CHANGES IN VASCULAR AND HEMODYNAMIC PARAMETERS FOLLOWING ACUTE EXERCISE AND ANTIOXIDANT SUPPLEMENTATION

A Thesis
by
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Submitted to the Graduate School
Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2010
Department of Health, Leisure and Exercise Science

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ABSTRACT

VASCULAR AND HEMODYNAMIC PARAMETERS FOLLOWING ACUTE EXERCISE AND ANTIOXIDANT SUPPLEMENTATION
(May 2010)

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Objective: The objective of this study was to investigate the potential additive effects of the multi-flavonoid plus fish oil combination and aerobic exercise on post exercise hypotension (PEH). We hypothesize that the use of a multi-flavonoid plus fish oil supplement in combination with aerobic exercise will show an additive effect on favorable blood pressure (BP) and pulse wave velocity (PWV) changes while increasing arterial distensibility. **Design:** Hemodynamic and vascular measurements were performed pre and post exercise and before and after double blinded placebo or supplementation of 1000 mg quercetin (Q) with 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, and 400 mg omega-3 fatty acids from fish oil (Q-EGCG). **Setting:** Visits took place at the Vascular Biology and Autonomic Studies lab or the Human Performance lab at Appalachian State University. **Participants:** 20 young, healthy, pre-hypertensive subjects not on any medication, including aspirin or birth control, and without known cardiovascular disease, diabetes, or hypertension were recruited. **Interventions:** Two weeks of Q-EGCG or placebo. **Main Outcomes:** The dependent variables in this study were PWV, BP, Pulse wave analysis (PWA), Augmentation index (AIx) and ORAC and FRAP levels. **Results:** No significant

differences were found in PWV (central or peripheral), resting systolic blood pressure (SBP), diastolic blood pressure (DBP) or ORAC. There were significant differences found in AIx, FRAP and BP from pre to post supplementation in the post exercise SBP measurements. **Conclusions:** Two weeks of supplementation on Q-EGCG produced significant decreases in AIx, post exercise SBP and an increase in FRAP.

ACKNOWLEDGEMENTS

The author would like to offer a big thank you to Dr. Scott Collier. I would not have started or completed a thesis without your support, encouragement, and your belief in my abilities as a researcher and as a student. You believed in me when I wasn't sure I did.

Thank you to my thesis committee, Dr. Jeff Soukup and Dr. Dave Morris for all of their hard work and time spent reading and revising my thesis. Your suggestions and comments meant a lot to me and I greatly appreciate the time you took out of your schedule to sit down with me to review my writing.

My research would have never been completed if it wasn't for the 20 subjects who spent hours of their time undergoing testing. I am also grateful for the many people who generously spent long hours donating their time to help with my research: Chelsea Curry, Janice Welsh, Martin Hubner, Carol Cook, and Kristin Campbell.

I would not be here if it wasn't for my wonderful family and friends. I am so blessed to have the love of so many people. I am especially thankful to my parents, who have always encouraged and supported me unconditionally. Without your love, I would not be here.

This research was possible with the help of Appalachian State University's Graduate Student Association Senate Research Grant, Cratis D. Williams Graduate Student Research Grant, and BeActive NC/Appalachian Partnership Grant.

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Chapter 1
Introduction

Hypertension is a chronic medical condition in which there is an elevation in blood pressure (BP) of 140/90 mmHg or above (Chobanian et al., 2003) and is considered to be a risk factor for cardiovascular disease (Cockcroft, Webb, & Wilkinson, 2000). Untreated hypertension is a risk factor for stroke, heart attacks, heart failure and arterial aneurysm and is the leading cause of renal failure (Pierdomenico et al., 2009). Epidemiological studies have shown that individuals with untreated, severely high blood pressure (250/130 mm Hg) can expect to live no more than a few years unless medically treated (Guyton & Hall, 2005).

Beginning at a systolic pressure of 115 mmHg and a diastolic pressure of 75 mmHg, cardiovascular disease risk doubles for each increment of 20/10 mmHg (Chobanian et al., 2003). In addition, the prevalence of childhood hypertension in increasing (Honzikova et al., 2006) and regardless of age onset, early detection is important to begin a treatment plan that could prevent long term damage (Gan, Loh, & Seet, 2003).

Following an acute bout of exercise, there is a drop in blood pressure, and chronic exercise leads to a more sustained fall in BP. Post-exercise hypotension (PEH) can be defined as a sustained reduction in blood pressure in both normotensive and hypertensive subjects following a bout of exercise. The length of the hypotensive response in individuals with pre-to stage-one hypertension (>120/80 mm Hg) can last up to 24 hours, comparatively,

in normotensives the PEH response typically lasts 1 hour (Halliwill, 2001). It is generally agreed that PEH reflects a fall in peripheral vascular resistance that is not completely offset by an increase in cardiac output in the post-exercise period (Halliwill, 2001). Numerous studies have confirmed the existence of PEH in normotensive and hypertensive individuals and in various animal models (Halliwill, 2001; Kenney & Seals, 1993; MacDonald, 2002; Martin, Dubbert, & Cushman, 1990; Rossow et al., 2010).

The reduction in peripheral vascular resistance is mediated by nitric oxide (NO), adenosine, and the alteration of sympathetic outflow. NO, also known as endothelium-derived relaxing factor (EDRF), acts as a potent vasodilator by promoting vascular smooth muscle relaxation, which can reduce vascular resistance to blood flow, therefore decreasing blood pressure (Boone & Corry, 1996). NO expression has been shown to be upregulated following exercise, due to rapid blood flow through arteries causing shear stress on the endothelial cells, which use NO to signal to the surrounding smooth muscle to relax, resulting in vasodilation (Guyton & Hall, 2005). Reductions in NO bioavailability may be caused by decreased expression of the endothelial cell NO synthase (eNOS), a deficiency of substrate or cofactors for eNOS, modification of cellular signaling so that eNOS is not activated, or NO degradation by reactive oxygen species (ROS, i.e.: oxidative stress) (Cai & Harrison, 2000). Exogenous antioxidant supplementation has been shown to reduce inflammation and oxidative stress, which will increase the bioavailability of NO (Barton, Ni, & Vaziri, 2001; Eiserich, Butler, van der Vliet, Cross, & Halliwell, 1995; Forstermann, Boissel, & Kleinert, 1998; Kingwell, 2000; Ohta, Nanri, Matsushima, Sato, & Ikeda, 2005) and recent studies have shown that antioxidants can reduce blood pressure (Edwards et al., 2007). Flavonoids in particular are strong antioxidants due to their low redox potential and

capacity to donate several electrons or hydrogen atoms. They consist of a group of polyphenolic substances that occur naturally in fruits, vegetables, grains, herbs, tea and fruit juices. The most widespread of all flavonoids is quercetin, an antioxidant with a variety of bioactive effects including anti-inflammatory, anti-pathogenic, anti-oxidant, and immunoregulatory influences. Quercetin supplementation in high dosages for an extended period of time has not been linked to any adverse effects in humans or rodents, and some researchers suspect a high quercetin intake from food may reduce risks of ischemic heart disease, type 2 diabetes, asthma, and various types of cancer including lung, colorectal, pancreatic, and prostate (Nieman et al., 2009). There are few studies that have investigated the effects of antioxidants on human vasculature, and to this author's knowledge, no studies have investigated the effects of a multi-flavanoid and fish-oil supplement with or without exercise intervention.

The current study investigated hemodynamic and vascular responses and blood antioxidant levels following a 2-week multi-flavonoid and fish oil supplement or placebo in 20 young healthy males and females (18-26 y) without known cardiovascular disease. All subjects underwent hemodynamic and vascular measurements before and after exercise as well as prior to, and following a 2 week oral supplementation regimen of the compounded multi-flavonoid and fish oil supplement.

Statement of the Problem

Hypertension is a risk factor for stroke, myocardial infarction, heart failure and arterial aneurysm and is the leading cause of renal failure (Pierdomenico et al., 2009). Finding an effective and non pharmacological treatment for hypertension would help reduce

health care costs by the use of exercise and supplementation instead of prescription medications, which in turn would decrease insurance costs. Therefore, the purpose of this study was to investigate the potential additive effects of the multi-flavonoid plus fish oil supplement and aerobic exercise on post-exercise blood pressure and arterial homeostasis.

Hypothesis

We hypothesize that the use of a multi-flavanoid plus fish oil supplementation in combination with aerobic exercise will show further decreases in blood pressure during post-exercise hypotension. Further, we hypothesize that the treatment group will show greater decreases in pulse wave velocity (PWV) and augmentation index.

Significance of the Study

The pharmacological treatment of hypertension can be costly and in some cases ineffective, and produce many negative side effects (Collier, 2008). Due to the increasing numbers of hypertensive individuals, there has been a substantial increase in costs pertaining to diagnosing and treating hypertension. This study provides clinical and economical value since hypertensives could possibly reduce their blood pressure with supplementation and exercise. It also could reduce the dependency of hypertensive individuals on medication, which can be unsuccessful and have multiple negative side effects. Not only could exercise and antioxidant supplementation be a treatment, but this treatment may also be used as a preventative measure.

Definition of Terms
(Berne, 2001; Brooks G, 2000)

Blood pressure (BP) – the pressure exerted by the blood on the walls of the blood vessels.

Systolic blood pressure (SBP) - the highest pressure in the arteries during the contractile phase of the cardiac cycle

Diastolic blood pressure (DBP) - the lowest pressure during the resting phase of the cardiac cycle

Baroreceptor Reflex – One of the body's mechanisms for maintaining blood pressure. It is a negative feedback loop in which an elevated blood pressure causes a decrease in vasomotor tone due to an increase in relaxing factors. A decreased blood pressure depresses the baroreflex, causing blood pressure to increase.

Mean arterial pressure (MAP) - the average arterial pressure during a single cardiac cycle and can be approximated by the equation MAP = DBP + 1/3(SBP-DBP)

Arterial distensibility (AD) – defined as change in an artery's diameter/change in blood pressure within that artery

Arterial stiffness - Measures of arterial stiffness estimate the artery's ability to expand and contract with cardiac pulsation and relaxation.

Pulse wave velocity (PWV) - The velocity of travel of a pressure wave along an artery is related to the stiffness of an arterial segment between measurement sites. PWV measures arterial stiffness indirectly since it is influenced by a number of factors, including wall thickness, vessel radius, or blood density independent of arterial stiffness. PWV = Distance (m) / time (s)

Augmentation Index (AIx) - The percentage of central pulse pressure (the difference between systolic and diastolic blood pressure) that is reflected back to the heart, derived with pulse

wave analysis and BP measurements. AIx is considered to be an index that is linked to arterial stiffness and cardiac afterload.

Chapter 2
Literature Review

Hypertension, commonly referred to as elevated blood pressure (≥140/90 mmHg), is widely prevalent in the western world and it imposes a massive burden on health care costs in the United States. The cost of hypertension, both direct and indirect, were estimated to be $69.4 billion for the year of 2008, making it the second most costly cardiovascular related disorder (Lynch, Markosyan, Melkonian, Pesa, & Kleinman, 2009). Therefore, it is important to investigate new pharmacological and non-pharmacological modalities for treating this problem. Currently, most hypertensives are being treated with some form of prescription medication. However, non-pharmacological medical treatments are in demand in order to ease the burden on healthcare and insurance costs in addition to lessening the negative side effects associated with prescription medications. It is commonly known that an acute bout of exercise leads to a decrease in blood pressure (Hamer, 2006), making exercise an effective treatment for hypertension. In recent literature, antioxidants have been studied for their effect on blood pressure and have been shown to increase NO levels, reducing inflammation and oxidative stress (Barton et al., 2001; Eiserich et al., 1995; Forstermann et al., 1998; Kingwell, 2000). An increase in the bioavailability of NO with a concomitant

decrease in vascular inflammation leads to augmented levels of vasodilation, subsequently decreasing systemic blood pressure. Therefore, the purpose of this study is to determine the effects of a combined exercise and antioxidant supplementation regimen on blood pressure and pulse wave velocity, when compared to placebo.

