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The effect of garlic supplementation on flow-mediated dilation after acute maximal exercise

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Dedication

This thesis is dedicated to Dr. M. Kent Todd and Dr. Chris Womack, who despite their own busy schedules and commitments, were present for 5:30am trials ready to help.

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Abstract

Antioxidative compounds found in garlic may overcome exercise-induced oxidative stress (OS) and attenuate any associated decrease in endothelial function, perhaps leading to performance improvements in high intensity exercise. The purpose of the current study was to investigate whether or not a single dose garlic supplement induced positive changes to flow-mediated dilation (FMD), a measure of nitric oxide mediated endothelial function, in young, trained adults before and after maximal exercise. Maximal oxygen consumption (VO_{2max}) was measured to determine if any changes in FMD were associated with performance improvements. A total of 11 trained participants (aged 20.64 ± 2.25 yrs) were included in the analysis. Participants were randomized to receive either the placebo, which contained 900 mg of all-purpose flour, or 900 mg of powdered garlic three hours prior to data collection. Flow-mediated dilation measurements were taken following 20 min rest (PRE), immediately following a treadmill VO_{2max} test (IPE), and 1-hour post-exercise (1HR). All FMD measurements were taken in the right arm. This protocol was repeated with either the placebo or garlic supplement (depending upon randomization) at least two weeks after the initial test date.

Although relative (garlic = 61.20 ± 6.44 ml·kg·min^{-1}, placebo = 59.09 ± 6.96 ml·kg·min^{-1}, p = 0.021) and absolute (garlic = 4.57 ± 0.61 L·min^{-1}, placebo = 4.44 ± 0.52 L·min^{-1}, p = 0.045) VO_{2max} were higher in the garlic trial, no significant differences were found between PRE, IPE, or 1HR- FMD (PRE garlic: 0.068 mm ± 0.032 mm, PRE placebo: 0.071 mm ± 0.051 mm, IPE garlic: 0.091 mm ± 0.119 mm, IPE placebo: 0.071 mm ± 0.084 mm, 1HR garlic: 0.084 mm ± 0.097 mm, 1HR placebo: 0.108 mm ± 0.097 mm; p = 0.479). In addition, there were no significant differences found between PRE, IPE, and 1HR peak lumen diameter measurements (p = 0.524), or between PRE, IPE, and 1HR time to peak diameter measurements (p = 0.641). The results of the current study suggest that a single-dose garlic supplement improves VO_{2max} in young, healthy subjects but that the improvements are not related to alterations in NO bioavailability as measured by FMD.

Chapter 1: Introduction

Background

There is evidence that garlic elicits cardiovascular benefits through the enhanced availability of nitric oxide (NO), one of the more powerful vasodilation substances released from the endothelium. Nitric oxide is synthesized from L-arginine by NO synthase (eNOS). Garlic's influence on eNOS has been proposed as a potential mechanism for this enhanced availability, and a single dose may be enough to elicit this response (Morihara et al, 2002; Morihara et al, 2006). Das et al (1995) found that the active compounds in garlic can increase eNOS activity intracellularly, subsequently increasing NO production. Similar findings by Morihara et al (2006) showed that aged garlic extract (AGE) significantly increased NO levels in the plasma when compared to baseline values. This enhanced production of NO may lead to a number of secondary outcomes, including increased antioxidant capacity and decreased exercise-induced oxidative stress (OS) (Harris et al, 2008), improved blood flow, and enhanced oxygen and nutrient delivery (Bloomer et al, 2010; Maiorana et al, 2003). The endothelial benefits of this response may be primarily in the enhancement of aerobic sports performance and recovery as well as in the prevention of cardiovascular disease (CVD).

Known for its vasodilating effects, NO is increasingly used as an ergogenic aid to assist in endothelial function, as it has been suggested that the increase in blood flow resulting from NO synthesis may improve the recovery process of the activated tissue while increasing nutrient delivery (Bescós et al, 2012; Bloomer, 2010). There are a number of sports supplements available on the market that claim to enhance NO synthesis, however analysis of these supplements reveals a “cocktail” of ingredients such as creatine, carbohydrates, and amino acids that may have an ergogenic benefit in and of themselves (Burke et al, 2004; Schoch et al, 2006; Tipton et al, 2008). Furthermore, very little research has been done to test the efficacy of these supplements, with only one published study being identified. This study found that the ability of supplements to increase NO or improve performance is limited (Bloomer, 2010). The amino acid L-arginine is also utilized as an NO enhancing supplement, as it synthesizes NO via eNOS (Bescós et al, 2012; Morihara et al, 2002), however L-arginine does not appear to be the rate limiting component in the reaction (Bloomer et al, 2010). Rather, it appears that eNOS enzymes are the most important components (Bloomer et al, 2010). As garlic has been associated with increased eNOS activity (Das et al, 1995), it may be that garlic could provide the benefits that purportedly result from NO supplements, and be used as an alternative to enhance exercise performance and recovery.

Garlic’s capacity to increase NO availability and improve endothelial function may be related to its antioxidant properties. Superoxide radical, a product of normal oxidative metabolism, provides one example in which antioxidants may be of importance. Under normal conditions, superoxide is inactivated by superoxide dismutase (Jackson et al, 1998). However, superoxide radical production is elevated with the increased oxygen uptake that occurs during acute exercise, causing exercise-induced OS (Harris et al, 2008). This additional superoxide is able to react with NO to form peroxynitrite, effectively consuming available NO at a rate that is three times faster than the catabolism of superoxide by superoxide dismutase (Napoli et al, 2006).

It has been proposed that an individual's resistance to this OS may be related to exercise-induced changes in NO availability and endothelial function (Harris et al, 2008; Munoz et al, 2010; Schneider et al, 2005). Ji (1999) established that active individuals and those with a higher capacity of antioxidant defense have a greater resistance to acute exercise-induced OS, and additional studies have shown that exercise training may enhance antioxidant capacity (Harris et al, 2008; Ji, 1999; Silvestro et al, 2002). It appears that exercise training is able to improve endothelial function by improving endothelium-dependent vasodilation via up-regulation of NO activity (Hauk and Hosey, 2006).

Despite the neutralizing effect that exercise training may have on OS, there is some evidence that high intensity exercise may increase the appearance of reactive oxygen species (ROS) to a level that overwhelms the antioxidant capacity found in trained individuals and decreases availability of NO (Bergholm et al, 1999; Goto et al, 2003; Harrison et al, 2006; Matsuoka, 2001; Napoli et al, 2006; Vincent et al, 2004). Johnson et al (2012) showed that exercise intensity equal to or greater than 50% of VO_{2max} favors the accumulation of ROS. The OS brought on by acute exercise at these intensities has been found to interfere with the vasodilatory benefits of NO; and, the effects appear to reach a peak up to 1-hour post-exercise (Harris et al, 2008; Rognmo et al, 2008). Recent evidence supports the ability of exogenous antioxidants to limit the presence of superoxide by reacting with it before it is able to react with and consume NO (Jackson et al, 1998). Hence the potential benefit of garlic supplementation. Garlic contains phytochemicals that act as antioxidants (Borek, 1997) and may be able to help protect against exercise-induced OS. If garlic supplementation attenuates OS, then it is plausible that garlic may at least indirectly enhance NO bioavailability and any associated improvements in blood flow during and for up to 1-hour post-exercise

If garlic improves NO bioavailability during high intensity exercise then it is possible that it may simultaneously improve blood flow to the vascular beds in the active muscle tissue. If this is true, it is also reasonable to propose that oxygen consumption may increase due to delivery of a greater volume of oxygenated blood in the regions of increased demand (Bloomer, 2010).

