THE EFFECTS OF A MULTI-FLAVONOID SUPPLEMENT ON CARDIAC AUTONOMIC REGULATION

A Thesis
by
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FOREWORD

The research detailed in this thesis will be submitted to the journal of *Clinical Autonomic Research*, an international peer-reviewed journal for research and treatment related to autonomic function and dysfunction owned by Springer, a subsidiary of Springer-Verlag GmbH, and published by Dr. Dietrich Steinkopff Verlad.

ABSTRACT

THE EFFECTS OF A MULTI-FLAVONOID SUPPLEMENT ON CARDIAC AUTONOMIC REGULATION (May 2012)

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Antioxidant supplementation has been shown to stimulate positive effects on cardiovascular health including a reduction in blood pressure (BP) by alleviating arterial stiffness, but little is known of the benefits of antioxidants on the autonomic nervous system (ANS). The ANS plays a role in regulating BP via changes in vagal tone. Aerobic exercise has proven to be very beneficial to the ANS by reducing sympathetic modulation and increasing vagal modulation providing an overall antiarrhythmic effect on the heart [28]. *Purpose* This study aims to look at the effects of a multi-flavanoid supplement combined with an acute bout of exercise and its effects on heart rate variability and autonomic function in pre-hypertensive, middle-aged individuals. *Methods* 20 pre-hypertensive middle-aged individuals between 40-60 years were randomly assigned to the placebo (50.778 ± 1.829 years, n =10) or treatment group (51.667 ± 1.829 years, n =10). The multi-flavonoid antioxidant supplement included 1000 mg quercetin, 120 mg epigallocatechin 3-gallate (EGCG), 400 mg isoquercetin, and 400 mg omega-3 fatty acids. Subjects reported to the lab for baseline measurements. Beat-to-beat BP was measured on each subjects left arm and heart rate (HR) was monitored using a three lead electrocardiogram (CM5 configuration) to attain low frequency (LF), high frequency (HF), total power (TP), and other cardiac

autonomic modulation variables. Subjects were lying on a motorized tilt table in a quiet, dimly lit room, supine for 5 minutes, tilted head-up for 5 minutes and were lying supine again for 5 minutes, while measurements were being taken. Subjects supplemented for two weeks and returned for post measurements, identical to the procedure used earlier. *Results* There was a statistically significant decrease in normalized low frequency (nLF) in the treatment group (pre 0.647 ± .064 to post 0.567 ± 0.045, $p > 0.05$), with no changes in the placebo group (pre 0.483 ± 0.077 to post 0.556 ± 0.061 $p > 0.05$), pre to post supplementation. There was a large increase in normalized high frequency (nHF) in the treatment group (pre 0.342 ± 0.08 to post 0.417 ± 0.07, $p = 0.07$), but it was not statistically significant. There were no significant changes in total power (TP), root mean squared of the standard deviation between successive ventricular depolarization (R-R) intervals (RMSSD), or the area differences between R-R intervals (pNN50) for both treatment and placebo. There was a large decrease in percent (%) change in LF:HF ratio after tilt in the treatment group, but it was not significant. *Conclusion* The multi-flavonoid antioxidant supplementation showed favorable effects on the ANS by lowering the sympathetic outflow, and there was no additive effect of exercise and supplementation following the acute bout of aerobic exercise.

ACKNOWLEDGMENTS

I would like to thank my mentor, Dr. Scott R. Collier, for his support throughout my graduate study at Appalachian State University. I would also like to express my appreciation to the members of my thesis committee, Dr. Steve McAnulty and Dr. Lisa McAnulty, for their involvement in the completion of my thesis. Lastly, I would like to thank all of my fellow graduate students for the wonderful friendship and support throughout my graduate years.

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INTRODUCTION

The autonomic nervous system (ANS) integrates two divisions (sympathetic and parasympathetic) of the central nervous system (CNS) to regulate cardiovascular responses and adaptations to varying physiological and environmental conditions. Heart rate variability (HRV) is the variation of both instantaneous heart rate (HR), or the distance between successive ventricular depolarization (R-R) intervals [on an electrocardiogram (EKG)] and can be used to measure cardiac autonomic function. Recently, HRV has become a promising marker to help associate increased sympathetic activity or reduced vagal tone with lethal arrhythmias, which develop as a result of cardiovascular disease (CVD) [15]. Reductions in HRV have been correlated with the development of many cardiovascular conditions; whereas, a HR with wide variability that is responsive to stressors is believed to grant a survival advantage. In contrast, reduced HRV is found in many different diseased populations such as diabetics, those who have suffered myocardial infarctions, and those with hypertension or other CVD [15]. All of these diseases, which result in increased sympathetic drive, can lead to a fatal, sudden arrhythmia [24]. This autonomic dysfunction is a result of dysregulation of both branches of the ANS. Regular aerobic exercise has been shown to increase parasympathetic influence, thereby reducing blood pressure (BP) and eliciting numerous benefits on overall cardiovascular health in adults. In those who are borderline hypertensive, it has been reported that an acute bout of exercise can reduce systolic (SBP) and diastolic blood pressure (DBP) 10 and 16 mmHg, respectively [11]. The speculated

mechanisms for blood pressure reductions during post exercise hypotension could be part by increased parasympathetic outflow coupled with peripheral vasodilation.

