

THE EFFECTS OF A MULTI-FLAVONOID SUPPLEMENT ON VASCULAR AND
HEMODYNAMIC PARAMETERS IN OLDER PRE-HYPERTENSIVES

A Thesis
by
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Department of Health, Leisure, and Exercise Science

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FOREWORD

The research presented in this thesis will be submitted to the *European Journal of Applied Physiology*, an international peer-reviewed journal dedicated to furthering the field of applied physiology. This journal is owned by Springer, a subsidiary of Springer-Verlag GmbH, and published by Dr. D.S. Verlad. This thesis has been prepared according to the guidelines set forth by the Graduate School of Appalachian State University.

ABSTRACT

THE EFFECTS OF A MULTI-FLAVONOID SUPPLEMENT ON VASCULAR AND HEMODYNAMIC PARAMETERS IN OLDER PRE-HYPERTENSIVES. (May 2012)

Chelsea Dawn Curry, B.A., Appalachian State University

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Chairperson: Scott R. Collier

Antioxidants have been shown to increase vasodilation leading to increases in vascular distensibility which would decrease blood pressure (BP) in individuals with elevated BP; therefore an additive effect would be expected when combined with exercise. The purpose of this study was to investigate the potential additive effects of an acute aerobic exercise bout paired with two weeks of anti-oxidant supplementation on post-exercise BP in middle-aged (40-60 year old) pre-hypertensives. Methods: 18 subjects (51.7 ± 1.8 , 50.8 ± 1.8 years old, treatment and placebo group respectively) were randomly assigned to either the supplement or the placebo group in double-blinded fashion. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), Augmentation Index (AIx), central and peripheral pulse wave velocity (cPWV and pPWV, respectively) were assessed pre- and post-exercise prior to and following 2 weeks of supplementation in a double-blind, counterbalanced design. Results: Following two weeks of supplementation, there was a significant decrease in SBP (132.2 ± 5.4 pre-supplementation to 124.9 ± 5.4 post-, $p < 0.05$) and MAP (100.2 ± 2.2 pre-supplementation to 94.6 ± 4.4 post-, $p < 0.05$) following supplementation. No significant differences were shown in DBP, AIx, cPWV, or pPWV. Conclusion: Two weeks of multi-flavonoid supplementation elicited a significant

decrease in SBP and MAP in the treatment group with no changes in vascular parameters.

ACKNOWLEDGMENTS

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INTRODUCTION

Stage-1 essential hypertension (resting blood pressure $\geq 140/90$ mmHg) is one of the most significant diseases in modern countries (Mayet and Hughes, 2003). Possibly even more problematic is the newer classification of pre-hypertension (130/80-139/89 mmHg) which continues the disease trend towards stage-1 hypertension, yet is not a clinical diagnosis but a warning that an individual is progressing towards patienthood. Making the diagnosis more difficult, the underlying causes of hypertension are multifactorial. However, one common factor is increased arterial stiffness (Gedikli et al., 2010). Increased stiffening of the arteries leads to a consequent increase in operating blood pressures, due to increased arterial resistance, and ultimately to hypertension and further cardiovascular complications.

Although there are many factors that can lead to stiffening of the arteries, one of the most common is aging. As a person ages, there is a deterioration in the amount of elastin within the vessel wall matrix which is replaced with collagen, a less compliant tissue, as a protective mechanism to prevent rupturing of the vessels. However, this mechanism also elicits an increase in blood pressure (BP) due to the decreased ability of the vessel wall to expand (Wagenseil and Mecham, 2012).

Aerobic exercise training has been shown to reduce central hemodynamics by reducing arterial stiffness (Collier et al., 2008). Therefore, aerobic exercise is the cornerstone in the prevention and treatment of elevated BP. The changes elicited by aerobic exercise training may be linked to the post-exercise hypotensive (PEH) response. PEH is defined as a decrease in BP following exercise (Halliwill, 2001). This phenomenon is due to peripheral

vasodilation that is not compensated for by a timely increase in cardiac output. Acute moderate intensity aerobic exercise (65-70% of determined maximal aerobic capacity, [VO_2 peak]) results in PEH, during which the baroreflex, a stretch receptor responsible for BP regulation, is able to reset to lower operating pressures (Overhaus et al., 2003).

