrin film assay	HUVECs cultured with garlic powder at 37°C for 18 h Fibrin film plate with fibrinogen, thrombin, plasminogen, tPA and garlic	↔ in release of tPA or PAI-1 from HUVECs ↑1.8x in tPA ACT on fibrin plate in the presence of garlic ↑8.7x increase in efficacy of enzyme reaction between tPA and plasminogen in the presence of garlic

FA= fibrinolytic activity (measured by euglobin clot lysis time), FPB β= Fibrinopeptide Bβ, tPA ACT= tissue plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1= plasminogen activator inhibitor 1, HUVEC=human umbilical vein endothelial cells

The Effects of Acute Exercise on Fibrinolytic Potential

Exercise accelerates fibrinolytic activity (Davis et al., 1979, Wheeler et al., 1986, Drygas 1988, El-Sayed 1990). This acceleration is primarily attributed to elevations in tPA (El-Sayed, 1990). The magnitude of the increase in tPA is dependent upon both the exercise intensity and duration. A study by Hansen et al. (1990) observed fibrinolytic responses to seven healthy male volunteers who ran 1.7 km, 4.8 km, and long 10.5 km distances at speeds close to their maximal capacity. tPA antigen levels rose substantially across all trials, but, the greatest response occurred in the 10.5 km run, with the 4.8 km run eliciting the second greatest response. A study by Van Den Burg et al. (1997) also demonstrated the influence of duration. In this study, 20 healthy men cycled at 70% of VO_2max for 25 minutes followed by a graded test to volitional exhaustion. During the 25 minutes of steady state cycling there was a linear rise in tPA ACT and tPA AG. However, at the onset of the graded exercise test to maximal exhaustion there was a dramatic increase in tPA ACT and tPA AG. Although the separate influence of duration on fibrinolytic activity is important, the primary determent is intensity of exercise. A study by Womack & colleagues (2006) best demonstrates this principle. In their study they observed that tPA activity and tPA antigen were significantly higher following 20 minutes of exercise above lactate threshold (LT) as compared to an isocaloric, longer duration exercise test below LT. Similarly, Weiss et al. (1998) observed about a 3x larger increase in tPA antigen after a 1 hour run at 83% VO_2max as compared to after a 1 hour run at 68% VO_2max.

The mechanisms for the exercise-induced increases in tPA are not completely understood and numerous factors have been proposed to be responsible for the exercise-induced increases in tPA. One of the most strongly cited mechanisms is increased adrenergic stimulation. Chandler and colleagues (1992) observed that the time course and dose response of tPA release was similar for epinephrine infusion and exercise. However, the absolute rate of increase of tPA versus epinephrine concentration was 2x as fast during exercise as with infusion. This lead Chandler and colleagues to attribute approximately 50% of the increase in tPA directly to increased levels of

epinephrine. Yet if epinephrine only accounts for 50% of the observed increases in tPA, then there must be at least one other mechanisms to contribute to the remaining 50%. A second potential mechanism is vasopressin. Vasopressin itself stimulates the fibrinolytic system (Hariman et al., 1989) and there is a rise in plasma levels of vasopressin during exercise (El-Sayed et al., 1990). However, there is no correlation between the exercise induced rise in vasopressin and the exercise induced rise in fibrinolysis (El-Sayed, 1990). A third commonly cited mechanism is changes in blood lactate. Wheeler and colleagues (1986) observed a strong correlation ($r=0.81$, $P< 0.001$) between postexercise fibrinolytic activity and postexercise blood lactate concentrations. However, eight minutes after exercise lactate levels continued to rise but fibrinolytic activity decreased rapidly. Similarly, Drygas (1988) demonstrated that 60 minutes of submaximal exercise could increase fibrinolytic activity without changing blood pH. A fourth potential mechanism may be diminished hepatic clearance. Clearance of tPA is achieved through the mannose receptor located on hepatic endothelial cells and a low-density lipoprotein receptor-related protein that is a hepatic receptor for both free tPA and the inactive tPA/PAI-1 complex (Narita et al., 1995). Blood flow to the liver is reduced during exercise, which in turn decreases hepatic clearance (Bounameaux et al., 1986; De Boer et al., 1992). This may allow for the accumulation of tPA in the blood during exercise. Research by Hersch et al. (1987) supports this theory as they observed elevated tPA levels in patients with decreased clearance due to liver cirrhosis.

At the termination of exercise tPA levels begin to rapidly decline (Davis et al., 1976). tPA remains only slightly elevated above base line within 30 minutes after exercise (Hansen et al., 1990; Van Den Burg et al., 1995). By one hour post-exercise there is no longer any enhancement of the fibrinolytic system (Paton et al., 2004; Womack et al., 2006). Cooper et al. (2004) examined the time course changes of tPA after acute maximal exercise in eight healthy men. Blood samples were collected before exercise and at 1, 2, 4, 5, 6, and 10 minutes post

exercise. It was observed that plasma tPA activity and antigen reached had significantly declined within four minutes after exercise.

PAI-1 has been shown to decrease with exercise by some (Paton et al., 2004; Womack et al., 2006) and to remain unchanged with exercise by others (Hilberg et al., 2003). The response by PAI-1 has also been shown to vary within the same study depending upon if PAI-1 activity or PAI-1 antigen was being examined (Hilberg et al. 2003; Womack et al., 2006). This suggests that PAI-1 is not appreciably altered by exercise. Moreover, statistical power calculations performed by Cooper & colleagues (2004) reveal that at least 37 participants are necessary to yield a statistical power of 0.80 for PAI-1 activity. Since most of the studies examining the effect of exercise on PAI-1 had a participant pool of <37 they lacked sufficient statistical power to properly examine PAI-1.

Table 2.2.: The Effects of Acute Exercise on Fibrinolytic Potential

Study	Problem	Participants	Design	Finding(s)
Davis et al. (1976)	Fibrinolytic changes during and after maximal exercise	10 healthy males	Subjects performed a graded exercise test to physical exhaustion	↑FA ≈175% immediately post-maximal exercise ↔ FA at intensities <50-60% MHR ↑FA exponentially at intensities >60-70% MHR
Wheeler et al. (1986)	Changes in hemostatsis associated with acute exercise	19 healthy males	Subjects performed a branching multistage treadmill protocol to maximum	↑ FA 505% postexercise ↓ FA 25% from postexercise value 8 min post-exercise Postexercise FA ∝ postexercise blood lactate, r=0.81
Hersch et al. (1987)	Pathogenesis of accelerated fibrinolysis in liver cirrhosis	30 liver cirrhosis patients	Blood samples of 30 liver cirrhosis patients were taken	↑tPA AG ≈428% in patients with liver cirrhosis
Hamouratidis et al. (1988)	Effect of exercise on plasma fibrinolytic activity in healthy males	30 healthy males	Exercise testing on a treadmill according to the Bruce protocol	↓ 51% euglobulin clot lysis time in healthy men post-exercise

FA= fibrinolytic activity, MHR= maximum heart rate, tPA AG= tissue plasminogen activator antigen

Table 2.2: Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Drygas (1988)	Changes in FA in response to moderate, exhaustive, and prolonged exercise	47 healthy men	Subjects performed isometric exercise, 18 min of submaximal exercise, 60 min of submaximal exercise, and repeated 60 s bouts of maximal exercise	↔ in FA with isometric exercise ↑ FA 25% after 18 min of submaximal exercise ↑ FA 58% after 60 min of submaximal exercise ↑63% after maximal bouts of exercise ↔ in pH with isometric or 60 min of submaximal exercise ↓ pH 0.5% and 2% with 18 min of submaximal exercise and repeated maximal exercise, respectively
Hariman et al., (1989)	Physiological concentrations of vasopressin on the fibrinolytic system	9 male volunteers	Infusion of saline for 30 min followed by infusion with vasopressin for 1 hour	↑ tPA ACT ≈28x 60 min post-infusion ↓ PAI-1 ACT ≈19% 60 min post-infusion

FA= fibrinolytic activity, tPA ACT= tissue-type plasminogen activator activity, PAI-1 ACT=plasminogen activator inhibitor activity

Table 2.2. Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
El-Sayed (1990)	Exercise intensity related responses of fibrinolysis and vasopressin	16 normal healthy subjects	Subjects cycled at 40%, 70% and 100% of VO_2max	No correlation between exercise-induced changes in FA and vasopressin ↑ in FA in response to exercise is directly related to fractional workload (%VO_2max) ↑ in exercise-related FA is mainly due to elevated tPA levels
Hansen et al. (1990)	Procoagulant and fibrinolytic activities in circulation after strenuous physical exercise	7 healthy recreationally active men	Subjects participated in short (1.7km), middle (4.8 Km) and long (10.5 km) runs at a speed close to their maximal capacity	↑≈ 300%, 200%, 180% in tPA AG at long, middle, and short distances respectively tPA AG ↓ to baseline by 30 min post-exercise
De Boer et al. (1992)	Liver Blood Flow as a Determinant of tPA Clearance	6 healthy males 8 male Wistar rats	Subjects performed a submaximal exercise test while receiving an infusion of ICG and rtPA Rat liver perfused through portal vein with rtPA at different flow rates	↑rTPA ACT and AG 119% and 91% respectively during exercise ↑ICG 133% during exercise ↓liver blood flow 53% during exercise Live blood flow returned to normal 25 min post-exercise Halving or doubling flow rate of perfused rate liver causes proportional changes in rtPA clearance

FA= fibrinolytic activity, tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor activity, ICG= tricarboycyanine dye indocyanine green,

Table 2.2. Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Chandler et al. (1992)	Fibrinolytic response during exercise and epinephrine infusion in the same subjects	14 healthy men	Participants were studied during epinephrine infusion and graded supine bicycle exercise to exhaustion.	↑388% tPA ACT at point of exhaustion over baseline ↑PAI-1 ACT 24% at point of exhaustion over baseline ↑ 218% tPA ACT at maximal epinephrine infusion rate over baseline ↔ in PAI-1 or tPA/PAI-1 complex during infusion Linear relationship between tPA and epinephrine levels observed