The reduction in BP in response to exercise may be related to improvements in autonomic function due to the positive effects both mechanisms elicit after exercise [9]. Previous studies have shown that exercise training increases vagal tone by inducing resting bradycardia, indicating that exercise can produce an antiarryhthmic effect [24]. Furthermore, it has been demonstrated that those who aerobically train display an overall higher HRV and a lower mean resting heart rate compared to those who are sedentary [12]. Overall, higher HRV equates to enhanced vagal activity, thereby reducing myocardial demand in central BP.

While reductions in BP following exercise training are often linked to increases in parasympathetic tone, new strategies in BP reduction study the combination of aerobic exercise and dietary antioxidant supplementation for a potential additive effect. Antioxidant supplementation has been shown to improve arterial elasticity which, in turn, can help lower BP and increase baroreflex sensitivity in middle aged individuals [26]. Quercetin is a type of flavonoid antioxidant derived from plant pigment found in many different types of fruits and vegetables. Quercetin has been shown to help with asthma, inflammation, and with the prevention of CVD. Shanely et al. have shown that quercetin alone is ineffective in decreasing oxidative stress; however, it has been speculated that combinations of several antioxidants may result in greater absorption into the bloodstream which would result in greater BP benefits [25]. Therefore, the purpose of this study was to investigate a combination supplement that included the antioxidants quercetin, iso-quercetin, and epigallocatechin 3-gallate (EGCG) with omega-3 fatty acids (from fish oil) on HRV in response to an acute bout of aerobic exercise. We hypothesized that the combination of both

antioxidant supplementation and aerobic exercise would decrease overall sympathetic input, increase parasympathetic input and thus increase HRV.

METHODS

Subjects

Twenty healthy, pre-hypertensive (BP > 120/80) males and females between the ages of 40-60 years (10 men, 10 women) were recruited for this study. Subjects were not currently prescribed antihypertension or any other medication, were non-smokers, and had no risk factors for CVD. Both pre and post menopausal women were recruited; however, pre-menopausal women were measured during the identical phase of menstruation as their pre-measures. Before participation, each subject completed a health history questionnaire and was fully informed of the nature of the study. Participants then provided written consent to participate.

Research Design

Each subject reported to the lab on three different occasions. The first visit included the completion of a health history questionnaire and informed consent and a laboratory familiarization. Each subject then had a baseline BP measurement manually recorded along with descriptive measures of height, weight and body composition (BIA). The subject then performed a maximal exercise test (VO_2 max) to assess fitness capacity and determine maximal exercise HR. For visit two, each subject came to the lab 3-4 hours postprandial and had weight, bioelectrical impedance analysis (BIA), and baseline BP measurements were taken like in visit one. BP and HR were continuously measured both supine and during heads-up, 80 degree tilt in 5 minute epochs. The subject then performed 30 minutes of aerobic exercise on a treadmill at 65-70% of previously determined maximal HR. After the

completion of the exercise, a post exercise BP was taken, and the previous measurements on the tilt table were repeated. At the end of visit two, subjects were randomly assigned to either the treatment (Trt) or the placebo (Pla) group. The supplement included the active ingredients quercetin, iso-quercetin, EGCG, and omega-3 fatty acids from fish oil. The placebo was identical in composition as previously mentioned, without the active ingredients. Subjects underwent supplementation for two weeks and were instructed to ingest four chews; two chews in the morning and at night for six weeks before returning for visit three. Subjects then reported to the lab and completed the identical procedure from visit two to compare pre- and post- supplementation measurements. Each subject completed a total of two, three-day food diet records before visits two and three. Each set of records was completed on two weekdays and one weekend to assess diet.