MacDonald (2002) has shown that following an acute bout of moderate intensity aerobic exercise, normotensive (< 120/80 mmHg) individuals usually exhibit a drop in BP averaging 8/9 mmHg (systolic / diastolic) for up to an hour. However, in populations that are pre- to stage-one hypertensive, the PEH response following the aforementioned exercise is more exaggerated and longer lasting, with decreases of 14/9 mmHg and 10/7 mmHg in pre-hypertensive and hypertensive populations, respectively. During PEH, the baroreflex is reset to lower operating pressures which elicits a decrease in resting BP. Lower operating pressures result from a decrease in arterial resistance which, in turn, lead to decreased levels of arterial stiffness, an emerging risk factor for cardiovascular disease. More importantly, this post-exercise drop in BP may last as long as 24 hours in the pre- to stage-one hypertensive populations which has proven to decrease myocardial stress (MacDonald, 2002). Due to the resetting of the baroreflex, PEH can be clinically significant for normotensive, pre-hypertensive, and hypertensive populations as either prevention or treatment for cardiovascular disease.

Recently, multiflavonoid supplementation has shown beneficial effects in young, pre-hypertensive individuals (Kappus et al., 2011). However, due to the greater incidence of elevated BP in older populations, the effect of this supplementation course may be most beneficial in an older pre- to stage-one hypertensive cohort. However, no previous studies have documented the effects of multiflavonoid supplementation combined with an acute bout

of aerobic exercise on central hemodynamics and arterial stiffness. Therefore, the purpose of this study was to elucidate the effects of an acute bout of aerobic exercise following 2 weeks of multiflavonoid supplementation on BP and arterial stiffness in older, pre-hypertensive individuals. We hypothesize that the combination of an acute aerobic exercise and a 2 week treatment regimen of multiflavonoid supplementation will induce beneficial decreases in BP and pulse wave transit times in an older, pre-hypertensive cohort.

METHODOLOGY

Subjects

Eighteen healthy, non-smoking males ($n = 8$) and females ($n = 10$) between the ages of 40 and 60 years old were recruited from the local community. All subjects were unmedicated (including aspirin) and refrained from dietary supplementation one week prior to and throughout data collection. Subjects were excluded if they had a history of diabetes, heart, or kidney disease. All of the subjects read and signed an informed consent (Appendix B), and the study was approved by the Appalachian State University Institutional Review Board (Appendix A).

Experimental design

Subjects reported to the Vascular Biology and Autonomic Studies Laboratory for a preliminary visit followed by three visits for data collection. The first visit included informed consent, a health history questionnaire (Ainsworth et al., 1993), and a 12-hour fasted blood draw. Visit one also included basic anthropometric measurements, VO_2 peak assessment, and familiarization with laboratory equipment and procedures. Height was measured to the nearest 0.5 cm using a stadiometer, and weight was measured to the nearest 1 kg. Body mass index (BMI) was calculated using the formula: weight (kg) divided by height (m) squared. Bioelectrical Impedance Analysis (BIA) was performed using the Tanita (Arlington Heights, IL) to indicate body fat percentage.

Visits two and three included measurements of vascular and hemodynamic variables pre- and post-30 minutes of aerobic exercise on a treadmill at 65-70% of the subject's

determined VO₂ peak. These visits were scheduled at the same time of day pre- and post- 2 weeks of multi-flavonoid supplementation. Subjects were randomly assigned to either the supplement ($n = 9$) or placebo group ($n = 9$).

