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Daily Blueberry Consumption Improves Blood Pressure and Arterial Stiffness in Postmenopausal Women with Pre- and Stage 1-Hypertension: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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ABSTRACT

Background Postmenopausal women have a high prevalence of hypertension and often develop arterial stiffness thereby increasing cardiovascular disease risk. Although antihypertensive drug therapies exist, increasing numbers of people prefer natural therapies. In vivo studies and a limited number of clinical studies have demonstrated the antihypertensive and vascular-protective effects of blueberries.

Objective To examine the effects of daily blueberry consumption for 8 weeks on blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension.

Design This was an 8-week, randomized, double-blind, placebo-controlled clinical trial.

Participants/setting Forty-eight postmenopausal women with pre- and stage 1-hypertension recruited from the greater Tallahassee, FL, area participated.

Intervention Participants were randomly assigned to receive either 22 g freeze-dried blueberry powder or 22 g control powder.

Main outcome measures Resting brachial systolic and diastolic blood pressures were evaluated and arterial stiffness was assessed using carotid-femoral pulse wave velocity and brachial-ankle pulse wave velocity. C-reactive protein, nitric oxide, and superoxide dismutase were measured at baseline, 4 weeks, and 8 weeks.

Statistical analyses performed Statistical analysis was performed using a split plot model of repeated measures analysis of variance.

Results After 8 weeks, systolic blood pressure and diastolic blood pressure (131±17 mm Hg [$P<0.05$] and 75±9 mm Hg [$P<0.01$], respectively) and brachial-ankle pulse wave velocity (1,401±122 cm/second; $P<0.01$) were significantly lower than baseline levels (138±14 mm Hg, 80±7 mm Hg, and 1,498±179 cm/second, respectively), with significant ($P<0.05$) group×time interactions in the blueberry powder group, whereas there were no changes in the group receiving the control powder. Nitric oxide levels were greater (15.35±11.16 μmol/L; $P<0.01$) in the blueberry powder group at 8 weeks compared with baseline values (9.11±7.95 μmol/L), whereas there were no changes in the control group.

Conclusions Daily blueberry consumption may reduce blood pressure and arterial stiffness, which may be due, in part, to increased nitric oxide production.

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HYPERTENSION IS A KNOWN MAJOR, YET preventable, risk factor for the development of cardiovascular disease (CVD), the leading cause of death in the United States. According to the American Heart Association, in the United States approximately 77.9 million, or one in three adults, have hypertension.[1,2] Although the prevalence of hypertension is associated with aging in both sexes, the increased incidence of high blood pressure, particularly systolic blood pressure (SBP), in women after menopause exceeds that of men.[3,4] Endothelial dysfunction has been suggested to play an important role in the increases in blood pressure that occur after menopause. Endothelial dysfunction is defined as changes in both vascular tone and in endothelium-derived substances that

influence vasodilation, such as a reduction in nitric oxide production and bioavailability,[5] and vasoconstriction,[6,7] such as an increase in the production of endothelin-1,[8] angiotensin II,[9] and reactive oxygen species (ROS).[10,11] For instance, the self-perpetuating cycle between oxidative stress and inflammation that often occurs in a postmenopausal and/or a hypertensive state leads to the upregulation of the enzyme nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), which increases the production of ROS such as superoxide anions in the arteries and the kidneys. These ROS are then able to react with nitric oxide to form peroxynitrate leading to a reduction in nitric oxide bioavailability and subsequently impaired nitric oxide-dependent vasodilation that can result in hypertension.[12,13] In addition, endothelial dysfunction is known to increase arterial stiffness, which is involved in the development and progression of both hypertension and CVD.[14,15] Therefore, endothelial function and arterial stiffness should be targeted in the prevention and treatment of hypertension.

The recommended intervention for controlling blood pressure in pre- and stage 1-hypertension is not pharmaceutical interventions but rather lifestyle modifications, including dietary approaches,[16] and there is evidence that many cases of hypertension can be prevented and treated through diet and lifestyle changes.[17,18] Considering the prevalence of hypertension in the United States,[19] preventive strategies such as dietary modifications (eg, functional foods and dietary supplements) that aim to improve hypertension and its related complications are warranted.[20] Epidemiologic evidence suggests that dietary intake of certain flavonoids is associated with a reduced risk of CVD risk factors, including hypertension.[21] In fact, Cassidy and colleagues[22] reported an 8% reduction in the risk of hypertension in individuals in the highest quintile of anthocyanin intake (primarily from blueberries and strawberries) compared with those in the lowest quintile (relative risk 0.92; 95% CI 0.86 to 0.98; $P<0.03$). Berries, including blueberries, and their polyphenols have been reported[23] to improve several surrogate markers of cardiovascular risk, including blood pressure, endothelial function, and arterial stiffness. Among all fruits, blueberries are one of the richest sources of phenolic compounds, including flavonoids, phenolic acids, and stilbenes,[24-26] which are known to have biological activity and high antioxidant capacity[24,27,28] and they are a promising functional food with respect to vascular health. For instance, Basu and colleagues[29] reported that the consumption of 50 g freeze-dried highbush blueberries significantly lowered blood pressure in obese men and women with metabolic syndrome after 8 weeks. The vascular-improving effects of blueberries has been, in part, attributed to the ability of its circulating polyphenol metabolites to inhibit NADPH oxidase, which will enhance nitric oxide bioavailability leading to enhanced endothelial-dependent vasodilation.[30]

Despite the existence of the aforementioned studies, there is a paucity of clinical studies investigating the antihypertensive and vascular-protective effects of blueberries, particularly in postmenopausal women with pre- and stage 1-hypertension. In addition, to our knowledge, there are presently no studies that have investigated the effects of blueberry consumption on arterial stiffness as measured by pulse wave velocity, which is considered the gold standard marker of arterial damage and is predictive of CVD risk.[31] Therefore, the objective of this study was to bring forth evidence that blueberry consumption would reduce blood pressure and cardiovascular risk factors, including endothelial dysfunction and arterial stiffness, in postmenopausal women with pre- and stage 1-hypertension. This randomized, double-blind, placebo-controlled clinical trial tested the hypothesis that daily blueberry consumption for 8 weeks would reduce arterial stiffening and improve endothelial function resulting in reduced blood pressure.