Hypertension

Hypertension is an increasing problem in today's society. In 2006, 73.6 million American adults were affected by hypertension, which increased from 65 million in 2000 (Lynch et al., 2009) and it is predicted to affect a third of the world's population by 2025 (Hamer, 2006). In 2000, over 150,000 people died from hypertension, which was a primary or secondary cause attributed in their death (Hajjar, Kotchen, & Kotchen, 2006). When left untreated, hypertension will increase the risk of coronary artery disease and stroke (Hajjar et al., 2006). The disease results from two major factors; elevated cardiac output and/or increased peripheral resistance and eventually the heart will abnormally remodel and heart failure risk will increase due to a decrease in left ventricular function (Mayet & Hughes, 2003).

Arterial Stiffness

Healthy large arteries contain elastin, smooth muscle, and collagen, which allow them to be acquiescent and buffer the pressure change due to ventricular ejection (Cockcroft et al., 2000). When degradation occurs in their walls, the arteries are not as compliant and are unable to absorb the high energy of the blood outflow. Arterial stiffness is the "hardening"

or loss of elasticity in the arteries, which can accelerate the atherosclerotic process and is considered to be a predictor of cardiovascular disease (Maki-Petaja & Wilkinson, 2009). When arteries lose their elasticity, this reduces their ability to buffer the pressure change in the cardiac pulse, leading to an increase in systolic blood pressure and a decrease is diastolic blood pressure, or an overall increase in pulse pressure, which is an important predictor of cardiovascular risk (Cockcroft et al., 2000). It is recognized that arterial stiffness results from a loss of elastin fibers in the vasculature and is modulated by endothelium derived mediators, such as nitric oxide and endothelin-1. The destruction of elastin leads to the stiffening of collagen fibers, since the collagen fibers bear the pressure that the elastin can no longer handle (Maki-Petaja & Wilkinson, 2009). The severity of coronary artery disease is associated with aortic stiffness (Cockcroft et al., 2000), and because of this, arterial stiffness has received increasing attention in recent literature (Kingwell, Berry, Cameron, Jennings, & Dart, 1997; Williams et al., 2007). Non-distensible arteries contribute to increased peripheral resistance, higher pulse pressures and increased ventricular afterload (Benetos et al., 2002; Dart & Kingwell, 2001; Kingwell & Gatzka, 2002; Kingwell, Waddell, Medley, Cameron, & Dart, 2002). This is of clinical importance because increases in the stiffness of central elastic arteries such as the carotid and aorta have been associated with increased mortality and morbidity and is now recognized as independent risk factors for cardiovascular disease (Mackenzie, Wilkinson, & Cockcroft, 2002).

Arterial stiffness is an important assessment of vascular health and can be measured noninvasively using pulse wave velocity measurements. When the heart contracts it generates a pulse or energy wave that travels through the circulation. The velocity of travel of this pulse wave, measured in meters per second, is related to the time it takes for the pulse

wave to cover a specific distance, which reflects the stiffness of the arteries. Pulse wave velocity (PWV) is dependent upon the distensibility of the vasculature (Cockcroft et al., 2000), and a higher PWV is indicative of a higher aortic stiffness (less distensibility), which increases the risk of a cardiovascular event (Mitchell et al.). Distensibility is measured by PWV using the distance/time equation whereas the pulse wave distance is measured in an arterial segment and the time travelled by this blood bolus is ascertained by Doppler.

Another important measure of arterial stiffness is the Augmentation Index (AIx) which is the percentage of the central pulse pressure that is attributed to the refracted pulse wave. AIx is an estimate of aortic vessel elasticity and is derived by pulse wave analysis (PWA and BP measurements (Kelly, Hayward, Avolio, & O'Rourke, 1989)). Nurnberger et al. (2002) found that AIx supports a positive correlation with cardiovascular risk and mortality, which may demonstrate the need for using this measure where measurements of arterial stiffness can be clinically significant (Nurnberger et al., 2002).

Sung et al. (2009) studied the effects of exercise on arterial stiffness among patients with coronary artery disease and healthy individuals. Arterial stiffness was measured using pulse wave velocity, and it was shown that following exercise there were significant decreases in pulse wave velocity, with a larger decrease shown in the patients with coronary artery disease. However, arterial distensibility measurements (PWV) have not been measured on normotensive subjects after an acute bout of exercise. Although normotensive subjects might have some breakdown in the elasticity of their vasculature, it would not be as pronounced as in hypertensive individuals, so there could potentially be a difference in the distensibility of arteries post exercise.

Post Exercise Hypotension

Following an acute bout of exercise there is a decrease in blood pressure termed post exercise hypotension (Hamer, 2006). The mechanisms and durations of PEH are not fully understood, but because of this decrease in BP, PEH has the potential for preventing and treating hypertension (MacDonald, 2002). Hypertension is less prevalent in people with high fitness levels due to the effects of chronic exercise on resting blood pressure and reductions in blood pressure immediately post exercise (Hamer, 2006). Current interventions used to treat hypertension include pharmacological and psychological therapies, exercise and weight loss, or any variety of these strategies. By far, the major therapy used is prescription medication and these medical therapies can have significant negative side effects. Because of PEH, moderately intense exercise has been studied as a treatment and a prevention of hypertension that has no long term side effects (Hagberg & Brown, 1995). Even moderately intense exercise as short as 10 minutes has been shown to decrease resting blood pressure and also have benefits as a non-pharmacological aid to the treatment of hypertension (MacDonald, MacDougall, & Hogben, 2000).

Halliwell (2001) confirmed the existence of PEH due to reductions in vascular resistance mediated by the autonomic nervous system and the release of local vasodilators. It was shown that PEH was more pronounced and long lasting in people with hypertension. Hamer (2006) explored differences between acute and chronic mechanisms and showed that exercise treatment of hypertension should be individualized depending on each person's response to exercise and level of disease status. Furthermore, it was shown that with exercise there was a decrease in blood pressure, but it wasn't clear what the intensity, duration, and frequency of the exercise should be in order to cause this drop in blood pressure. However, a

recent investigation from Rossow et al. (2010) showed that amongst endurance trained men and women, the drop in blood pressure following exercise was the same whether high or moderate intensity exercise was performed.

Acute aerobic exercise leads to a reduction in both systolic and diastolic blood pressure with a drop of 18 to 20 mm Hg and 7 to 9 mm Hg, respectively, in hypertensives (Kenney & Seals, 1993). Rueckert et al. (1996) found that with hypertensive individuals there was a post exercise hypotensive response that lasted up to two hours after an exercise bout. Ten minutes after exercise there was a significant decrease from baseline systolic pressure, mean arterial pressure, total peripheral resistance, and calf vascular resistance. Systolic blood pressure (SBP) and mean arterial pressure (MAP) were maintained for two hours post exercise and total peripheral resistance (TPR) returned to baseline within 20 minutes (Rueckert, Slane, Lillis, & Hanson, 1996). However, this study did not examine the effects of PEH on normotensive individuals.

Mechanisms

The most influential factor of short term blood pressure control is the baroreflex feedback loop (Overhaus, Ruddel, Curio, Mussgay, & Scholz, 2003), which are pressure sensors that regulate BP through a negative feedback loop. Individuals with hypertension demonstrate cardiac autonomic dysfunction and altered baroreflex sensitivity (BRS) (Pikkujamsa et al., 1998). The baroreceptor system adapts to the high pressure within days by increasing the stimulation threshold of the pressure receptor (AHA, 2003), so hypertensives develop less sensitive baroreflexes than healthy individuals. Cardiac

autonomic dysfunction results from changes in adrenergic modulation and dysregulation of parasympathetic and tonic cardiovascular control, demonstrated by heart rate variability (HRV) measurements (Lakatta, 1993). The baroreflex in hypertensive individuals also resets to maintain a higher blood pressure, due to a vagal tone decrease and a sympathetic outflow increase (Prakash, Madanmohan, Sethuraman, & Narayan, 2005). As blood pressure increases further with chronic hypertension, baroreflex sensitivity will continue to decrease (Pikkujamsa et al., 1998). This is of clinical importance since reductions in HRV and BRS have been associated with increased mortality and morbidity and an increase in the prevalence of cardiovascular diseases (La Rovere, Specchia, Mortara, & Schwartz, 1988; Nishiue et al., 1999; Singh et al., 1998; Tsuji et al., 1996). It is believed that the decrease in BRS is a cause and maintaining factor of hypertension (Overhaus et al., 2003).

As mentioned earlier, exercise provides an antihypertensive hemodynamic effect (Arakawa, 1993; Arroll & Beaglehole, 1993; Kelley & McClellan, 1994; Martin et al., 1990). These changes in blood pressure may be associated with changes in autonomic function which is controlled within the higher centers of the brain through the division of parasympathetic (vagal modulation and decrease in heart rate) vs. sympathetic (increase in heart rate) branches of the nervous system. It has been shown that cardiovascular autonomic modulation and blood pressure can be improved with exercise training in pre-hypertensive individuals (Collier et al., 2009; Ketelhut, Franz, & Scholze, 2004; Lucini et al., 2002; Timmers, Wieling, Karemaker, & Lenders, 2004). It is also more judicious and safer to study pre-hypertensive individuals during exercise training since they have less risk factors, yet their hemodynamic responses liken that of hypertensives where we would extrapolate the results.

The structure of the vasculature does not determine arterial stiffness by itself; the endothelium releases vasoactive substances such as nitric oxide (NO) and endothelin (Cockcroft et al., 2000). Some other mechanisms of post exercise hypotension are mediated by adenosine and the alteration of sympathetic outflow (Boone & Corry, 1996). Boone & Corry (1996) showed that NO acts as a powerful vasodilator which can reduce vascular resistance to blood flow, thus decreasing blood pressure, and is upregulated following exercise.

Shear stress and pressure on the endothelium lead to signaling of vasodilators such as nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factors (EDHF) (Rush & Ford, 2007). NO is a vasodilator and its bioavailability is dependent on its synthesis and destruction. Endothelial nitric oxide synthase (eNOS) is prevalent in the endothelium and myocardium and generates NO when stimulated by physical and chemical factors, such as shear stress and Angiotensin II (Ang II) (Izzo, Sica, & Black, 2008). NO must then diffuse to vascular smooth muscle to cause relaxation and dilation of the blood vessel, decrease vascular resistance and increase blood flow. NO induced endothelium dependent relaxation has a direct vasodilatory effect on the surrounding smooth muscle, but NO also has several indirect vasodilatory effects such as the downregulation of Ang II, counteracting agonist-induced vasoconstriction and the inhibition of endothelin-1, a powerful vasoconstrictor. The inhibition of NO upregulates Angiotensin Converting Enzyme (ACE), increases Ang II, increases a toxic free radical, superoxide (O_2^-), and induces vasoconstriction, which ultimately leads to hypertension (Izzo, Sica, & Black, 2008). NO can also be degraded when it interacts with ROS. Several studies have shown that in sedentary individuals, there is a higher presence of reactive oxygen species (ROS) which has

been shown to decrease NO (Barton et al., 2001; Eiserich et al., 1995). Normally, cells are protected from ROS damage by enzymes like superoxide dismutase (SOD) and antioxidants prevent ROS damage by scavenging free radicals. Under normal conditions, ROS are produced in low concentrations and regulate vascular homeostasis, but in hypertensive individuals, there is an elevation in ROS which decreases NO bioavailability and leads to vascular dysfunction (Rush & Ford, 2007).

Exercise and oxidative stress

Reactive oxygen species (ROS), reactive nitrogen species (RNS) and their by-products are termed free radicals and have been linked to premature vessel aging and disease by modifying cellular components (Goto, Naito, Kaneko, Chung, & Radak, 2007). ROS are composed of two main groups: free radicals and nonradicals. Free radicals such as superoxide (O_2^-), hydroxyl (OH) and nitric oxide (NO) have one or more unpaired electrons which contribute to the instability of the compound; while nonradicals like hydrogen peroxide (H_2O_2), ozone (O_3) and peroxynitrite ($ONOO^-$) are more stable compounds because they do not have a single unpaired electron. Nonradicals are reduced when its electrons are accepted into orbitals and function as a strong oxidizing agent.