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor activity

Table 2.2. Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Szymanski & Pate (1994)	Fibrinolytic responses to moderate exercise in physically active and inactive men Fibrinolytic responses during morning and evening exercise	I) 14 inactive men II) 12 active men	Two 30 minute submaximal exercise sessions at 50% of VO_2max	↑ tPA ACT ≈71% with evening exercise in active group ↑tPA ACT ≈28% with morning exercise in active group ↑ tPA ACT ≈25% with evening exercise in inactive group ↑tPA ACT ≈8% with morning exercise in inactive group ↔ in PAI-1 ACT with exercise in either group at either time point
Van Den Burg et al. (1995)	Haemostatic changes following strenuous physical exercise	29 healthy sedentary men	Exercise test consisted of 4 phases on a cycle ergometer: 1) 25 minutes of cycling at 70% VO2max 2) graded cycling to VO2max, 3)10 min of active recover, 4) 15 minutes of passive recovery	↑183% tPA AG at maximal exercise ↑tPA ACT 892% at maximal exercise ↓tPA ACT 61% from maximal exercise value by 25 min of recovery ↓ tPA ACT 42% from maximal exercise value by 25 min of recovery Coagulant and fibrinolytic activity during submaximal exercise are = up to 70% VO_2max

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor activity

Table 2.2. The Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Szymanski et al. (1996)	Factors affecting fibrinolytic potential	46 healthy white men	Maximal graded exercise tests on a treadmill	↑217% in tPA ACT, ↓31% PAI-1 ACT following maximal exercise
Van Den Burg et al. (1997)	Effect of endurance training on seasonal fluctuations on coagulation and fibrinolysis	20 healthy sedentary men	Exercise tests were performed in 4 phases on a cycle ergometer: 1) 25 minutes of cycling at 70% VO2max, 2) graded cycling to VO2max, 3)10 min of active recovery, 4 15 minutes of passive recovery.	tPA ACT and tPA AG ↑ linearly with time during submaximal exercise tPA ACT and tPA AG↑ exponentially during graded exercise tests
Weiss et al. (1998)	Coagulation and fibrinolysis after moderate and very heavy exercise	12 young men	Two treadmill tests at 68% and 83% VO_2max for 1 h	↑tPA AG ≈300% and ≈380% after 30 min and 60 min of running at 83% VO_2max ↑tPA AG 100% and 160% after 30 min and 60 min of running at 68% VO_2max ↓ tPA AG to base line 1 h post-83% VO_2max run Fibrinolytic system ↑to a higher degree during heavy exercise than the coagulant system Fibrinolytic potential ↓ at faster rate than coagulation potential post-exercise

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor

Table 2.2. The Effects of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Hedge et al. (2000)	Changes in clotting and fibrinolytic activity during the 1 h after a submaximal run	10 healthy males	Subjects completed two treadmill tests at either 70-75% VO2max or at 1.2 mph for 30 minutes each.	↑200% tPA ACT after 30 min of running ↓ tPA ACT 33%, 44%, and 49% 20 min, 40 min, and 60 min post-exercise
Hilberg et al. (2003)	Blood coagulation and fibrinolysis after extreme short-term exercise	15 healthy trained males	Three maximal cycle ergometer tests with duration of 15, 45, and 90s and a control day without an exercise	↑ tPA AG 83%, 149%, and 293% after 15s, 45s, and 90s respectively Changes in tPA AG returned to baseline 1 h post-exercise
Hilberg et al. (2003)	Blood coagulation and fibrinolysis after long duration treadmill exercise controlled by anaerobic threshold	16 healthy males	Treadmill ergometer test at intensity of 90% of individual anaerobic threshold lasting 60-120 min	↑ tPA ACT 32% post exercise over baseline ↓ tPA ACT to baseline value 2 h post-exercise ↑tPA AG 737% immediately post exercise ↓ tPA AG 82% 2 h post-exercise from initial post-exercise value ↓PAI-1 ACT 69% post-exercise. ↑ PAI-1 AG 26% 2 h post-exercise over post-exercise value

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor

Table 2.2.The Effect of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Womack et al. (2003)	Coagulation and Fibrinolytic Response to Manual vs Automated Snow Removal	13 health male subjects	Subjects performed a maximal ramped treadmill test, shoved a driveway clear of snow, and removed snow from a driveway with an automated snow thrower	↑tPA AG 227% immediately post-exercise with treadmill test ↓PAI-1 31% immediately post-exercise with treadmill test
Cooper et al. (2004)	Temporal changers in tPA and PAI-1 after maximal exercise	8 healthy males	Subjects performed a graded exercise test to volitional exhaustion on a treadmill	↑ tPA ACT 19x from baseline to post-exercise ↑tPA AG 5x from baseline to post-exercise ↓PAI -1 ACT ≈55% from baseline to post-exercise
Paton et al. (2004)	Changes in von Willebrand factor and fibrinolysis following a post-exercise cool-down	10 healthy males	Subjects performed two maximal treadmill tests. Each test was followed by either an active cool down or a passive cool down	↑ tPA ACT and tPA AG ≈15x and ≈4x respectively Fibrinolytic potential returned to baseline 1 h post exercise

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor

Table 2.2.The Effect of Acute Exercise on Fibrinolytic Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Womack et al. (2006)	Changes in fibrinolysis following exercise above and below lactate threshold	15 healthy males	Subjects performed cycle ergometry tests >LT threshold and equicaloric cycle ergometry <LT	↑tPA ACT 145% in the >LT trial ↑tPA AG 71% and 22% in the >LT and <LT trials Post-exercise tPA AG 37% higher in >LT trial ↓PAI-1 ACT 33% and 13 % in both the >LT and <LT trials ↔ changes in tPA or PAI-1 levels 1 h post-exercise

tPA ACT= tissue-type plasminogen activator activity, tPA AG= tissue plasminogen activator antigen, PAI-1 ACT=plasminogen activator inhibitor, LT= lactate threshold

The Effects of Acute Exercise on Coagulation Potential

Exercise induces a hypercoagulant state (Davis et al., 1976; Wheeler et al., 1986; El-Sayed, 1993; Hedge et al., 2001). As with fibrinolytic activity, markers of coagulation rise substantially after the onset of exercise (Davis et al., 1976; Wheeler et al., 1986; Hansen et al., 1990; Van Den Burg et al., 1995; Van Den Burg et al., 1997). This rise in coagulation activity parallels the aforementioned rise in fibrinolytic potential up to 70% VO_2max (Van Den Burg et al., 1995). At higher intensities there is an even more pronounced increase in fibrinolytic potential that exceeds the increase in coagulant activity (Van Den Burg et al., 1995; Weiss et al., 1998). This suggests a reduced thrombotic tendency at these higher intensities.

Post-exercise there is a marked difference in the coagulant response as compared to the fibrinolytic response. As mentioned in the previous section, there is an immediate decrease in firbinolysis post-exercise with values rapidly returning to baseline (Weiss et al., 1998). However, with coagulant activity, markers either persistently rise (Wheeler et al., 1986, Weiss et al., 1997; Van Den Burg et al., 1997; Hilberg et al., 2003), remain elevated (Hedge et al., 2000; Paton et al., 2004), or decline slowly with levels remaining significantly elevated above baseline up to 10 hours later (Hansen et al., 1990).

The mechanisms responsible for the exercise-induced increase in coagulant activity are not fully understood. This is inherently due to the intricate nature of the coagulation cascade itself. However, some mechanisms may be described, albeit not fully. The increase in clotting activity, as measured by a fall in the activated partial thromboplastin time (aPPT), can be attributed to an increase in FVIII activity. Hedge et al. (2000) examined the clotting activity during the hour after a submaximal run at 70-75% VO_2max in 10 healthy men and observed that aPPT decreased by approximately 2 seconds and remained shortened during the entire 1 hour recovery period. They also observed that this shortening in aPTT was significantly correlated (r=-0.7) to increases in FVIII activity. Yet, the mechanism(s) for the increased FVIII activity are not fully understood. Since FVIII is composed to two proteins, factor VIII:C and Von Willebrand

Factor (vWF), vWF can be used to elucidate changes in coagulation potential as well. Von Kanel et al. (2003) demonstrated that beta-adrenergic stimulation and blockade respectively increases and inhibits the release of vWF from endothelial cells. Since exercise produces substantial elevations in epinephrine (Chandler et al., 1992), perhaps increased concentrations of catecholamines is the mechanism for enhanced coagulant activity. However, this is unlikely to be a lone mechanism as catecholamines do not display a continual increase post-exercise (Dimsdale et al., 1984). Another proposed mechanism is reduced hepatic blood flow. FVIII is cleared at the liver by a low-density lipoprotein-related receptor (Saenko et al., 1999). Paton et al. (2004) examined changes in vWF following a post-exercise cool-down in 10 healthy men. They observed that an active cool-down resulted in reduced levels of vWF post-cool down and 1 hour post-exercise. Since active cool-downs are used to prevent venous pooling and improve venous returns, this may suggest that hepatic clearance can play a role. However, hepatic blood flow returns to normal levels rapidly post-exercise (De Boer et al., 1992). Therefore, it seems unlikely that reduced hepatic clearance is a primary cause of the persistent coagulant activity post-exercise. Additional proposed hypotheses include the neogenesis of FVIII from the vascular endothelium (El-Sayed, 1993), increased thrombin activation (El-Sayed, 1993), and lactic acidosis (Crowell & Houston, 1961). Clearly future research is needed to elucidate the exact mechanisms of the exercise-induced enhancement in coagulant activity.

Table 2.3. The Effects of Acute Exercise on Coagulation Potential.

