EFFICACY OF PROVEXCV TO REDUCE HYPERTENSION IN INDIVIDUALS WITH METABOLIC SYNDROME

by

Shara Biesinger

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition

College of Health

University of Utah

December 2011

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Shara Biesinger**

has been approved by the following supervisory committee members:

Thunder Jalili	, Chair	**06/28/2011** Date Approved
Stacie Lynn Wing-Gaia	, Member	**06/28/2011** Date Approved
Rodney Seymour Badger	, Member	**06/28/2011** Date Approved

and by **Eldon Wayne Askew** , Chair of the Department of **Nutrition**

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

Hypertension is one of the major causes of cardiovascular disease, which is the leading cause of death in America. A major risk factor of hypertension is metabolic syndrome. A promising method of treatment for reducing hypertension in individuals with metabolic syndrome is supplementation with polyphenolic compounds. Studies have shown that supplements of quercetin, grape seed extract (GSE), and green tea can reduce blood pressure. However, little research exists on the synergistic effects of these phytochemicals. It was hypothesized that a cocktail of GSE, resveratrol, green tea, and quercetin would decrease blood pressure in hypertensive individuals. Eighteen individuals with metabolic syndrome and hypertension were enrolled in a 10-week, randomized, double-blind, placebo-controlled, crossover study to test whether this cocktail of phytochemicals, ProvexCV, would reduce hypertension.

Differences between the effects of ProvexCV and placebo were analyzed using individual t-tests with a p-value of < 0.05. Although there was no significant difference between ProvexCV and placebo in regard to systolic blood pressure (SBP), there was a significant decrease in diastolic blood pressure (DBP) and mean arterial pressure (MAP) ($p < 0.05$). Additionally, there was a trend toward increased nitrate concentration with treatment of ProvexCV when compared to placebo ($p = 0.057$). This trend was slightly correlated with a decrease in MAP.

While we hypothesized that there would be a significant difference in platelet aggregation, angiotensin-converting enzyme (ACE) activity and inflammatory activity, our data did not support this hypothesis. However, there was a significant decrease in MAP and DBP, as well as a near significant increase in nitric oxide. The decrease in MAP may be related to increased nitric oxide availability and/or production. Although there was a significant decrease in DBP, our data do not show a synergistic effect between the components of ProvexCV. However, the main component of ProvexCV, GSE, is known to affect blood pressure and nitric oxide levels. Therefore, although it seems that the other ingredients of ProvexCV are ineffective, it is possible that this is due to the low dosage of these ingredients. Thus, it appears that it is no more beneficial to supplement with ProvexCV than to supplement with GSE.

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INTRODUCTION

Hypertension and Metabolic Syndrome

The leading cause of death in the United States today is heart disease (1). One of the major contributing factors of heart disease is hypertension. Between 2003 and 2006, an estimated 32% of Americans, ages 20 and older had hypertension (2). In 2006, an additional 28% had prehypertension (systolic BP 120–139 mm Hg or diastolic BP 80-89 mm Hg) (2). High blood pressure increases the risk of heart attack, stroke, and heart failure.

A common risk factor for hypertension is metabolic syndrome (3). Metabolic syndrome is characterized by insulin resistance, large waist circumference, high blood pressure, high triglyceride levels and low levels of high density lipoprotein (HDL) cholesterol. Although no specific standards have been set for diagnosing metabolic syndrome, the American Heart Association, along with the National Heart, Lung and Blood Institute recommend that metabolic syndrome be identified by the presence of three or more of the aforementioned characteristics (Table 1).

Metabolic syndrome is a prevalent public health problem in the United States and afflicts an estimated fifty million Americans. The symptoms of metabolic syndrome defined in Table 1 act to increase the risk of cardiovascular disease (CVD) such as cardiac hypertrophy, coronary artery disease, and

Table 1. Metabolic Syndrome Characteristics;

Presence of 3 or more indicates metabolic syndrome.

Elevated Blood Pressure	>130/85 mmHg
Elevated Waist Circumference	Men ≥ 40” (102 cm) Women ≥ 35” (88 cm)
Elevated Triglycerides	≥ 150 mg/dL
Elevated Fasting Glucose	≥ 100 mg/dL
Reduced HDL Cholesterol	Men ≤ 40 mg/dL Women ≤ 50 mg/dL

myocardial infarction. To this end, any treatment or intervention that can reduce symptoms of metabolic syndrome such as high blood pressure, plasma lipids and glucose, and body weight will help reduce risk of CVD (4).

The American Heart Association estimates that 75 million Americans have either Stage 1 or Stage 2 hypertension with many more categorized as "prehypertensive" (Table 2). Hypertension increases the risk for cardiac hypertrophy, specifically left ventricular hypertrophy (LVH), congestive heart failure, coronary artery disease, myocardial infarctions and death (Figure 1) (5). According to the Framingham Heart Study, LVH is associated with a 6-fold increase in coronary heart disease mortality and an 8-fold increase in cardiovascular mortality (6). Currently, about 300,000 deaths occur each year due to heart failure (7).