To measure the potential impact of garlic on endothelial function as it relates to NO availability for exercise performance and recovery, brachial artery flow-mediated dilation (FMD) may be both a reliable and accurate tool (Harris et al, 2008). The degree of NO availability will in part dictate the degree of brachial artery vasodilation. Therefore, if NO availability is increased, FMD should increase as well. As a measure of NO dependent vasodilation FMD can be used to measure endothelial function non-invasively (Engler et al, 2004; Harris et al, 2008). Moreover, any improvements in NO bioavailability may be associated with an increase in oxygen delivery to the active muscle tissue, making the measurement of oxygen consumption a valuable tool in assessing the potential performance enhancing benefits of single dose garlic consumption.

The proposed study will examine the role of a single dose garlic supplement in enhancing NO availability before, immediately after, and 1-hour post acute maximal exercise. Maximal oxygen consumption will also be measured as a means of assessing the potential performance benefit of garlic supplementation. Measuring resting FMD before acute maximal exercise will allow baseline values to be obtained for garlic alone, before the influence of exercise. Based on evidence indicating increased NO availability and the vasodilatory effects of garlic (Das et al, 2995; Morihara et al, 2002; Morihara et al, 2006; Williams et al, 2005), it is anticipated that resting FMD values with garlic supplementation will be greater than resting FMD values obtained with placebo. The anticipated results for immediate post-exercise FMD are less clear, although based on limited data that the antioxidant capacity may attenuate exercise-induced OS (Harris et al, 2008; Munoz et al, 2010; Schneider et al, 2005), it is important to include this time-point in our measurements. Flow-mediated dilation measurements will also be recorded 1-hour post-exercise, as there is evidence that garlic may be able to attenuate the anticipated decrease in endothelial function brought on by exercise-induced OS at this time-point (Harris et al, 2008; Rognmo et al, 2008).

Purpose of the Study

The purpose of this study was to observe the effects of a single dose garlic supplement on FMD and maximal oxygen consumption in healthy trained young adults before and after maximal exercise.

Need for the Study

An analysis of the current research on endothelial function reveals few studies combining both a dietary intervention and acute exercise. This study takes what has already been confirmed in previous studies about the independent relationships of garlic and exercise to endothelial function and attempts to determine if acute garlic supplementation improves exercise performance via enhanced endothelial function. This study may also provide insight about the therapeutic properties of garlic alone.

Hypotheses

1. Garlic supplementation will increase resting FMD when compared to a placebo.
2. Immediately following exercise FMD in the garlic supplementation trial will be higher than FMD in the placebo trial.
3. Garlic supplementation will mitigate the expected decrease in FMD 1-hour post-exercise.
4. Garlic supplementation will increase maximal oxygen consumption during graded exercise testing.

Assumptions

1. It is assumed that participants are truthful about their current physical activity levels.
2. It is assumed that participants follow correct dietary protocol for the duration of the study.

Delimitations

1. Participants are healthy, young adult males aged 18-30 years old, living in the Shenandoah Valley region of Virginia.

Limitations

1. This study is a cross-section design and any interference in regards to the effect of exercise training are limited to the time of the study, as measurements were not taken before and after the exercise intervention within the same group of participants.
2. The results of this study cannot be extrapolated to females or those outside of the specified age range.

Chapter 2: Literature Review

Current data related to the influence of garlic, acute exercise and exercise training on endothelial function is reviewed below. The data provided is intended to help elucidate some of the mechanisms under investigation, as well as to provide explanation for the selections made in regard to protocol, sample population, and supplement use.

Garlic and Endothelial Function

Garlic's relationship to endothelial function has not been widely researched, and only a few of the existing studies are actual intervention trials. Three published articles were found pertaining to the proposed study. These articles are summarized in **Table 2.1**.

All three studies used AGE as a supplement. Both studies by Morihara et al (2002, 2006) examined the effects of single-dose garlic supplementation on NO production in mice, while Williams et al (2005) measured changes to FMD over two weeks of garlic supplementation in males with evidence of coronary artery disease (CAD). Morihara et al (2002, 2006) observed significant increases in NO production compared to the control. The authors made it clear that the arginine present in AGE was not responsible for this increase, providing evidence for the ability of AGE to work independently of other vasodilating substances (Morihara et al, 2002). It was also observed that while AGE rapidly increased NO production over baseline, levels returned to baseline within 120 min after administration (Morihara et al, 2006). Williams et al (2005) concluded that short-term supplementation with AGE may improve endothelial function in men with CAD. While these studies do not use the same sample population as used in the present study, they do provide evidence that AGE influences endothelial function via enhanced NO production.

Table 2.1: Previous studies conducted on the relationship of garlic and endothelial function and/or FMD

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Morihara et al, 2002	Aged garlic extract enhances production of nitric oxide	Investigate the effect of aged garlic extract (AGE) on NO production by measuring the NO metabolites nitrite and nitrate in the plasma of mice	Male, 6 week old ddY mice	2.86 g • kg AGE	NO production	NO production: significant increase compared to control ($p < 0.05$)	AGE increases NO production by activating cNOS, but not iNOS; the arginine contained in AGE was not responsible for the effect
Morihara et al, 2006	Aged garlic extract maintains cardiovascular homeostasis in mice and rats	To examine the effects of AGE on NO production and on oxidative stress induced by peroxynitrite	Male, 5 week old ddY mice	2.86 g • kg AGE	NO production	NO production: significant increase compared to control ($p < 0.05$) % NO increase over time: 15 min = 44%, $p < 0.01$; 30 min = 37%, $p < 0.05$; 60 min = 44%, $p < 0.01$	AGE can rapidly increase NO production over baseline by ~30-40% after administration, but will return to baseline at 120 min after administration
Williams et al, 2005	Aged garlic extract improves endothelial function in men with coronary artery disease	Determine the effect of two weeks supplementation with AGE on FMD, serum ox-LDL concentrations, and other circulating markers of oxidant stress and systemic inflammation in men with coronary artery disease (CAD)	Males: n=15 59 ± 7 years old, angiographically proven CAD	Randomized, placebo-controlled, cross-over design with 2-week treatment of 2.4 g AGE • day^{-1} or placebo	FMD, serum ox-LDL concentrations	FMD: significantly higher than baseline at end of AGE treatment as compared to placebo treatment ($p = 0.03$) No significant changes in other markers of oxidant stress or systemic inflammation	Suggest that short-term treatment with AGE may improve impaired endothelial function in men with CAD

Exercise Training and Endothelial Function

Exercise has long been associated with improvements in endothelial function. There are hundreds of studies published addressing the many ways in which exercise affects and enhances endothelial function, but the studies selected highlight the main ideas of importance in the current study- that healthy populations may experience increased endothelial function from exercise training, and that OS may be present even in healthy populations. **Table 2.2** summarizes the effect of exercise on FMD.