Study	Problem	Participants	Design	Finding(s)
Crowell & Houston (1961)	Effect of acidity on blood coagulation	15 dogs	Samples of heparinized blood had their pH altered, ranging from 6.0-7.4, via the addition of lactic acid to the sample	Lowering the pH of heparinized blood to ≈6.7 reduced clotting time
Davis et al. (1976)	Fibrinolytic changes during and after maximal exercise	10 healthy males	Subjects performed a graded exercise test to physical exhaustion	↔ FVIII at intensities <60-70% MHR ↑FVIII exponentially at intensities >70% MHR Peak ↑FVIII, 125%, occurred 5 min post-exercise
Dimsdale et al. (1984)	Changes in catecholamines postexercise	10 healthy males	Subjects performed a graded exercise test to exhaustion	↑ NEPI and EPI ≈880% and ≈241% 3 minutes post-exercise, respectively ↓NEPI and EPI ≈48% ≈36% from 3 min value by 6 min
Wheeler et al. (1986)	Pyhsiological changes in hemostasis associated with acute exercises	19 health male subjects	Subjects performed a branching multistage treadmill protocol to maximum	↑FVIII activity ≈180% post-exercise ↑FVIII activity ≈300% 8 min post-exercise as compared baseline value

F VIII= Factor VIII, FVIII= Factor VIII, NEPI= norepinephrine, EPI= epinephrine

Table 2.3 The Effect s of Acute Exercise on Coagulation Potential (Continued)

Study	Problem	Participants	Design	Finding(s)
Hansen et al. (1990)	Procoagulant and fibrinolytic activities in circulation after strenuous physical exercise	7 healthy recreationally active men	Subjects participated in short (1.7km), middle (4.8 Km) and long (10.5 km) runs at a speed close to their maximal capacity	↑vWF 194%, 95.9%, 95.9% in short, middle, and long runs, respectively, immediately post-exercise vWF remained ≈30%, 60%, and 80% elevated in short, middle, and long runs 10 h after exercise
El-Sayed (1993)	Hemostatic responses after resistance exercise	Seven healthy subjects	Subjects performed 3 exercise sessions: low volume resistance exercises at 90-100% 1RM, high volume resistance exercises at 70-80% 1RM, or no exercise control	↑ FVIII 100% post low volume resistance exercise ↑FVIII 160% post high volume resistance exercise
Van Den Burg et al. (1995)	Haemostatic changes following strenuous physical exercise	29 healthy sedentary men	Exercise test consisted of 4 phases on a cycle ergometer: 1) 25 minutes of cycling at 70% VO2max 2) graded cycling to VO2max, 3)10 min of active recover, 4) 15 minutes of passive recovery	Coagulant and fibrinolytic activity during submaximal exercise are = up to 70% VO_2max ↑ FVIII, FXII, FIX, and FVII 80%, 10%, 17%, and 4% over baseline at maximal exercise ↑Factor VIII 148% over baseline 25 min following maximal exercise ↓aPTT 15% over baseline at maximal exercise ↓aPTT16.3% over baseline 25 min following maximal exercise

vWF=von Willebrand factor, aPTT= Activated partial thromboplastin time, F VIII= Factor VIIIFVIII= Factor VIII, FXII= Factor XII, FIX= Factor IX, and, FVII= Factor VII,

Table 2.3 The Effects of Acute Exercise on Coagulation Potential (Continued)

Study	Problem	Participants	Design	Finding(s)
Van Den Burg et al. (1997)	Effect of endurance training on seasonal fluctuations on coagulation and fibrinolysis	20 healthy sedentary men	Exercise test consisted of 4 phases on a cycle ergometer: 1) 25 minutes of cycling at 70% VO2max 2) graded cycling to VO2max, 3)10 min of active recover, 4) 15 minutes of passive recover	↑ FVIII ≈100% and ≈126% immediately following maximal exercise and 25 min following maximal exercise, respectively ↓aPTT ≈14% and remained ↓to this extent 25 min later

F VIII= Factor VIII, aPTT= Activated partial thromboplastin time

Table 2.3. The Effects of Acute Exercise on Coagulation Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Weiss et al. (1998)	Coagulation and fibrinolysis after moderate and very heavy exercise	12 young men	Two treadmill tests at 68% and 83% VO_2max for 1 h	↑FPA ≈100%, and ≈155% over baseline after 30 and 60 min of running at 83% VO_2max run ↑FPA ≈144% over baseline 1 h post-83% VO_2max run ↑TAT ≈87% over baseline after 60 min of running at 83% VO_2max ↑TAT 47% over baseline 1 h post-83% VO_2max run ↓aPTT 8% and 13% from baseline 30 and 60 min post-83% VO_2max run ↓aPTT 11% from baseline 1 h post-83% VO_2max run Fibrinolytic system ↑ to a higher degree during heavy exercise than the coagulant system Fibrinolytic potential ↓ at faster rate than coagulation potential post-exercise period
Saenko et al., (1999)	Catabolism of factor VIII by LPR	Cultured mouse embryonic fibroblasts	Cell-mediate ligand internalization and degradation assays	FVIII specifically binds to LPR LPR mediates internalization and degradation of FVIII

aPTT= Activated partial thromboplastin time, TAT= Thrombin Antithrombin III Complex, FPA= Fibrinopeptide A, LPR= Low density lipoprotein receptor-related protein

Table 2.3. The Effects of Acute Exercise on Coagulation Potential (Continued).

Table 2.3. The Effects of Acute Exercise on Coagulation Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Hedge et al. (2000)	Changes in clotting and fibrinolytic activity during the 1 h after a submaximal run	10 healthy males	Subjects completed two treadmill tests at either 70-75% VO2max or at 1.2 mph for 30 minutes each.	↑ FVIII ≈40% after 30 min of running FVIII remained ≈40% elevated 1 h post-exercise ↓8% in aPTT immediately after submaximal run showed significant negative correlation (r=-0.7) with ↑ in FVIII
Von Kanel et al. (2000)	Effects of nonspecific β-adrenergic stimulation and blockade on blood coagulation in hypertension	I) 15 hypertensive subjects and 21 normotensive subjects II) 13 member subgroup (3 hypertensives)	I) Subjects received isoproternol infusion II) Subjects were studied twice, once after 5 days of placebo treat and once after 5 days of 100 mg/day of propranolol	Isoproternol infusion led to ↑ in plasma vWF across all subjects. Propranolol completely abolished the ↑ in wVF elicited by isoproternol
Hilberg et al. (2003)	Blood coagulation and fibrinolysis after extreme short-term exercise	15 healthy trained males	Three maximal cycle ergometer tests with duration of 15, 45, and 90s and a control day without an exercise	↓ aPTT 10%, 17%, and 20% after15s, 45s, and 90s. Changes in aPTT not fully reversed after 1 h ↑ TAT 53% immediately post-exercise Remained elevated 2 h post-exercise ↑F1+2 34% immediately post exercise

vWF=von Willebrand factor, aPTT= Activated partial thromboplastin time, F1+2= Prothrombin Fragment, FVIII= Factor VIII,

Table 2.3. The Effects of Acute Exercise on Coagulation Potential (Continued).

Study	Problem	Participants	Design	Finding(s)
Hilberg et al. (2003)	Blood coagulation and fibrinolysis after long duration treadmill exercise controlled by anaerobic threshold	16 healthy males	Treadmill ergometer test at intensity of 90% of individual anaerobic threshold lasting 60-120 min	↓aPTT 14% and 11% immediately post-exercise and 2 h post-exercise, respectively, as compared to baseline ↑ TAT 53% immediately post-exercise ↑F1+2 34% and 16% immediately post exercise and 2 h post-exercise respectively
Paton et al. (2004)	Changes in von Willebrand factor and fibrinolysis following a post-exercise cool-down	10 healthy males	Subjects performed two maximal treadmill tests. Each test was followed by either an active cool down or a passive cool down	↑vWF: ≈ 120% and ≈88% post-cooldown and 1 h post-exercise, respectively, as compared to baseline
Womack et al. (2003)	Coagulation and Fibrinolytic Response to Manual vs Automated Snow Removal	13 health male subjects	Subjects performed a maximal ramped treadmill test, shoved a driveway clear of snow, and removed snow from a driveway with an automated snow thrower	↑vWF 76% immediately post-exercise with treadmill test

vWF=von Willebrand factor, aPTT= Activated partial thromboplastin time, F1+2= Prothrombin Fragment, TAT= Thrombin Antithrombin III Complex

Methodological Considerations for Fibrinolytic Research

Participants are an important methodological consideration for conducting research involving fibrinolysis. Research has shown that activation of the fibrinolytic system varies across the menstrual cycle (Cederblad et al., 1977; Giardina et al., 2004). Therefore, one either needs to avoid using female participants or control for differences in the menstrual cycle status. The physical activity status of the participants also needs to be taken under consideration. Physically active men tend to have lower resting PAI-1 values and slightly higher tPA activity values post-exercise as compared to physically inactive men (Symanski & Pate, 1994).

A second point to consider is time of day. There are significant circadian variations in the fibrinolytic system (Angleton et al., 1988). PAI-1 activity and tPA antigen are highest in the morning compared to the evening. In contrast, tPA activity is lowest in the morning and highest in the evening. The fibrinolytic response to physical activity is also influenced by time of day as physical activity in the evening elicits a substantially greater fibrinolytic response than physical activity in the morning (Symanski & Pate, 1994). Therefore, to avoid time day confounding the data, it is necessary have each of a participant's treatment trials occur at the same time of day.

Posture must also be standardized when conducting experiments involving fibrinolytic potential. Winther & colleagues (1992) examined the effects of posture on fibrinolytic potential in 12 healthy men. Participants went from the supine to upright posture, the upright to supine posture, and the supine to upright posture across the time course of a morning. Participants held the remained in the new posture after each switch for 90 minutes. Significant variations in tPA antigen levels were noticed 10 minutes following the supine to upright and upright to supine posture shifts. No statistical differences were seen in either postural shift after the new posture had been assumed for the next time point (90 minutes). This suggests that when evaluating tPA antigen levels, it is necessary to control for postural effects by standardizing pre-trial positions for a minimum of greater than 10 minutes.

The timing of post-exercise blood sampling is also an important methodological consideration as the half-life of tPA is approximately 3 minutes (Korninger et al., 1981; Nilsson et al., 1984) and fibrinolytic activity declines post-exercise have been noted in as little as 5 minutes (Davis et al., 1976). A study by Cooper et al. (2004) examined the temporal changes in tPA and PAI-1 after maximal exercise. tPA activity did not change from 1 to 2 minutes post-exercise but decreased significantly by 4 minutes post-exercise. They also observed PAI-1 decrease from pre to post-exercise but PAI-1 values did not change during 10 minutes post-exercise. This suggests that to accurately assess the tPA response to acute exercise, blood samples need to be collected within 2 minutes after the cessation of exercise.