Hypertension is also found in association with elevated markers of inflammation that regulate atherosclerosis. In a 2001 study, Chae et al. found that as blood pressure increased, so too did inflammatory factors such as soluble intercellular adhesion molecule-1 (sICAM-1) and interleukin-6 (IL-6) (8). Inflammatory markers such as C-reactive protein (CRP) are increased in individuals with metabolic syndrome and hypertension. CRP has shown many proatherogenic effects such as an increase in VCAM, ICAM-1, MCP-1 and other proinflammatory cytokines (9). These cytokines and adhesion molecules stimulate monocyte infiltration in the intima and can contribute to an increase in fatty streak formation and eventually atherosclerosis (Figure 2) (10). This combination of hypertension and atherosclerosis can lead to coronary artery

Table 2. Hypertension Classification

Category	**Systolic**		**Diastolic**
Normal	<120	and	<80
Prehypertensive	120-139	or	80-89
Stage 1 Hypertensive	140-159	or	90-99
Stage 2 Hypertensive	≥160	or	≥100

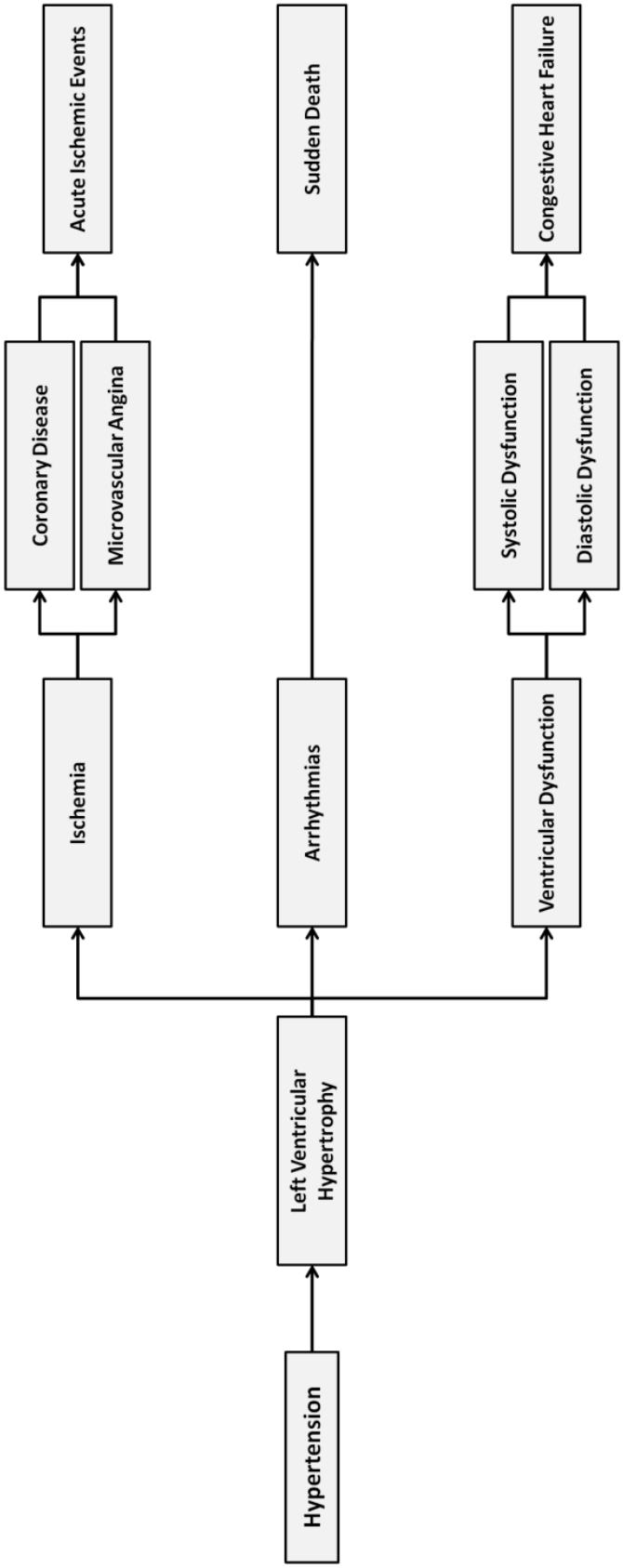

Figure 1. Consequences of Hypertension and Hypertensive LVH
Adapted from Arthram et al.