The significance of superoxide production is that is can lead to the impairment of endothelium dependent relaxation by increasing vascular oxidative stress, leading to eNOS uncoupling and a shift to production of superoxide instead of NO by the oxidation of tetrahydrobioprotein (BH_4). ROS are scavenged by antioxidant systems such as glutathione

peroxidase (GTP) and SOD, however when this balance is disturbed the physiological consequence is vascular-oxidative stress (Izzo, Sica, & Black, 2008).

Free radicals, and more specifically ROS levels, increase due to inflammation produced from an acute exercise bout. This leads to an increase of oxidative stress and cellular damage (Goldfarb, Patrick, Bryer, & You, 2005). It has been shown that 30 minutes of aerobic and anaerobic exercise can increase biomarkers of oxidative stress (Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005). The damage resulting from oxidative stress has been suggested to contribute to many diseases, such as atherosclerosis, Parkinson's disease, heart failure, heart attacks, and Alzheimer's disease (de Diego-Otero et al., 2009). Vascular oxidative stress is elevated in hypertensives, leading to the elevation of ROS levels which impairs NO dependent dilation. Antioxidant therapy has been shown to buffer this increase of ROS and therefore restore NO dependent dilation to the vasculature (Rush & Ford, 2007). Antioxidants could protect cellular components from ROS (Goldfarb et al., 2005) and it has been shown that the antioxidant supplementation of Vitamin C can protect against exercise induced oxidative stress (Goldfarb et al., 2005). Two weeks of an antioxidant supplement reduces exercise induced oxidative stress in both males and females (Goldfarb, McKenzie, & Bloomer, 2007). However, most of these studies have used vitamins, such as Vitamin C, D, or E, which have been shown to possess antioxidant capabilities, yet have used only a single vitamin in high concentrations. This study will be one of the first to study the effects of a multi-flavonoid compound plus fish-oil supplement on cardiovascular hemodynamics and arterial stiffness.

Antioxidants

Flavonoids are strong antioxidants because of their low redox potential and capacity to donate several electrons or hydrogen atoms. Flavonoids are found in common foods such as onions, apples, dark chocolate, green tea, red wine, and grapefruit. Antioxidant supplementation reduces acute exercise induced inflammation and oxidative stress, which increases the bioavailability of NO (Barton et al., 2001; Eiserich et al., 1995; Forstermann et al., 1998; Kingwell, 2000). It has also been shown that antioxidant supplementation is beneficial in offsetting the negative effects of vascular oxidative stress in elderly people by increasing blood flow to skeletal muscle, thereby enhancing the benefits of exercise. Increased blood flow and decreases in vascular oxidative stress has been shown to reduce cardiovascular risks by further decreases in blood pressure (Galan et al., 2006).

Quercetin

Quercetin is a plant-derived flavonoid commonly used as a nutritional supplement and is found in foods such as onions, apples, red wine, broccoli and tea (McAnulty et al., 2008). Laboratory studies show that quercetin may have anti-inflammatory and antioxidant properties, and it is currently being investigated for a wide range of potential health benefits (Davis, Murphy, Carmichael, & Davis, 2009; Stewart et al., 2008). There is also research that has been done that suggests that quercetin reduces blood pressure in hypertensive subjects (Edwards et al., 2007). In rat models, severe oxidative damage produced from swimming was decreased due to quercetin supplementation, showing that quercetin may be effective for the prevention and treatment of oxidative damage (Haleagrahara,

Radhakrishnan, Lee, & Kumar, 2009). In a recent study, supplementation of quercetin plus Vitamin C was shown to increase plasma quercetin levels but have no influence on oxidative stress or antioxidant capacity measures (Shanely et al., 2009). Chronic quercetin ingestion has not been proven to protect against exercise induced oxidative stress or inflammation (McAnulty et al., 2008). However, the supplement used in the present investigation was a mixture of several antioxidants and multi-flavonoids in addition to quercetin, which may have a synergistic effect resulting in a decrease in oxidative stress, leading to an upregulation of NO which in turn augments vasodilation and decreases blood pressure.

EGCG

EGCG (epigallocatechin 3-gallate) or green tea extract, has beneficial effects on cardiovascular and metabolic health and it has been suggested that EGCG may contribute to the maintenance of health and treatment of diseases (Wolfram, 2007). EGCG also decreased stroke and mortality in stroke-prone, spontaneously hypertensive rats (Uchida et al., 1995). In relation to blood pressure, EGCG has been shown to decrease systolic blood pressure and enhance endothelial function and insulin sensitivity of spontaneous hypertensive rats. It also decreased infarct volume and improved ventricular function after ischemia reperfusion (Potenza et al., 2007).

Fish Oil

Fish oil has been shown to have many benefits concerning cardiovascular health. It was first researched when the Greenland Inuits, who have a diet high in n-3 polyunsaturated fatty acids (like fish oil) were found to have low mortality from cardiovascular disease (Leaf, Kang, & Xiao, 2008). People with a high risk of ischemic heart disease and/or hypertension could benefit from eating fish as clinical trials have shown that undergoing supplementation of fatty acids like fish oil can improve arterial elasticity in subjects with diabetes or dyslipidemia and even improved large arterial elasticity in overweight hypertensive patients (Wang et al., 2008). A combination of fish oil and aerobic exercise has been shown to be more effective compared with separately to decrease triglycerides, increase high-density lipoprotein (HDL) and improve endothelium dependent arterial vasodilation (Hill, Buckley, Murphy, & Howe, 2007). However, results from fish oil students are equivocal. A study by Lofgren, et al. (1993) found no change from pre-supplementation in systolic or diastolic blood pressure.

Because quercetin, EGCG, and fish oil have shown positive effects on arterial health separately, it is possible that a combination could be more effective on vasculature parameters such as PWV, an increase in vasodilation leading to a decrease in blood pressure.

Measurements of Antioxidant Capacity

FRAP and ORAC Assays

The Ferric Reducing Ability of Plasma (FRAP) assay measures the ferric reducing ability within the blood in the presence of antioxidants whereas the Oxygen Radical Absorptive Capacity (ORAC) assay measures the capacity of an antioxidant to directly reduce free radicals. The ORAC assay has a high sensitivity for the absorbed oxygen radicals and provides considerable information concerning the antioxidant capacity of biological samples such as melatonin, dopamine, and flavonoids, as well as tea, fruits, vegetables, and animal tissues (Cao & Prior, 1998).

There is little correlation between the ORAC and FRAP assay because they use different methodologies of determining antioxidant capacity. ORAC uses an inhibition method in which a blood sample is added to a system that generates free radicals, the inhibition of the free radical action is calculated and related to the antioxidant capacity of the sample. FRAP, however, uses no free radicals or oxidants in the assay (Cao & Prior, 1998). Therefore, ORAC measures the inhibition of free radicals generated and the FRAP assay uses antioxidants as reductants in a redox-linked reaction to determine the total antioxidant power of the blood.

Summary

Hypertension is a serious yet treatable condition. However, if it remains untreated, it can lead to many health problems, cardiovascular disease and a decreased life span. Currently, the most common treatment is medication, which can lead to multiple side effects or can interact negatively with other medications. It is apparent in the literature that exercise leads to a decrease in blood pressure which can be effective in treating hypertension. Antioxidants have been shown to increase NO, which in turn increases vasodilation, which leads to a decrease in the pressure of blood traveling through the arteries. A combination of PEH and the anti-hypertensive effects of antioxidants could possibly lead to an effective treatment for hypertensives that does not require medication. Assuming this treatment is safe and effective in a young population, the research problem would then be applied to a hypertensive population. This research is important due to the issues that hypertension presents both socially and economically.

Chapter 3
Methodology

Subjects

Twenty subjects (2 females and 18 males) were recruited in the age range of 18-26 years and were moderately active as classified by Ainsworth et al. (1993). Subjects were recruited through posters/fliers located throughout the University. During initial screening, subjects were asked about any known cardiovascular disease, with special emphasis on sudden cardiac death in any young first, or second degree relatives. Subjects with any such family history were excluded. Any subject with known cardiovascular disease, diabetes, hypertension, or currently taking any medication, including birth control and aspirin, was also excluded. Procedures were reviewed by Appalachian State University's Internal Review Board. All subjects read and signed informed consent forms to show willingness to participate in the research study and were allowed to withdraw at any time.

Research Design

Subjects underwent a total of four visits, as outlined below:

Visit 1 consisted of lab familiarization, informed consent, a graded exercise test performed on a treadmill to determine VO_{2peak}, and a body composition assessment. The subjects underwent a fasting blood draw on their second visit.

Upon entering the laboratory for Visit 3, subjects were seated for 5 minutes and had a resting blood pressure taken in their left arm using a manual sphygmomanometer and a stethoscope, as well as pulse wave analysis measurements. The subjects then rested in a supine position while pulse wave velocity measurements were taken, after which the subjects were left in a quiet, darkened room for 10 minutes while beat to beat blood pressure measurements were recorded. After beat to beat measurements, the subjects underwent a pre-exercise blood draw, followed by a run on the treadmill at 65-70% of their VO_{2peak} for 30 minutes. Immediately following completion of exercise, a second blood draw was performed and then a seated manual blood pressure and pulse wave analysis measurements were obtained. Finally, the subjects were placed in a supine resting position and had PWV and beat to beat BP measurements. All above described measurements were repeated 30 minutes following exercise.

After the third visit, the subjects started on a fourteen day supplementation of an antioxidant mixture of quercetin, omega-3, and EGCG or a placebo, which was similar in composition and taste but without the active ingredients. The daily dose of four treatment supplements provided 1000 mg quercetin (Q) with 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, and 400 mg omega-3 fatty acids from fish oil (Q-EGCG). The subjects were instructed to take four doses a day, with two taken in the morning and two taken in the evening. Once the fourteen day supplementation was completed, the subjects reported back to the lab within 24 hours for their fourth and final visit. The procedures for the fourth visit were identical to the third visit, except they were post-supplementation.

The female subjects had to arrange their supplementation and visits around their menstrual cycle to control for fluctuating hormones, due to the cardioprotective effect of

estrogen. Both Visit 3 and 4 were performed between day 1 and day 5 of their menstrual cycle. Following visit 3, supplementation was withheld in an attempt to allow visit 4 to occur between day 1 and 5 of their cycle. Retrospectively, both female subjects were tested at Visit 3 and 4 on the second day of their menstrual cycle.

Analyses: Biomarkers of Oxidative Stress

Fasted blood samples were drawn from an antecubital vein with subjects in a seated position. The blood samples were centrifuged in sodium heparin or EDTA tubes, and plasma was aliquoted and then stored at -80°C prior to analysis for plasma antioxidant parameters (FRAP and ORAC).

FRAP

Total plasma antioxidant potential was determined by the ferric reducing ability of plasma (FRAP) assay according to the methodology of Benzie and Strain (1996). The general principle of this assay is that water soluble reducing agents (antioxidants) in the plasma will reduce ferric ions to ferrous ions, which then react with an added chromogen. Samples and standards were analyzed in duplicate and expressed as ascorbate equivalents based on an ascorbate standard curve (0-1000 μmol). Intra-assay and inter-assay coefficients of variation were less than 5% and 7%, respectively.