Table 2.4. Methodological Considerations for Fibrinolytic Research

Study	Problem	Participants	Design	Finding(s)
Davis et al. (1976)	Fibrinolytic changes during and after maximal exercise	10 healthy males	Subjects performed a graded exercise test to physical exhaustion	↓FA ≈15% from post-exercise maximum by 5 min post-exercise
Cederblad et al. (1977)	Variations in blood coagulation and fibrinolysis across the menstrual cycle	30 normal women	Blood samples collected across the menstrual cycle	FA is greatest during menstruation and luteal phase FA is the smallest during the follicular phase and ovulatory phase
Korninger et al. (1981)	Turnover of Human tPA	7 New Zealand white rabbits	Injected tPA from purified cell culture fluids radiolabelled with ^{125}I	Half-life of injected tPA is ≈2-3 min tPA is mainly cleared by the liver
Nilsson et al. (1984)	In vivo metabolism of human tPA	2 healthy males	Injected tPA from purified cell culture fluids radiolabelled with ^{125}I	Half-life of injected tPA is ≈3-4 min tPA is mainly cleared by the liver
Angleton et al. (1989)	Diurnal variation on fibrinolytic potential	I) 33 healthy men II) 15 patients with previous MI or unstable angina	Resting blood samples were taken in the morning and evening in groups I and II.	↓PAI-1 ACT 44% and 48% in groups I and II between morning and evening ↑ tPA ACT 80% and 113% in groups I and II between morning and evening ↓tPA AG 15% and 23% in groups I and II between morning and evening

FA= Fibrinolytic Activity, tPA ACT= Tissue Plasminogen Activator Activity, tPA AG= Tissue Plasminogen Activator Antigen, PAI-1 ACT= Plasminogen Activator Inhibitor 1 Activity, PAI-1 AG= Plasminogen Activator Inhibitor Antigen, FA= Fibrinolytic Activity

Table 2.4. Methodological Considerations for Fibrinolytic Research (Continued).

Study	Problem	Participants	Design	Finding(s)
Winther et al. (1992)	Effects of upright posture on fibrinolytic activity	12 male volunteers	Subjects assume the upright posture for 90 min, lay supine for 45 min, and then returned to upright posture for 90 min.	↑tPA AG 64% by moving from a supine posture to an upright posture ↓tPA AG 17% by moving from upright to supine posture ↑FA 27% in upright posture versus supine posture
Szymanski & Pate (1994)	Fibrinolytic responses to moderate exercise in physically active and inactive men Fibrinolytic responses during morning and evening exercise	I) 14 inactive men II) 12 active men	Two 30 minute submaximal exercise sessions at 50% of VO2max	↑ tPA ACT ≈71% with evening exercise in active group ↑tPA ACT ≈28% with morning exercise in active group ↑ tPA ACT ≈25% with evening exercise in inactive group ↑tPA ACT ≈8% with morning exercise in inactive group ↔ in PAI-1 ACT with exercise in either group at either time point

tPA ACT= Tissue Plasminogen Activator Activity, tPA AG= Tissue Plasminogen Activator Antigen, PAI-1 ACT= Plasminogen Activator Inhibitor 1 Activity, PAI-1 AG= Plasminogen Activator Inhibitor Antigen, FA= Fibrinolytic Activity

Table 2.4. Methodological Considerations for Fibrinolytic Research (Continued).

Study	Problem	Participants	Design	Finding(s)
Cooper et al. (2004)	Temporal changers in tPA and PAI-1 after maximal exercise	8 healthy males	Subjects performed a graded exercise test using a treadmill ramped protocol until volitional exhaustion	↑tPA ACT 19x from pre-exercise to 1 min post-exercise period ↔ tPA ACT from 1 to 2 min post-exercise tPA ACT↓ significantly by 4th min post-exercise as compared to 1st min post-exercise value ↓PAI-1 ACT 56% from pre-exercise to 1 min post-exercise. ↔ changes observed in PAI-1 ACT from 1 min post-exercise to any other post-exercise time point
Giardina et al. (2004)	Variability of Hemostatic and Fibrinolytic Factors in Young Women	20 pre-menopausal women	Underwent blood testing across the menstrual cycle	Difference in amount of estradiol between follicular and luteal phase mean Δ= 30 pg/mL Change in estradiol associated with change in PAI-1, mean Δ= 14 ng/mL Change in estradiol associated with change in D-Dimer, mean Δ=57 ng/mL

tPA ACT= Tissue Plasminogen Activator Activity, tPA AG= Tissue Plasminogen Activator Antigen, PAI-1 ACT= Plasminogen Activator Inhibitor 1 Activity, PAI-1 AG= Plasminogen Activator Inhibitor Antigen, FA= Fibrinolytic Activity

Chapter Three

Methods

Participants

Twenty trained males, between the ages of 18-25, from the James Madison University community and the surrounding Harrisonburg area, agreed to take part in this study. Two of these subjects dropped out of the study. The remaining 18 completed both treatment trials. Subject characteristics are summarized in table 3.1. Exclusion criteria included: any known cardiovascular, pulmonary, or metabolic disease; current tobacco use; current use of any medication known to influence fibrinolysis; infection; fever, or illness within 2 weeks prior to testing; garlic or flour allergy; and any other medical condition that could compromise safety. The questionnaire used to determine this information can be found in Appendix A. Participants were provided with written and verbal information about the experimental procedures, including potential risks, prior to completing the informed consent form (Appendix B). All procedures were approved by the James Madison University Committee on Research Involving Human Subjects prior to testing.

Table 3.1: Subject Characteristics

	Age (yrs)	Height (cm)	Weight (kg)	BMI
Mean ± SD	20.9 ± 2.2	178 ± 7.7	75.5 ± 9.6	23.8 ± 2.3

Determination of Training Status

The short-form International Physical Activity Questionnaire (IPAC) was administered prior to testing (Appendix C). The IPAQ short form has acceptable reliability and criterion validity in men (Kurtze et al., 2008). The criterion for being considered trained in the current study was: A) 3 or more days of vigorous-intensity activity of at least 20 min per day or B) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-min*wk^{-1}.

Supplementation

Participants were given a packet containing either the placebo treatment (PT), which consisted of three gel capsules containing flour, or the garlic treatment (GT), which consisted of three 300 mg capsules of Kyolic Brand powdered aged garlic extract (Mission Viejo, CA). These treatment packets were administered in a randomized, double-blind, cross-over fashion to ensure that an equal number of participants performed the placebo treatment and the garlic treatment first. Participants were instructed to ingest all of the tablets with a glass of water three hours prior to testing. Participants were also required to fast for 12 hours and abstain from alcohol consumption for 24 hours prior to testing. Adherence to the dietary restrictions was confirmed by a 24 hour food recall questionnaire administered prior to testing. All tests were performed before 10AM to minimize the effect of diurnal variations (Angleton et al., 1989) and each participant was tested at the same time of day for both trials.

Exercise Tests

Treatment Trials

Participants reported to the Human Performance Laboratory on the campus of James Madison University's in the morning after having supplemented with 900 mg of powdered garlic or a placebo. To control for any postural effects on fibrinolysis, participants assumed a semi-recumbent position for 20 min prior to obtaining the baseline blood sample (Winther et al., 1992). Participants then completed a treadmill graded exercise test to volitional fatigue. Post-exercise blood samples were taken within two minutes of the cessation of exercise to avoid an immediate decline in tPA (Cooper et al, 2004). The final blood samples were taken one hour after exercise. Participants repeated the protocol with the other treatment two weeks later.

Maximal Exercise Test

Each participant performed a graded treadmill test on a Stairmaster Clubtrack 612 treadmill (Kirkland, WA). The test began at an initial speed of 2.5 mph. Speed was then increased at a rate of 0.5 mph min^{-1} until 6.0 mph was reached. At this point, the treadmill speed remained

constant while the elevation increased at a rate of 3.0% * min^{-1} until 15.0% grade was reach. Speed was then increased once again at a rate of 0.5 mph min^{-1} until volitional exhaustion. VO_2 was continuously monitored with a Sensormedics Spectra metabolic cart (Yorba Linda, CA). This protocol has been previously shown to elicit a large fibrinolytic response (Paton et al., 2004; Cooper et al., 2004).

Blood Sampling

For each blood sample 5 ml of venous blood was collected in a tube treated with acidified citrate (Biopool International; Ventura, Calif) and an additional 5 ml of blood was collected in an EDTA tube. Whole blood collected in EDTA was analyzed for hematocrit using the microhematocrit method. The remainder of the EDTA sample and the blood collected in an acidified citrate tube were spun in a refrigerated centrifuge (4°C) at 10,000 rpm for 20 min to obtain platelet-poor plasma. Plasma was alliquoted and stored at -80°C until assayed. Fibrinolytic potential was determined by assaying for tPA activity, tPA antigen, and PAI-1 activity. tPA activity was determined by using an amidolytic activity assay (Biopool International; Ventura, Calif). tPA antigen and PAI-1 activity were determined using an enzyme linked immunosorbancy assay (ELISA) (American Diagnostica, Greenwich, Conn and Diapharma respectively). All values for tPA and PAI-1 were corrected for plasma volume changes (Van Beaumont et al., 1972).

Dietary and Exercise Controls

Throughout the duration of the study participants were instructed to: 1) choose a standard "self-selected" meal plan for the 24 hours preceding the two treatment trials, 2) consume the last meal of the day no later than 12-hours prior to the treatment trials, 3) avoid consumption of alcohol 24-hours prior to each treatment trial, and 4) avoid exercise immediately prior to each treatment trial. Compliance with the dietary restrictions was confirmed via the administration of a 24 hour food re-call questionnaire prior to teach treatment trial. Compliance was defined as a 75% match between the garlic and the placebo trials' food logs. Twelve of the 18 participants met

this criterion. However, the participants who did not meet this criterion did not report eating garlic the day prior to testing. Compliance to the exercise restrictions was confirmed verbally before each treatment trial. Participants were allowed to drink water *ad libitum* throughout the duration of the study.

Statistical Analysis

A two-factor (treatment and time) repeated measures analysis of variance (ANOVA) was used to assess changes in tPA activity, tPA antigen, and PAI-1 activity. The two within-subject factors were treatment condition (placebo trial (PT) and garlic trial (GT)) and time (pre-exercise, post-exercise, and 1 hour post-exercise). Post hoc tests were performed using two-tailed t-tests with a bonferroni correction factor. Differences in participant weight, resting hematocrit, VO_2max, respiratory exchange ratio (RER), and treadmill time between the two treatment conditions were assessed via two-tailed paired t-tests. An alpha level of $P<0.05$ was set as the a priori level of statistical significance.