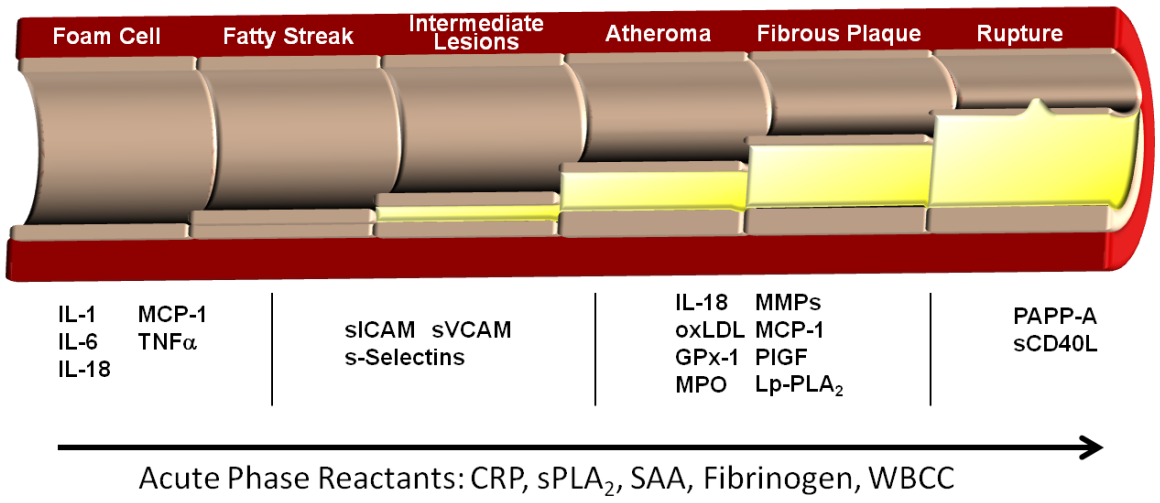

Figure 2. Biomarkers of Atherosclerotic Plaque
Adapted from Koenig, et al.

disease, stroke, heart attack, and heart failure. Furthermore, increased platelet aggregation in hypertensive individuals can increase the risk of myocardial infarction. In a hypertensive individual, the rate of platelet aggregation is higher than the rate in a nonhypertensive individual (11). This is significant because if platelets aggregate at a faster rate, there is increased risk for arterial blockage in areas of atherosclerotic plaques.

Several approaches can be used to reduce hypertension. Pharmacological interventions such as angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers (ARBs), diuretics, beta-blockers and calcium channel blockers often result in decreased blood pressure, but these treatments also carry with them adverse side effects such as weakness, leg cramps, insomnia, depression, loss of taste and dry cough (12). Dietary treatments have also been found to be beneficial. One such treatment is the American Heart Association's DASH (dietary approaches to stop hypertension) diet that focuses on reducing sodium and high-fat dairy while increasing fruit and vegetable consumption. In addition to drugs and diet, aerobic exercise and weight loss have also been used to successfully reduce blood pressure.

An additional approach that has been gaining popularity is nutrient supplementation. Studies have shown that certain phytochemicals, which occur naturally in fruits and vegetables, have successfully decreased hypertension in animals as well as humans. Specifically, resveratrol, quercetin, grape seed extract, and green tea extract have demonstrated a reduction in blood pressure (13, 14). These compounds have been combined in a new commercially

available supplement called ProvexCV. In addition, phytochemicals such as those found in ProvexCV have been shown to have an anti-platelet effect, have an anti-inflammatory effect and reduce ACE activity, all of which are risk factors for CVD (15-17). While these phytochemicals have been well studied individually, the synergistic effects of these phytochemicals, as found in ProvexCV, have yet to be established.

The goal of this study was to determine if ProvexCV can reduce blood pressure in patients with metabolic syndrome. An important secondary goal was to determine if ACE activity, platelet aggregation, and circulating inflammatory cytokines are also reduced by supplementation since they are important risk factors for CVD that are elevated in patients with metabolic syndrome.

MATERIALS AND METHODS

Participants and Recruitment Criteria

The study was approved by the University of Utah Internal Review Board and written consent was obtained from each participant. The study was conducted on a sample of 18 prehypertensive and hypertensive individuals. The exclusion criteria were as follows: consumes > 12 alcoholic drinks weekly; no longer has high blood pressure once on antihypertensive medication; pregnant; BMI over 40; diabetes; liver disease; renal insufficiency; history of prior cardiovascular event; chronic disease that might interfere with participation; an unwillingness to stop current dietary supplement intake or use of calcium/magnesium antacids. Participants were recruited via flyers, radio, internet ads, public transportation ads, health screenings and by word of mouth.

Study Design

This double-blind, placebo-controlled study took place over a 10-week period, consisting of a 4-week supplemental phase and a 4-week placebo phase which were separated by a 2-week washout phase. Before starting treatment, participants were provided with an ambulatory blood pressure cuff to wear for the duration of 24 hours that recorded their blood pressure every 30 minutes during the daytime hours.