The studies highlighted in **Table 2.2** all use either brachial artery FMD (BAFMD), posterior tibial artery FMD, or forearm blood flow as outcome measures for improved endothelial function. Treatment ranged from four weeks to 12 weeks, and consisted of primarily aerobic training with the exception of the study by Clarkson et al (1999), in which the researchers used a combination of aerobic and strength training. Sample demographics also varied across all studies, with some including young, trained males (Clarkson et al, 1999), young, sedentary males (Allen et al, 2004; Goto et al, 2003), and older males with impaired endothelial function (Gokce et al, 2002; Lavrenčič et al, 2000). Brachial artery FMD was improved in all studies except for Gokce et al (2002), in which improvements were only seen in the posterior tibial artery. Goto et al (2003), who used forearm blood flow to indicate increased NO production, determined that improvements were only observed after moderate-intensity (50% VO_2max) training, and that high-intensity exercise may have increased OS. Combined, these studies give evidence that not only can exercise training improve endothelial function in those with impairment, but that exercise training can elicit benefits in healthy individuals, as well.

There is also limited evidence that OS is present at high exercise intensities, even in healthy subjects (Goto et al, 2003). Thus, it seems reasonable to investigate the ability of garlic supplementation to overcome OS in trained young males after an acute bout of maximal exercise.

Table 2.2: Previous studies conducted on the relationship of exercise training and endothelial function and/or FMD

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Allen et al, 2004	Time course of improved flow-mediated dilation after short-term exercise training	Examine the influence of a unilateral exercise training protocol on brachial artery flow-mediated dilation (BAFMD)	n=15; sedentary; 26 ± 5.7 yr of age	4-wk non-dominant arm training protocol	Change in BAFMD; time course of change in BAFMD	Percent and absolute change in BAFMD were significantly elevated by 62% and 70% respectively; flow mediated dilation (FMD) is modified early in a training program with evidence of a significant change in both the percent and absolute change in BAFMD after 4 weeks training	Localized, short-term exercise training will result in BAFMD in the trained arm as compared to the untrained arm
Clarkson et al, 1999	Exercise training enhances endothelial function in young men	Assess whether exercise training can enhance endothelium-dependent dilatation in healthy young men	Treatment: n=25; male military recruits; average fitness level; 16-25 yrs of age Control: n=20; matched civilians	10 weeks aerobic and anaerobic training: daily 3-mile runs and upper-body strength and endurance exercises	Change in BAFMD	BAFMD improved from 2.2 ± 2.4% to 3.9 ± 2.5% (p = 0.01)	Ten weeks of generalized exercise training leads to improvements of brachial artery endothelium-dependent responses in healthy subjects

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Gokce et al, 2002	Effect of exercise on upper and lower extremity endothelial function in patients with coronary artery disease	Explore the effects of endurance exercise training on the peripheral vasculature of patients with CAD	Exercise: n=40;78% male; 58 ± 10 yrs of age Control: n=18; 71% male; 58 ± 11 yrs of age	Exercise: supervised 10-week exercise regimen consisting of moderate-intensity, symptom-limited, lower extremity training using a treadmill or stationary cycle; at an intensity of 45% - 85% of heart rate reserve; 30 – 40 minutes, 3 times/week Control: no intervention	Brachial artery and posterior tibial artery FMD	29% increase in functional capacity (7.3 ± 2.2 vs. 9.4 ± 2.7 METs, p <0.001); significant improvement in endothelium-dependent, flow-mediated dilation in a conduit artery of the leg, but not the arm	Exercise improves endothelial function in peripheral conduit arteries of patients with CAD
Goto et al, 2003	Effect of different intensities of exercise on endothelium-dependent vasodilation in humans	To determine the role of different intensities of exercise on endothelial function	n=26; healthy Japanese men; 25 ± 2.5 yrs of age Mild intensity: n=10 Moderate intensity: n=8 High intensity: n=8	30 minutes of bicycle ergometer 5 to 7 times • $week^{-1}$ for 12 weeks Mild intensity: 25% VO_2max Moderate intensity: 50% VO_2max High intensity: 75% VO_2max	Forearm blood flow responses to acetylcholine (Ach) and isosorbide dinitrate	Moderate intensity: significantly increased Ach-induced vasodilation (p < 0.05) NG-monomethyl-L-arginine abolished the moderate-intensity exercise-induced increase of the forearm blood flow response to Ach	Suggest that moderate-intensity aerobic exercise augments endothelium-dependent vasodilation in humans through the increased production of nitric oxide and that high-intensity exercise possibly increases oxidative stress

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Lavrenčič et al, 2000	Physical training improves flow-mediated dilation in patients with the polymetabolic syndrome	Investigate the effect of physical training on BAFMD in asymptomatic patients with the polymetabolic syndrome	Training: n=14; 53 ± 5 yrs of age Control: n=15; 51 ± 7 yrs of age	Training group: aerobic exercise 3 times weekly for 12 weeks; 20 min warm-up followed by 30 min sessions intense exercise on a bicycle ergometer at 80% of maximum heart rate Control group: maintain their habitual activities	Changes in BAFMD; changes in physical fitness	Training induced an increase of 18% in physical fitness; BAFMD increased from 5.3 ±2.8% - 7.32.7% ($p < 0.05$)	Physical training has beneficial effects on early functional atherosclerotic changes of the arterial endothelium in patients with the polymetabolic syndrome