ORAC

The ORAC assay was performed on a microplate reader using a modification of the methodology of Ou et al. (2001). The ORAC assay is based upon the inhibition of the peroxyl-radical-induced oxidation initiated by thermal decomposition of azo-compounds such as 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH). Briefly, trolox standards were made from a trolox stock solution. A serial dilution of a 50 μM trolox solution was made with phosphate buffer solution to produce 25, 12.5, 6.25 μM trolox standards. A fluorescein (Aldrich Chemicals, St.Louis, MO) working solution was made by pipetting 800 μL of stock solution into 50 mL phosphate buffer in a 50 mL conical tube. Before use, the solution was incubated in the water bath at 37°C until thoroughly heated. The AAPH solution was made by dissolving 0.108 g of AAPH (Wako Chemical) into 5 mL of incubated phosphate buffer immediately before the start of the assay. The microplate was prepared and loaded in a "forward-then-reverse" order to avoid possible positional errors. The edge wells were left empty or blank (phosphate buffer working solution) to reduce the impact of "edge effect" on samples and standards particularly from temperature effects on the outside wells. Twenty μL of sample, blank, and trolox standard solutions were pipetted into appropriate wells. Then, 200 μL fluoroscein working solution were added to each well using an 8 channel micropipettor. A cover was placed on the microplate and the plate and contents incubated at 37 ^{o}C for at least 20 minutes. Then, 20 μL AAPH working solution were added using an 8 channel micropipettor as quickly as possible. Final ORAC values (uM/L trolox) were made from the plate reader derived area under the curve (AUC). Then, the final ORACFL values were calculated by using a quadratic regression equation $x = -b \pm \sqrt{b^2 - 4ac + 4cy}$. Excitation wavelength was 485 nm and emission wavelength was

520 nm. Intra-assay and inter-assay coefficients of variation were less than 5% and 7%, respectively.

Procedures

Visit 1

Dual Energy X-ray Absorptiometry (DEXA). Body composition was assessed determined using a DEXAscan. The subject laid in a supine position on the DEXA scan table while a low dose X-ray scanned the full body.

VO_2peak exercise testing. Peak oxygen consumption was assessed using a progressive exercise test performed to fatigue on a motor driven treadmill. The subject began the test on a level treadmill at a comfortable running pace (between 5.0 and 6.0 miles per hour) with the incline increasing 2.5% every two minutes until volitional fatigue was reached. If the subject did not reach fatigue after 2 minutes at a treadmill grade of 10%, the speed was then increased by 0.5 mph every minute. Heart rate was measured using a Polar Heart Rate Monitor (Polar Electro, Inc., Woodbury, NY, USA) and was recorded at the end of each stage, along with RPE. Expired gases were analyzed using a Quark b^2 breath-by-breath metabolic system (Cosmed, Rome, Italy). Maximal effort was considered to have been reached when subjects met three of the following four criteria: a) an RER of 1.15 or greater; b) a plateau in HR despite an increase in workload; c) a final RPE score of 17 or greater on the Borg scale (scale 6-20); and/or d) a plateau in oxygen uptake despite an increase in workload.

Visit 3 and Visit 4

Pulse Wave Velocity (PWV). All measurements were conducted in accordance with guidelines set forth by the Clinical Application of Arterial Stiffness, Task Force III. A pressure tonometer was used to transcutaneously record the pressure pulse waveforms in the underlying artery. The pressure pulse waveform was recorded simultaneously with an ECG signal, which provided an R-wave timing reference. Pressure pulse recordings were performed consecutively at two superficial artery sites. The tonometer was used to obtain the pulse wave between: (1) left common carotid artery and the left femoral artery, and (2) between the left femoral and the left dorsalis pedis artery. Distance from the carotid sampling site to the midpoint of the manubrium sterni, manubrium sternum to femoral artery, and femoral artery to dorsalis pedis was measured between these points as straight lines with a tape measure. The distance from the carotid artery to the manubrium sterni was subtracted from the manubrium to femoral artery distance. PWV was determined from the foot-to-foot flow wave velocity. The foot of the pressure wave was identified visually as the point of systolic upstroke. The time delay between a minimum of 15 simultaneously recorded flow waves was averaged. PWV was then calculated using the mean time difference and the arterial path length between the two recording sites as follows: PWV = D / t (m/s); where D is distance in meters and t is the time interval in seconds. Values attained from carotid to femoral artery were taken as an index of central compliance while values attained from the femoral to dorsalis pedis were taken as an index of peripheral compliance. All data were stored and analyzed off-line after completion of testing.

Hemodynamic Monitoring. Beat-to-beat blood pressures were determined via the Finometer (FMS, Amsterdam, The Netherlands). With subjects in a supine position in a

quiet and darkened room, beat-to-beat blood pressures were recorded for 10 minutes via finger plethysmography. This noninvasive measurement of the change in blood pressure has been shown to be reliable when compared with measurements of intra-arterial blood pressure (Imholz, Wieling, Langewouters, & van Montfrans, 1991). The brachial blood pressure was obtained using an integrated brachial blood pressure cuff and brachial BP waveforms were reconstructed from finger arterial waveforms by applying an inverse transfer function, a waveform filter, a level correction, and a level calibration (Guelen et al., 2003). This has been shown to increase the correlation between finger and proximal arterial blood pressure values, allowing pressure values to remain within the American Association for Medical Instrumentation (AAMI) standards for the evaluation of automated sphygmomanometers (Parati et al., 2003).

Supplementation. Using a double blind methodology, supplementation was provided to the 20 subjects, with 10 subjects ingesting the supplement and 10 ingesting the placebo. Supplement and placebo were provided in a chew form for 14 days, with two chews taken in the morning and two chews taken in the evening. The total daily dose of the active ingredient was 1000 mg quercetin (Q) with 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, and 400 mg omega-3 fatty acids from fish oil (Q-EGCG).

Statistical analysis

A pairwise *t*-test was employed to determine if there were differences between subject characteristics. A 3 x 2 way ANOVA with repeated measures (time [baseline vs. pre vs. post] by group [trt vs. placebo]) was performed on all dependent variables. If a

significant interaction was found then the measures were followed with a Bonferroni post-hoc test. Statistical analysis software (SPSS, Version 17.0; SPSS, Inc., Chicago, IL) was used with a significance level of $p \leq 0.05$.

Results

Subject characteristics are presented in Table 1. No significant differences were found in PWV (central or peripheral), resting SBP, DBP, heart rate (HR) or ORAC between groups. As seen in Figure 1, significant differences were found in 30 minute post exercise systolic blood pressure measurements following supplementation in the post exercise. As shown in Figure 2, AIx also showed a significant reduction following supplementation. In Figure 3, a significant increase was shown in FRAP in the supplementation group in the pre-exercise measurements. Following exercise, there were no significant differences in FRAP levels in supplement or placebo groups.

Table 1: Participant characteristics

Variable	Treatment	Placebo
N	10	10
Age (yr)	21.6 ± 0.62	20.7 ± 0.30
Height (cm)	177.4 ± 2.08	177.3 ± 2.57
Body mass (kg)	76.05 ± 3.24	75.55 ± 6.05
Peak oxygen consumption (ml/kg/min)	53.9 ± 3.88	47.9 ± 3.01

Figure 1: SBP Change: Pre to post exercise and pre to post supplementation

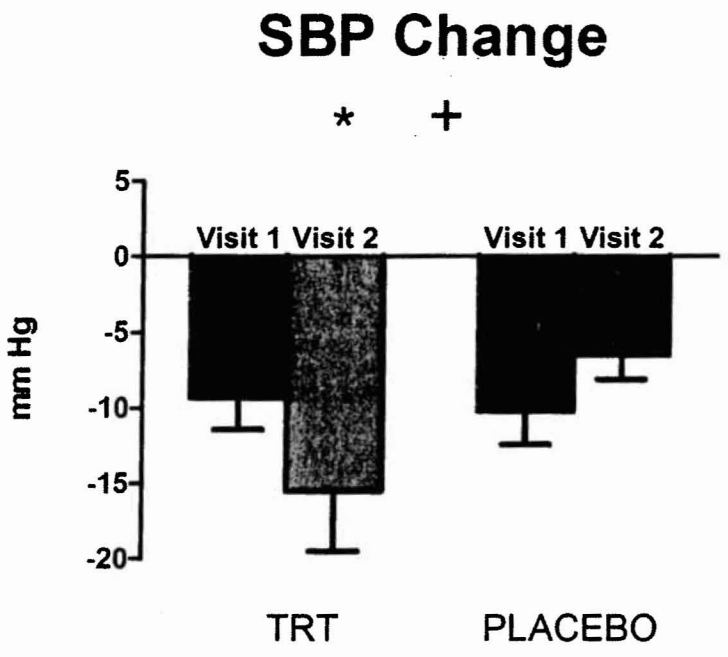


Figure 2: AIx Change: Pre to post exercise and pre to post supplementation

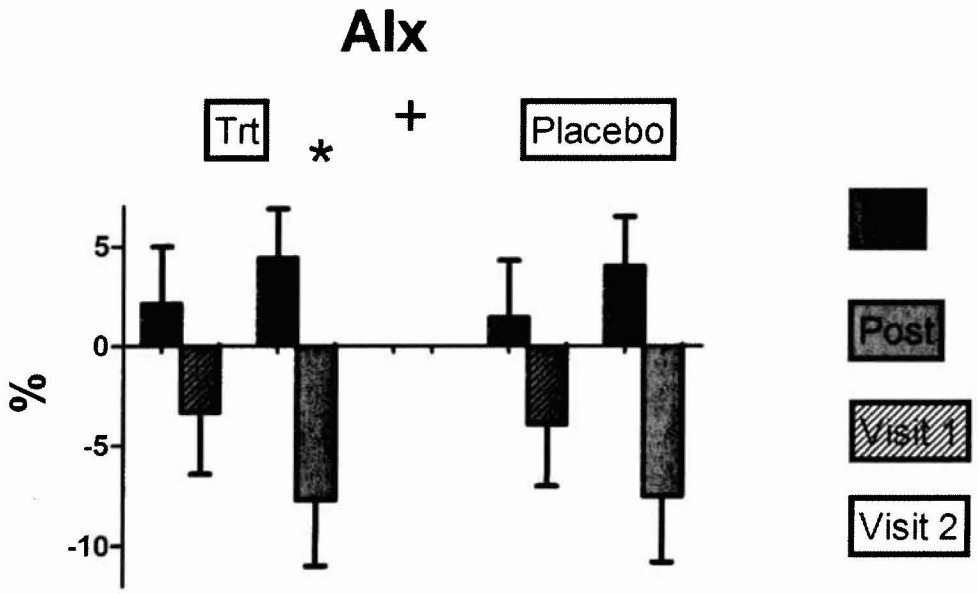
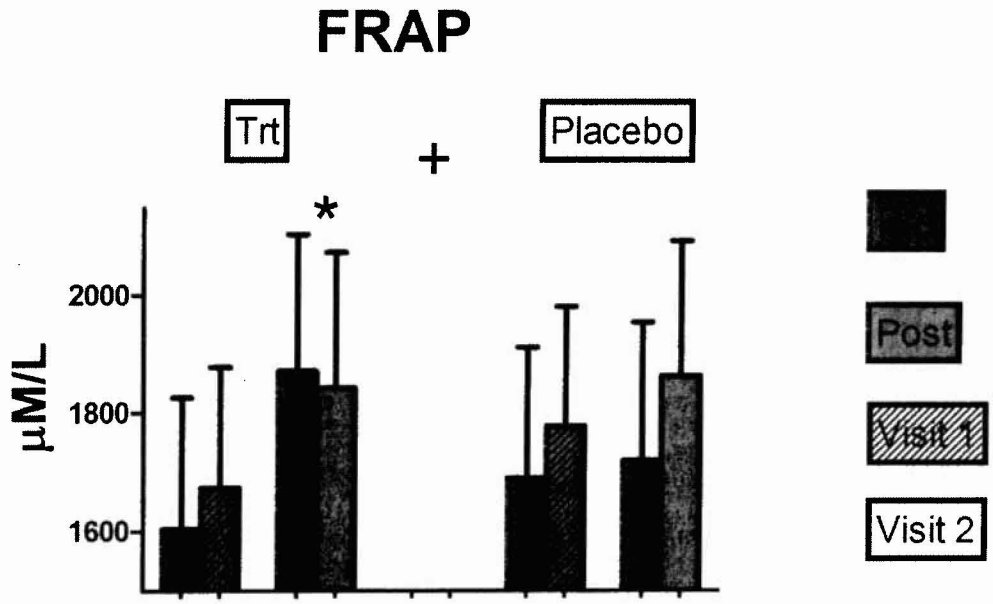


Figure 3: FRAP Change: Pre to post exercise and pre to post supplementation



Discussion

Results from the present investigation support the theory that ingesting a multi-flavonoid plus fish oil supplement in combination with aerobic exercise significantly reduces SBP 30 minutes post-exercise, AIx, and significantly increases resting levels of FRAP in post supplementation levels compared with pre-supplementation.