Chapter Four

Results

Exercise Performance

No significant difference ($P > 0.05$) was found between the two treatment conditions for weight [PT = 75.5 ± 9.6 kg, GT = 75.4 ± 9.8 kg], resting hematocrit [PT = 42.4 ± 10.9%, GT = 44.9 ± 2.6%] or treadmill time [PT = 14.23 ± 1.3 min, GT = 14.15 ± 1.4 min]. One participant's nose clip fell off during a trial and was therefore excluded from the VO_2max analysis. A significant difference ($P < 0.05$) was found between the two treatment conditions for absolute VO_2max [PT = 4.48 ± 0.47 L min^{-1}, GT = 4.61 ± 0.59 L min^{-1}] and relative VO_2max [PT = 59.8 ± 6.7 ml kg^{-1} min^{-1}, GT = 61.4 ± 6.6 ml kg^{-1} min^{-1}]. No significant difference ($P > 0.05$) was found between the two treatment conditions for respiratory exchange ratio (RER) at VO_2max [PT = 1.13 ± 0.05, GT = 1.13 ± 0.05].

Fibrinolytic Potential

Two participants' post-exercise blood samples were not obtained during the 2-min post-exercise timeframe. These participants were excluded from the analysis of fibrinolytic variables. One participant was a consistent outlier (greater than two standard deviations above the mean) for tPA antigen and PAI-1 activity and was excluded from the analysis of fibrinolytic variables. A Shapiro-Wilk test demonstrated that the data was not normally distributed for tPA antigen or PAI-1 activity for at least one time point. These data were log-transformed prior to ANOVA. Further analysis revealed that PAI-1 activity was still abnormally distributed after the log-transformation. The next highest outlier was removed from the analysis of fibrinolytic variables and normalcy was then achieved for PAI-1. The final sample size was 14 participants.

Figure 1 and 2 displays the mean tPA activity and tPA antigen response for the PT and GT. A significant ($P < 0.000$) main effect was observed for time for tPA activity. tPA activity significantly increased from pre-exercise [PT = 0.74 ± 0.08 IU ml^{-1}, GT = 0.76 ± 0.07 IU ml^{-1}] to

post-exercise [PT = 19.5 ± 2.8 IU ml^{-1}, GT = 19.8 ± 3.3 IU ml^{-1}]. Compared to pre-exercise values, there was no elevation in tPA activity 1-h post-exercise [PT = 0.69 ± 0.10 IU ml^{-1}, GT = 0.69±0.06 IU ml^{-1}]. There was no main effect for treatment (P = 0.91) and no treatment x time interaction (P = 0.99) for tPA activity. A significant (P < 0.000) main effect for time was observed for tPA antigen. tPA antigen significantly increased from pre-exercise [PT = 3.6 ± 0.46 ng ml^{-1}, GT = 4.8 ± 1.0 ng ml^{-1}] to post-exercise [PT = 15.4 ± 1.8 ng ml^{-1}, GT = 20.0 ± 3.1 ng ml^{-1}]. One hour post-exercise tPA antigen was reduced to a level not significantly different than pre-exercise values [PT = 4.0 ± 0.52 ng ml^{-1}, GT = 4.8 ± 0.80 ng ml^{-1}] There was no main effect for treatment (P = 0.16) and no treatment x time interaction (P = 0.89) for tPA antigen.

Figure 3 displays the mean PAI-1 activity response for the PT and GT. A significant (P < 0.000) main effect was observed for time. PAI-1 activity significantly decreased from pre-exercise [PT = 0.63 ± 0.10 ng ml^{-1}, GT = 0.92 ± 0.22 ng ml^{-1}] to post-exercise [PT = 0.16 ± 0.05 ng ml^{-1}, GT = 0.32 ± 0.13 ng ml^{-1}]. There was no difference in pre-exercise PAI-1 activity and PAI-1 activity 1-h post-exercise [PT = 0.88 ± 0.17 ng ml^{-1}, GT = 0.97 ± 0.21 ng ml^{-1}]. There was no significant main effect for treatment (P = 0.40) and no treatment x time interaction (P = 0.617) for PAI-1 activity.

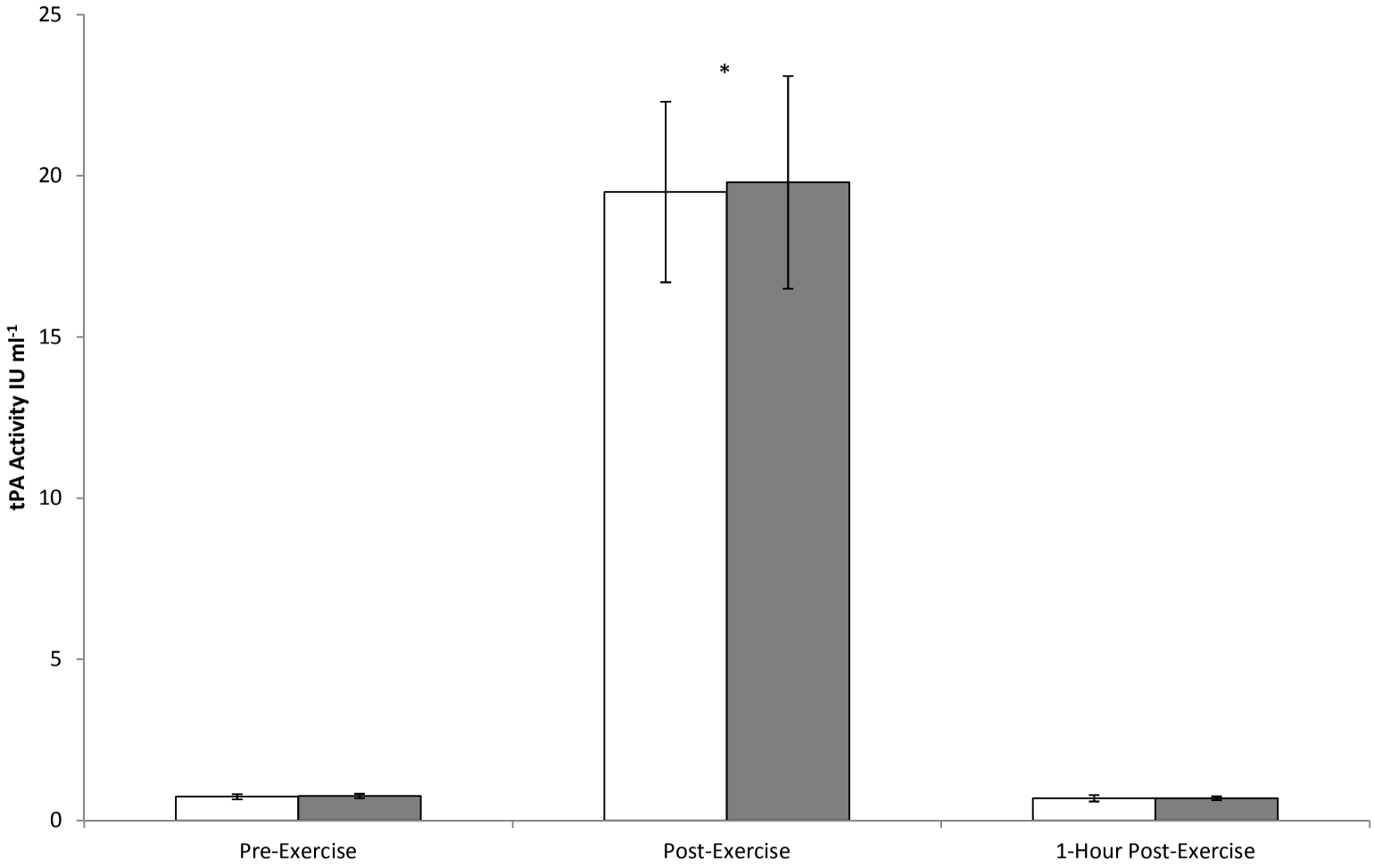

Figure 1. Placebo (white bars) and garlic (gray bars) mean (SE) plasma levels of tPA Activity (IU ml^{-1}) pre-exercise, post-exercise, and 1-hour post-exercise. *- Significantly higher than pre-exercise (P<0.05).