Acute Exercise and Endothelial Function

Several studies that address the effects of a single bout of exercise on FMD are summarized in **Table 2.3**. The studies vary in sample demographics and interventions, yet they provide an overall picture of the effects of acute exercise on FMD. A number of the studies provide evidence of increased OS. Dawson et al (2008) observed elevated OS and inflammation after subjects participated in the London Marathon, suggesting reduced NO bioavailability and partially explaining the reduced femoral FMD values detected. Rognmo et al (2008) observed increased NO and antioxidant levels following high-intensity interval running in endurance trained athletes and sedentary controls, however, FMD values were still reduced when measured at 1-hour post-exercise. Results from Farsidfar et al (2008) showed decreased FMD values at peak exercise, and along with Dawson et al (2008) and Rognmo et al (2008), verify the findings of those by Goto et al (2003)- that enhanced endothelial function from acute exercise may only be expected after moderate intensity exercise. Johnson et al (2012) measured thiobarbituric acid reactive substances (TBARS) as an indicator of OS, and found that levels were elevated immediately following high-intensity (80% VO_{2peak}) exercise, confirming once again that increased OS may be expected after maximal, high-intensity exercise. Harris et al (2008) also observed increased OS in the form of significantly elevated concentrations of the inflammatory marker interleukin-6 (IL-6) in participants following moderate (50% VO_{2peak}) and high (75% VO_{2peak}) intensity exercise. However, FMD values were still increased in active participants as compared to sedentary participants (Harris et al, 2008). The authors suggest that this result may be the result of a higher capacity of antioxidant defense in active participants, as well as the ability of the dietary antioxidant intake of the participants to overcome the exercise-induced OS (Harris et al, 2008). Combined, these studies give reason to expect decreased FMD values in response to an acute bout of maximal exercise. However, there is also indication that elevated antioxidant levels may be able to attenuate this decrease (Harris et al, 2008), providing rationale for garlic supplementation in the proposed study.

Table 2.3: Previous studies conducted on the relationship of acute exercise and endothelial function and/or FMD

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Dawson et al, 2008	Changes in vascular and cardiac function after prolonged strenuous exercise in humans	To investigate the impact of an acute bout of prolonged strenuous exercise on vascular and cardiac function and the appearance of biomarkers of cardiomyocyte damage in 15 male (32 ± 10 yr) non-elite runners	n=15; male non-elite runners age 32 ± 10 yr (range 23– 63)	London Marathon	Changes in endothelium-dependent vasodilation of the brachial and superficial femoral arteries before and after the London marathon	Femoral FMD was significantly (P = 0.04) reduced; no change in brachial artery function, as determined by FMD Concomitant reduction in femoral artery function and left ventricular diastolic function, as well as the appearance of markers of cardiac damage in nearly all runners	Suggests a period of depressed cardiovascular function and elevated cardiac risk soon after running a marathon; impaired femoral, but not brachial, endothelial function following a marathon suggest that if elevated oxidative stress or inflammation are responsible for reduced NO bioactivity, then may be isolated to the active muscle

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Farsidfar et al, 2008	Effects of different intensities of acute exercise on flow mediated dilation in patients with coronary heart disease	To evaluate anaerobic threshold and peak oxygen uptake level of acute exercise on BAFMD in stable CAD	Study group: n=32; 29 males; documented CAD by angiography Control group: n=14; 13 males; no evidence of CAD	VO_{2max} tests performed on three separate occasions	Change in BAFMD at anaerobic threshold and peak level	Endothelium-independent vasoreactivity showed an upward trend at threshold level exercise; BAFMD was significantly decreased at peak level exercise ($p < 0.01$)	Moderate intensity exercise can enhance endothelial function
Harris et al, 2008	The flow-mediated dilation response to acute exercise in overweight active and inactive men	Investigate the interaction of IL-6 and TNF-α on the BAFMD response to acute exercise of different intensities in overweight men exhibiting different physical activity profiles	Active: n=8; 59.9 ± 2.8 yrs of age Inactive: n=8; 56.9 ± 2.6 yrs of age	Either a low (25% VO_{2peak}), moderate (50% VO_{2peak}), or high (75% VO_{2peak}) intensity treadmill walking session for 45 min; treatments separated by ≥2 days to eliminate any training effect	Changes in BAFMD; concentrations of IL-6 and TNF-α	Active group had 24% increase ($p = 0.034$) in FMD following acute exercise compared to a 32% decrease ($p = 0.010$) in the inactive group; Elevated ($p < 0.001$) concentrations of IL-6 following moderate and high intensity acute exercise in both groups; TNF-α concentrations were unchanged in response to acute exercise ($p = 0.584$)	BAFMD response to acute exercise is enhanced in overweight active men as compared to the decrease observed in their inactive counterparts

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Johnson et al, 2012	The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men	To determine whether changes in oxidative stress and endothelial function following acute aerobic exercise are dose-dependent	n=10; aerobically trained males; 26.5 ± 0.7 yrs of age	Four acute aerobic running exercise bouts on separate days: (1) 50% VO_{2peak} for 30 min (moderate intensity moderate duration, MIMD); (2) 80% VO_{2peak} for 30 min (high intensity moderate duration, HIMD); (3) 50% VO_{2peak} for 60 min (moderate intensity long duration, MILD); (4) 80% VO_{2peak} for the duration required to expend the equivalent number of kilocalories as MIMD (approximately 17–22 min) (high intensity short duration: HISD)	Thiobarbituric acid reactive substances (TBARS) measured as an index of oxidative stress; brachial artery flow-mediated dilation assessed as an index of endothelial function; measurements taken immediately (post-exercise, 1-hour post, and 2-hours post	No significant correlation between the absolute change from baseline TBARS and the absolute change from baseline %FMD ($p = 0.94$)	The main findings of the present study are that TBARS was greatest immediately following high-intensity aerobic running exercise regardless of duration, and FMD was greatest immediately following exercise volumes equivalent to MIMD regardless of intensity or duration

Investigators	Title	Purpose	Sample Demographics	Intervention	Outcome Measures	Results	Comments
Llewellyn et al, 2012	The relationship between shear rate and flow-mediated dilation is altered by acute exercise	To compare brachial artery endothelial function at rest and post-exercise in young, healthy subjects and to compare the data expressed as a percent change and normalized to shear rate (SR)	n=15; 7 males; 19-30 yrs of age	VO_{2max} test; FMD measurements	% change in FMD; SR	Strong correlation between FMD and SR_{AUC} at rest (p < 0.001); very weak correlation between FMD and SR_{AUC} after exercise (p = 0.6)	Suggests that post-exercise FMD should not be normalized to SR_{AUC}, nor does FMD depend on SR
Rognmo et al, 2008	Endothelial function in highly endurance-trained men: effects of acute exercise	To compare the influence of high vs. normal aerobic capacity and a single bout of high-intensity interval running on arterial diameter, peak blood flow, endothelium-dependent dilation in the brachial artery, bioavailability of nitric oxide, and antioxidant status in well-trained and sedentary healthy men.	Endurance trained: n=10; 23.5 ± 0.9 yrs of age Sedentary: n=7; 25.4 ± 1.2 yrs of age	High-intensity interval running consisting of 15-min warm-up, (running at 60-70% HR_{max}), 5x5 min with last 3 min of every bout >90% HR_{max}, 2 min active recovery between bouts at 60-70% HR_{max}	Arterial diameter; peak blood flow; endothelium-dependent dilation of the brachial artery; bioavailability of nitric oxide; antioxidant status	No significant differences in FMD at baseline; resting and max arterial diameters 10-15% larger in trained; peak blood flow after cuff release 28-35% greater in trained; NO level increased by 93% in trained, 63% in sedentary 1 hour post training; circulating antioxidant levels increased 7% in sedentary, 10% in trained	Differences in artery diameter, and not vessel ability to dilate, may be responsible for transport capacity of blood in athletes' arteries

Chapter 3: Methodology

This study was done in conjunction with another study designed to investigate the influence of acute garlic supplementation on fibrinolytic potential. The following methodology mirrors that of other study with the exception of FMD measurements.