The attenuation of AIx, a measure linked to aortic arterial stiffness, was likely due to the significant increase in FRAP which may have led to greater expression of NO within the elastic modulus of the aortic vessel. Since the amount of reflected wave was decreased, the amount of elastic "cushion" had to be augmented to absorb the pulsatile bolus of blood being ejected from the left ventricle. The increased expression of NO following flavonoid and fish oil supplementation has been shown in many aforementioned studies. However, It was interesting to note that despite the decrease in AIx, there was no decrease in PWV as both are considered to be indicators or measures of arterial stiffness. This finding is not surprising since it has been shown on several occasions that AIx is not proportional to PWV in systolic hypertension, but is inversely related (Izzo, Sica, & Black, 2008). In one study by Vyas et al. (2007), AIx was inversely related to aortic PWV and weakly related to aortic compliance and an increase in AIx is not a reliable replacement for increased aortic stiffness. However, it did show that higher stiffness (increased PWV and lower compliance) was associated with a lower AIx (Vyas et al., 2007). The increased distensibility that resulted in a more favorable augmentation index could be due to the composition of the matrix of the vessel. The aorta has been shown to have the largest amount of eNOS potential and the supplement may have the greatest potential to upregulate this synthase pool.

Although the antioxidant supplementation did not have any effect on vascular distensibility (PWV) or resting SBP, the magnitude of PEH was significantly decreased in the 30 minute post exercise period after supplementation for the treatment condition when compared to the placebo without showing a correlating decrease in HR or PWV. Although resting SBP did not change with supplementation, there was a significant decrease in the systolic component of PEH. Because there was no change in PWV, one can only postulate that arterial distensibility was not the cause of this reduction in blood pressure. One possible explanation is that the supplementation caused a decrease in oxidative stress, potentially due to an increase in antioxidant power (increase in FRAP). A reduction in ROS would increase the bioavailability of NO, stimulating vasodilation. A second theory would be a change in sympathovagal regulation in which the supplement may induce a sympatho-lysing effect on sympathetic tone, thereby increasing the vagal tone.

The results of the present investigation support research done by Edwards et al. (2007) who demonstrated a reduction in resting blood pressure in hypertensive versus pre-hypertensive patients following an antioxidant supplementation regimen of quercetin. Many studies have shown a beneficial decrease in resting blood pressure in hypertensives, showing the possibility that this decrease in blood pressure can affect the hypertensive patient but not a normotensive or pre-hypertensive patient. This also supports research demonstrating that antioxidants (like quercetin and Vitamin C) increased plasma quercetin levels but had no influence on oxidative stress or antioxidant capacity measures (Shanely et al., 2009) and that chronic quercetin ingestion does not protect against exercise induced oxidative stress or inflammation (McAnulty et al., 2008). Without this protective mechanism against oxidative stress, it is possible that NO was not increased. Much of the research concerning antioxidant

use has been shown to have beneficial effects in animal models (Duarte, Galisteo et al., 2001; Duarte et al., 2002; Duarte, Perez-Palencia et al., 2001; Duarte, Perez-Vizcaino, Zarzuelo, Jimenez, & Tamargo, 1993, 1994; Haleagrahara et al., 2009; Ibarra et al., 2003; Ibarra et al., 2002), but there is a lack of benefits shown in human models. This is potentially because the dosages ingested are not large enough to induce any benefits physiologically and because in large quantities, most antioxidants are not able to be absorbed and are simply excreted out.

The significant increase in FRAP in the treatment group demonstrates that the total plasma antioxidant capacity increased following supplementation which may be related to the further decrease in systolic blood pressure during the post-exercise period. This increase in antioxidant capacity could lead to an increase of free radical scavenging by antioxidants, thereby decreasing oxidative stress and potentially increasing NO levels. An increase of NO in the vasculature would lead to vasodilation and a further decrease of the systolic blood pressure post exercise. Interestingly, resting blood pressure following two weeks of supplementation was unchanged, demonstrating that a decrease in blood pressure in the 30 minute post exercise period from antioxidant supplementation occurred in conjunction with post exercise vasodilation.

Figure Legends

Figure 1. The change in systolic blood pressure from pre and post exercise for Visit 3 (pre supplementation/placebo) and Visit 4 (post supplementation/placebo) amongst the trt group (n=10) and the placebo group (n=10). Values are means $\pm$ SEM. Figure 2. Augmentation index pre and post exercise for visit 1 (pre supplementation/placebo) and visit 2 (post supplementation/placebo) for Trt group (n=10) and placebo group (n=10). Values are means

± SEM. Figure 3. FRAP assay results pre and post exercise for Visit 3 (pre supplementation/placebo) and Visit 4 (post supplementation/placebo) amongst the trt group (n=10) and the placebo group (n=10). Values are means ± SEM.

Bibliography

AHA. (2003). *Hypertension Primer: The Essentials of High Blood Pressure* (3 ed.). Dallas: Lippincott Williams and Wilkins.

Ainsworth, B. E., Jacobs, D. R., Jr., & Leon, A. S. (1993). Validity and reliability of self-reported physical activity status: The Lipid Research Clinics questionnaire. *Med Sci Sports Exerc, 25*(1), 92-98.

Arakawa, K. (1993). Antihypertensive mechanism of exercise. *J Hypertens, 11*(3), 223-229.

Arroll, B., & Beaglehole, R. (1993). Exercise for hypertension. *Lancet, 341*(8855), 1248-1249.

Barton, C. H., Ni, Z., & Vaziri, N. D. (2001). Enhanced nitric oxide inactivation in aortic coarctation-induced hypertension. *Kidney Int, 60*(3), 1083-1087.

Benetos, A., Waeber, B., Izzo, J., Mitchell, G., Resnick, L., Asmar, R., et al. (2002). Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens, 15*(12), 1101-1108.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem, 239*(1), 70-76.

Berne, R., & Levy, M. (2001). *Cardiovascular Physiology*. St. Louis: Mosby.

Bloomer, R. J., Goldfarb, A. H., Wideman, L., McKenzie, M. J., & Consitt, L. A. (2005). Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J Strength Cond Res, 19*(2), 276-285.

Boone, J. B., Jr., & Corry, J. M. (1996). Proenkephalin gene expression in the brainstem regulates post-exercise hypotension. *Brain Res Mol Brain Res, 42*(1), 31-38.

Brooks, G., White, T., and Baldwin, K. (2000). *Exercise Physiology.* Boston: McGraw Hill.

Cai, H., & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. *Circ Res, 87*(10), 840-844.

Cao, G., & Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem, 44*(6 Pt 1), 1309-1315.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jr., et al. (2003). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension, 42*(6), 1206-1252.

Cockcroft, J. R., Webb, D. J., & Wilkinson, I. B. (2000). Arterial stiffness, hypertension and diabetes mellitus. *J Hum Hypertens, 14*(6), 377-380.

Collier, S. R. (2008). Sex differences in the effects of aerobic and anaerobic exercise on blood pressure and arterial stiffness. *Gend Med, 5*(2), 115-123.

Collier, S. R., Kanaley, J. A., Carhart, R., Jr., Frechette, V., Tobin, M. M., Bennett, N., et al. (2009). Cardiac autonomic function and baroreflex changes following 4 weeks of resistance versus aerobic training in individuals with pre-hypertension. *Acta Physiol (Oxf), 195*(3), 339-348.

Dart, A. M., & Kingwell, B. A. (2001). Pulse pressure--a review of mechanisms and clinical relevance. *J Am Coll Cardiol, 37*(4), 975-984.

Davis, J. M., Murphy, E. A., Carmichael, M. D., & Davis, B. (2009). Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *Am J Physiol Regul Integr Comp Physiol, 296*(4), R1071-1077.

de Diego-Otero, Y., Romero-Zerbo, Y., el Bekay, R., Decara, J., Sanchez, L., Rodriguez-de Fonseca, F., et al. (2009). Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. *Neuropsychopharmacology, 34*(4), 1011-1026.

Duarte, J., Galisteo, M., Ocete, M. A., Perez-Vizcaino, F., Zarzuelo, A., & Tamargo, J. (2001). Effects of chronic quercetin treatment on hepatic oxidative status of spontaneously hypertensive rats. *Mol Cell Biochem, 221*(1-2), 155-160.

Duarte, J., Jimenez, R., O'Valle, F., Galisteo, M., Perez-Palencia, R., Vargas, F., et al. (2002). Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. *J Hypertens, 20*(9), 1843-1854.

Duarte, J., Perez-Palencia, R., Vargas, F., Ocete, M. A., Perez-Vizcaino, F., Zarzuelo, A., et al. (2001). Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br J Pharmacol, 133*(1), 117-124.

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., & Tamargo, J. (1993). Vasodilator effects of quercetin in isolated rat vascular smooth muscle. *Eur J Pharmacol, 239*(1-3), 1-7.

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., & Tamargo, J. (1994). Inhibitory effects of quercetin and staurosporine on phasic contractions in rat vascular smooth muscle. *Eur J Pharmacol, 262*(1-2), 149-156.

Edwards, R. L., Lyon, T., Litwin, S. E., Rabovsky, A., Symons, J. D., & Jalili, T. (2007). Quercetin reduces blood pressure in hypertensive subjects. *J Nutr, 137*(11), 2405-2411.

Eiserich, J. P., Butler, J., van der Vliet, A., Cross, C. E., & Halliwell, B. (1995). Nitric oxide rapidly scavenges tyrosine and tryptophan radicals. *Biochem J, 310 (Pt 3)*, 745-749.

Forstermann, U., Boissel, J. P., & Kleinert, H. (1998). Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). *FASEB J, 12*(10), 773-790.

Galan, A. I., Palacios, E., Ruiz, F., Diez, A., Arji, M., Almar, M., et al. (2006). Exercise, oxidative stress and risk of cardiovascular disease in the elderly. Protective role of antioxidant functional foods. *Biofactors, 27*(1-4), 167-183.

Gan, S. K., Loh, C. Y., & Seet, B. (2003). Hypertension in young adults--an under-estimated problem. *Singapore Med J, 44*(9), 448-452.

Goldfarb, A. H., McKenzie, M. J., & Bloomer, R. J. (2007). Gender comparisons of exercise-induced oxidative stress: Influence of antioxidant supplementation. *Appl Physiol Nutr Metab, 32*(6), 1124-1131.

Goldfarb, A. H., Patrick, S. W., Bryer, S., & You, T. (2005). Vitamin C supplementation affects oxidative-stress blood markers in response to a 30-minute run at 75% VO2max. *Int J Sport Nutr Exerc Metab, 15*(3), 279-290.

Goto, S., Naito, H., Kaneko, T., Chung, H. Y., & Radak, Z. (2007). Hormetic effects of regular exercise in aging: correlation with oxidative stress. *Appl Physiol Nutr Metab, 32*(5), 948-953.

Guelen, I., Westerhof, B. E., Van Der Sar, G. L., Van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H., et al. (2003). Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Press Monit, 8*(1), 27-30.

Guyton, A., & Hall, J. (2005). *Textbook of Medical Physiology* (7th ed.). Philadelphia: Elsevier-Saunders.

Hagberg, J. M., & Brown, M. D. (1995). Does exercise training play a role in the treatment of essential hypertension? *J Cardiovasc Risk, 2*(4), 296-302.

Hajjar, I., Kotchen, J. M., & Kotchen, T. A. (2006). Hypertension: Trends in prevalence, incidence, and control. *Annu Rev Public Health, 27*, 465-490.