The subjects were encouraged to maintain their regular diet and physical activity. To ensure diet consistency, participants completed a 3-day food record which was entered and analyzed using the Food Processor dietary analysis program (ESHA Research, Salem, OR). Participants were invited to come to the University of Utah a total of four times for all study procedures. After the initial screening, the subjects were assigned to obtain either the treatment or placebo pill. The following timeline describes the events that took place at each meeting:

Phase 1

1. Day 0:
    a. Measured weight using BIA, and height
    b. Obtained fasting (10-12 hours prior) blood and urine samples
    c. Tested cholesterol and glucose levels with Cholestech
    d. Provided participant with ambulatory blood pressure monitor (ABP)
    e. Provided participant with either placebo or ProvexCV
    f. Provided participant with instructions and a food record form to be filled out over 3 days
2. Day 1:
    a. Obtained ABP from participant
3. Day 27:
    a. Provided participant with ambulatory blood pressure monitor (ABP)
    b. Obtained remaining placebo or ProvexCV

4. Day 28:
    a. Obtained ABP from participant
    b. Measured weight using BIA
    c. Obtained fasting (10-12 hours prior) blood and urine samples
    d. Tested cholesterol and glucose levels with Cholestech
5. Two-week wash-out period

Phase II

6. Repeated Phase I
7. Provided participant with paperwork for compensation

Measurements

Blood Pressure and Heart Rate

An Omron automated blood pressure cuff was used to measure blood pressure and heart rate during the screening process.

Ambulatory Blood Pressure

An A&D Medical ambulatory blood pressure monitor was placed on the participant's upper left arm for a 14-hour period from 9 a.m. until 11 p.m. The cuff measured blood pressure every 30 minutes.

Dietary Records

Subjects were given a 3-day food record to be filled out during the second week during both the placebo phase and the supplemental phase. Each subject was instructed on how to fill out the diet records. Data was entered and analyzed

using the Food Processor dietary analysis program (ESHA Research, Salem, OR).

Blood Sample

Fasting subjects had their blood drawn by a certified phlebotomist in the University of Utah's Nutrition Lab. A venous sample of 12 mls was drawn into two serum separator tubes. The blood was allowed to sit for 1 hour before being centrifuged at 1000 RCF for 15 minutes at 4º. Plasma was then aliquoted into eight 500 μl portions. An additional 6 mls venous sample was drawn into two sodium citrate tubes. This sample was then used to assess platelet aggregation using a Chrono-log Whole Blood Aggregometer.

Lipids

Total cholesterol, LDL, HDL VLDL, triglycerides was measured at each clinic visit using Cholestech machines.

Cytokines

Serum Cytokines (IL-1alpha, IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-10, Interferon gamma, TNF alpha) were measured by Quansys Biosciences using multiplex kits from Quansys Biosciences (Logan, Utah).

Angiotensin-Converting Enzyme (ACE)

ACE activity was measured in serum samples using Buhlmann Assay Kit according to manufacturer's instructions. Briefly, substrate was added to calibrator, controls and serum samples after which calibrator, controls and

samples were mixed and incubated at 37° C. Blanks consisted of Milli-Q water. Absorption was read using a plate reader at 340nm at zero and 10 minutes.

Urinary Nitrates and Nitrites

Urinary nitrates and nitrites were measured using Nitrate/Nitrite Colorimetric Assay Kit according to manufacturer's instructions. After creating a nitrate standard curve, Enzyme Cofactor and Enzyme Reductase were added to standard curve and urine samples. The plate was then allowed to sit for 1 hour at room temperature before the addition of Griess Reagent R1 and Griess Reagent R2. After an additional 10 minutes of incubation at room temperature, absorbance was read at 540 nm using a plate reader.

Statistical Analysis

Blood pressure, ACE activity, lipids, inflammatory activity, and nitrates were analyzed using paired t-tests (SPSS) to detect differences between the placebo and ProvexCV treatment phase. Significance was accepted at $p < 0.05$.

RESULTS

Patient characteristics are summarized in Table 3. Six of the 18 participants had a prehypertensive blood pressure ranging from 120-130 mm Hg systolic or 80-89 mm Hg diastolic. Ten of the participants were stage 1 hypertensive while the remaining two participants were stage 2 hypertensive. Lipid and glucose data are summarized on Table 4. No statistically significant changes were seen in nutrient composition between placebo and treatment phases (Table 5).

Ambulatory Blood Pressure

While no statistical significance was seen in systolic blood pressure or heart rate, there was a statistically significant difference between placebo and treatment in reduction of diastolic blood pressure (5 mmHg decrease in treatment; $p=0.022$) as well as mean arterial blood pressure (5 mmHg decrease in treatment; $p=0.04$) (Figure 3, Table 6).

Platelet Aggregation, ACE Activity, Nitrate/Nitrite Concentration

No statistically significant differences were seen in either platelet aggregation or ACE activity (Figures 4 and 5). However, analysis of urinary nitrates showed a trend toward increased nitrate concentrations after the

Table 3. Distribution of Baseline Participant Characteristics

Gender (n=18)	
Male	15
Female	3
Age (n=18)	
20-29	2
30-39	4
40-49	4
50-59	5
60-69	2
70-79	1
Weight (lbs) (n=18)	
100-150	1
151-200	5
201-250	6
251-300	5
301-350	1
BMI (n=18)	
Normal (18.5-24.9)	3
Overweight (25-29.9)	3
Obese Class 1 (30.0-34.9)	5
Obese Class 2 (35.0-39.9)	4
Obese Class 3 (≥40)	3
SBP (n=18)	
Normal <120	1
Prehypertensive (120-139)	5
Stage 1 (140-159)	10
Stage 2 (≥160)	2
DBP (n=18)	
Normal <80	4
Prehypertensive (80-89)	10
Stage 1 (90-99)	2
Stage 2 (≥100)	2