Participants

A total of 20 trained subjects between 18-30 years of age were recruited from the James Madison University community and the surrounding Shenandoah Valley region of Virginia. Participants were asked to complete a health status questionnaire, and exclusion criteria included: known cardiovascular, pulmonary, or metabolic disease; current tobacco use; infection; fever or illness within two weeks prior to testing; garlic or gluten allergy; any other medical condition that could compromise safety (**Appendix A**). Participant characteristics are summarized in **Table 4.1**. All participants were provided written and verbal information explaining the experimental procedures and were made aware of any potential risks prior to completing the informed consent form (**Appendix B**). The James Madison University Committee on Research Involving Human Subjects approved all testing procedures.

The short-form International Physical Activity Questionnaire was administered prior to testing (**Appendix C**). The criterion for trained 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

Administration of the placebo, which contained 900 mg of all-purpose flour, and the supplement, which contained 900 mg of powdered garlic was randomized. The placebo consisted of all-purpose flour contained in a gel capsule identical to the garlic supplement. These packets were administered in a double-blind, cross-over fashion. Participants were instructed to ingest all capsules with eight ounces of water three hours prior to testing. Additionally, participants were required to fast and abstain from exercise for 12-hours and abstain from alcohol consumption for the 24-hours leading up to testing (Harris et al, 2010). Adherence to dietary restrictions was confirmed by a 24-hour food recall questionnaire administered by a registered dietitian prior to testing (**Appendix D**).

Exercise Tests

Treatment Trials

On their scheduled test date, participants reported to the Human Performance Laboratory on the James Madison University campus. To limit changes in orthostatic pressures that can influence hemodynamics, participants were asked to relax in a semi-recumbent position for 20 min prior to obtaining the resting FMD measurement (Harris et al, 2008). After 20 min a blood draw was obtained to address the purposes of the corresponding study and the first occluded FMD measurement followed. Participants then completed a treadmill VO_{2max} test. Within two minutes of completion of the treadmill VO_{2max} test, another blood draw was obtained. An FMD measurement was then taken upon completion of the blood draw. The participants were then asked to remain semi-recumbent for the duration of one hour, at which point the third and final blood draw and FMD measurement were performed. All FMD measurements were taken in the right arm. Participants repeated this protocol with either the placebo or garlic supplement (depending upon randomization) at least two weeks after the initial test date.

Cardiorespiratory Fitness (VO_2max)

All participants performed a ramped treadmill VO_{2max} test on a Stairmaster Clubtrack 612 treadmill (Kirkland, WA). The test began at an initial speed of 2.5 mph and was increased at a rate of 0.5 mph $\cdot$ min^{-1} until 6.0 mph was reached. From this point on, the treadmill speed remained constant while the incline was increased at a rate of 3.0% $\cdot$ min^{-1} until volitional exhaustion. Participants were instructed to straddle the treadmill belt or press the “Stop” button upon achievement of volitional exhaustion. Oxygen uptake and heart rate were continuously monitored with a Sensormedics Spectra metabolic cart (Yorba Linda, CA) and a Polar heart rate monitor (Lake Success, NY), respectively.

Flow-Mediated Dilation

Flow-mediated dilation of the brachial artery was measured on three separate occasions during each trial: at resting prior to the exercise test, beginning within two minutes post-exercise test, and one hour post-exercise test. PerformancePlus ECG Diagnostic Electrodes (Vermed: Bellows Falls, VT) for an electrocardiograph (ECG) were placed on each participant at the left arm, right arm, and left hip for the purposes of coordinating blood flow with cardiac cycles. The distance between the right olecranon process and the elbow was measured, and a DC-6 Diagnostic Ultrasound System (ShenZhen Mindray Bio-Medical Electronics Co., Ltd.: ShenZhen, China) probe was placed at approximately 1/3 of this distance from the elbow to locate the brachial artery. Once the brachial artery was located and an adequate image achieved, the ultrasound probe's position was marked lightly on the participant's skin using a permanent marker to allow for quick relocation of the artery during the trial and standardization of the measurements. For each FMD measurement, a DS400 Aneroid Sphygmomanometer (Hokanson: Bellevue, WA) was placed on the forearm, distal to the right brachial artery (Betik et al, 2004). The cuff was inflated to 200-250 mmHg (≥25 to 50 mm Hg above systolic arterial pressure) and kept inflated for a period of five minutes (Kooijman et al, 2008). Blood flow measurements were initiated at least ten seconds prior to cuff release and post occlusion data collection was continued for ≥ 2 minutes (Harris et al, 2010). The cuff was removed after completion of each FMD measurement during the trial.

All data was collected with the use of Microsoft AM Capture software (Version 8 downloaded from Pennacle Systems Division of Avid Technologies, Inc. New York) at a rate of eight frames per second. Microsoft AM Capture files were converted using Movavi Video Converter software (Version 10 downloaded from Movavi, Novosibirsk Province, Russian Federation) and analyzed using Brachial Analyzer for Research software (Medical Imaging Applications, LLC. Coralville, IA).

Baseline vessel diameters were recorded as the mean diameter recorded during the ten seconds prior to cuff release. This method for measuring baseline diameter values has been successfully used elsewhere (Betik et al, 2004; Dyson et al, 2006; Gill et al, 1985; Parker et al, 2006; Pyke et al, 2008; Pyke et al, 2007). Post-occlusion diameter measurements were recorded and averaged at four-second intervals (32 frames) during the first 20 seconds post-occlusion and at five-second intervals (40 frames) for the remainder of the two minutes. Peak diameter was recorded as the highest average diameter measured from among the four- or five-second intervals during the two minutes of measurement following cuff release (Harris et al, 2010). Time to peak diameter was estimated as the midpoint of the interval in which peak diameter was recorded. For example, if peak diameter was found in the interval between 60 and 65 seconds the time to peak diameter was recorded as 62.5 seconds. This method for measuring vessel diameters and computing FMD has been shown to be equally valid and reliable as QRS-gating brachial artery diameter measurements (Kizhakekuttu et al, 2010). Diameter measurements analyzed with the Brachial Analyzer for Research software were used to calculate FMD, which was calculated as [(peak diameter – baseline diameter) / baseline diameter] and recorded as a percent of change.