Procedures

HRV

All HRV measurements from our laboratory have been previously described [5]. To measure beat-to-beat HR, subjects were monitored on a three lead EKG (CM5 configuration) and a sampling rate of 1000 Hz, to analyze R-R intervals and obtain statistical measures such as root mean squared of successive differences (RMSSD) and spectral analysis including total power (TP), high frequency (HF), low frequency (LF) and the ratio of low frequency and high frequency (LF:HF ratio) variables. All data were stored off line on a computer to be analyzed at a later time. Data were visually inspected for ectopic beats and artifacts and linearly interpolated to provide a continuous data stream. HR peaks were automatically detected via an established QRS (atrial to ventricle depolarization and repolarization) detection algorithm and used to generate an R-R interval time event series (WinCPRS;

Absolute Aliens Oy, Turku, Finland). Data were continuously streamed and resampled at 5 Hz and passed through a low-pass filter with a cutoff frequency of 0.5 Hz [6]. Power spectral analysis was performed using a maximum entropy method [2]. The optimum order of the autoregressive model was determined by Akaike's information criterion. This method yielded a model order greater than 16 to avoid possible shifting of the spectral peaks [3].

The power was calculated by measuring the area under the peak of the power spectral density curve. Three peaks were revealed and their corresponding bandwidths defined as follows: an HF region (0.15 - 0.40 Hz) caused by arrhythmic respiratory oscillations that is indicative of parasympathetic modulation of the heart; an LF region (0.04 - 0.15 Hz) related to baroreflex activity and thermoregulatory components that is mediated by both the sympathetic and parasympathetic arms of the ANS; and a very-LF component (< 0.04 Hz) resulting from non-harmonic fractal oscillations of unknown origin [15]. The very-LF component (VLF) was not used in this study other than in the calculation of normalized LF and HF HRV. The power spectra were calculated in both absolute and normalized units to represent the relative value of each power component as a proportion of the TP. TP was taken as an index of overall variability and was used as a global marker of vagal modulation [19]. The LF:HF ratio was used as an indicator of sympathovagal balance [18]. All data acquisition and post-acquisition analyses were carried out in accordance with standards put forth by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology [15].

Blood pressure

Beat-to-beat BP was measured using an upper arm cuff placed on the left arm and a finger plethysmography placed on the middle finger of the left hand (Finometer; FMS,

Amsterdam, the Netherlands). The finometer is a non-invasive technique that measures beat-to-beat BP wave forms through the pulse in the finger to analyze hemodynamic parameters such as cardiac output (CO), HR. and BP.

Tilt Testing

While beat-to-beat HR and BP were being measured, subjects were lying supine on a tilt table for 5 minutes, tilted head-up at 80 degrees for 5 minutes, and returned to supine for 5 minutes to assess HR and BP responses to a head-up tilt. An 80 degree head-up tilt was in accordance to Sharma et al. who determined that a 70 degree head-up tilt was enough to elicit a change in HRV and an increase in sympathetic predominance compared to lying supine [27]. Therefore, 80 degrees should be able to elicit the same response.

Descriptive Characteristics

All blood pressure measurements were attained using a manual BP cuff and a sphygmomanometer. Height and weight were both measured using a manual weight scale. BIA was measured using the Tanita scale (Tanita, Arlington Heights, Illinois, USA).

Graded Exercise Testing

A VO_2 max text was performed using the modified Balke protocol [22]. Subjects began walking at a comfortable speed for two minutes. During the test, intensity was increased by increasing speed, until a comfortable pace was met. The grade was then increased by 2% every two minutes until the subject reached volitional fatigue. HR was recorded every two minutes using a Polar Heart Rate Monitor (Polar Electro, Woodbury, NY, USA). A rating of perceived exertion (RPE) was also assessed per each stage using 6-20 Borg Scale[4]. Expired gas was analyzed using a breath by breath metabolic system (ParvoMedics, Sandy, Utah). Maximal effort was attained when subjects reached three of the

following four criteria: (1) respiratory exchange ratio (RER) > 1.15; (2) RPE of 17 or greater; (3) volitional fatigue; and (4) or greater than 85% of max.

Treatment of the data

All data were analyzed by SPSS version 19.0 (IBM Armonk, NY) using a two by two ANOVA with repeated measures group by time (pre versus post supplementation and placebo versus supplement group). If significance was found, an appropriate post hoc test was employed. Significance was set at $p < 0.05$.

RESULTS

Subjects

Subject characteristics by group are presented in Table 1. No significant differences were found between groups for any variables measured.

