5-17-2017

The Acute Effects of Beetroot Powder and Arginine Supplementation on Blood Pressure in Normotensive Adults

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May 17, 2017

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PURPOSE. Hypertension is one of the top risk factors for cardiovascular disease, which is the leading cause of death in America. Beetroot is high in nitrates that could potentially be beneficial for hypertension. Beetroot comes in many forms including juice, juice concentrate, powdered, and encapsulated. The most studied form of beetroot is beetroot juice. Our study examined the encapsulated form of beetroot powder. L-arginine supplementation has also been suggested to have a strong association with blood pressure due to the nitric oxide pathway. The purpose of this graduate student research study was to investigate the effects of beetroot powder and arginine supplementation on blood pressure in normotensive adults.

METHODS. Participants included students and faculty of Loma Linda University between the ages of 20 and 90 years with normal blood pressure readings. Participants were randomized into three groups: received beetroot powder and arginine supplement, received beetroot powder with arginine placebo, or received only placebo capsules. The supplements were taken in the morning for three consecutive days with blood pressure readings at four specific times during the day.

RESULTS. There were no significant differences over time or between groups for systolic (Day 1 $p = .82$; Day 2 $p = .97$; Day 3 $p = .62$) or diastolic (Day 1 $p = .50$; Day 2 $p = .97$; Day 3 $p = .59$) blood pressure.

CONCLUSION. Our study concluded that encapsulated beetroot powder combined with arginine supplementation would not be useful for reducing blood pressure in normotensive adults.

KEY WORDS: Hypertension, Arginine, Beetroot powder.

Hypertension is a major health care problem in the United States. About 70 million people, one in three people, have high blood pressure.[1] Hypertension has a strong association with cardiovascular disease, coronary artery disease, and heart failure, which is the number one leading cause of death in in both men and women in America.[2] Hypertension is also one of the most common risk factors for stroke and a contributor to the development of renal insufficiency and end-stage renal disease. With the aging population and the obesity epidemic, it is estimated that over 23 million people will die from cardiovascular disease by 2030.[3] Yet, out of the 70 million Americans who have hypertension, only 52% have their blood pressure under control.[4]

Beetroot is high in nitrates that promote cardiovascular health and are potentially beneficial for hypertension. There are many forms of beetroot products in the industry today, such as beetroot juice, beetroot powder, beetroot tablets, and beetroot enriched bread products. The most popular and the most studied form is beetroot juice. Many studies have suggested that beetroot juice is capable of lowering blood pressure in healthy and hypertensive adults.[5,6] Beetroot juice was shown to decrease the blood pressure in healthy individuals in as little as three hours after consumption of 500 milliliters of beetroot juice.[7] Another study measured blood pressure of hypertensive adults in three different settings including in the clinic, ambulatory, and at home. In three settings subjects showed a decrease in blood pressure after consuming 250 milliliters of beetroot juice every morning for four weeks.[8] Both populations of normotensive and hypertensive adults showed responsiveness to the beetroot juice supplementation in the ranges of 250 milliliters to 500 milliliters. Also, in healthy normotensive adults, beetroot enriched products, 100, 250, and 500 grams of the bread product produced a reduction in blood pressure.[9] These recent research articles have found beetroot juice and beetroot enriched bread

products to be effective in lowering blood pressure in adults. It is unknown whether supplementation of the powder form of beetroot lowers blood pressure.

Nitrate that is found in beetroots is a precursor to the chemical compound nitric oxide, which is made by the endothelial cells that line the blood vessels.[4] The endothelium of the blood vessels use nitric oxide to relax the smooth muscle causing vasodilation which increases blood flow resulting in a decrease in blood pressure. When cholesterol levels are too high, nitric oxide is destroyed and the blood vessels are unable to relax. When this happens, the endothelial cells try to compensate by producing more nitric oxide, however, even with adequate nitrate, endothelial cells cannot keep up with the demand for nitric oxide because the cells run out of L-arginine, another precursor to nitric oxide.[4]

Several studies have suggested that there is a strong association between oral L-arginine supplementation and reduction in blood pressure.[10-11] L-arginine is an amino acid that is used in the biosynthesis of protein and is a conditionally essential amino acid found in a wide variety of food items including both animal and plant sources.[12] As a precursor to nitric oxide, L-arginine may help to improve blood pressure by improving dilation of the endothelium.[13] A meta-analysis of 11 randomized, double-blind, placebo-controlled trials concluded that there is evidence that oral L-arginine supplementation significantly lowers both systolic and diastolic blood pressures.[14]

The current prevention strategy for heart disease is a healthy, balanced diet with low sodium consumption and regular exercise. Along with a diet full of fruits and vegetables, most adults are looking for natural and easier ways to lower their blood pressure. Nitrates in the form of beetroot juice have been shown to reduce blood pressure in many recent studies. This study may benefit the community by providing a convenient way to improve blood flow and decrease

the risk of heart disease. The purpose of this graduate student research study is to investigate the effects of beetroot powder and arginine supplementation on blood pressure in normotensive adults. We hypothesize that beetroot powder supplementation alone may decrease blood pressure, and beetroot powder supplementation along with arginine supplementation may have a greater effect on lowering blood pressure.