Haleagrahara, N., Radhakrishnan, A., Lee, N., & Kumar, P. (2009). Flavonoid quercetin protects against swimming stress-induced changes in oxidative biomarkers in the hypothalamus of rats. *Eur J Pharmacol, 621*(1-3), 46-52.

Halliwill, J. R. (2001). Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc Sport Sci Rev, 29*(2), 65-70.

Hamer, M. (2006). The anti-hypertensive effects of exercise: integrating acute and chronic mechanisms. *Sports Med, 36*(2), 109-116.

Hill, A. M., Buckley, J. D., Murphy, K. J., & Howe, P. R. (2007). Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. *Am J Clin Nutr, 85*(5), 1267-1274.

Honzikova, N., Novakova, Z., Zavodna, E., Paderova, J., Lokaj, P., Fiser, B., et al. (2006). Baroreflex sensitivity in children, adolescents, and young adults with essential and white-coat hypertension. *Klin Padiatr, 218*(4), 237-242.

Ibarra, M., Moreno, L., Vera, R., Cogolludo, A., Duarte, J., Tamargo, J., et al. (2003). Effects of the flavonoid quercetin and its methylated metabolite isorhamnetin in isolated arteries from spontaneously hypertensive rats. *Planta Med, 69*(11), 995-1000.

Ibarra, M., Perez-Vizcaino, F., Cogolludo, A., Duarte, J., Zaragoza-Arnaez, F., Lopez-Lopez, J. G., et al. (2002). Cardiovascular effects of isorhamnetin and quercetin in isolated rat and porcine vascular smooth muscle and isolated rat atria. *Planta Med, 68*(4), 307-310.

Imholz, B. P., Wieling, W., Langewouters, G. J., & van Montfrans, G. A. (1991). Continuous finger arterial pressure: Utility in the cardiovascular laboratory. *Clin Auton Res, 1*(1), 43-53.

Izzo, J., Sica, D., & Black, H. (2008). *Hypertension Primer* (Fourth ed.). Philedelphia: Lippincott Williams & Wilkins.

Kelley, G., & McClellan, P. (1994). Antihypertensive effects of aerobic exercise. A brief meta-analytic review of randomized controlled trials. *Am J Hypertens, 7*(2), 115-119.

Kelly, R., Hayward, C., Avolio, A., & O'Rourke, M. (1989). Noninvasive determination of age-related changes in the human arterial pulse. *Circulation, 80*(6), 1652-1659.

Kenney, M. J., & Seals, D. R. (1993). Postexercise hypotension. Key features, mechanisms, and clinical significance. *Hypertension, 22*(5), 653-664.

Ketelhut, R. G., Franz, I. W., & Scholze, J. (2004). Regular exercise as an effective approach in antihypertensive therapy. *Med Sci Sports Exerc, 36*(1), 4-8.

Kingwell, B. A. (2000). Nitric oxide-mediated metabolic regulation during exercise: Effects of training in health and cardiovascular disease. *FASEB J, 14*(12), 1685-1696.

Kingwell, B. A., Berry, K. L., Cameron, J. D., Jennings, G. L., & Dart, A. M. (1997). Arterial compliance increases after moderate-intensity cycling. *Am J Physiol, 273*(5 Pt 2), H2186-2191.

Kingwell, B. A., & Gatzka, C. D. (2002). Arterial stiffness and prediction of cardiovascular risk. *J Hypertens, 20*(12), 2337-2340.

Kingwell, B. A., Waddell, T. K., Medley, T. L., Cameron, J. D., & Dart, A. M. (2002). Large artery stiffness predicts ischemic threshold in patients with coronary artery disease. *J Am Coll Cardiol, 40*(4), 773-779.

La Rovere, M. T., Specchia, G., Mortara, A., & Schwartz, P. J. (1988). Baroreflex sensitivity, clinical correlates, and cardiovascular mortality among patients with a first myocardial infarction. A prospective study. *Circulation, 78*(4), 816-824.

Lakatta, E. G. (1993). Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev, 73*(2), 413-467.

Leaf, A., Kang, J. X., & Xiao, Y. F. (2008). Fish oil fatty acids as cardiovascular drugs. *Curr Vasc Pharmacol, 6*(1), 1-12.

Lofgren, R. P., Wilt, T. J., Nichol, K. L., Crespin, L., Pluhar, R., & Eckfeldt, J. (1993). The effect of fish oil supplements on blood pressure. *Am J Public Health, 83*(2), 267-269.

Lucini, D., Milani, R. V., Costantino, G., Lavie, C. J., Porta, A., & Pagani, M. (2002). Effects of cardiac rehabilitation and exercise training on autonomic regulation in patients with coronary artery disease. *Am Heart J, 143*(6), 977-983.

Lynch, W. D., Markosyan, K., Melkonian, A. K., Pesa, J., & Kleinman, N. L. (2009). Effect of antihypertensive medication adherence among employees with hypertension. *Am J Manag Care, 15*(12), 871-880.

MacDonald, J. R. (2002). Potential causes, mechanisms, and implications of post exercise hypotension. *J Hum Hypertens, 16*(4), 225-236.

MacDonald, J. R., MacDougall, J. D., & Hogben, C. D. (2000). The effects of exercise duration on post-exercise hypotension. *J Hum Hypertens, 14*(2), 125-129.

Mackenzie, I. S., Wilkinson, I. B., & Cockcroft, J. R. (2002). Assessment of arterial stiffness in clinical practice. *Qjm, 95*(2), 67-74.

Maki-Petaja, K. M., & Wilkinson, I. B. (2009). Anti-inflammatory drugs and statins for arterial stiffness reduction. *Curr Pharm Des, 15*(3), 290-303.

Martin, J. E., Dubbert, P. M., & Cushman, W. C. (1990). Controlled trial of aerobic exercise in hypertension. *Circulation, 81*(5), 1560-1567.

Mayet, J., & Hughes, A. (2003). Cardiac and vascular pathophysiology in hypertension. *Heart, 89*(9), 1104-1109.

McAnulty, S. R., McAnulty, L. S., Nieman, D. C., Quindry, J. C., Hosick, P. A., Hudson, M. H., et al. (2008). Chronic quercetin ingestion and exercise-induced oxidative damage and inflammation. *Appl Physiol Nutr Metab, 33*(2), 254-262.

Mitchell, G. F., Hwang, S. J., Vasan, R. S., Larson, M. G., Pencina, M. J., Hamburg, N. M., et al. (2010). Arterial Stiffness and Cardiovascular Events. The Framingham Heart Study. *Circulation, 121*(4), 505-11.

Nieman, D. C., Henson, D. A., Maxwell, K. R., Williams, A. S., McAnulty, S. R., Jin, F., et al. (2009). Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. *Med Sci Sports Exerc, 41*(7), 1467-1475.

Nishiue, T., Tsuji, H., Tarumi, N., Tokunaga, S., Tamura, K., Masaki, M., et al. (1999). Heart rate variability and left ventricular dilatation early after myocardial infarction. *J Electrocardiol, 32*(3), 263-268.

Nurnberger, J., Keflioglu-Scheiber, A., Opazo Saez, A. M., Wenzel, R. R., Philipp, T., & Schafers, R. F. (2002). Augmentation index is associated with cardiovascular risk. *J Hypertens, 20*(12), 2407-2414.

Ohta, M., Nanri, H., Matsushima, Y., Sato, Y., & Ikeda, M. (2005). Blood pressure-lowering effects of lifestyle modification: possible involvement of nitric oxide bioavailability. *Hypertens Res, 28*(10), 779-786.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem, 49*(10), 4619-4626.

Overhaus, S., Ruddel, H., Curio, I., Mussgay, L., & Scholz, O. B. (2003). Biofeedback of baroreflex sensitivity in patients with mild essential hypertension. *Int J Behav Med, 10*(1), 66-78.

Parati, G., Ongaro, G., Bilo, G., Glavina, F., Castiglioni, P., Di Rienzo, M., et al. (2003). Non-invasive beat-to-beat blood pressure monitoring: new developments. *Blood Press Monit, 8*(1), 31-36.

Pierdomenico, S. D., Di Nicola, M., Esposito, A. L., Di Mascio, R., Ballone, E., Lapenna, D., et al. (2009). Prognostic value of different indices of blood pressure variability in hypertensive patients. *Am J Hypertens, 22*(8), 842-847.

Pikkujamsa, S. M., Huikuri, H. V., Airaksinen, K. E., Rantala, A. O., Kauma, H., Lilja, M., et al. (1998). Heart rate variability and baroreflex sensitivity in hypertensive subjects with and without metabolic features of insulin resistance syndrome. *Am J Hypertens, 11*(5), 523-531.

Potenza, M. A., Marasciulo, F. L., Tarquinio, M., Tiravanti, E., Colantuono, G., Federici, A., et al. (2007). EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. *Am J Physiol Endocrinol Metab, 292*(5), E1378-1387.

Prakash, E. S., Madanmohan, T., Sethuraman, K. R., & Narayan, S. K. (2005). Cardiovascular autonomic regulation in subjects with normal blood pressure, high-normal blood pressure and recent-onset hypertension. *Clin Exp Pharmacol Physiol, 32*(5-6), 488-494.

Rossow, L., Yan, H., Fahs, C. A., Ranadive, S. M., Agiovlasitis, S., Wilund, K. R., et al. (2010). Postexercise Hypotension in an Endurance-Trained Population of Men and Women Following High-Intensity Interval and Steady-State Cycling. *Am J Hypertens, 23*(4), 358-67.

Rueckert, P. A., Slane, P. R., Lillis, D. L., & Hanson, P. (1996). Hemodynamic patterns and duration of post-dynamic exercise hypotension in hypertensive humans. *Med Sci Sports Exerc, 28*(1), 24-32.

Rush, J. W., & Ford, R. J. (2007). Nitric oxide, oxidative stress and vascular endothelium in health and hypertension. *Clin Hemorheol Microcirc, 37*(1-2), 185-192.

Shanely, R. A., Knab, A. M., Nieman, D. C., Jin, F., McAnulty, S. R., & Landram, M. J. (2009). Quercetin supplementation does not alter antioxidant status in humans. *Free Radic Res, 44*(2), 224-231.

Singh, J. P., Larson, M. G., Tsuji, H., Evans, J. C., O'Donnell, C. J., & Levy, D. (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension, 32*(2), 293-297.

Stewart, L. K., Soileau, J. L., Ribnicky, D., Wang, Z. Q., Raskin, I., Poulev, A., et al. (2008). Quercetin transiently increases energy expenditure but persistently decreases circulating markers of inflammation in C57BL/6J mice fed a high-fat diet. *Metabolism, 57*(7 Suppl 1), S39-46.

Sung, J., Yang, J. H., Cho, S. J., Hong, S. H., Huh, E. H., & Park, S. W. (2009). The effects of short-duration exercise on arterial stiffness in patients with stable coronary artery disease. *J Korean Med Sci, 24*(5), 795-799.

Timmers, H. J., Wieling, W., Karemaker, J. M., & Lenders, J. W. (2004). Cardiovascular responses to stress after carotid baroreceptor denervation in humans. *Ann N Y Acad Sci, 1018*, 515-519.

Tsuji, H., Larson, M. G., Venditti, F. J., Jr., Manders, E. S., Evans, J. C., Feldman, C. L., et al. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation, 94*(11), 2850-2855.

Uchida, S., Ozaki, M., Akashi, T., Yamashita, K., Niwa, M., & Taniyama, K. (1995). Effects of (-)-epigallocatechin-3-O-gallate (green tea tannin) on the life span of stroke-prone

spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol Suppl, 22*(1), S302-303.

Vyas, M., Izzo, J. L., Jr., Lacourciere, Y., Arnold, J. M., Dunlap, M. E., Amato, J. L., et al. (2007). Augmentation index and central aortic stiffness in middle-aged to elderly individuals. *Am J Hypertens, 20*(6), 642-647.