Heart rate variability

Pre and post supplementation data are presented in Table 2. Before the exercise intervention, there was a significant interaction between both the Trt and Pla groups in normalized LF (nLF). A univariate test was run to determine where the underlying significance occurred, and a significant decrease was discovered in nLF from pre to post supplementation (pre 0.647 ± .064 to post 0.567 ± 0.045, $p = 0.05$), indicating an overall decrease in sympathetic input. However, there was no significant change in the Pla group (pre 0.483 ± 0.077 to post 0.556 ± 0.061, $p > .05$; Figure 1). There was an overall increase in normalized HF (nHF) (pre 0.342 ± 0.08 to post 0.417 ± 0.07, $p = 0.07$) after treatment and before the exercise intervention in the Trt group indicating an increase in parasympathetic input. However, the change was not statistically significant. Changes to nHF in the Pla group were not significantly different (pre 0.415 ± 0.08 to post 0.418 ± 0.07, $p > 0.05$). There were overall increases in RMSSD pre-exercise and after treatment, indicating increases in variability, but there were no statistically significant differences (pre 36.3 ± 8.71 to post 44.5 ± 11.55, $p > 0.05$). There was a large drop in RMSSD (Figure 2) pre-exercise for the Pla group, indicating a decrease in variability, but again there was no significant difference (pre

50.9 ± 21.84 to post 38.5 ± 9.23, $p > 0.05$). There was no significant difference in the area differences between R-R intervals (pNN50) pre-exercise and after treatment for both the Trt group (pre 14.39 ± 6.83 to post 15.5 ± 6.58, $p > 0.05$) and the Pla group (pre 17.79 ± 8.94 to post 10.21 ± 2.68, $p > 0.05$). There was a large increase in TP for the Trt group (pre 1676.9 ± 1476 to post 1809.4 ± 1973, $p > 0.05$), and a large decrease for the Pla group (pre 1726.2 ± 643.7 to post 959.3 ± 134.2, $p > 0.05$), respectively, but no significant differences were found after treatment and before exercise. When the subjects underwent the head-up tilt there was a large increase in the differences in LF:HF ratio pre to post supplementation before exercise for both the Trt (supine 113.21 ± 254.6 to tilt 456.93 ± 1381, $p > 0.05$) and Pla (supine 152.91 ± 523.5 to tilt 433.96 ± 1162.1, $p > 0.05$) group, but the increase was not statistically significant. When the subjects underwent the head-up tilt after exercise, there was no significant difference in the differences in LF:HF ratio pre to post supplementation for the Trt group (Figure 3A) or the Pla group (Figure 3B), although there was a large decrease in the change in LF:HF ratio for both groups.

Table 1. Subject characteristics

Variables	Treatment Mean SE ±	Placebo Mean SE ±
Age (years)	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
VO_2 peak (ml/kg/min)	33.056 ± 8.768	36.789 ± 7.655

All data are reported as mean ± standard (SE) for $n = 20$. Baseline values were taken at the initial lab visit. *BMI* body mass index, *BIA* bioelectrical impedance analysis, *VO_2 peak* aerobic fitness

Table 2. Autonomic variable changes between treatment groups

	Treatment		Placebo	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
nHF	0.342 ± 0.084	0.417 ± 0.065	0.415 ± 0.084	0.418 ± 0.065
RMSSD (ms)	36.3 ± 16.627	44.5 ± 10.455	50.9 ± 16.627	38.5 ± 10.455
pN550 (%)	14.39 ± 7.596	15.5 ± 5.027	17.79 ± 7.956	10.21 ± 5.027
TP (ms)	1676.9 ± 562.363	1809.4 ± 451.268	1726.2 ± 562.363	959.3 ± 451.268

All data reported as mean ± standard error (SE) for $n = 20$. *nHF* normalized high frequency, *RMSSD* root mean squared of successive differences, *pNN50* area differences between R-R intervals, *TP* total power

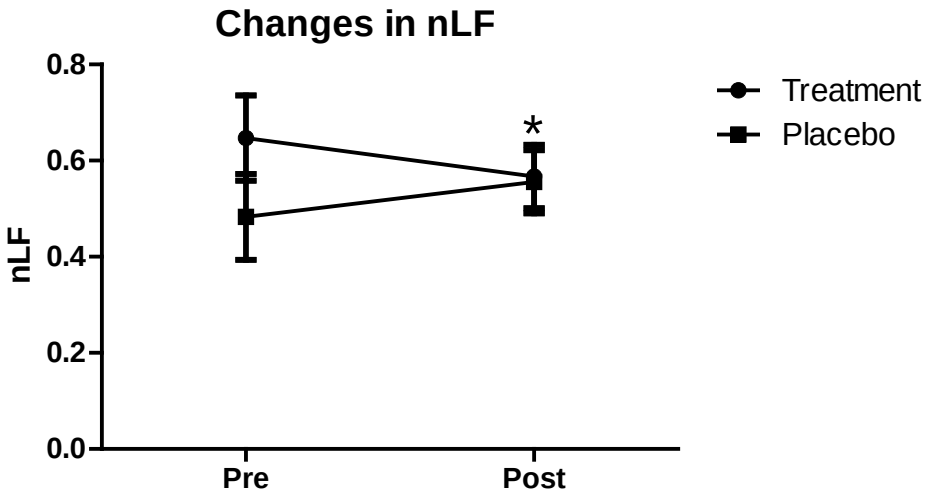

Figure 1. Values reported as mean ± standard error (SE) for $n = 20$. *nLF* normalized Low Frequency, determined by heart rate variability (HRV), changes from pre to post supplementation ($p < 0.05$); *pre* pre-supplementation; *post* post-supplementation