Methods

Participants

We recruited students and faculty at Loma Linda University. Participants were recruited by posting flyers in Nichol Hall, the Loma Linda University dorms, and around campus. Recruitment flyers were emailed to the students, faculty, and staff of Loma Linda University. Recruitment was also done by presenting our study to classes in the Nutrition and Dietetics Department of the School of Allied Health Professions.

Individuals interested in participating were screened using the following inclusion and exclusion criteria. Potential subjects were included in this study if they were healthy adults between the ages of 20 and 90 years with a baseline blood pressure reading for systolic blood pressure between 90-139 mmHg and diastolic blood pressure between 60-89 mmHg. They were available on Monday's and Friday's and were able to check their blood pressure throughout the days on Tuesday, Wednesday and Thursday for one week. Potential subjects were excluded if they had a diagnoses of hypertension, low blood pressure, liver cirrhosis, herpes types one and two, asthma, guanidinoacetate methyltransferase deficiency, kidney stones, hepatic dysfunction, recent heart attack within six months, had recent surgery or upcoming surgery within six months, currently taking blood pressure medications, pregnant or lactating, were currently taking beetroot, arginine, or protein supplementation, consuming a diet high in nitrate rich foods, or had

beet allergies. Individuals who could not perform blood pressure measurements were also excluded.

Instruments

The contact sheet included the participant's name, phone number, email address, preferred method of contact, as well as appointment request for the returning Friday. The Master Identification sheet for the investigator's information included a code number, participant's name and participant's group assignment and was kept separate from all data collected from the participants. The log books and questionnaires contained only the participant's code. The lifestyle questionnaire, developed by the researchers, included questions about the participant's age, height, weight, family history of heart disease, exercise habits, and consumption frequency of nitrate rich foods and arginine rich foods. The logbook was given to the participants to record Tuesday, Wednesday, and Thursday blood pressure readings, time they took the supplement, and any additional comments for that day.

Upper arm automatic blood pressure monitors (Omron® 5 services arm blood pressure monitor) were loaned to participants by the Department of Nutrition and Dietetics of Loma Linda University to monitor their blood pressure. The blood pressure monitors were standardized to the blood pressure monitors at the cardiology department at Loma Linda Medical Center.

Supplementation

The supplementation and placebo pills were be obtained from Corona Specialty Compounding Pharmacy.[15] The study had two types of supplements with their corresponding placebos produced by the compounding pharmacy. The first supplement contained 500 mg beetroot powder in capsule form. The second supplement contained 1000 mg of arginine in capsule form. The arginine came in two capsules per serving since each capsule held 500 milligrams.

Procedure

This study was a randomized, single-blind, placebo-controlled study with repeated measures design to test multiple occasions and multiple groups. The protocol was approved by the Institutional Review Board of Loma Linda University.

The researchers went to various classes in the Nutrition and Dietetics Department to present the study to potential volunteers. The purpose of the study, the time requirements, the responsibilities, and inclusion and exclusion criteria were explained. A sign-up sheet was handed out to those interested in participating. After collection of the sign-up sheet, those who were interested were contacted to meet on a scheduled Monday to test their blood pressure, sign the consent form, and if they qualified, they continued to answer the lifestyle questionnaire and contact information to join the study. Identification numbers were randomized into three groups (A, B, C) prior to participant meeting. As the participants were enrolled into the study, they were given an ID number that was already randomized into their respective groups. Group A was given the experimental beetroot capsule along with the experimental arginine capsule. Group B was given the experimental beetroot capsule with placebo arginine capsule. Group C was the control group with placebo beetroot capsule and placebo arginine capsule.

At the Monday meeting, the researchers explained how to take their blood pressure, when to take the supplements, and how to log their blood pressure and daily activities in the comments section of the log book. They were given a logbook and a blood pressure monitor. On Tuesday, Wednesday, and Thursday, the participants took the supplements in the morning between 6AM and 10AM. They logged their blood pressure upon waking, and 3 hours, 6 hours, and 12 hours after consuming the supplement. Returning on Friday, the participant met the researchers to return the blood pressure monitors and log books. The participants were given a reminder of their

appointments by text message, phone call, or e-mail on Sunday and Thursday. One of the researchers was assigned to contact all the participants for a given day.

Data Analysis

Collected data were analyzed using SPSS-PC version 24 software. Gender, age, height, weight, BMI, and exercise habits were summarized using frequencies and means (SD) by group. A mixed factorial ANOVA with time as a repeated measures factor and group as an independent factor was used to compare blood pressure values. The level of significance was set at .05.