Visit one

Height, weight, BMI, and BIA were measured upon arrival. Seated resting BP was taken manually at the brachial artery on the left arm. VO₂ peak was measured on a treadmill using a Parvomedic True One metabolic analyzer (Sandy, UT), as previously reported (Kappus et al., 2011). A modified Balke protocol was used, during which the subjects started exercising at a comfortable speed at which point the incline was increased by 2% every 2 minutes for each stage of the test. Heart rate (HR) was continuously monitored for the duration of the test using a Polar heart rate monitor (Polar Electro Inc., Woodbury NY) and recorded at the end of each 2 minute stage, along with Rating of Perceived Exertion (RPE). Subjects were considered at VO₂ peak if three of the following four criteria had been met: (a) no change in HR with a change in workload; (b) a final rating of perceived exertion (RPE) score of 17 or greater on the Borg scale (scale 6-20); (c) a respiratory exchange ratio (RER) greater than 1.15; and (d) a “plateau” (increase of no more than 150 ml) in oxygen uptake with an additional incremental increase in workload.

Each subject was instructed to keep two 3 day diet records and maintain specific dietary restrictions for the duration of the study. Subjects attended visits two and three a minimum of 4 hours post-prandial. Subjects were also instructed to avoid alcohol, caffeine, and foods high in nitrates and nitrates completely for 24 hours prior to visits two and three.

Visit two

Visit two included measurements of weight, BMI, and BIA. Next, vascular and hemodynamic measurements were taken. Seated resting BP was taken manually at the brachial artery in the left arm. Pulse Wave Analysis (PWA) was measured using the SphygmaCor (Sydney, Australia) at the left radial artery while the subject was in a seated position. Augmentation Index (AIx) and Aortic Systolic Pressure (ASP) were recorded and stored on a computer and analyzed at a later date.

Central and peripheral Pulse Wave Velocity (PWV) were measured using the SphygmaCor (Sydney, Australia), with the subject in a supine position. Waveforms were captured simultaneously, with an electrocardiogram (ECG) as a reference for time. Central PWV (cPWV) was collected at the carotid and femoral arterial sites. Distance from the carotid site to the midpoint of the manubrium sterni was measured using a fabric tape measure and was subtracted from the distance between carotid and femoral sites. Peripheral PWV (pPWV) was collected at the femoral and distal arterial sites. Distance from the midpoint of the manubrium sterni to the femoral site was measured and subtracted from the distance between the midpoint of the manubrium sterni and the distal site. PWV was then calculated as the distance traveled divided by time in meters per second (m/s). All waveforms were collected on the left side of the body.

Beat-to-beat BP (Systolic blood pressure [SBP], diastolic blood pressure [DBP], mean arterial pressure [MAP]) and cardiac output were collected from the left middle finger in a supine position for five minutes using the Finometer (FMS, Amsterdam, The Netherlands) simultaneously with a 3-lead ECG to record the variation between successive ventricular contraction (R-R) intervals. All data were recorded in real time, stored on a computer, and

analyzed at a later date.

A blood sample was collected prior to each subject performing thirty minutes of aerobic exercise on the treadmill at 65-70% of the maximum heart rate determined by the VO₂ peak test at visit one. Following exercise, another blood draw was taken followed by an identical series of vascular and hemodynamic measurements post-exercise to be compared to pre-exercise. The first 3-day diet record was reviewed, and dietary instructions and guidelines during supplementation were explained.

Subjects were randomly assigned to either the supplement or the placebo group in a double-blind fashion. The active ingredients in each supplement chew included 250 mg quercetin, 30 mg epigallocatechin gallate (EGCG), 100 mg isoquercetin, and 100 mg n3-polyunsaturated fatty acids (PUFA), 55mg eicosapentaenoic acid (EPA), and 45mg docosahexaenoic acid (DHA) from fish oil. Each supplement chew also contained filler ingredients. The placebo chews contained identical filler ingredients to the supplement but did not include the active ingredients. Dosage included two chews in the morning and two chews in the evening for two weeks. Each dose was to be taken thirty minutes pre- and post-consumption of any food or beverages, especially dairy products.