Dietary and Exercise Controls

To insure standardization, the participants were instructed to: 1) choose a standard "self-selected" meal plan for the 24-hour time frame leading up to each treatment trial; 2) consume the last meal of the day no later than 12-hours prior to the scheduled trials; 3) avoid consumption of alcohol 24-hours prior to each trial; and 4) avoid exercise 12-hours prior to each trial. Adherence to dietary restrictions was confirmed by a 24-hour food recall questionnaire administered by a registered dietitian prior to testing (**Appendix D**). Participants were permitted to drink water *ad libitum* throughout the study. Compliance was defined as a 75% match between the trial one and trial two food logs. Six of the final 11 participants with complete data sets met this criteria, however no participants were excluded based on this compliance as none reported high consumption of garlic or onions before either trial.

Statistical Analysis

Changes in FMD were tested for normality (Shapiro-Wilkes) and evaluated using a two-factor (2x3) repeated measures analysis of variance (ANOVA). The within-subject factors were testing condition (garlic or placebo) and time (pre-exercise, post-exercise, 1-hour post-exercise). Post-hoc testing was performed using two-tailed test for multiple comparisons with a Bonferroni correction factor. Maximal oxygen consumption between the garlic and placebo trials was compared with a paired T-test. A priori statistical significance was determined at $p < 0.05$. Data is reported as means +/- standard deviations.

Chapter 4: Results

A total of 18 participants (aged 20.64 ± 2.25 yrs) were included in the data collection, however 11 were included in the analysis. Data was excluded if video files were unable to be analyzed by the Brachial Analyzer for Research Software (Medical Imaging Applications, LLC. Coralville, IA) due to poor image quality or if there were any missing data points for any subject. In addition, 12 participants were originally tested for normality using the Shapiro-Wilkes method. When log transformation failed do normalize the data, further examination revealed two outlying data points (approximately three standard deviations from the mean) belonging to the same participant. Once removed, data from the remaining 11 participants were shown to be normally distributed. Demographic data for all 11 participants is summarized in **Table 4.1**. There were no significant differences found between participants for height (p = 0.289), weight (p = 0.283), BMI (p = 0.430), or total time on treadmill (p = 0.953). The VO_{2max}values, however, whether adjusted for between trial differences in body mass (ml · kg · min^{-1}; p = 0.021) or presented as absolute values (L · min^{-1}; p = 0.045) were significantly different.

Table 4.1: Participant Characteristics & Exercise Performance Data

	Garlic	Placebo	*p*
Age (yrs)	20.5 ± 2.17	20.5 ± 2.17	NA
Avg. Height (cm)	176.5 ± 8.94	176.9 ± 9.08	0.289
Avg. Weight (kg)	74.6 ± 11.2	75.1 ± 11.1	0.283
Avg. BMI ($kg \cdot {}^{1}/m^{2}$)	23.87 ± 2.84	24.10 ± 2.80	0.430
VO_{2max}($ml \cdot kg \cdot min^{-1}$)	61.20 ± 6.44	59.09 ± 6.96	0.021
VO_{2max}($L \cdot min^{-1}$)	4.57 ± 0.61	4.44 ± 0.52	0.045
Treadmill Time (min)	13.53 ± 1.40	13.52 ± 1.21	0.953

Average percent change in FMD data for all 11 participants is summarized in **Table 4.2**. No significant main effect for treatment ($p = 0.943$) or between resting, immediate post, or 1-hour post measurements ($p = 0.364$) or treatment by time interactions were found ($p = 0.479$).

Table 4.2: Mean FMD ± Std. Dev.

Time-point	Garlic FMD (%)	Placebo FMD (%)
Resting	6.8 ± 3.2	7.0 ± 5.1
Immediate post-exercise	9.1 ± 11.9	7.1 ± 8.4
1-hour post-exercise	8.4 ± 9.7	10.9 ± 9.7

Peak diameter data for all 11 subjects at each time point is summarized in **Table 4.3**. No significant main effect for treatment ($p = 0.309$) or between resting, immediate post, or 1-hour post measurement observation times ($p = 0.483$) or treatment by time interactions were found ($p = 0.524$).

Table 4.3: Mean Peak Diameters ± Std. Dev.

Time-point	Garlic Peak Diameter (mm)	Placebo Peak Diameter (mm)
Resting	4.241 ± 0.388	4.337 ± 0.516
Immediate post-exercise	4.307 ± 0.383	4.322 ± 0.348
1-hour post-exercise	4.293 ± 0.443	4.463 ± 0.285

Time to peak diameter data for all 11 subjects at each time point is summarized in **Table 4.4**. No significant main effects for treatment ($p = 0.935$) or treatment by time interactions were found ($p = 0.641$). Although a significant main effect for time ($p = 0.032$) was found, the lack of a significant interaction combined with a similar pattern of change in time to peak diameter indicates that the responses were not different between the garlic and placebo trial. Follow-up multiple comparison indicated that there was a significant increase in time to peak between rest and immediate post-exercise ($p = 0.012$). Comparison between rest and 1-hour post-exercise trended towards significance ($p = 0.052$), but no differences between immediate post exercise and 1-hour post-exercise ($p = 0.165$) were observed.

Table 4.4: Mean Time to Peak Diameter ± Std. Dev.

Time-point	Garlic Time to Peak (sec)	Placebo Time to Peak (sec)
Resting	56.36 ± 19.24	51.82 ± 16.77
Immediate post-exercise	66.36 ± 13.43	69.09 ± 12.81
1-hour post-exercise	60.00 ± 14.66	61.36 ± 13.05

Chapter 5: Discussion

The current study examined the possible effect of single-dose garlic supplementation on FMD following an acute exercise bout. If garlic was shown to increase resting baseline FMD and immediate post-exercise FMD as well as minimize the expected decrease in 1-hour post-exercise FMD, the proposed benefits would include improved exercise performance (e.g., VO_{2max}) and recovery from exercise. The novel finding in this study was that VO_{2max} was higher with garlic supplementation when compared to placebo, independent of any measured changes in FMD.

Nitric oxide enhancement for exercise training and performance has become increasingly popular in the nutritional supplements industry, with both aerobic and anaerobic athletes looking to increase NO production in an attempt to gain better results from their workouts. These supplements are classified for "pre-workout" use and most companies will claim that a single use will lead to dramatic increases in circulating nitric oxide, resulting in improved blood flow and exercise performance (Bloomer et al, 2010). Recent evidence has indicated NO-mediated glucose uptake during exercise via recruitment of glucose transporter GLUT-4 as a potential benefit (Kingwell, 2000), however there is inconclusive evidence as to the ability of these supplements to effectively increase NO production or to improve exercise performance (Bloomer, 2010). The current study attempted to identify garlic as an effective mediator of NO in the event that NO does in fact deliver on its claimed benefits.