* nLF significantly decreased pre to post $p < 0.05$

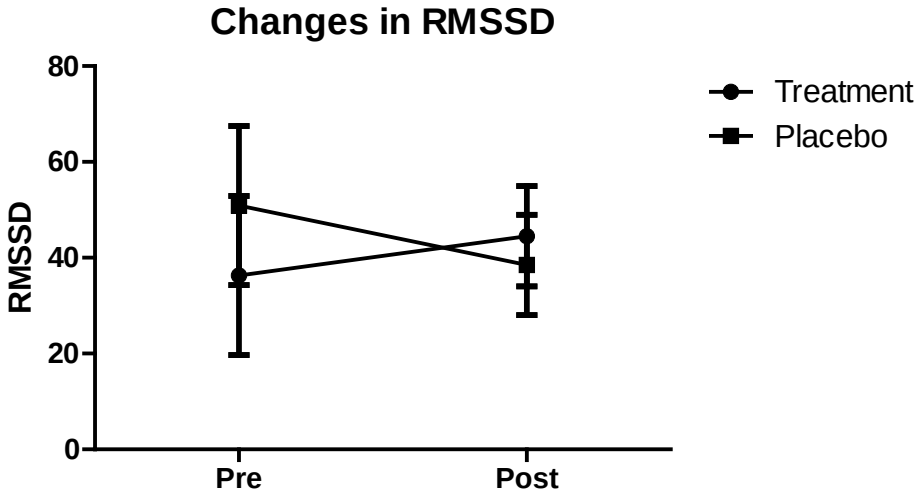

Figure 2. Values reported as mean ± standard error (SE) for $n = 20$. *RMSSD* Root Mean Square of Successive Differences, determined by heart rate variability (HRV) measurements, changes from pre to post supplementation ($p > 0.05$); *pre* pre-supplementation; *post* post-supplementation