Subjects returned for visit three 24-48 hours after their last dose. Pre-menopausal women were scheduled for visits two and three according to their menstrual cycle in order to control for hormonal fluctuations. Visits two and three took place between days 1 through 5 of their menstrual cycle. Supplementation was timed so that visits two and three took place on the same day of the two consecutive menstrual cycles. The first dosage of supplementation was ingested two weeks prior to visit three.

Visit three

Visit three took place within 48 hours of completing supplementation. Procedures for visit three were identical to those for visit two and included vascular and hemodynamic measurements pre- and post- 30 minutes of treadmill exercise.

Blood analyses

Blood samples from visits two and three were analyzed for biomarkers of antioxidant capacity. Ferric Reducing Ability of Plasma (FRAP) was used to measure total plasma reducing potential. Oxygen Radical Absorbance Capacity (ORAC) was performed to examine integrated plasma antioxidant capacity. Blood samples taken pre- and post-exercise both before and following supplementation were analyzed.

Treatment of the data

All data were analyzed using a 2 x 2 ANOVA with repeated measures, group (treatment versus placebo) by time (pre- versus post-) using SPSS version 19 (IBM, Chicago, IL). All data were reported as mean \pm standard error (SE).

RESULTS

At the onset of the study, there were no significant differences in age, height, weight, BMI, percent (%) body fat, VO₂ peak, SBP, or DBP between groups (Table 1). Two weeks of multi-flavonoid supplementation had no effect on weight, BMI, or % body fat. VO₂ peak did not change significantly between visits as this was not a training study. Furthermore, there were no changes seen in DBP (Fig 2) following supplementation. Resting SBP decreased by 7.3 (SE ± 5.4) mmHg in the treatment group compared to 1.4 (SE ± 5.4) mmHg in the placebo group following supplementation (Table 2, Fig 1). This was further supported by a decrease in MAP of 5.6 (SE ± 3.3) mmHg in the treatment group compared to 2.3 (SE ± 2.5) mmHg in the placebo group. No significant changes were seen in AIx, cPWV, or pPWV pre- or post-exercise following supplementation.

DISCUSSION

The main finding of the present study was a clinically significant decrease in SBP and DBP following two weeks of supplementation. No outcome variables were found to be significant between intervention and placebo conditions.

It is well known that a key factor contributing to arterial stiffness relates with the production of reactive oxygen species (ROS) which are known to cause oxidative damage (Goto et al., 2007). An increase in ROS generally leads to a decrease in nitric oxide (NO), a powerful vasodilator involved in the changes in blood flow to skeletal muscle (Barton et al., 2001). Current research has shown antioxidant supplementation to increase the bioavailability of NO. Antioxidants donate electrons to ROS to reduce inflammation and oxidative stress (Al-Gubory et al., 2010) consequently increasing the amount of NO available.

In agreement with our findings, Knab et al. (2011) studied the effect of 12 weeks of quercetin supplementation (500 mg or 1000 mg) on cardiovascular risk factors including BP and blood-borne markers of inflammation and oxidative stress in male and female subjects of various fitness levels grouped by age (18 - 40, 40 - 65, 65+ years old). Subjects showed significant decreases in MAP in both supplementation groups (500 mg and 1000 mg) at rest following the course of supplementation. No changes in MAP were seen in the placebo group. Furthermore, no significant decreases were seen in plasma C-reactive protein or plasma cytokines amongst all groups with the exception of a 0.01 picogram per milliliter

(pg/mL) decrease in interleukin-6 in the 1000 mg group (Knab et al., 2011). These results reveal a disconnect between decreases in BP and inflammatory markers.

It has been well-documented that quercetin supplementation does not elicit decreases in inflammatory markers. A recent study using quercetin supplementation and acute exercise-induced inflammation in runners (11 men, 9 women, age 38.4 ± 2.1 year) also found no differences in C-reactive protein or nine other inflammatory cytokines including IL-6 and IL-10 between the treatment group and placebo group after ingestion of 1000 mg of quercetin 15 minutes before an acute exercise bout (Konrad et al., 2011). Furthermore, in a study conducted in pre- to stage 1 hypertensive men and women (Edwards et al., 2007), there were no changes seen in inflammatory markers in any groups despite significant reductions in systolic, diastolic, and mean arterial pressure in the stage-one hypertensive treatment group following 28 days of quercetin supplementation. No changes in BP were observed in the pre-hypertensive group.