TRG (mg/dl) (n=14)	
<150	6
150-199	4
200-249	1
250-299	1
≥300	2
TC (mg/dl) (n=18)	
<200	6
200-249	10
250-299	1
≥300	1
GLU (mg/dl) (n=14)	
80-99	6
100-119	7
120-129	0
130-139	1
140-159	0
HDL (mg/dl) (n=16)	
20-29	5
30-39	7
40-49	2
≥50	2
LDL (mg/dl) (n=12)	
<150	6
150-199	5
200-249	1
≥250	0

Table 4. Total Cholesterol, HDL, LDL, Triglycerides, Glucose, Weight and BMI

	Placebo		ProvexCV	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**
Lipids (mg)				
Total Cholesterol	213 ± 8.9	221.9 ± 11.5	216.7 ± 13	219.7 ± 10.7
HDL	46.5 ± 4.8	49.3 ± 3.7	45.7 ± 4.8	45.4 ± 4.9
LDL	134.9 ± 6.8	143.6 ± 8.2	150.9 ± 10.7	156 ± 9.1
Triglycerides	211.6 ± 29.7	225.8 ± 32.8	246 ± 27.8	243.6 ± 24.9
Glucose	98.2 ± 1.7	100.3 ± 3.2	100.3 ± 2.5	100.4 ± 2.6
Anthropometrics				
Weight (kg)	104.5 ± 12.9	104.9 ± 13.1	105.7 ± 13.4	106 ± 13.7
BMI	33 ± 1.5	33.1 ± 1.5	33.4 ± 1.6	33.7 ± 1.6

Table 5. Nutrient Composition

	Placebo	ProvexCV
Nutrients		
Calories (kcal)	2982.1 ± 246.4	3115.5 ± 396.6
Protein (g)	111.1 ± 9.8	114.3 ± 10.6
Carbohydrates (g)	366.6 ± 28	358.4 ± 48.1
Fiber (g)	24.3 ± 3.5	27.2 ± 4.4
Fat (g)	122.5 ± 12.5	138.7 ± 20.2
Vitamin A (IU)	7032.8 ± 1336.9	7497.4 ± 2212.4
Vitamin E (mg)	3.7 ± 0.5	4.6 ± 1.6
Vitamin K (µg)	28.6 ± 4.4	33.9 ± 9
Calcium (mg)	1085.7 ± 124.2	1401.2 ± 188.6
Magnesium (mg)	194.8 ± 23.7	209.5 ± 26.4
Potassium (mg)	2058.9 ± 229.4	2066.2 ± 285.3
Sodium (mg)	5253.3 ± 540	6182.8 ± 643.2

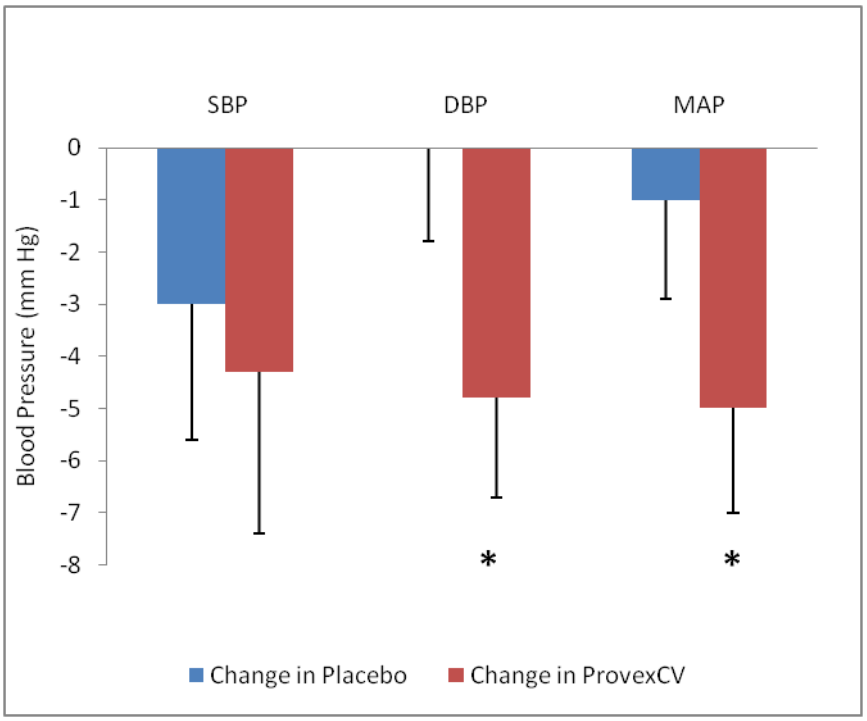

Figure 3. Difference in Systolic, Diastolic and Mean Arterial Pressure**.**

*P<0.05. Bars represent standard error.