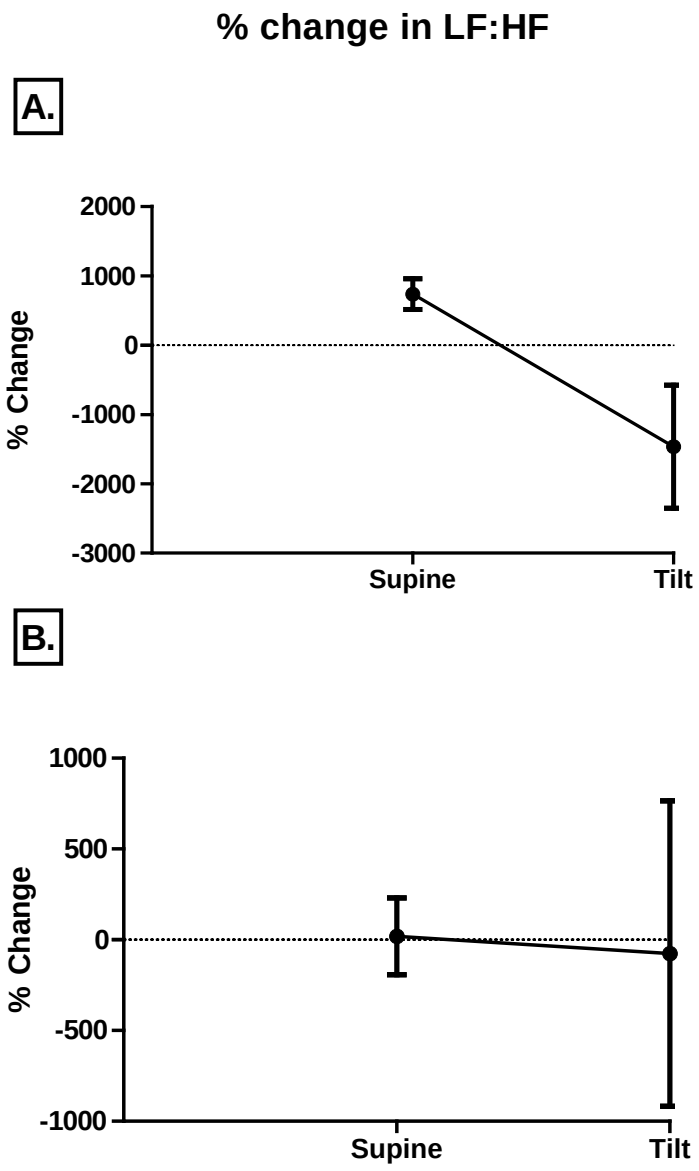

Figure 3. Values reported as mean ± standard error (SE) for $n = 20$. Percent (%) changes in LF:HF ratio pre to post supplementation while lying supine and during tilt for the treatment ($p > 0.05$; A) and placebo ($p > 0.05$; B) groups.

DISCUSSION

The main findings of our study have shown favorable benefits in cardiac autonomic modulation by decreasing nLF and favorably lowering central BP. These beneficial reductions in central hemodynamics may decrease the incidence of all causes mortality, arrhythmias, and sudden cardiac death as well the progression of hypertension.

Our study supports other finding including Edwards et al. who showed that supplementation with 730 mg/day of quercetin could reduce systolic, diastolic, and mean arterial pressure in pre-hypertensive individuals [7]. Other recent studies have also reported that quercetin significantly decreases SBP and plasma low density lipoprotein (LDL) concentrations in 25- 50 year old hypertensive subjects over a 6 week period of supplementation [8]. These previous studies support our study results indicating reductions in BP following antioxidant dosing. The overall reduction in BP may be due to the decrease in sympathetic outflow and an increase in overall variability.

In a four week aerobic exercise program, middle-aged individuals demonstrated a decrease in LF power, indicating an overall decrease in sympathetic modulation [5]. Although our study demonstrated a trend toward decreases in nLF in middle-aged pre-hypertensive individuals, this trend was only found post supplementation. Yet the acute bout of exercise showed no additive effect. This may be due to the fact that an acute bout of exercise is not enough to elicit an effect on the ANS, and long term exercise training may be more beneficial. Alom et al. conducted a study with adolescent males (12-18 years) and

observed that a regular exercise training program over a year induced a resting bradycardia, which is associated with changes as well as adaptations in the ANS [1].

While sympathetic markers showed an overall decrease, our study found no significant changes in nHF. Similar studies using exercise training as a treatment condition have resulted in decreases in HF modulation which have been reported to induce resting bradycardia [24]. The result of aerobic exercise training has been known to increase vagal tone which, in turn, has been proven to result in an antiarryhthmic sinus rhythm [24]. Sandercock et al. also reported increased power in the HF component which consequentially presents a greater parasympathetic input in response to exercise [24]. Our study results coincide with Sandercock et al. showing an increase in nHF post exercise, which is clinically significant, yet our sample size was determined to be to small to achieve statistical significance [24].

When expressing our HRV in time domain measures, our study showed no significant additive effects in TP, pNN50, or RMSSD with the combination of supplementation and exercise. A similar study by Raczak et al. with young, untrained male subjects performing a single bout of mild exercise without supplementation showed increases in several parasympathetic measurements including standard deviation between R-R intervals in the time domain (SDNN; from 81 ± 44 milliseconds to 96 ± 53 milliseconds), pNN50 (from 43 ± 27 % to 48 ± 26 %), and RMSSD (from 89 ± 66 milliseconds to 109 ± 90 milliseconds) [23]. Although large increases were observed in all of these variables, the changes were only significant with SDNN ($p = 0.02$). These data suggest that even mild physical activity has a positive autonomic affect, since the SDNN variation increased with exercise training. Our study demonstrated large increases in RMSSD, pNN50, and TP in the treatment group,

indicating an increase in overall variability, yet our findings were not statistically significant due to the large standard errors and small sample size.

Head-up tilting is an orthostatic tolerance test and ANS stressor predominantly used to measure clinical autonomic function and HRV. Sharma et al., using a perturbation of a 70 degree head-up tilt, showed that HF power significantly decreases during tilt which decreases vagal dominance, lending to increases in sympathetic stimulation [27]. This group also showed significant decreases in pNN50 corresponding to short term HRV changes. These are considered normal responses in a physiological system that is making instantaneous changes in an effort to maintain homeostasis. With age, the ANS has a decline in functioning leading to decreased sensitivity of postural changes altering the autonomic responses. Aging responses have been documented, and Laitinen et al. compared young individuals, middle-aged, and elderly subjects and concluded that as humans age, there is a decrease in TP, LF, and HF to a 70 degree head-up tilt [14]. The decrease in LF power was also significantly less when compared to younger subjects. In our study, the Trt group demonstrated less sympathetic influence during the head-up tilt test post supplementation. Although the scale of change in LF:HF ratio was not significant, the small decrease in sympathetic response could be due to a lysing of sympathetic outflow, resulting in decreased BP. La Rovere et al. used a similar tilt table test to evaluate trained and untrained subjects who had suffered a myocardial infarction [13]. The conclusion was that exercise training led to significantly greater LF increases and significantly greater HF decreases during the tilt. Limited data exist on the effects of an acute bout of aerobic exercise on an individual's autonomic response while undergoing an ANS stressor, yet La Rovere et al. suggests that trained patients who had