Studies have shown epigallocatechin 3-gallate (EGCG) supplementation can aid in the maintenance of cardiovascular health and prevention of disease (Wolfram, 2007). A study by Uchida et al. (1995) showed that EGCG supplementation effectively decreased stroke and mortality in spontaneously hypertensive rats. Furthermore, Potenza et al. (2007) concluded that EGCG supplementation was able to effectively decrease SBP in hypertensive rats.

PUFA supplementation has been investigated as a potential method for BP reduction as well. A review investigating the effects of omega-3 fatty acid supplementation found that supplementation was able to decrease SBP and DBP as well as resting heart rate due to a variety of possible mechanisms including a reduction in vagal tone, improvement of ventricular filling during diastole, increasing nitric oxide production consequently increasing

vessel radius, and more (Mozaffarian and Wu, 2011). However, the review also determined that the effect of omega-3 fatty acid supplementation on inflammation is still an area in need of further investigation.

Antioxidant supplementation, as well as omega-3 fatty acid supplementation, has been shown to decrease transit times of arterial pulse waves, thereby increasing arterial distensibility (Kappus et al., 2011). For example, Wang et al. (2008) showed that omega-3 fatty acid supplementation led to reductions in large arterial elasticity in overweight, hypertensive individuals. In contrast, our study showed no significant changes in several indices of arterial stiffness. AIX, cPWV, and pPWV did not show significant changes, suggesting that supplementation of the aforementioned multiflavonoid was not able to decrease arterial stiffness. Past studies have found similar results with no changes seen in these variables following supplementation with quercetin or omega-three fatty acids alone. Sanders et al. (2011), found no reductions in arterial stiffness after 12 months of daily PUFA supplementation in 45-70 year old males and females. However, Kappus et al. (2011) conducted a study assessing the effects of a multiflavonoid supplement on similar variables in a pre-hypertensive, college-aged cohort. No changes were seen in cPWV, pPWV, resting SBP, DBP, or ORAC pre- to post-supplementation. However, there was a significantly greater decrease in SBP following an acute aerobic exercise bout seen in the treatment group. Furthermore, a decrease in AIX was also seen following supplementation in the treatment group.

The differing responses seen with identical supplementation can be attributed to the subject population, as our study investigated 40-60 year old pre- to stage-1 essential hypertensives as opposed to college-aged young adults. Differences seen in vessel

composition as a consequence of the aging process may cause the old and young populations to respond differently to the supplementation. For example, Kappus et al. (2011) showed increases in FRAP, helping to explain the favorable changes elicited following supplementation in the younger population. However, no significant increases in FRAP were present in our subject data, suggesting that the older population did not respond similarly. This could explain why the same favorable changes were not exhibited in our subject pool. However, we also suspect that our population was already very healthy in general, which did not allow room for substantial improvement. Still, our findings are consistent with current literature suggesting that although quercetin supplementation increases plasma quercetin levels, there are no changes seen in FRAP and ORAC (Shanely et al., 2010).

Our study specifically aimed to investigate the effects of multiflavonoid supplementation on PEH, the decrease in BP seen following an acute aerobic exercise bout due to peripheral vasodilation. Kappus et al. (2011) showed that SBP decreased at a greater magnitude in the treatment group when compared to the control group following two weeks of supplementation. Therefore, we anticipated a similar, potentially exaggerated response in our subject pool as changes in the blood vessels with aging tend to elicit gradual increases in BP, and PEH is greater in magnitude and duration with increases in resting BP levels (MacDonald, 2002). However, as previously stated, our subject pool was generally healthier than expected, with the treatment group barely classifying as pre-hypertensive. These healthy operating BP levels may be responsible for the lack of improvements exhibited following supplementation.