Results

Study Participants

Fifteen participants qualified for the study. There was three groups; beetroot plus arginine (A), beetroot only (B) and control (C), with five participants per group. Thirteen participants were female and two participants were male. Group A had one male and four females, Group B had one male and four females, and Group C had five females. The majority of the subjects did not have a history of family heart disease. Participant's regular physical activity ranged from one day a week to seven days a week, with all groups having a similar range of activity level. The mean BMI of all three groups was 24.1 kg/m^2. Group A had a mean BMI of 24.1 kg/m^2, Group B had a mean BMI of 26.1 kg/m^2 and Group C had a mean BMI of 22.2 kg/m^2.

Systolic Blood Pressure

The mixed factorial ANOVA found no significant interaction (Day 1, $p = .82$; Day 2, $p = .97$; Day 3, $p = .62$). There was no significant effect due to either group or time for any of the three days (see Table 1).

Diastolic Blood Pressure

The mixed factorial ANOVA found no significant interaction (Day 1, $p = .50$; Day 2, $p = .97$; Day 3, $p = .59$). There was no significant effect due to either group or time for any of the three days (see Table 2).

Discussion

As far as we are aware, this is the first study to look at the combined effects of beetroot powder and arginine supplementation on blood pressure in normotensive adults. This study used 500 mg of beetroot powder and 1000 mg of arginine supplementation in encapsulated form. Participants were asked to take the supplements in the morning for three days and record their blood pressure at four specific times during the day. No significant difference in blood pressure was observed over time or between groups. In contrast, several previous studies have found that beetroot products and arginine supplementation taken separately for longer periods of time in similar or higher dosage significantly reduce blood pressure in both normotensive and hypertensive adults.[5-14] The result from these studies suggest the potential for a natural low cost approach for the prevention and management of high blood pressure.

The research had several limitations. The first limitation of this study was the sample size. The sample consisted of only 15 participants divided evenly between three groups. Secondly, the research was conducted in a short time frame of only three days to assess the effects of the dietary nitrate intervention compared to the time frame of other studies. Thirdly, the participants were asked to manually record their daily blood pressure readings, which may introduce a bias in the recording of blood pressure measurements. Fourthly, we did not take into consideration the lifestyle factors such as physical activity, stress, or intake of caffeine. Lastly, we did not obtain blood samples in this study to assess plasma nitrite levels or biomarkers of

nitric oxide bioactivity, such as cyclic guanosine monophosphate, arginine, or methylated arginine.

Future studies may consider altering the dosage to double or triple the amount of supplementation to result in more significant findings. One thousand milligrams of arginine was a relatively small dosage and doubling the amount to two thousand milligrams may be more effective. Five hundred milligrams of beetroot powder was also a small dosage and future research may want to consider tripling this dose. Another method to test is beetroot juice or beetroot concentrate with arginine rather than the powder form that was tested. Since beetroot and arginine in our study was safe to test on normotensive adults, future research should consider testing hypertensive adults. Controlling for other variables such as physical activity, caffeine intake, other diet related factors, and even stress should be documented. These factors may have a large influence on the blood pressure readings and may play a part in the results. This study only supplemented and tested blood pressure for three days, future studies may consider testing for longer periods of time.

Conclusion

Five hundred mg of beetroot powder combined with 1,000 mg of arginine supplementation showed no significant reduction in blood pressure compared to 500 mg of beetroot powder alone or the placebo in the normotensive general population. There were also no observed significant changes in blood pressure over time.

Our findings were not significant, and therefore encapsulated beetroot powder combined with arginine supplementation would not be recommended for reducing blood pressure in normotensive adults.

Table 1: Comparison of Systolic Blood Pressure among Beetroot plus Arginine, Beetroot, and Control Groups at Four Specified Times.

Day	Beetroot + Arginine Mean (SD)	Beetroot Mean (SD)	Control Mean (SD)	*p*-value **
Day 1				
SBP1*	111.0 (14.6)	112.4 (16.7)	110.8 (5.4)	
SBP2*	108.8 (12.8)	114.2 (14.5)	109.3 (6.2)	.79
SBP3*	111.2 (12.0)	120.2 (11.5)	109.8 (8.2)	
SBP4*	112.2 (16.2)	114.2 (20.9)	111.3 (10.9)	
p-value**	.67	.67	.67	
Day 2				
SBP1*	111.0 (15.5)	119.0 (16.9)	118.7 (6.5)	
SBP2*	107.4 (13.0)	112.8 (17.8)	114.7 (8.4)	.59
SBP3*	110.6 (9.0)	117.2 (16.2)	120.3 (7.6)	
SBP4*	105.4 (10.0)	116.4 (22.2)	114.0 (13.1)	
p-value**	.19	.19	.19	
Day 3				
SBP1*	106.2 (16.0)	108.8 (20.3)	111.5 (6.6)	
SBP2*	106.8 (12.7)	118.0 (18.8)	111.0 (12.6)	.77
SBP3*	113.4 (6.9)	118.0 (19.3)	112.0 (11.0)	
SBP4*	108.6 (17.5)	116.5 (13.1)	110.5 (10.7)	
p-value**	.20	.20	.20	

* SBP1, blood pressure upon waking; SBP2, blood pressure three hours after supplement; SBP3, blood pressure after six hours after supplement; SBP4, blood pressure twelve hours after supplement.