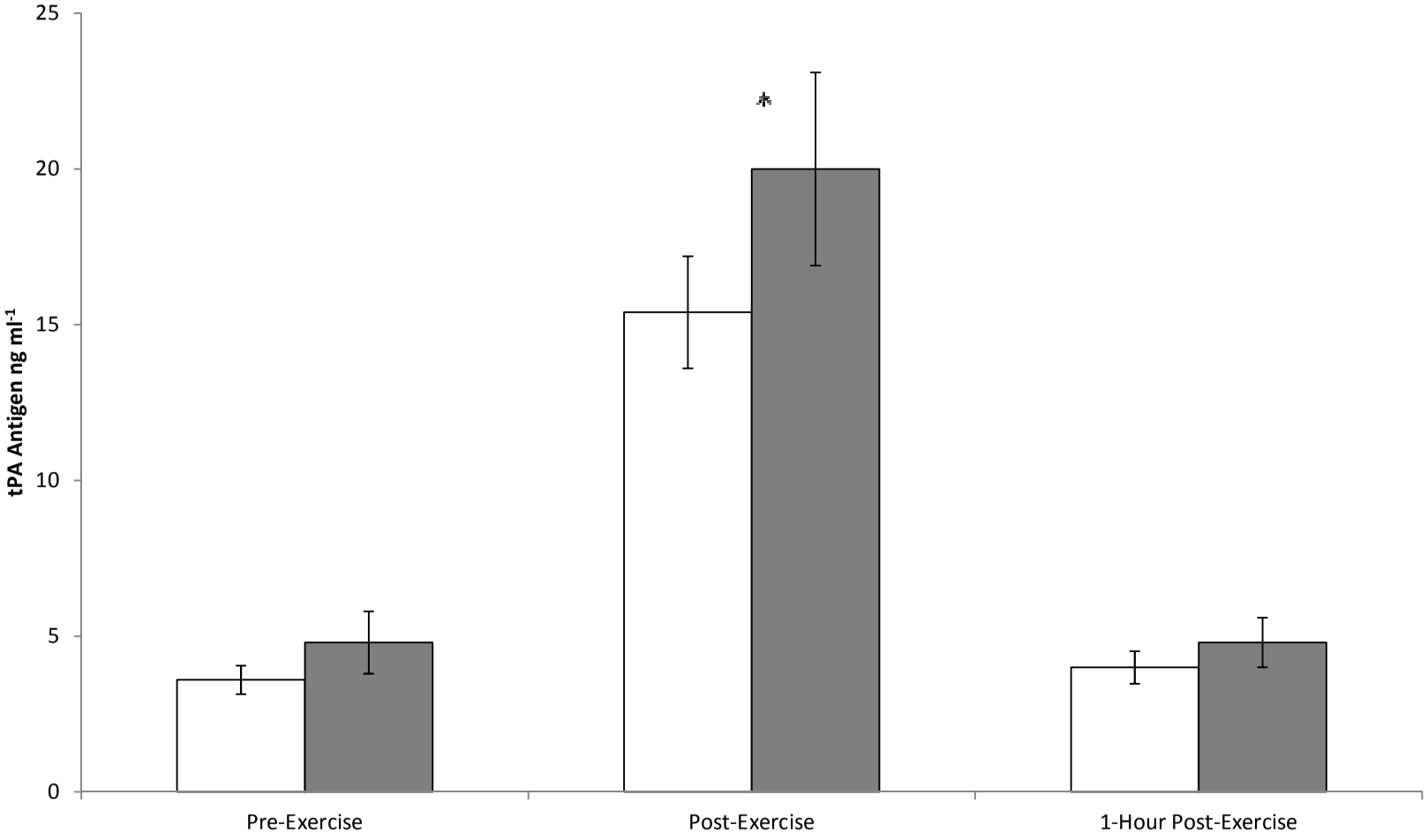

Figure 2. Placebo (white bars) and garlic (gray bars) mean (SE) plasma levels of tPA antigen (ng ml^{-1}) pre-exercise, post-exercise, and 1-hour post-exercise. *- Significantly higher than pre-exercise (P<0.05).

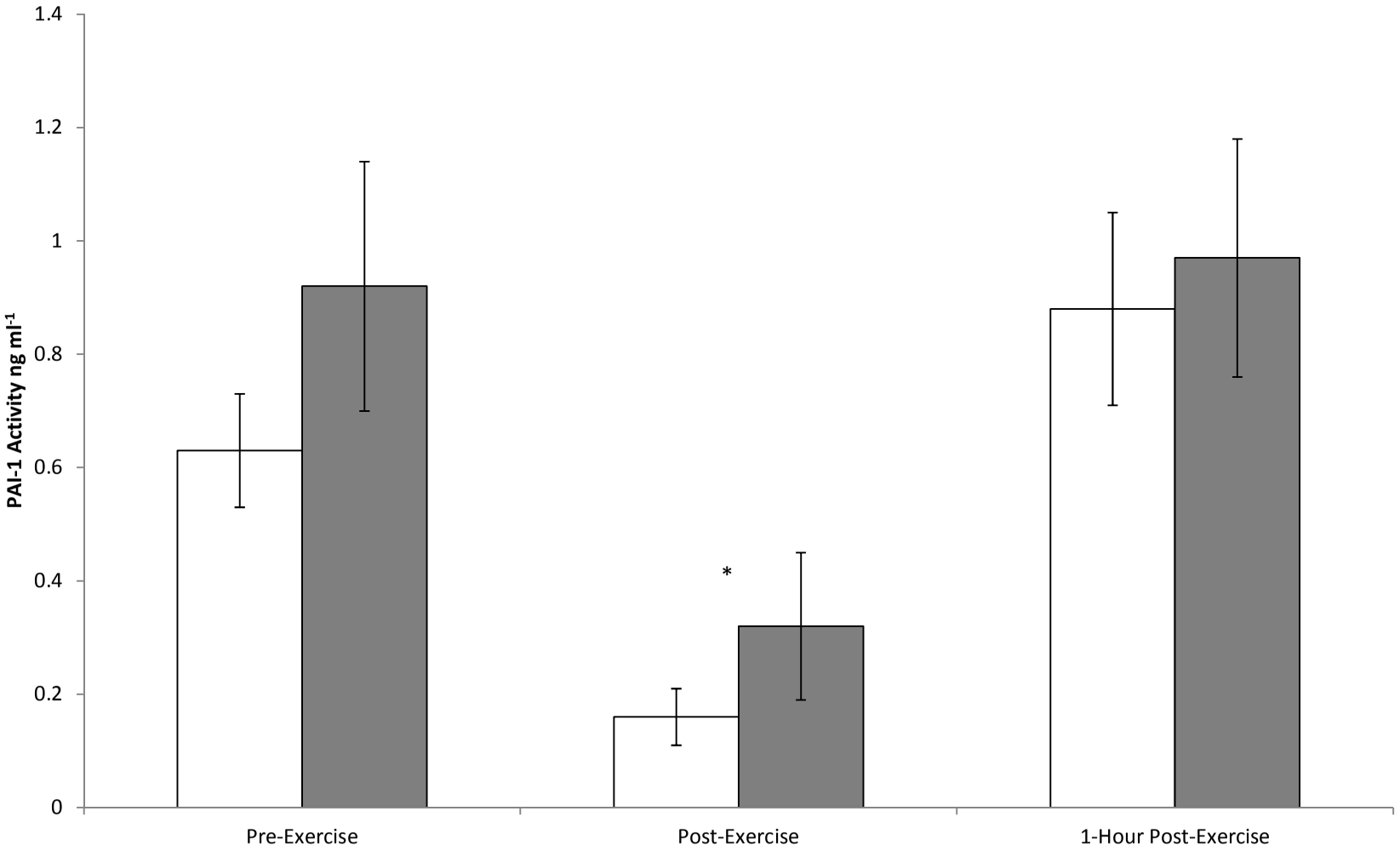

Figure 3. Placebo (white bars) and garlic (gray bars) mean (SE) PAI-1 activity (ng ml^{-1}) pre-exercise, post-exercise, and 1-hour post-exercise. *- Significantly higher than pre-exercise (P<0.05).

Chapter Five

Discussion

The primary finding of this study is that acute garlic supplementation does not improve the fibrinolytic potential or the fibrinolytic response to exercise in young, healthy, endurance-trained men. These results support the findings of another randomized, placebo controlled, double blind study by Legnani and co-workers (1993) where no improvements in tPA activity were observed with acute garlic supplementation. Both studies had a young, healthy, participant pool which supplemented with 900 mg of garlic and had their fibrinolytic profile examined after approximately 4 hours.

In contrast, several studies have described enhancement of the fibrinolytic system within a few hours after the consumption of garlic. Bordia and colleagues (1975) measured global fibrinolytic activity, using the euglobulin clot lysis method, following the administration of either 50 grams of garlic along with 100 grams of butter or only 100 g of butter. After 3 hours there was an observed 15% increase in fibrinolytic activity in the garlic trial but an observed 49% decrease in fibrinolytic activity in the butter only trial. Chutani & Bordia (1981) found that fibrinolytic activity, also measured via the euglobulin clot lysis method, increased 72% and 62% after the administration of raw and fried garlic, respectively. Moreover, they saw the enhancement maintained for up to 12 hours. Kiesewetter et al. (1990) measured fibrinolytic potential following acute garlic supplementation and observed significant increases in tPA activity five hours after the administration of 300 mg and 600 mg of garlic (56% and 51%, respectively). Kiesewetter et al. (1990) also observed 600 mg of garlic to produce significant elevations in tPA activity in all participants. Jung and coworkers (1991) measured fibrinolytic potential following acute garlic supplementation and observed about an 86% increase in tPA activity after five hours.

The current study did not confirm these findings, possibly due to differences in the form of garlic administered. Bordia et al. (1975) and Chutani & Bordia (1981) administered raw garlic. The main sulfur compound in raw garlic is alliin (Amagase et al., 2001). When garlic is cut or crushed, the enzyme alliinase rapidly lyses aliin to another sulfur compound known as aliicin (Block, 1985). Alliicin is extremely unstable and cannot be detected in the blood or urine after the consumption of raw garlic (Lawson et al., 1992). Allicin, perfused into rat livers, shows a large first-pass effect whereby allicin is converted to the metabolite diallyl disfulfide (DADS) (Egen-Schwind et al., 1992). Similar metabolites of allicin include the sulfides diallyl sulfide (DAS) and diallyl trisulfide (DAT) (Amagase et al., 2001). Kiesewetter et al. (1990) and Jung et al. (1991) administered commercial preparations of powdered garlic that had a standard 1.3% alliin content. Garlic powder retains the same ingredients as raw garlic and, when exposed to water, allinase rapidly converts alliin to allicin and subsequently the metabolites DAS, DADS, and DAT (Amagase et al., 2001). Like allicin, these sulfur containing compounds are metabolized quickly (Lawson et al., 1992), and therefore, they seemed unlikely to be the active ingredient that enhanced the fibrinolytic system acutely. The supplement used in the present study, is a form of aged garlic extract (AGE) that is standardized to s-allyl cysteine (SAC). SAC is formed from the catabolism of γ-glutamyl cysteine, a sulfur containing compound separate from alliin (Amagase et al., 2001). SAC is a stable, odorless, and water soluble compound garlic (Amagase et al., 2001). Unlike DADS, SAC can be detected in the plasma, liver, and kidney after the oral ingestion (Nagae et al., 1994). Because it is detectable in the blood and increases quantitatively following the oral intake of garlic, it is used as a compliance marker in human studies (Steiner & Li, 2001). SAC is also found in small quantities in raw garlic and garlic powder (Amagase et al., 2001). Thus, it is possible that differences in the active ingredient alter the hemostatic changes that occur with this form of garlic supplementation.