Our study is the first to investigate the effects of a multiflavonoid supplement in an older population. The results indicate that an older population may respond differently to

supplementation when compared to younger individuals, as there were decreases seen in resting SBP in the treatment group but no changes in FRAP. Furthermore, there were no changes seen in any of the indices of arterial stiffness, suggesting that the decreases in BP may not be a consequence of favorable changes in the elasticity of the arteries.

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	Treatment		Placebo	
	Mean	SE \pm	Mean	SE \pm
Age (yrs)	51.667	1.829	50.778	1.829
Height (in)	65.583	1.09	68.083	1.049
Weight (kg)	69.911	5.295	78.289	5.295
BMI (kg/m²)	25.396	1.498	25.952	1.498
BIA (%)	29.144	3.109	27.744	3.109
VO2 peak (ml/kg/min)	33.056	8.768	36.789	7.655

Table 1. Descriptive Characteristics. All data are represented as mean \pm SE. There were no significant differences between subject populations ($p > 0.05$). *BMI* Body Mass Index, *BIA* Bioelectrical Impedance Analysis, *VO2 peak* maximal aerobic capacity.

	Treatment				Placebo			
	Pre-		Post-		Pre-		Post-	
	Supplementation		Supplementation		Supplementation		Supplementation	
	Pre- Exercise	Post- Exercise	Pre- Exercise	Post- Exercise	Pre- Exercise	Post- Exercise	Pre- Exercise	Post- Exercise
SBP	132.2	124.8	124.9	125.3	127.6	116.4	126.2	123.6
(mmHg)	±5.4	±4.2	±5.4	±4.2	±5.4	±4.4	±5.4	±4.4
DBP	76.9	80.2	72.3	81.1	68.9	79.3	68.1	77.3
(mmHg)	±4.2	±2.9	±4.2	±3.0	±3.1	±2.9	±3.1	±3.0
AIx	22.2	17.7	23.8	20.4	22.7	16.4	21.8	16.0
(%)	±2.8	±3.2	±2.8	±3.0	±3.0	±3.2	±3.0	±3.0
cPWV	8.5	8.3	8.8	8.6	9.2	8.5	8.6	7.8
(m/s)	±0.6	±0.42	±0.6	±0.83	±0.5	±0.42	±0.5	±0.83
pPWV	9.3	10.1	10.1	9.4	10.0	9.6	9.0	8.3
(m/s)	±0.6	±0.68	±0.6	±0.82	±0.7	±0.68	±0.7	±0.82

Table 2. Vascular and hemodynamic data pre- and post- supplementation. All data are represented as mean ± SE. There were no significant differences ($p > 0.05$). *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *AIX* Augmentation Index, *cPWV* central pulse wave velocity, *pPWV* peripheral pulse wave velocity.