When analyzing the effect of garlic supplementation on brachial artery FMD, a significant change in resting baseline FMD values between trials was expected; however, no differences were found. Evidence has shown that garlic supplementation improves endothelial function via increased synthesis of NO (Das et al, 1995; Morihara et al, 2002; Morihara et al, 2005). Das et al (1995) investigated the extent to which garlic enhances the production of nitric oxide synthase (NOS), the enzyme that leads to NO synthesis. Both *in vivo* and *in vitro* tests were performed, and the authors concluded that eNOS activity was increased significantly in both settings (Das et al, 1995). In addition, Morihara et al (2002) conducted a study in which AGE was shown to increase NO metabolism significantly in mice. Morihara et al (2006) further confirmed these findings, showing that AGE increased NO production by ~30%-40% after administration, although production returned to basal level ~120 min after administration (Morihara et al, 2006). The authors suggest that this may be due to the fact that S-allylcysteine, a major compound in garlic, is rapidly absorbed by the gastrointestinal tract within 15 min of administration (Morihara et al, 2006). Moreover, the mice were supplemented with 2.86 g · kg body weight, giving them a much greater relative dose than the 900 mg (~0.012 g · kg body weight) provided to the participants in the current study (Morihara et al, 2002, 2006). This may explain why, in the current study, resting FMD values measured three hours after garlic supplementation showed no significant differences as compared to the resting FMD values measured after the placebo. However, Kleijnen et al (1989) has identified a number of studies in which measurements were taken up to two hours post-administration of garlic and compounds were still found to be active. In addition, a study by Jung et al (1991) observed a 55% increase in capillary skin perfusion five hours after intake of 900 mg garlic, providing evidence that both dosage and timing have been sufficient in previous studies.

No differences in FMD were found between the garlic and placebo immediately following exercise. This was in contrast to the expected finding that FMD in the garlic supplementation trial would be higher than FMD in the placebo trial. The hypothesis was based on limited evidence suggesting that an increase in FMD values may be observed due to the antioxidant properties of garlic (Harris et al, 2008). Garlic contains phytochemicals that function as antioxidants to scavenge ROS (Borek, 1997). Morihara et al (2006) attempted to identify the antioxidant mechanism responsible for this effect and demonstrated that AGE significantly suppresses peroxynitrite-induced hemolysis in a dose-dependent manner. Aged garlic extract contains an S-allyl moiety or an allel sulfoxide moiety, which have been shown to be important in the prevention of peroxynitrite-induced hemolysis by scavenging peroxynitrite and stabilizing the erythrocyte membrane (Moriguchi et al, 2001; Morihara et al, 2006). It has been suggested that these antioxidant effects of garlic are sufficient enough to overcome exercise-induced OS (Williams et al, 2005). In the current study there was no change in immediate post-exercise FMD with either garlic or placebo, indicating that contrary to the hypothesis, garlic did not stimulate FMD or further alter any changes in FMD that may have already been in effect as a result of exercise.

The failure of the garlic supplement to alter immediate post-exercise FMD may also be explained by data reported by Johnson et al (2012), who demonstrated that increased FMD values are only expected at an aerobic exercise volume equivalent to 30 min at 50% VO_{2peak}. The threshold for OS does not appear to occur until 50-70% of

VO_{2peak} has been achieved, suggesting that OS may not inhibit positive changes in FMD at lower intensities (Johnson et al, 2012). In the same study, higher exercise intensities (such as that achieved by a VO_{2max} test) resulted in decreased FMD values, a result that the authors attribute to OS (Johnson et al, 2012). If significant OS was present, as the data by Johnson et al (2012) suggest, the efficacy of garlic may be dependent on the dose and the timing of ingestion (Morihara et al, 2002, 2006).

It was also hypothesized that garlic supplementation would minimize the anticipated decrease in FMD measured 1-hour post-maximal exercise. Decreased values in the placebo trial were anticipated based on the results of a study by Rognmo et al (2008), in which a 38% reduction in FMD values of trained participants was observed 1-hour post-exercise. Rognmo et al (2008) also found that despite the reduction in FMD, NO was increased 93% in the trained group at 1-hour post-exercise, leading the investigators to acknowledge that oxidative radicals produced as a result of exercise-induced OS could have caused a reduction in the bioavailability of NO through uncoupling of eNOS and quenching of NO.

Rognmo et al (2008) also suggested that a different mechanism may have been responsible for the reduction in FMD. One such potential mechanism is based on evidence that endothelial dependent vasodilation in large arteries is proportionally less than that observed in smaller arteries (Silber et al, 2001; Silber et al, 2005). Rognmo et al (2008) observed that the large arterial diameters in their trained subjects at 1-hour post-exercise, combined with high levels of circulating NO in the plasma, may have dilated the vessel to maximal or near maximal diameter prior to FMD measurement. This would then reduce shear stress and reactive hyperemia, and may subsequently attenuate FMD values (Silber et al, 2001; Silber et al, 2005). It is possible that the trained participants in the current study also experienced this effect, as percent change in FMD did not change at the 1-hour post-exercise time-point with placebo or garlic.

Evidence from Silber et al (2005) may also explain why there was no significant difference ($p = 0.524$) in peak diameters across all time-points. The current study used young, healthy, trained males similar in characteristics to the trained athletes in the study by Rognmo et al (2008). The trained athletes in the study by Rognmo et al (2008) had larger brachial diameters than the sedentary controls, and were less prone to increased dilation due to increased shear stress as their vessel diameters were already large enough to accommodate increased blood flow to exercising muscles. As the participants in the current study were of similar fitness level, it is probable that they too had relatively large brachial artery diameters. If larger arteries dilate to a lesser extent than smaller arteries (Silber et al, 2005), there would be less change in dilation from resting to peak dilation.

It is worth noting that independent of garlic, exercise caused the time to peak diameter to significantly increase immediately following exercise. With garlic supplementation, the immediate post-exercise time to peak diameter took 18% longer than at rest. With placebo, the immediate post-exercise time to peak diameter took 33% longer than at rest. This may suggest that following exercise, FMD tends to be lower and time to peak diameter appears to increase. Given the percent differences in time to peak between immediate post-exercise and rest time-points and the potential for interaction, we excluded the 1-hour post in the attempt to identify a treatment by time interaction; however, none was observed (p=0.339).

The VO_{2max} values were higher in response to the garlic supplementation trial when compared to placebo, though the difference could not be attributed to changes in FMD or other indices of blood flow (vessel diameter and time to peak diameter) measured in this study. Garlic may have a specific impact on microvascular circulation that it does not have on conduit arteries (Jung et al, 1991; Wolf et al, 1990). If microvascular flow increases independently of conduit artery flow, then VO_2 may increase due to improvements in local perfusion (Jung et al, 1991; Wolf et al, 1990). These changes in microcirculation would not be detectable through FMD measurements. In addition, if garlic does influence blood flow, it may be amplified in the active musculature, thus suggesting a potential limitation in this study since FMD was measured in the brachial artery and the loaded muscle tissue was in the lower extremities. It is also possible that flow increased in the conduit arteries without changes in FMD. This could improve local perfusion, suggesting that in future studies, flow data should be collected and analyzed.