Table 6. Mean Arterial Pressure and Heart Rate

	Placebo		**ProvexCV**	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**
Mean Arterial Pressure (mmHg)				
MAP	106.8 ± 2.9	105.6 ± 2.7	108 ± 2.3	104.9 ± 2.1
HR	80.9 ± 2.9	83.8 ± 2.5	85 ± 3.2	82.5 ± 2.9

*P<0.05. Values are mean ± SE

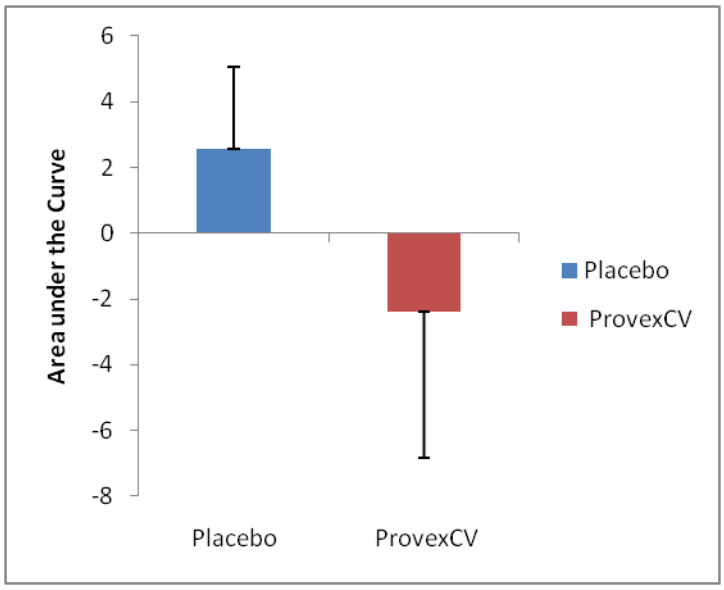

Figure 4. Platelet Aggregation**.** Bars represent standard error.

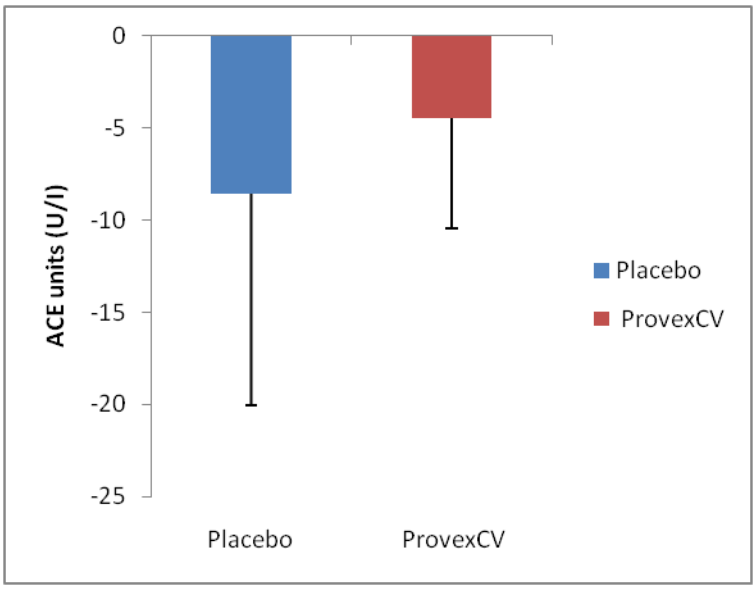

Figure 5. Angiotensin-Converting Enzyme (ACE) Activity**.**

Bars represent standard error.

ProvexCV phase vs. Placebo (p=0.057). This trend is slightly correlated with a decrease in MAP (R^2 = 0.11, Figures 6,7).

Inflammatory Markers

Results from inflammatory assays showed no significant differences in inflammatory markers between placebo and treatment groups (Table 7).

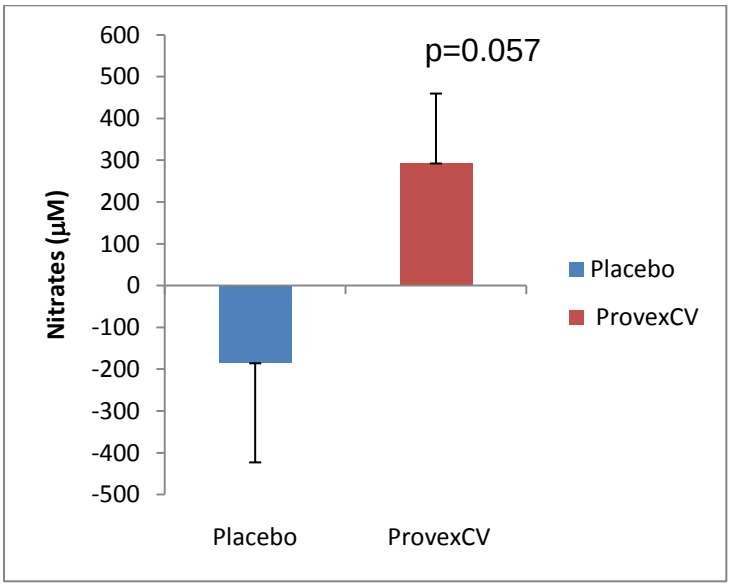

Figure 6. Urinary Nitrates. Bars represent standard error.