SBP Change

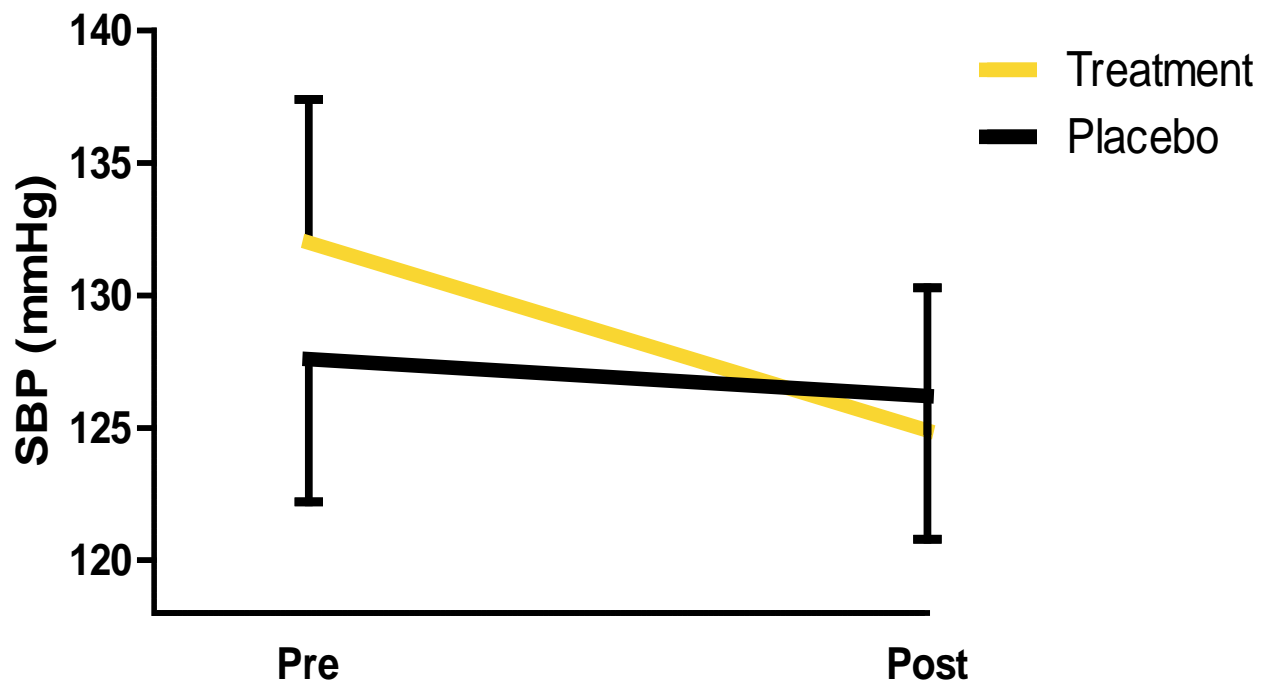


Fig 1. Systolic blood pressure (SBP) change pre- and post-supplementation.