Wang, S., Ma, A. Q., Song, S. W., Quan, Q. H., Zhao, X. F., & Zheng, X. H. (2008). Fish oil supplementation improves large arterial elasticity in overweight hypertensive patients. *Eur J Clin Nutr, 62*(12), 1426-1431.

Williams, M. A., Haskell, W. L., Ades, P. A., Amsterdam, E. A., Bittner, V., Franklin, B. A., et al. (2007). Resistance exercise in individuals with and without cardiovascular disease: 2007 update: A scientific statement from the American Heart Association Council on Clinical Cardiology and Council on Nutrition, Physical Activity, and Metabolism. *Circulation, 116*(5), 572-584.

Wolfram, S. (2007). Effects of green tea and EGCG on cardiovascular and metabolic health. *J Am Coll Nutr, 26*(4), 373S-388S.

APPENDIX A

Informed Consent

Consent Form

Appalachian State University

Consent / Authorization Form

Title of Study: Changes in vascular and hemodynamic parameters following acute exercise and antioxidant supplementation

Background/Purpose:

You are being asked to participate in a research study because you are a healthy person between 18 and 24 years of age, with no diabetes, kidney or heart problems, no history of stroke and no other known cardiovascular risk factors. Scott Collier, PhD (Department of Health, Leisure and Exercise Science at Appalachian State University) is conducting this study.

The purpose of this study is to examine the potential methods of modulating blood pressure response to aerobic exercise training. In addition we are interested in examining how aerobic exercise affects your body's ability to change blood pressure, heart rate and blood vessel size.

During exercise, it is important for your arteries to be able to expand to let more blood flow through them to get to the working muscles. We know that certain biological molecules modulate this distensibility. This may also depend upon the level of reactive oxygen species, henceforth, referred to as ROS, in the vessel wall. We would like to investigate the effects of an antioxidant molecule, commonly known as a mixed flavanoid, in modulating vascular response after an acute bout of aerobic exercise.

The purpose of this research study includes:

General: Study the effects of an antioxidant molecule, commonly known as a flavonoid, in modulating vascular response after an acute bout of aerobic exercise. Each supplement contains 1000 mg quercetin (Q) with 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, and 400 mg omega-3 fatty acids from fish oil (Q-EGCG), and was formulated and tested in collaboration with Quercegen Pharma (Newton, MA). The supplement has been shown to decrease the reactive oxygen species in the blood which can lead to a decrease in mean blood pressure and increase the rate at which your kidneys filter blood.

Specific: To assess the changes in blood vessel stiffness/expandability following an acute bout of aerobic training before, and after 2 weeks of oral flavanoid supplementation.

If you have a known cardiovascular heart disease, such as congenital heart disease, hypertrophic cardiomyopathy, complex supraventricular or ventricular dysrhythmias at rest or dysrhythmias that worsen with exercise, this study is not appropriate for you. The remainder of this form will explain the study in greater detail. If you have any questions, feel free to ask.

Study Procedures:

If you choose to participate, you will be asked:

1. To report to the Human Performance Laboratory at Appalachian State University (Room 054 of the Holmes Convocation Center) on 3 separate occasions for about 1 to 1.5 hours per visit for a blood draw, hemodynamic and exercise measures.

At visit 1 (Initial visit)

During this visit, you will be familiarized to the study instruments and procedures in the Human Performance Laboratory at Appalachian State University. All tests will be completed in the Human Performance Laboratory, room 054 of the Holmes Convocation Center at 111 Rivers Street, Appalachian State University. You will be asked to answer a medical and

exercise history questionnaire. Then we will measure your height, weight and percentage body fat and evaluate your cardio-respiratory fitness.

Blood Draw: We will draw approximately 20 ml of blood to use for the detection of flavonoids and reactive oxygen species concentrations. A trained technician will draw the blood.

Percentage body fat: Your present body fat will be measured using the DEXA scan. You will be lying comfortably on a cushioned bed wearing shorts and a t-shirt while the DEXA estimates your body's composition of fat and muscle.

Graded exercise test: You will be evaluated for cardio-respiratory fitness using the graded exercise test on a treadmill. In this test, you will start jogging on a treadmill at about 5 to 6 miles per hour and every 2 minutes the speed or grade will be increased slightly until you get tired. This test is designed to make you tired in about 10 to 12 minutes. You will be wearing a facemask (so that we can collect and analyze your expired air) and a heart rate monitor to measure your heart rate. This test will determine your maximum oxygen consumption (VO2) which is your ability to take oxygen out of the air, to the working muscles. This will help us determine the correct starting point for your aerobic exercise prescription.

You will also be familiarized with other equipment in the laboratory necessary to make the measurements of pulse wave velocity, reactive hyperemia, and blood pressure.

At visit 2-3

At the second visit, you will be asked to undergo all of the above testing noted above. This helps us to establish good baseline measures for you. Then you will be subjected to an acute bout of aerobic training at 65% of your maximum capacity for 30 minutes as determined during the first visit. At the end of exercise pulse wave velocity, reactive hyperemia, and beat-to-beat variation of blood pressure will be measured again. After completion of this session you will be asked to ingest 4 soft chews of the flavonoid supplement orally to equal a dose of 1000 mg/day for 2 weeks, and then return to lab for the 3rd and final visit.

The various measurements during visit 2 will be as follows:

1) **Pulse Wave Velocity**—A small sensor resembling a pen is placed over your carotid artery (side of your neck) and over the femoral artery (top of your leg). A transducer (like a microphone) uses ultrasound waves (sound waves which bounce off the blood in the blood

vessel) to measure the speed and direction of blood flow through an artery. No physical discomfort should be experienced during this test. Your privacy will be upheld with great care during the assessment of the femoral artery, as this is best located near the pubic area. There are no known risks associated with the Doppler ultrasound used in this technique.

2) **Occlusion test of the forearm**—This test measures blood flow through your forearm. A blood pressure cuff is placed on your upper arm, and a smaller cuff around your wrist. The upper cuff will be inflated to a pressure above your systolic blood pressure (the first number when a doctor reads your blood pressure) and remain inflated at this pressure for approximately five minutes. The wrist cuff will be inflated to a pressure above your systolic blood pressure one-minute before the five-minute time period has lapsed. After five minutes, the upper cuff pressure will be released quickly. An elastic strap with a sensor placed on the lower part of the same arm will measure the blood volume changes in your arm after the cuff pressure has been released. Once the cuff has been released we will measure changes in blood volume in the arm for three minutes, while maintaining the wrist cuff pressure above the systolic blood pressure. Upon completion of this three-minute time period, the wrist cuff pressure will be released.

3) **Blood Pressure Assessment** – Systolic and diastolic blood pressure will be measured using the Portapres. This technique uses a tiny cuff worn around your middle finger that can measure blood pressure on a heart beat-to-heart beat basis.

Visit 3

The components of visit 3 will be identical to those of visit 2.

Risks:

The risks and discomforts involved with participating in this study are:

Exercise testing and training: The risks associated with exercise testing and training include increased blood pressure and possible heart arrhythmias (abnormal heart beats). There is a very small risk of a heart attack during the exercise testing and training. To minimize this risk, we will have you answer questions regarding your medical history and family history to screen for any significant heart disease that might exist asymptomatically.

Although, the electrocardiogram (EKG) poses minimal risk, occasionally a person is allergic to the adhesive on the electrodes and may develop a local skin irritation.

Individuals may experience localized fatigue during the exercise testing/ training, and possibly some muscle soreness after the exercise testing/training. This should subside within 24-48 hours after testing. Soreness is rare in normal, healthy individuals. Rest breaks will be

incorporated into the training to help minimize possible soreness associated with exercise training. We will also try to minimize this risk by taking you through a series of light stretches after testing is completed.

Another possible risk may be abnormal changes in your heart rate and blood pressure. We will attempt to minimize this risk by carefully monitoring your heart rate and blood pressure responses during aerobic training.

DEXA testing: The DEXA machine is used to assess body composition. This technique is safe, however subjects are exposed to very small amounts of radiation. The radiation equivalent is equated to an individual taking a flight from the East coast to the West coast, very minimal. A trained operator will be present to conduct the test and can help minimize exposure by using the shortest test time possible.

Pulse-wave velocity: There are no known risks associated with the Doppler ultrasound used in this test. No physical discomfort should be experienced during this test. Again, your privacy will be upheld with great care during the assessment of the femoral artery, as this is best located near the pubic area.

Occlusion test of the forearm: You may feel discomfort during the occlusion as your arm may fall asleep, which is similar to the 'pins & needles' feeling you may have experienced when a limb (e.g. leg, hand, foot) fell asleep. This feeling will be alleviated almost immediately when the pressure cuff is released allowing normal blood flow to return. It is possible that you may find this test painful. We can stop the test if this occurs. Rarely does this procedure cause bruising.

Blood pressure assessment: There may be slight discomfort due to pressure felt in the finger that the cuff is placed on. However, this slight pressure is only felt for about one minute while the measurement is being taken.

Blood Draw: The subject may experience slight discomfort and bruising associated with the blood draw, however we will use trained technicians to minimize the risk of discomfort and bruising.

The investigators involved in this project have extensive experience in exercise testing, which should minimize the above risks.

Blood drawing: It may cause pain and/or bruising at the location on your arm where the blood was taken. On rare occasions, it may cause lightheadedness or fainting and an infection.

Answering Questionnaires: should not pose any risk to you.

The investigators involved in this project have extensive experience in exercise testing, which should minimize the above risks.

Benefits:

You will benefit from having a personal fitness assessment which will give you information about your current aerobic fitness level. Additionally, you will receive information on how well your heart and blood vessels respond to exercise.

The information learned may also help others in the future.

Voluntary Participation:

Your participation in this study is entirely voluntary and you may refuse to participate or discontinue participation at any time without penalty or loss of benefits to which you would normally be entitled. Your decision about whether or not to participate in the study will not affect your relationship with Appalachian State University or The Watauga Medical Center.

Alternatives:

You are free to choose not to participate in this study.

Costs/Payments:

There are no costs to you and/or your insurance carrier for participating in this study. You will not be paid for your participation. In addition, you will not be reimbursed for any parking costs incurred, however all efforts will be made to measure and train you after hours where no parking costs will be incurred.

Questions:

If you have any questions about the research, or in the event of a research-related injury, please contact Scott Collier, PhD at (828) 262-7145. If you have any questions about your rights as a research subject, please contact Jay Cranston, M.D. at the Appalachian State University Institutional Review Board Office at (828) 262-2692.

In Case Of Injury:

In the event of illness or physical injury resulting from taking part in this research study, medical treatment will be provided at Watauga Medical Hospital. You will be responsible for any costs not paid by your insurance company. No other compensation is offered by Appalachian State University. We have no plans to give you money if you are injured. You have not waived any of your legal rights by signing this form.

Confidentiality of Records and Authorization to Use/Share Protected Health Information for Research:

If you agree to participate in this research, identifiable health information about you will be used and shared with others involved in this research. For you to be in this research we need

your permission to collect and share this information. Federal law protects your right to privacy concerning this information.

When you sign this consent form at the end, it means that you have read this section and authorize the use and/or sharing of your protected health information as explained below. Your signature also means you have received a copy of Appalachian State Universities Notice of Privacy Practices.

Individually identifiable health information under the federal privacy law is considered to be any information from your medical record, or obtained from this study, that can be associated with you, and relates to your past, present, or future physical or mental health or condition. This is referred to as protected health information.

Your protected health information will be kept confidential. Your identity will not be revealed in any publication or presentation of the results of this research.

Why is it necessary to use/share your protected health information with others?

The main reason to use and share your health information is to conduct the research as described in this consent form. Your information may also be shared with people and organizations that make sure the research is being done correctly, and to report unexpected or bad side effects you may have.

In addition, we may be required by law to release protected health information about you; for example, if a judge requires such release in a lawsuit, or if you tell us of your intent to harm yourself or others.

What protected health information about you will be used or shared with others as part of this research?

We may use and share the results of tests, questionnaires, and interviews. We may also use and share information from your medical and research records. We will only collect information that is needed for the research.