In addition to feeding 50 grams of garlic along with 100 grams of butter to 10 healthy men, Bordia and coworkers (1975) also extracted garlic's essential oil from 50 g of garlic and

then administered the essential oil to the same participant population. After 3 hours there was an observed 15% increase in fibrinolytic activity. The essential oil of garlic is commonly obtained by one of two methods. The first method is steam distillation whereby whole garlic is put into boiling water and the rising steam from the vessel is captured (Block, 1985). The second method involves grinding whole garlic cloves, adding them to water, and then having the oil fraction extracted in an organic solvent (Block, 1985; Amagase et al., 2001). Both of these processes completely eliminate the water-soluble components of garlic, such as SAC, but yield DAS and DADS (Block, 1985; Amagase et al., 2001). This suggests the acute enhancement in fibrinolytic potential by garlic may be derived from DAS and DADS rather than SAC. Further research should be conducted to better understand the relationship between the sulfur metabolites of alliin and their effects on fibrinolytic potential. If the sulfur metabolites of alliin are the active compounds in garlic which enhance fibrinolytic potential acutely, then inconsistencies in the literature would be explained as these metabolites are difficult to standardize and metabolize quickly. For instance Legnani & co-workers (1993) administered a comparable dosage of the same commercial garlic preparation to a comparable young, healthy, participant sample across a similar time course as Kiesewetter et al. (1990) and Jung et al. (1991), yet, could not confirm their results.

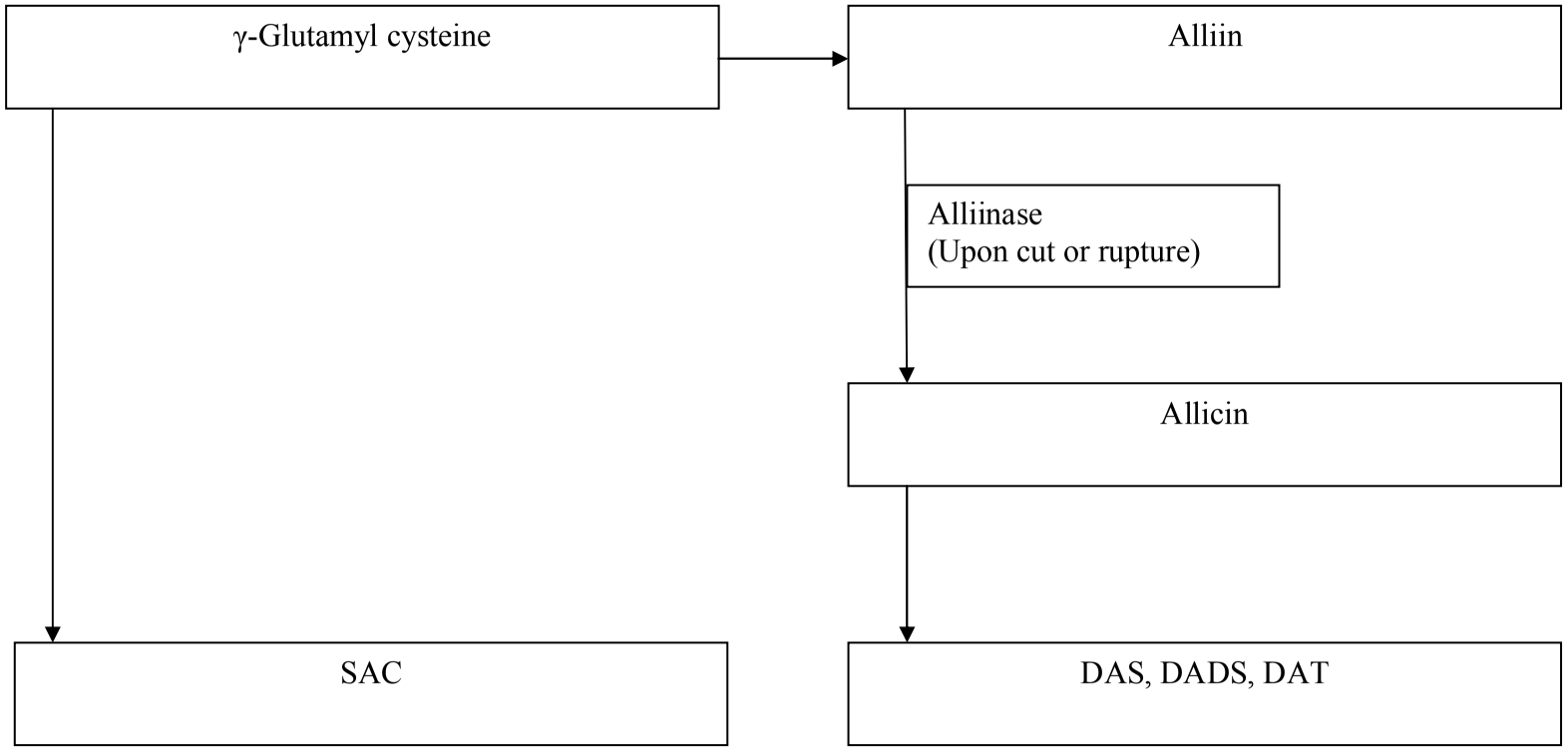

Figure 5.1: The chemical changes in two sulfur compounds of garlic. Garlic contains γ-glutamyl cysteine. Through the process of hydrolysis and oxidation, γ-glutamyl cysteine can be converted to alliin. After cutting or rupturing of the garlic bulb, alliinase rapidly lyses alliin to allicin. Allicin decompose rapidly to the other sulfur containing compounds diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DAT). γ-glutamyl cysteines may also be converted to S-allylcysteine (SAC) via a separate pathway.

It is unclear as to whether or not our selected participant population contributed to the lack of an observed effect. Bordia & colleagues (1975) used a population of ten healthy males between the ages of 30-50 years and observed a 15% increase in fibrinolytic activity three hours after the ingestion of garlic. Chutani & Bordia (1981) used an older population of males, mean age 47.9 years, with a history of coronary artery disease and observed a 72% increase in fibrinolytic activity 6 hours after the ingestion of garlic. These two studies suggest age may have precluded an observed enhancement in fibrinolysis in the present study. In addition, the findings of Chutani & Bordia (1981) suggest a difference in health status may also have contributed to our observed lack of an effect of garlic. However, the comparison of our study's participants to the participants in these studies is problematic. The outcome measure employed by Bordia & colleagues (1975) and Chutani & Bordia (1981) was the euglobulin clot lysis time, which does not directly measure changes in fibrinolytic variables. Two of the studies which measured tPA activity did observe an increase in plasma tPA activity in a participant population similar to ours. Kiesewettier & co-workers (1990) examined ten apparently healthy participants, three women and seven men, with a mean age of 26.9 years and observed about a 50% increase in tPA activity after 5 hours. Jung & co-workers (1991) also examined ten apparently healthy participants, seven men and three women, with a mean age of 26.9 years and observed about an 86% increase in tPA activity after 5 hours. However, Legnani & coworkers (1993) also examined ten apparently health participants, three women and seven men, with a mean age of 25.5 years and did not observe an enhancement in tPA activity or tPA antigen or a decrease in PAI-1 activity after 2,4, or 6 hours. No details were given about the cardiorespiratory function of the participants in either the Kiesewettier & co-workers (1990) study the Jung & co-workers (1991), or the Legnani & coworkers (1993) study. Future research should be conducted to better evaluate the relationships between age, training status, and the effects of acute garlic supplementation on fibrinolytic potential.

An unexpected secondary finding of the present study is that acute garlic supplementation causes a slight but significant increase in VO_2max in young, healthy, trained males without significantly increasing their treadmill time. Of the 17 participants whose gas exchange data was analyzed, 13 of them achieved a higher relative VO_2max during the garlic trial than during the placebo trial. The average relative VO_2max was 59.8 ml kg^{-1} min^{-1} and 61.4 ml kg^{-1} min^{-1} for the placebo and garlic trials, respectively. Ince & colleagues (2000) administered a single dose of garlic to 10 college aged endurance athletes. Five hours after the ingestion of the garlic tablets, subjects underwent an incremental treadmill test according to the Bruce protocol. A significant difference was observed for VO_2max, 57.3 ml kg^{-1} min^{-1} for the garlic trial and 55.6 ml kg^{-1} min^{-1} for the placebo trial, and treadmill time, 1033.6 s for the garlic trial and 990 s for the placebo trial. It was speculated that the observed differences between the garlic and placebo trials were due to improvements in the fluidity of the blood. They argue for a "rheological advantage" whereby whole-blood viscosity is decreased due to garlic-induced reductions in fibrinogen concentration. It was suggested that this reduction in fibrinogen concentration translates into better oxygen supply to the working musculature. Fibrinogen concentration was not measured by Ince & colleagues (2000) nor was it measured in the current study. But, Stuart & Kenny (1980) suggest hematocrit is the single most important determinant of whole-blood viscosity and in the present study there was no significant difference observed in hematocrit values at any of the time point across the two trials.

Nitric oxide (NO) is an important cellular messenger involved in the functioning of the cardiovascular system which is synthesized from L- arginine by NO synthases (NOS) found in endothelial cells, smooth muscle cells, and platelets (Moncada et al., 1991). A special type of NOS, called endothelial constitutive (cNOS), is responsible for vasomotor tone (Moncada et al., 1991). When cNOS is stimulated, NO is released from the endothelium (Kerwin et al., 1995). This causes a rapid and transient relaxation of arteries and veins to improve flow through vessels (Ignarro et al., 1991). Morihara & coworkers (2002) investigated the effect of AGE on NO

production via measurement of NO metabolites in the plasma of mice. An increase in NO production by 30-40% was observed from 15 to 60 minutes following the administration of AGE. It was also noted that a selective cNOS inhibitor, given to the mice prior to AGE, neutralized the enhanced NO production. This indicates that AGE increases NO production by activation of cNOS. In a subsequent study by Morihara & coworkers (2006), they examined the effects of AGE on NO production in rats engaging in repeated endurance exercise on a mechanical treadmill 5 times per week for 4 weeks. AGE significantly increase NO metabolites 2-fold as compared to the exercising control group which did not receive AGE. These studies suggest that AGE enhances NO production resulting in dilation of blood vessels. This may in turn enhance skeletal muscle perfusion and increase VO_2max values. However, unpublished work from our lab did not find enhanced flow mediated dilation in the brachial artery following garlic supplementation, suggesting that it did not affect endothelium-derived NO. However, others have observed enhanced vasodilation with acute garlic supplementation. Jung et al. (1991) observed an increase in the diameter of erythrocyte column by 8.6% due to the vasodilation of pre-capillary arterioles. Similarly, Wolf et al. (1990) observed a slight dilation of conjunctival vessels due to garlic supplementation. Consequently AGE induced enhancement in vessel diameter may be limited to the microcirculation only.