DBP Change

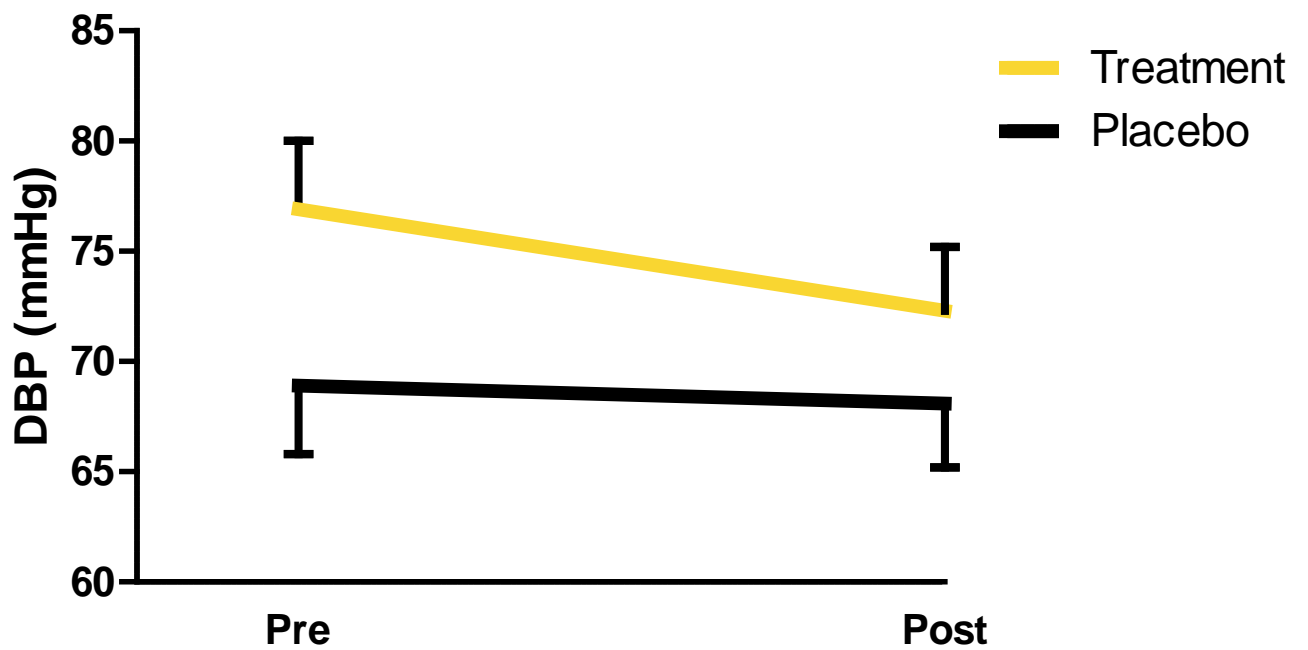


Fig 2. Diastolic blood pressure (DBP) change pre- and post-supplementation.

Appendix A: Institutional Review Board Approval

To: Scott Collier
Health, Leisure And Exercise Sci
CAMPUS MAIL

From: Dr. Timothy Ludwig, Institutional Review Board

Date: 6/28/2010

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)

Study #: 09-0270

Study Title: Changes in vascular and hemodynamic parameters following acute exercise and anti-oxidant supplementation

Submission Type: Modification

Expedited Category: (2) Collection of Blood Samples According to Guidelines,(4)
Collection of Data through Noninvasive Procedures Routinely Employed in Clinical Practice

Approval Date: 6/28/2010

Expiration Date of Approval: 6/28/2010

This submission has been approved by the Institutional Review Board for the period indicated. It has been determined that the risk involved in this modification is no more than minimal.

Investigator's Responsibilities:

Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date. You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented. Should any adverse event or unanticipated problem involving risks to subjects occur it must be reported immediately to the IRB.

CC:

Chelsea Curry, Health, Leisure And Exercise Sci

Appendix B: Informed Consent

Appalachian State University

Consent / Authorization Form

Title of Study: Changes in vascular and hemodynamic parameters following acute exercise and anti-oxidant supplementation

Background/Purpose:

You are being asked to participate in a research study because you are a healthy person between 40 and 60 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 flavanoid, in modulating vascular response after an acute bout of aerobic exercise. Each supplement contains 250 mg quercetin, 250 mg vitamin C, 10 mg niacinamide, 200 µg folic acid, 30 mg EGCG from green tea extract, 100 mg isoquercetin, and 100 mg n3-PUFAs (55 mg EPA, and 45 mg DHA) from fish oil, 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 flavanoids and reactive oxygen species concentrations. A trained technician will draw the blood.

Percentage body fat: Your present body fat will be measured using a Tanita bio-electrical impedance scale. You will stand on this scale akin to a regular home weight scale yet this one will emit a signal from one foot and be read on the opposing foot. This piece of study equipment estimates your body's composition of fat and muscle. This is accomplished by measuring the reactance (muscle) and resistance (fat) to the signal. Since muscle is composed of about 70% water, it will be a good conductor of the signal, however fat is a poor conductor of the signal so the differences between these readings will determine the percentages of fat and muscle mass.

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 walking on a treadmill at about 2.5 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 flavanoid 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 asymptotically.

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.

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.

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. Questions regarding the protection of human subjects may be addressed to the IRB Administrator, Research and Sponsored Programs, Appalachian State University, Boone, NC 28608 (828) 262-2130, irb@appstate.edu .

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 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:

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 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.

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

Timothy Ludwig	828-262-2692	irb@appstate.edu
Administrator,	IRB Telephone	e-mail

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VITA

Chelsea Dawn Curry was born in Winston-Salem, NC to Michael and Kelly Curry. Shortly after, she moved to Advance, NC, where she graduated from Davie County High School. In May of 2010, Ms. Curry earned a Bachelor of Science degree in Exercise Science with a minor in Biology and Psychology from Appalachian State University. Also in May of 2010, Ms. Curry began working towards a Master of Science degree in Exercise Science with a concentration in research. She worked as a research assistant through the Graduate Research Associate Mentoring program with Dr. Scott R. Collier in the Vascular Biology and Autonomic Studies Laboratory. Ms. Curry completed her Master of Science degree in May of 2012.