There has been some dispute in the literature as to whether or not using the mean diameters recorded during the ten seconds prior to cuff release as the baseline vessel diameter is the best method for measuring FMD. It has been suggested that using unoccluded baselines may provide more accurate results (Black et al, 2008; Nishiyama et al, 2007; Thijssen et al, 2007; Vita et al, 2008), as occlusion may modify the resting diameter through tissue displacement, imaging artifact (Thijssen et al, 2008) or pressure changes (Laughlin et al, 2008). Thijssen et al (2008) conducted a study that compared the two methods and found that baseline brachial diameters collected just prior to cuff release were significantly higher than the baseline brachial diameters collected pre-occlusion in children and healthy young adults. Thus we found it prudent to verify our findings using the alternative technique of measuring unoccluded baselines to calculate FMD.

Unoccluded baseline measurements were obtained from a total of six participants. Percent change in FMD data for the six subjects is summarized in **Table 5.1**. There were no significant differences found between resting, immediate post, or one-hour post measurements for any participant (p = 0.999). These results serve to further confirm our findings using all 11 subjects and occluded baseline measurements.

Table 5.1: Mean FMD ± Std. Dev. Using Unoccluded Baselines

Time-Point	Garlic FMD (%)	Placebo FMD (%)
Resting	4.2 ± 5.7	4.1 ± 10.6
Immediate post-exercise	1.9 ± 8.3	1.6 ± 3.7
1-hour post-exercise	5.2 ± 6.6	4.9 ± 7.6

The current study did not demonstrate any evidence of increased NO bioavailability, as measured by FMD, after acute garlic supplementation in combination with maximal exercise. However, recent research suggests that increased NO production may not be the only signaling mechanism by which garlic can enhance endothelial function. Benavides et al (2007) showed that garlic-derived organic polysulfides are converted by erythrocytes into H_2S, which will relax vascular smooth muscles. This will in turn induce vasodilation of blood vessels (Lefer, 2007). Zhao et al (2001) demonstrated that H_2S induced this relaxation through the opening of K_{ATP} channels. Zhao et al (2001) also showed that NO may actually regulate H_2S. It was found that NO may stimulate cystathionine □-lyase (CSE) activity, a protein with the ability to cleave L-cysteine to produce H_2S (Zhao et al, 2001). The authors also pointed out that the hypotensive effects observed were of short duration, explaining that this may have been the result of scavenging of H_2S by metalloproteins, disulfide-containing proteins, thio-*S*-methyl-transferase, or heme compounds (Zhao et al, 2001). It is possible that this mechanism was at work in the current study, and would most likely be observed in the six unoccluded baseline measurements as there was limited influence from NO and no additional influence from exercise. However, the current study found no difference (p = 0.967) in the six unoccluded baseline FMD measurements, though the timing of our measurements may have occurred too late to detect the short-term effects potentially elicited by H_2S.

The current study had a number of limitations. First, the participants were limited to healthy, young adult males 18-30 years of age. Participants were also required to be classified as trained, based on the following: A) 3 or more days of vigorous-intensity activity of at least 20 min · day^{-1} 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}. This may have interfered with the ability to detect significant changes in FMD via garlic supplementation, as trained subjects may have higher initial FMD values and it has been shown in the literature that changes are more easily observed in individuals with lower initial FMD values (Williams et al, 2005). In addition, the timing of measurements was also a factor limited by the requirements of the concurrent study, and it is possible that any NO- or H_2S-enahancing effects of garlic on FMD may have already returned to baseline by the time of the first measurement (Morihara et al, 2006; Zhao et al, 2001).

The current study did not observe any significant increases in percent change to FMD as a result of acute garlic supplementation. While there were no negative effects of garlic supplementation reported, we were unable to recommend garlic supplementation to improve cardiovascular function or exercise performance.

Chapter 6: Conclusion and Recommendations

The current study hypothesized the following:

1. Garlic supplementation will increase resting FMD.
2. Immediately following exercise FMD in the garlic supplementation trial will be higher than FMD in the placebo trial.
3. Garlic supplementation will mitigate the expected decrease in FMD values 1-hour post-exercise.
4. Garlic supplementation will increase maximal oxygen consumption during graded exercise testing.

The first three hypotheses listed above were not supported as garlic supplementation did not alter resting, immediate post-exercise or 1-hour post-exercise FMD values. Based on these observations, we were unable to demonstrate any positive benefits of taking garlic supplements as it relates to either cardiovascular health or exercise performance and recovery. However, it was found that VO_{2max} was higher with garlic supplementation when compared to the placebo, independent of any measured changes in FMD.

There were a number of recommendations that should be made for future investigations. Future studies should be conducted using untrained participants, as greater changes to FMD have been observed in individuals with lower initial FMD values (Williams et al, 2005). It is also possible that a larger dose of garlic may be needed to observe any positive changes in endothelial function (Morihara et al, 2002, 2006). Future protocols should also take into account the timing of measurements as they relate to metabolism of the active compounds in garlic (Morihara et al, 2006; Zhao et al, 2001), as well as the fact that most positive changes to endothelial function and antioxidant capacity are seen at moderate intensities (Johnson et al, 2012). In addition, it may also be advantageous to record and analyze flow data in the event of increased blood flow in conduit arteries after garlic supplementation.

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	f. Chest pain 1 2 3 4 5
b. Abdominal pain 1 2 3 4 5	g. Swollen joints 1 2 3 4 5
c. Low back pain 1 2 3 4 5	h. Feel faint 1 2 3 4 5
d. Leg pain 1 2 3 4 5	i. Dizziness 1 2 3 4 5
e. Arm or shoulder pain 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

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 Kent Todd, PhD & Leslie Redmond, BS, RD from James Madison University. The purpose of this study is to determine the effects of acute garlic supplementation on flow-mediated dilation 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" is intended to minimize these risks. In healthy individuals, the risk of death during vigorous exercise has been estimated at one death per year for every 18,000 individuals. CPR-certified individuals will perform exercise testing 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 (VO_{2max})

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 two separate occasions, separated by a ten-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. A monitor that wraps around your chest will monitor your heart rate.

$\underline{VO_{2max}\ Test}$: During the VO_{2max} 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 incline 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-hours 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-hour following the treadmill test. These blood samples will be obtained from an arm vein.

Flow-Mediated Dilation: We will perform an ultrasound analysis of flow-mediated dilation prior to the treadmill test, immediately after the treadmill test, and 1-hour following the treadmill test.

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 Leslie Redmond at redmonlc@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:

Leslie C. Redmond	Dr. Kent Todd, Advisor
Kinesiology	Kinesiology
James Madison University	James Madison University
redmonlc@dukes.jmu.edu	toddmk@jmu.edu

Questions about Your Rights as a Research Subject

Dr. David Cockley

Chair, Institutional Review Board

James Madison University

(540) 568-2834

cocklede@jmu.edu

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.

	Leslie C. Redmond
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 Re-Call

24-Hour Diet Recall

Please be as specific as possible. Include all beverages, condiments, and portion sizes.

Time	Food Item and Method of Preparation	Amount Eaten	Where

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