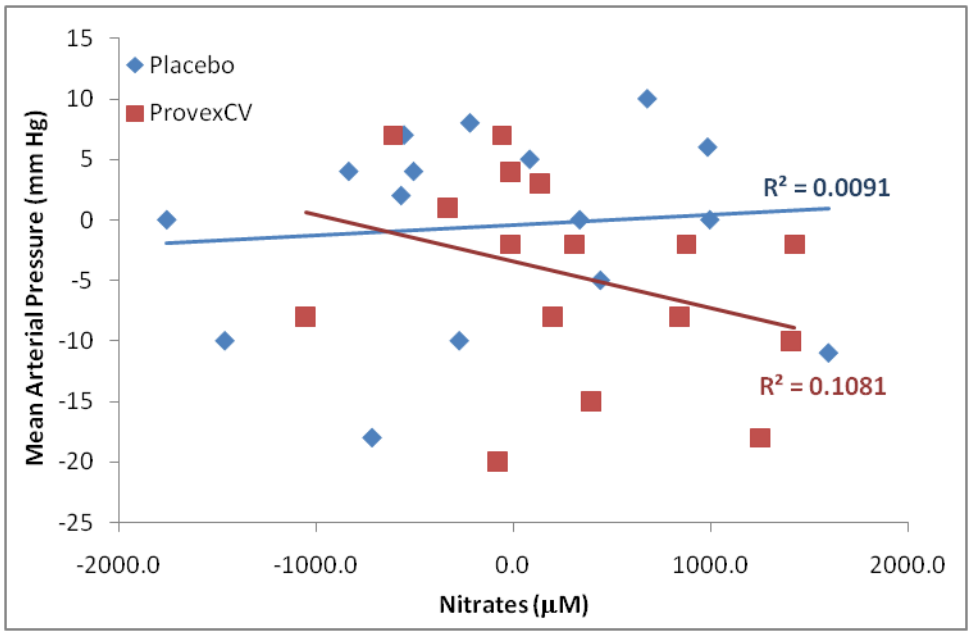

Figure 7. Correlation Between Nitrates and Mean Arterial Pressure

Table 7. Inflammatory Markers

	Placebo		ProvexCV	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**
	Inflammatory Activity (pg/ml)			
TIMP	6641.9 ± 1617.2	6204.5 ± 883.5	5398.4 ± 565.2	5590.1 ± 572.2
CRP	131.4 ± 13.2	133 ± 7	138.9 ± 8.5	128.5 ± 9
IL-8	9.3 ± 1.8	6.7 ± 1.3	6.7 ± 1.1	8.8 ± 2.5
IP-10	70 ± 14.1	53.8 ± 5.7	46.6 ± 4.6	48 ± 8.5
MCP-1	264.3 ± 35.2	248.9 ± 22.6	251.4 ± 23	259.3 ± 21.9

Values are mean ± SE

DISCUSSION

It was hypothesized that ProvexCV would decrease blood pressure in hypertensive individuals with metabolic syndrome. In agreement with this, our results demonstrate a significant reduction in diastolic and mean arterial pressure ($p=0.022$ and 0.04, respectively). These results are in line with previous studies in which blood pressure was reduced upon consumption of individual ProvexCV ingredients such as green tea, grape seed extract, resveratrol and quercetin.

Our results showed a significant decrease in diastolic and mean arterial pressure. The decrease in mean arterial pressure seen in this study is clinically significant because reductions of this magnitude are associated with a decreased risk of death due to CVD (18). As mentioned previously, hypertension increases the risk for cardiovascular diseases such as cardiac hypertrophy, coronary artery disease, and congestive heart failure (5).

It was hypothesized that ProvexCV would decrease platelet aggregation due to the presence of resveratrol and grape seed extract (15, 19). We hypothesized that ACE activity would be inhibited due to the activity of resveratrol (17). No significant decrease was seen in platelet aggregation or in ACE activity. Although previous studies suggest we would see an improvement in blood flow upon consumption of individual ProvexCV components, our results do not indicate that ProvexCV decreases risk of CVD by decreasing platelet

aggregation. While no significant decrease was seen in platelet aggregation or in ACE activity, it is possible that this did not occur because of inadequate dose. While the dosage of grape seed extract (GSE) is similar to the dosage used in studies in which a reduction of blood pressure was seen, the dosages of green tea, resveratrol and quercetin are all significantly lower than the dosages used in previous studies.