suffered a myocardial infarction (MI) had a functionally favorable ANS response during the tilt test compared with those who were untrained [13].

Our supplement was a combination of multiple antioxidants including quercetin, iso-quercetin, EGCG, and omega-3 fatty acids from fish oil. It is controversial whether the combination of several antioxidants may be more tolerable and culminate in greater physiological benefits when compared to single antioxidant treatments. However, flavonoids like EGCG have been shown to exert a positive effect on endothelial function which supports less arterial resistance and favorable decreases in resting BP. Being able to prevent and potentially treat arterial endothelial damage may help decrease the clinical manifestitation of elevated BP and resting HR, leading to greater HRV in pre-hypertensive adults [29]. Our supplement also contained omega-3 fatty acids which have been shown to produce favorable blood high density lipoprotein (HDL) profiles which in turn may help prevent and treat atherosclerotic plaques. In a recent study by Mozaffarian et al. healthy individuals > 65 years consumed fish and omega-3 fatty acids over the period of a year and showed a greater shift towards vagal predominance as well as improvements in baroreflex sensitivity (BRS) [17]. There were also increases in HF power as well as decreases in LF:HF ratio; thus fish oil could also potentially elicit improvements in HRV and reduce CVD risk factors. Also, a meta-analysis performed on clinical trials using supplementation of fish oil revealed a significant reduction in HR in humans of 1.6 beats per minute after supplementation for two supplement durations [16]. The decrease in HR with fish oil suggests that n-3 fatty acids possess a positive altering effect on at least one of the components that can help decrease cardiovascular risk. However, there have been no beneficial results shown with omega-3 fatty acid supplementation on any HRV variables. Park et al. found no association with

beneficial effects of omega-3 fatty acid as a sole supplement as an endpoint on autonomic function following the consumption of omega-3 fatty acids [20].

Additional studies on quercetin as a single dietary supplement have failed to show positive results on adult cardiovascular function following a supplementation regimen of 500 or 1000 mg [25]. A study performed with the dietary supplementation of quercetin using 1 gram per day dosage in apparently healthy subjects' for four weeks showed increased quercetin concentrations in the subjects' blood; however, BP, cholesterol, and resting HR showed no pre to post supplementation changes [21].

Recent literature reveals a combination supplement containing quercetin, EGCG, omega-3 fatty acids, and resveratrol showed beneficial effects with an identical design to our study [10]. Kappus et al. used two weeks of a combination of flavanol supplementation and acute exercise and measured resultant resting and post-exercise variables, and demonstrated a marked decrease in BP during post-exercise hypotension [10].

In conclusion, the present study has found that two weeks of a multi-flavonoid antioxidant supplement can have favorable effects on the ANS by lowering the sympathetic outflow and potentially decreasing BP. While supplementation caused significant effects on the ANS, an acute bout of exercise elicited little change, which could be contributed to the fact that an acute bout is not enough to elicit effects on the cardiovascular system. These data indicate that supplementing with a proprietary multi-flavonoid supplement with fish oil could result in beneficial effects on the ANS in pre-hypertensive individuals.

REFERENCES

1. Alom MM, Bhuiyan NI, Hossain MM, Hoque MF, Rozario RJ, Nessa W (2011) Physical training induced resting bradycardia and its association with cardiac autonomic nervous activities. Mymensingh Med J 20:665-670
2. Arai Y, Saul JP, Albrecht P, Hartley LH, Lilly LS, Cohen RJ, Colucci WS (1989) Modulation of cardiac autonomic activity during and immediately after exercise. Am J Physiol 256:H132-141
3. Boardman A, Schlindwein FS, Rocha AP, Leite A (2002) A study on the optimum order of autoregressive models for heart rate variability. Physiol Meas 23:325-336
4. Borg G (1982) Ratings of perceived exertion and heart rates during short-term cycle exercise and their use in a new cycling strength test. Int J Sports Med 3:153-158
5. Collier SR, Kanaley JA, Carhart R, Jr., Frechette V, Tobin MM, Bennett N, Luckenbaugh AN, Fernhall B (2009) Cardiac autonomic function and baroreflex changes following 4 weeks of resistance versus aerobic training in individuals with pre-hypertension. Acta Physiol (Oxf) 195:339-348
6. Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KU, Eckberg DL (1999) Human responses to upright tilt: a window on central autonomic integration. The Journal of physiology 517 (Pt 2):617-628
7. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T (2007) Quercetin reduces blood pressure in hypertensive subjects. J Nutr 137:2405-2411