Who will be authorized to use and/or share your protected health information?

The researchers, their staff and the staff of Watauga Medical Center participating in the research will use your protected health information for this research study. In addition, the Appalachian State Institutional Review Board (IRB) and the Watauga Medical Centers Institutional Review Board (IRB), committees responsible for protecting the rights of research subjects who supervise the way the research is done may have access to your protected health information.

The researchers and their staff will determine if your protected health information will be used or shared with others outside of Appalachian State University for purposes directly related to the conduct of the research.

With whom would the protected health information be shared?

Your protected health information may be shared with:

- Federal agencies that supervise the way the research is conducted, such as the Department of Health and Human Services' Office for Human Research Protections, or other governmental offices as required by law.
- If so desired, you can request your information be shared with your primary care physician

All reasonable efforts will be used to protect the confidentiality of your protected health information. However, not all individuals or groups have to comply with the Federal privacy law. Therefore, once your protected health information is disclosed (leaves Appalachian State University), the Federal privacy law may not protect it.

For how long will your protected health information be used or shared with others?

There is no scheduled date at which this information will be destroyed or no longer used. This is because information that is collected for research purposes continues to be used and analyzed for many years and it is not possible to determine when this will be complete.

Can you withdraw your authorization to collect/use/share your protected health information?

You always have the right to withdraw your permission (revoke authorization) for us to use and share your health information, by putting your request in writing to the investigator in charge of the study. This means that no further private health information will be collected. Once authorization is revoked, you may no longer participate in this research activity, but standard medical care and any other benefits to which you are entitled will not be affected. Revoking your authorization only affects uses and sharing of information obtained after your written request has been received, but not information obtained prior to that time.

Even after you withdraw your permission, Appalachian State University may continue to use and share information needed for the integrity of the study; for example, information about an unexpected or bad side effect you experienced related to the study.

Can you have access to your health information?

At the end of the study, you have the right to see and copy health information about you in accordance with the Appalachian State University policies; however, your access may be limited while the study is in progress.

Consent To Participate In Research & Authorization To Use And Share Personal Health Information:

I have read and understand the Informed Consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____ Date_____

Subject signature

_____ Date _____

Witness (Optional except for certain classes of subjects)

Should I have any questions about this research or its conduct, I may contact:

Scott Collier at 828.262.7145 or email him at colliersr@appstate.edu

Investigator(s) Telephone/e-mail

Faculty Advisor (if applicable) Telephone/e-mail

Jay W. Cranston, MD	**828-262-2692**	**irb@appstate.edu**
Administrator,	IRB Telephone	e-mail
Graduate Studies and Research		
Appalachian State University		
Boone, NC 26608		

APPENDIX B

APPALACHIAN STATE UNIVERSITY

Office Use Only_____-_____

REQUEST FOR REVIEW OF HUMAN PARTICIPANTS RESEARCH

Please type and submit one signed copy to irb@appstate.edu or mail to Research and Graduate Studies, John E. Thomas Building.

1. Date: April 23, 2009

2. Project Title: Changes in vascular and hemodynamic parameters following acute exercise and anti-oxidant supplementation

3. Principal Investigator(s): Scott Collier, PhD

4. Phone: (828)262-7145 Email: colliersr@appstate.edu

5. Post Office Address: 051 Holmes Convocation Center, ASU Box 32071

6. Academic Department/Unit: Dept of Health Leisure and Exercise Science

7. ASU Status: Faculty/Staff ☒ Graduate Student ☐ Undergraduate Student ☐ Other ☐

8. If student, name of faculty mentor

9. Faculty mentor's e-mail address:

   Faculty Post Office Address:

10. This is: Honors or Master's Thesis ☒ Capstone or Project of Learning ☐
    Dissertation ☐ Faculty Research ☐ Other

11. Plan to publish or present off-campus: Yes ☒ No ☐

12. Projected data collection dates 05/01/09 to 05/01/09

13. Proposals cannot be considered until the researchers have completed the online CITI Training (http://www.citiprogram.org/default.asp?language=english) required for human subject research. Do the investigators have documentation of completion on file in the IRB Office? Yes ☒ No ☐

14. Does this research involve any out-of-country travel? Yes ☐ No ☒

I have read Appalachian State University's Policy and Procedures on Human Subjects Research and agree to abide them. I also agree to report and significant and relevant changes in procedures and instruments as they relate to participants to the Chairperson of the Institutional Review Board

PI	Date	Co-investigator	Date
If PI is student, Faculty Mentor	Date	Co-investigator	Date

CHECKLIST FOR RESEARCH INVOLVING HUMAN PARTICIPANTS

1. Purpose of proposed research.

We will investigate the role of anti-oxidant flavanoid (Q-force immune, QFI) in modulating the vascular response to resistance exercise. Anti-oxidants have been demonstrated to increase bioavailability of NO. We hypothesize that use of QFI with aerobic exercise will prevent or reduce the increase in core BP and pulse wave velocity (measures of increased core BP), while maintaining vascular reactivity in brachial arteries owing to increased bioavailability of NO.

2. Briefly describe your subject population. Will any individuals be excluded solely on the basis of gender, race, color, or any other demographic characteristic? If so, please explain.

20 (10 males, 10 females) young healthy subjects (without known cardiovascular disease) in the age range of 18-24 years. Subjects will be recruited through posters/fliers located throughout Appalachian State University and word of mouth. During initial screening subjects will be asked about any known cardiovascular disease, with special emphasis on sudden cardiac death in any young first, or second degree relatives. Subjects with any such family history will be excluded. Any subject with known cardiovascular disease, diabetes, HTN, smoking, bony deformity, or currently taking any medication will be excluded.

3. Give a brief description of your research procedures as they relate to the use of human participants. This description should include, at least, the following:

- Procedures
- Name and description of data gathering instrument (attach copy, if applicable)
- How will the data be collected? (e.g., audio, video, written records)
- Sample size
- How long will the procedures take?
- What, if any, relationship exists between the researcher(s) and the participants?
- What, if any, relationship exists between the researcher(s) and the agencies (e.g., schools, hospitals, homes)?
- Attach statement of approval from any agencies (e.g., schools, hospitals, homes) that will be involved with recruitment of participants or data collection.

Visit 1 will consist of lab familiarization, informed consent, resting hemodynamic measurement and peak VO2 measures, and body composition assessment.

Visit 2 will consist of resting hemodynamics, pulse wave velocity, blood flow, and reactive hyperemia measurements. The subjects will then be asked to do weight training at 65% of their pre-determined peak for 30 minutes. The pre-measures will be repeated at immediately post-, 20, and 40 minutes following exercise. The subjects will then be started on oral supplementation of QFI 4-250mg chews/day for 2 weeks. After 2 weeks the subjects will return to the lab for visit 3 which will be identical to the measures taken at visit 2.

Visit 1

Bod Pod Testing: Whole body air-displacement will be evaluated using the DEXA (Hologic Incorporated, Concord, CA). During testing of body composition subjects will wear a swim cap and a bathing suit. Subjects sit quietly in the chamber of the Bod Pod while their body volume is measured. Thoracic gas volume is estimated by having the subjects breath normally for 3 breathing cycles through a tube while wearing the swim cap and nose clip. Body density is used to calculate percent body fat [illegible] using the Siri equation. Body weight will be measured to the nearest 0.01 kg using a calibrated Bod Pod electronic scale.

Maximal Exercise Testing: Resistance exercise capacity will be assessed using a standardized ASCM protocol.

The second visit will consist of the flowing testing measurements.

Pulse Wave Velocity (PWV): [illegible]

Blood Pressure Variability (BPV): [illegible]

Baroreflex Sensitivity (BRS): [illegible]

Forearm and calf blood flow: [illegible]

Vasodilatory Capacity of the Forearm: [illegible]

[illegible]

4. Is deception involved? YES ☐ NO ☒
If yes, please describe.

5. Do the data to be collected relate to any illegal activities (e.g. drug use, abuse, assault)?
YES ☐ NO ☒
If yes, please explain.

6. The benefits of this activity to the participants must outweigh the potential risks. To this end, please:

a. Describe the benefits to the individual participants and to society.

Subjects will receive information regarding their exercise capacity, body composition and fitness level.

b. Describe the potential risks to any individual participating in this project. Please explain any possible risks of psychological, legal, physical, or social harm. What provisions have been made to insure that appropriate facilities and professional attention necessary for the health and safety of the participants are available and will be utilized?

In the age group selected for this study, the cardiovascular complications are extremely unlikely. Subjects have an extremely low likelihood of cardiac arrhythmia and heart attack (myocardial infarction). In the extremely unlikely event of inclusion of a subject with rare syndromes of predisposition to cardiac arrhythmia (such as Brugada syndrome, ARVD, HOCM, long QT syndrome etc), there would be a risk of cardiac arrhythmia, and even sudden cardiac death. The risk will not be any higher than if that individual were to exercise on his/her own, without the knowledge of his/her underlying heart disease. To minimize any such risk, all subjects with any known pre-existing cardiovascular disease will be excluded. ASCM guidelines for exercise testing and training will be followed strictly by the investigator in determining exercise capacity of subjects to minimize risks of physical injury during exercise.

Bodpod: Bodpod is a device that measures body composition. The measurement is without risk, but may cause symptoms of claustrophobia to some subjects which may find the device unacceptable. During initial familiarization procedure subjects will be asked about such issues and given a chance to sit in the chamber prior to testing.

Reactive hyperemia: Subjects may experience 'pins and needles' in the arm being subjected to the test which quickly disappears once occluding cuff is deflated and blood flow returns to normal. Five minutes of complete occlusion is required in the standard method. No damage to tissue has ever been reported.

Pulse wave velocity: This is measured by placing Doppler probes over radial artery, carotid artery, femoral artery, and dorsalis pedis artery. The device uses ultrasound to measure velocity of blood flow, which has no known deleterious effect on tissue. Subject dignity will be strictly upheld while making measurements from the femoral artery, given that this would require probes to be placed in groin.

7. Please describe how participants will be informed of their rights and how informed consent will be obtained and documented. Attach a copy of the consent form and any materials used in the recruitment of participants.

Consent waiver, see attached

8. The confidentiality of all participants must be maintained. To this end, please respond to the following.

a. How will the confidentiality of participants be maintained?

Like any study, participation will involve some loss of privacy, but the information collected from each subject will be handled as confidentially as possible. The individuals file and hardcopy data will be locked in a file cabinet within a locked office in a secure building and each data file will be encoded without the individuals name to associate it. The blood samples will not have any names associated with them (decoded) prior to their packaging for shipment to the lab where the analyses are carried out.

b. How will confidentiality of data be maintained?

The data will be coded and entered into a computer spreadsheet and all individual identifiers will be removed.

c. Describe the process of final disposition of the data. How long will the data be stored and how will they be destroyed?

All hardcopy data will be destroyed and the computer files will be erased 5 years following the protocol, at which time all manuscript writing will cease.

d. How are participants protected from the future harmful use of the data collected in this protocol?

All data will be used only for the purpose of this study and no data will be shared with anyone outside the immediate study team. Further, all study data will be decoded so there will be no identifying features left on the spreadsheets.

Biographical Information

Rebecca Marie Kappus was born to Daniel and Emily Kappus on May 17, 1982 in Parma, Ohio. She attended St. Francis de Sales and St. Joan of Arc for her elementary and middle school years, and attended Kenston High School for 9th through 12th grade, where she ran track and cross country, placed in honors and AP science and English classes and was a member of the National Honor Society. In May 2000 she graduated and attended The University of Toledo where she majored in Biology and ran track and cross country for the college. After graduation in 2004, she started a job at the University of Michigan Hospital where she was an animal technician and supervisor of the Biomedical Science Research Building. In 2008, she left the hospital to begin graduate school at Appalachian State University. During her time there, she received four grants, worked in cardiopulmonary rehabilitation and did research. In May 2010, Rebecca graduated with a Master of Science and is working on her PhD.