We believed that ProvexCV would reduce inflammatory cytokines due to the presence of GSE (16). Inflammatory cytokines can increase risk of CVD as they lead to atherosclerotic plaque. As mentioned previously, as blood pressure increases, inflammatory markers such as CRP also increase which in turn increases cytokines that can contribute to increased arterial plaque (8, 9). This increased plaque can then lead to atherosclerosis (10). Although previous studies indicate that inflammatory markers would be decreased upon consumption of the components found in ProvexCV, consumption of ProvexCV itself did not result in a reduction of inflammation. However, there are several possibilities why we did not see a reduction in inflammatory markers. Again, dosage of individual components may have been too low. In 2009, Kar et al. found that when type 2 diabetics who were at risk for cardiovascular disease received 600 mg of GSE for 4 weeks, the inflammatory cytokine, CRP, was significantly reduced ($p = 0.0006$) (20). This dosage is nearly double that of the dosage found in ProvexCV. Additionally, the treatment phase was only a 4-week period in both studies. More time may have been needed to see a significant decrease in inflammatory markers.

A decrease of blood pressure and the correlated decrease in CVD is often associated with a concurrent decrease in platelet aggregation, ACE activity and inflammatory markers (8-11). ProvexCV treatment did not result in a significant decrease of platelet aggregation, ACE activity or inflammatory markers; however, ProvexCV treatment did result in a decrease in diastolic blood pressure and mean arterial pressure. This suggests that ProvexCV does not function to reduce blood pressure by modulating ACE activity, nor does it have an effect on platelet aggregation or inflammation. Urinary nitrates had a near significant increase ($p=0.057$). This increase suggests that higher levels of nitric oxide produced in the arteries may be responsible for lower blood pressure. Nitric oxide, released in the blood vessels by endothelial nitric oxide synthase, is a vasodilator and a platelet aggregation inhibitor. While a decrease in blood pressure is seen after supplementation with ProvexCV, the mechanism by which ProvexCV affects blood pressure does not completely correlate with those seen in previous studies, e.g., ProvexCV does not affect ACE activity, inflammation or platelet aggregation, whereas the individual components of ProvexCV were able to affect these pathways. This could be due to inadequate dose or an inadequate study length.

Research conducted on each component of ProvexCV demonstrates individual benefits in relation to decreasing blood pressure. Previous studies have demonstrated that a reduction in blood pressure can lead to a decreased risk of CVD (18). Although there was no reduction in ACE activity, platelet aggregation or inflammatory markers, we saw a clinically significant 5 mmHg

decrease in mean arterial pressure of a magnitude that has previously been shown to be correlated with reduced CVD risk (18). Our data indicate that this decrease may be related to increased nitric oxide availability and/or production. While the reduction in blood pressure is a positive finding, data from this study do not indicate that there is an additive effect of combined ingredients due to synergy between the components of ProvexCV. GSE, the main component of ProvexCV, has the potential to reduce high blood pressure in humans. In a double-blind, placebo-controlled, 4-week study, 27 hypertensive individuals with metabolic syndrome were randomly divided into 3 groups to receive a placebo, 150 mg or 300 mg dose of GSE. Blood pressure was tested at the end of the 4 weeks. The participants who received 150 mg and 300 mg doses of GSE showed a significant decrease in both systolic and diastolic blood pressure when compared to the placebo-controlled group ($p < 0.05$) Specifically, there was a 11 mmHg decrease in systolic blood pressure and a 7 mmHg decrease in diastolic blood pressure seen in participants who received 300 mg GSE (14). Although our results show a clinically significant reduction of 5 mmHg in diastolic blood pressure, it is minimal compared to the effects of GSE alone as reported in the 2009 study. It is possible that these differences are due to the difference in participant ages. The participant age range from the aforementioned study was 43±3 years while the age range in our study had a greater spread (44±13 years). Alternatively, there may be some interference in GSE effects from other ProvexCV components. Our data suggest that the other components of ProvexCV are ineffectual, possibly due to the low dosage of each component in

ProvexCV. Therefore, it is no more advantageous to supplement with ProvexCV than to supplement with GSE.

Strengths and Limitations

A major strength of this study was that it was a double-blinded study in that neither participants nor those directly involved with the research were aware of which treatment was placebo and which treatment was ProvexCV. An additional strength is that validated measures were used to assess blood pressure, platelet aggregation, ACE activity, nitrite and nitrate activity, inflammatory markers, lipids and body composition. The same research assistants took the measures each visit in order to standardize the results.

Participant recruitment was initially difficult despite proactive measures. However, after advertising for the study via public transportation, recruitment increased dramatically. Although a significant number of participants joined the study, it was difficult to retain them. Reasons for leaving the study included health problems, moving out-of-state, inability to get to the clinic, and general lack of motivation. A few of the participants failed to follow study protocol by eating before clinic appointments or by missing set clinic appointments. There are other factors that may have affected the results such as the events that took place on the day participants wore the 24-hour blood pressure cuff. For example, one participant had a family emergency on the night of wearing her 24-hour blood pressure cuff. After completing the study, one of the participants shared

that he had opened the treatment capsules before consumption. Behaviors such as this could have also negatively affected the study outcomes.

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