8. Egert S, Bosy-Westphal A, Seiberl J, Kurbitz C, Settler U, Plachta-Danielzik S, Wagner AE, Frank J, Schrezenmeir J, Rimbach G, Wolffram S, Muller MJ (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr 102:1065-1074

9. Green DJ, O'Driscoll G, Joyner MJ, Cable NT (2008) Exercise and cardiovascular risk reduction: time to update the rationale for exercise? J Appl Physiol 105:766-768

10. Kappus RM, Curry CD, McAnulty S, Welsh J, Morris D, Nieman DC, Soukup J, Collier SR (2011) The effects of a multiflavonoid supplement on vascular and hemodynamic parameters following acute exercise. Oxid Med Cell Longev 2011:210798

11. Kenney MJ, Seals DR (1993) Postexercise hypotension. Key features, mechanisms, and clinical significance. Hypertension 22:653-664

12. Kouidi E, Haritonidis K, Koutlianos N, Deligiannis A (2002) Effects of athletic training on heart rate variability triangular index. Clin Physiol Funct Imaging 22:279-284

13. La Rovere MT, Mortara A, Sandrone G, Lombardi F (1992) Autonomic nervous system adaptations to short-term exercise training. Chest 101:299S-303S

14. Laitinen T, Niskanen L, Geelen G, Lansimies E, Hartikainen J (2004) Age dependency of cardiovascular autonomic responses to head-up tilt in healthy subjects. J Appl Physiol 96:2333-2340

15. Malik M (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 93:1043-1065

16. Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL, Katan MB (2005) Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. Circulation 112:1945-1952

17. Mozaffarian D, Stein PK, Prineas RJ, Siscovick DS (2008) Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. Circulation 117:1130-1137

18. Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, Somers VK (1997) Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. Circulation 95:1441-1448

19. Parekh A, Lee CM (2005) Heart rate variability after isocaloric exercise bouts of different intensities. Med Sci Sports Exerc 37:599-605

20. Park SK, Tucker KL, O'Neill MS, Sparrow D, Vokonas PS, Hu H, Schwartz J (2009) Fruit, vegetable, and fish consumption and heart rate variability: the Veterans Administration Normative Aging Study. Am J Clin Nutr 89:778-786

21. Perez-Vizcaino F, Duarte J, Jimenez R, Santos-Buelga C, Osuna A (2009) Antihypertensive effects of the flavonoid quercetin. Pharmacol Rep 61:67-75

22. Pollock ML, Bohannon RL, Cooper KH, Ayres JJ, Ward A, White SR, Linnerud AC (1976) A comparative analysis of four protocols for maximal treadmill stress testing. Am Heart J 92:39-46

23. Raczak G, Pinna GD, La Rovere MT, Maestri R, Danilowicz-Szymanowicz L, Ratkowski W, Figura-Chmielewska M, Szwoch M, Ambroch-Dorniak K (2005) Cardiovagal response to acute mild exercise in young healthy subjects. Circ J 69:976-980

24. Sandercock GR, Bromley PD, Brodie DA (2005) Effects of exercise on heart rate variability: inferences from meta-analysis. Med Sci Sports Exerc 37:433-439

25. Shanely RA, Knab AM, Nieman DC, Jin F, McAnulty SR, Landram MJ (2010) Quercetin supplementation does not alter antioxidant status in humans. Free Radic Res 44:224-231

26. Shargorodsky M, Debby O, Matas Z, Zimlichman R (2010) Effect of long-term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q10 and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risk factors. Nutr Metab (Lond) 7:55

27. Sharma P, Paudel BH, Singh PN, Limbu P (2009) Heart rate variability: Response to graded head up tilt in healthy men. Kathmandu Univ Med J (KUMJ) 7:252-257

28. Soares-Miranda L, Sandercock G, Valente H, Vale S, Santos R, Mota J (2009) Vigorous physical activity and vagal modulation in young adults. Eur J Cardiovasc Prev Rehabil 16:705-711

29. Vita JA (2003) Tea consumption and cardiovascular disease: effects on endothelial function. J Nutr 133:3293S-3297S

VITA

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