Interestingly, Morihara & coworkers (2006) also examined the impact of AGE on succinate dehydrogenase (SDH) activity. SDH is a key enzyme involved in aerobic metabolism as it plays a role in both the Kreb's cycle and the electron transport chain (Oyedotun & Lemire, 2004). AGE significantly increased SDH activity by 40% in the gastrocnemius muscle of the rats engaging in repeated endurance exercise on a mechanical treadmill 5 times per week for 4 weeks as compared to the control exercising rats which did not receive AGE. This elevation in SDH activity may indicate AGE enhances aerobic metabolism of skeletal muscle during exercise, which in turn may account for the increase in VO_2max observed in the present study.

The functional importance of the observed improvements in VO_2max is uncertain. The present study found a 1.6 ml kg^{-1} min^{-1} increase in VO_2max without a corresponding improvement in treadmill time. Conversely, Ince & colleagues (2001) observed a 2 ml kg^{-1} min^{-1} increase in VO_2max as well as a 53.6 s increase in treadmill time. Both studies used groups of healthy trained college aged males. Future studies should seek to replicate the present findings to see if the lack of improved treadmill time persists.

Some limitations of the present study exist. The exact time course of garlic's enhancement of the fibrinolytic system is not known. Baseline fibrinolytic measurements were taken approximately three and a half hour after the administration of garlic. This assumes there was not a transient rise and fall in fibrinolytic potential during this initial time period. This limitation is unlikely, however, as the present study's time course was modeled on previous research which found no such transient rise and fall in fibrinolytic potential. The diet of the participants the day before each treatment trial was not stringently controlled in the present study. Dietary intake, particularly the intake of fat, can greatly influence fibrinolytic potential. Future research should control the dietary intake of participants by having them choose from a standardized meal plan option the day before each treatment trial. Participants in the present study were also young, healthy, trained males. Therefore, it is unknown if a similar result would be observed in untrained males, women, or clinical populations.

In summation, the present study does not support the hypothesis that acute supplementation with garlic enhances the fibrinolytic potential or fibrinolytic response to exercise in young, healthy, endurance trained males. Acute supplementation with garlic does result in a small but significant increase in VO_2max. It remains unclear if these improvements in VO_2max are of any functional importance.

Chapter Six

Summary

The primary aim of this study was to examine the potential effects of acute garlic supplementation on fibrinolytic potential and the fibrinolytic response to exercise in young, healthy, trained males with a randomized, double-blinded, crossover study design. We hypothesized that acute garlic supplementation would: 1) increase participants' plasma levels of tPA activity and tPA antigen at rest, within 2-minutes post-exercise, and 1-hour post-exercise, and 2) decrease participants' plasma levels of PAI-1 activity at rest, within 2-minutes post-exercise, and 1-hour post-exercise.

In contrast to our hypotheses, acute garlic supplementation was found not to increase plasma levels of tPA activity or tPA antigen nor was acute garlic supplementation found to decrease plasma levels of PAI-1 activity. A possible explanation for the lack of an effect may be due to the form of garlic administered to participants. Notwithstanding, the results of this study suggest that acute garlic supplementation does not alter the fibrinolytic potential or the fibrinolytic response to exercise in young, healthy, trained males. Unexpectedly, acute garlic supplementation was found to cause a small but statistically significant rise in VO_2max without a corresponding increase in treadmill time. This rise in VO_2max may be due to increased nitric oxide production in the microcirculation as well increase activity of the aerobic enzyme of succinate dehydrogenase. It is not yet clear if this increase in VO_2max is of any functional importance.

Appendix A
James Madison University
School of Kinesiology and Recreation Studies
Health Status Questionnaire

Instructions: Complete each question accurately. All information provided is **confidential.**
Part I: General Information

1. Study: The effects of acute garlic supplementation on the markers of fibrinolytic potential following a bout of maximal aerobic exercise in young healthy untrained males

2. Participant Number:

3. Gender (circle one) Male Female

4. Date of Birth (Month/ Day/ Year)

Part II: Medical History

5. Circle any that died of heart attack before age 50: Father Mother Brother Sister Grandparent

6. Date of last medical exam: _____ Last physical fitness test: _____

7. Circle operations you have had: Back Heart Kidney Eyes Joint Neck Ears Hernia

Lung Other _____

8. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism	Diabetes	Kidney Problems
Anemia (sickle cell)	Emphysema	Mental Illness
Anemia (other)	Epilepsy	Muscular Injury
Asthma	Eye Problems	Neck Strain
Back Strain	Gout	Obesity
Bleeding trait	Hearing Loss	Orthopedic Injuries
Bronchitis, chronic	Heart Problem	Phlebitis
Cancer	High Blood Pressure	Rheumatoid arthritis
Cirrhosis, liver	Hypoglycemia	Stroke
Concussion	Hyperglycemia	Thyroid problem
Congenital defect	Infectious Mononucleosis	Ulcer
Other _____		

9. Circle all medications taken in the last six months:

Blood thinner	Epilepsy medication	Nitroglycerin
Diabetic pill	Heart-rhythm medication	Weight Loss Medication
Digitalis	High-blood pressure medication	Other _____
Diuretic	Insulin	

10. Please list all vitamin, herbal, nutritional or other supplements that you are currently taking:

11. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

5 = Very often 4 = Fairly often 3 = Sometimes 2 = Infrequently 1= Practically never

a. cough up blood
1 2 3 4 5

b. abdominal pain
1 2 3 4 5

c. low back pain
1 2 3 4 5

d. leg pain
1 2 3 4 5

e. arm or shoulder pain
1 2 3 4 5

f. chest pain
1 2 3 4 5

g. swollen joints
1 2 3 4 5

h. feel faint
1 2 3 4 5

i. dizziness
1 2 3 4 5

j. breathless on slight exertion
1 2 3 4 5

Part III: Health Related Behavior

12. Do you smoke? Yes No

13. If you are a smoker, indicate the number of smoked per day:

Cigarettes:

40 or more 20-39 10-19 1-9

Cigars or pipes only:

5 or more or any inhaled less than 5, none inhaled

14. Do you exercise regularly? Yes No

15. How many times in a week do you spend at least 30 minutes in moderate to strenuous/vigorous exercise?

1 2 3 4 5 6 7 days per week

16. Can you walk 4 miles briskly without fatigue? Yes No

17. Can you jog 3 miles continuously at a moderate pace without discomfort? Yes No

18. Weight now: _____ lb. One year ago: _____ lb Age 21: _____ lb

19. Height: _____

Appendix B

Consent to Participate in Research

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Christopher J. Womack, Ph.D & David J. Lawton from James Madison University. The purpose of this study is to determine the effects of acute garlic supplementation on markers of blood clotting following exercise.

Potential Risks & Benefits

If you choose to participate in this study, you will perform two separate treadmill exercise tests. The investigator perceives the following are possible risks arising from your participation in the study: bad breath, belches with the taste of garlic, nausea, diarrhea, vomiting, discomfort, dizziness, and in rare occurrences, heart attack, stroke or death. The selection criteria used to obtain participants and the "Health Status Questionnaire" are intended to minimize these risks. In healthy individuals, the risk of death during vigorous exercise has been estimated at 1 death per year for every 18,000 individuals. Exercise testing will be performed by CPR-certified individuals with an automated defibrillator in the room. Risks from blood drawing include infection, bruising and mild discomfort.

Potential benefits from participation in this study include:

1) Contributing to the body of knowledge regarding the health benefits of garlic
2) Knowledge of your maximal aerobic capacity (VO2max)

Research Procedures

Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction. This study consists of two separate exercise tests performed on a treadmill. All testing will occur in Godwin Hall, room 209, on the campus of James Madison University. All tests will be performed on three separate occasions, separated by a 10-day washout period. You will also be breathing through a mouthpiece during all of the exercise tests so that we can analyze your expired air to determine how much oxygen you are using. Your heart rate will be monitored by a monitor that wraps around your chest.

VO2max Test: During the VO2max test, you will begin walking at a low intensity (2.5 mph). We will then increase the speed of the treadmill by 0.5 mph each minute thereafter until 6.0 mph is reached. After this point the treadmill speed will remain constant while the elevation is increased at a rate of 3.0% for each minute until you indicate that you can no longer continue. Between test preparation, completion of the exercise test, and the post-exercise period; this test should take approximately two hours.

Supplementation*:* Ten days prior to each test, you will be asked to refrain from eating garlic or foods that contain the active ingredients of garlic. Immediately prior to each test, you will be asked to refrain from food and beverages (except water) for 12 hours. In addition you will be asked to abstain from alcohol consumption for 24 h prior to these tests The night before each test you will be given a packet containing either a placebo or 900 mg of powdered garlic in gel tablets. Neither the investigator nor you will know which treatment you received. The investigator will obtain the pills from someone with the knowledge of whether you are receiving garlic or a placebo. That person will not make this known to the investigator until after all testing is complete. You must ingest all of the tablets with a glass of water three hours prior to testing.

Blood Sampling: We will obtain about 10 ml of blood (about 2 tablespoons) prior to the treadmill test, immediately after the treadmill test, and 1 h following the treadmill test. These blood samples will be obtained from an arm vein.

Confidentiality

The results of this research will be presented at conferences and published in exercise science journals. The results of this project will be coded in such a way that your identity will not be attached to the final form of this study. The researcher retains the right to use and publish non-identifiable data. However, you can ask that your data be removed from the study at any point prior to presentation and publication. While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole. All data will be stored in a secure location accessible only to the researcher. Final aggregate results will be made available to you upon request.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind. Your right to withdraw includes the right to request that your blood samples be discarded at any time.

Questions

You may have questions or concerns during the time of your participation in this study, or after its completion. If you have any questions about the study, contact David J. Lawton at lawtondj@dukes.jmu.edu

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact:

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Questions about Your Rights as a Research Subject

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Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

	David J. Lawton
Name of Participant (Printed)	Name of Researcher(s) (Printed)
Name of Participant (Signed)	Name of Researcher(s) (Signed)
Date	Date

Appendix C

International Physical Activity Questionnaire

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating

Appendix D

24 Hour Food Log and Re-Call

Daily Food Record

Record all foods and liquids consumed in a single day. Please be as specific as possible

Date: _____

Time	Food/Drink Consumed and Method of Preparation	Amount

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