** Mixed Factorial ANOVA with no interactions

Table 2: Comparison of Diastolic Blood Pressure among Beetroot plus Arginine, Beetroot, and Control Groups at Four Specified Times.

Day	Beetroot + Arginine Mean (SD)	Beetroot Mean (SD)	Control Mean (SD)	*p*-value **
Day 1				
DBP1*	74.4 (9.2)	76.4 (11.2)	79.3 (10.8)	
DBP2*	72.4 (8.6)	72.8 (11.4)	77.0 (12.0)	.88
DBP3*	72.8 (9.3)	77.6 (8.2)	76.3 (10.7)	
DBP4*	75.8 (10.9)	74.4 (12.8)	76.8 (11.1)	
p-value**	0.33	0.33	0.33	
Day 2				
DBP1*	72.2 (14.6)	77.8 (11.1)	84.3 (4.9)	
DBP2*	68.2 (9.0)	73.2 (12.3)	77.0 (15.9)	.47
DBP3*	71.6 (10.9)	74.8 (8.2)	80.7 (15.0)	
DBP4*	67.4 (8.4)	73.8 (10.9)	75.3 (18.6)	
p-value**	.12	.12	.12	
Day 3				
DBP1*	74.4 (15.8)	75.0 (4.4)	77.8 (13.1)	
DBP2*	72.4 (8.0)	74.3 (9.1)	74.0 (16.7)	.90
DBP3*	75.0 (6.3)	70.0 (12.5)	75.3 (16.4)	
DBP4*	66.6 (8.1)	71.0 (10.0)	74.25 (17.6)	
p-value**	.22	.22	.22	

* SBP1, blood pressure upon waking; SBP2, blood pressure three hours after supplement; SBP3, blood pressure after six hours after supplement; SBP4, blood pressure twelve hours after supplement.

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SBP4*	112.2 (16.2)	114.2 (20.9)	111.3 (10.9)	
p-value**	.67	.67	.67	
Day 2				
SBP1*	111.0 (15.5)	119.0 (16.9)	118.7 (6.5)	.59
SBP2*	107.4 (13.0)	112.8 (17.8)	114.7 (8.4)	
SBP3*	110.6 (9.0)	117.2 (16.2)	120.3 (7.6)	
SBP4*	105.4 (10.0)	116.4 (22.2)	114.0 (13.1)	
p-value**	.19	.19	.19	
Day 3				
SBP1*	106.2 (16.0)	108.8 (20.3)	111.5 (6.6)	.77
SBP2*	106.8 (12.7)	118.0 (18.8)	111.0 (12.6)	
SBP3*	113.4 (6.9)	118.0 (19.3)	112.0 (11.0)	
SBP4*	108.6 (17.5)	116.5 (13.1)	110.5 (10.7)	
p-value**	.20	.20	.20	

* SBP1, blood pressure upon waking; SBP2, blood pressure three hours after supplement; SBP3, blood pressure after six hours after supplement; SBP4, blood pressure twelve hours after supplement.

** Mixed Factorial ANOVA with no interactions

Table 2: Comparison of Diastolic Blood Pressure among Beetroot plus Arginine, Beetroot, and Control Groups at Four Specified Times.

Day	Beetroot + Arginine Mean (SD)	Beetroot Mean (SD)	Control Mean (SD)	*p*-value **
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DBP1*	74.4 (9.2)	76.4 (11.2)	79.3 (10.8)	
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DBP4*	75.8 (10.9)	74.4 (12.8)	76.8 (11.1)	
p-value**	.33	.33	.33	
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DBP3*	71.6 (10.9)	74.8 (8.2)	80.7 (15.0)	
DBP4*	67.4 (8.4)	73.8 (10.9)	75.3 (18.6)	
p-value**	.12	.12	.12	
Day 3				
DBP1*	74.4 (15.8)	75.0 (4.4)	77.8 (13.1)	
DBP2*	72.4 (8.0)	74.3 (9.1)	74.0 (16.7)	.90
DBP3*	75.0 (6.3)	70.0 (12.5)	75.3 (16.4)	
DBP4*	66.6 (8.1)	71.0 (10.0)	74.3 (17.6)	
p-value**	.22	.22	.22	

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