SUBJECTS AND METHODS

Subjects

A total of 81 healthy, postmenopausal women aged 45 to 65 years with pre- and stage 1-hypertension (seated blood pressure $\geq$ 125/85 mm Hg but $\leq$160/90 mm Hg at the screening visit) were recruited from Tallahassee, FL, and surrounding areas. Recruitment began in January 2012 and continued through March 2013 when the final patient finished the study. Those with diagnosed CVD; uncontrolled hypertension (>160/100 mm Hg); receiving hormone replacement therapy or insulin; active cancer, asthma, glaucoma, thyroid, kidney, liver, and pancreatic disease; and heavy smokers (>20 cigarettes/day) were excluded from the study. The Florida State University Institutional Review Board approved the study protocol and all participants provided written informed consent. This trial was registered at ClinicalTrials.gov: NCT01686282. After an initial prescreening over the telephone, qualified participants were invited to the study site for their first visit. During the first visit, written informed consent was obtained from all participants by the study coordinator. Brachial blood pressure measurements were taken in duplicate after 10 minutes of seated rest with an automatic device (Omron Healthcare, Inc). A complete medical and nutrition history was obtained from participants by a registered dietitian nutritionist for screening purposes. Based on inclusion and exclusion criteria, a total of 48 postmenopausal women qualified and participated in the study. Participants were asked to maintain their usual diet and physical activity pattern throughout the duration of the study.

Study Design and Intervention

Forty-eight postmenopausal women that met all inclusion criteria were recruited to participate in an 8-week, randomized, double-blind, placebo-controlled clinical trial. Using a statistician-pregenerated randomization list, eligible participants were randomly assigned by the study coordinator to one of the two intervention groups: 22 g freeze-dried blueberry powder or 22 g macronutrient-matched control powder. The rationale for choosing this dose is that 22 g freeze-dried blueberry powder equates to 1 cup fresh blueberries, which is feasible for people to consume on a daily basis (Table 1). The blueberry powder consisted of highbush freeze-dried blueberries (50/50 blend of tifblue [*Vaccinium virgatum*] and rubel [*Vaccinium corymbosum*]) and the placebo powder consisted of maltodextrin, fructose, artificial and natural blueberry flavoring, artificial purple and red color, citric acid, and silica dioxide. The nutritional composition of the freeze-dried blueberry powder and placebo powder was determined by Medallion Laboratories (Table 1). The participants were asked to consume half of the daily regimen (11 g) mixed with 1 cup (240 mL) water in the

Table 1. Nutrient composition of freeze-dried blueberry and placebo powders compared with fresh blueberries

	Placebo powder (per 22 g)[a]	Freeze-dried blueberry powder (per 22 g)[a]	Fresh blueberry (per c)[b]
Energy (kcal)	86	87	83
Fat (g)	0.02	0.26	0.48
Total carbohydrates (g)	20.82	20.57	21.02
Fiber (g)	0	4.73	3.50
Protein (g)	0.17	0.59	1.08
Vitamin C (mg)	0	2.27	14.10
Calcium (mg)	0	7.50	9.00
Potassium (mg)	0	103.18	112
Oxygen radical absorbance capacity (μmol TE[c]/g)	0	80.52	Unknown
Phenolics (mg/g)	0	8.45	Unknown
Anthocyanins (mg/g)	0	4.69	Unknown

[a]Analyzed by Medallion Laboratories.
[b]US Department of Agriculture National Nutrient Database for Standard Reference.
[c]TE=Trolox equivalents.

morning and the second half mixed with 1 cup (240 mL) water in the evening at least 6 to 8 hours apart. Participants were encouraged to add vanilla extract and/or Splenda to the regimen for added flavor based on their preference. The freeze-dried blueberry powder and placebo powder regimens used in this study were provided by the US Highbush Blueberry Council and were distributed to subjects on a biweekly basis. To monitor compliance, participants were given customized calendars and were asked to record the days they missed consuming the study regimen and return any unused portion for compliance monitoring purposes. Compliance was defined as missing $\leq$2 doses per week.

Anthropometric Assessments

Height without shoes was measured using a wall-mounted stadiometer to the nearest 0.5 cm and weight was assessed using a digital scale (Seca Corporation) to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms/height in meters^2. Midabdominal waist circumference was measured using a Gulick fiberglass measuring tape with a tension handle (Creative Health Products, Inc). With the exception of height measured at baseline, body weight and waist circumference were repeatedly measured at baseline, 4 weeks, and 8 weeks.

Blood Collection and Analysis

Fasting venous blood for plasma and serum was collected between 8:00 AM and 10:00 AM on a designated date from each participant in vacutainers with appropriate anticoagulants at baseline, 4 weeks, and 8 weeks. Serum and plasma were separated by centrifuging at 4,000 rpm for 15 minutes at 4°C within 2 hours of collection using an IEC CL31R multispeed centrifuge (Thermo Electron Corporation). Samples were then aliquoted and stored at −80°C until analyses. Serum levels of superoxide dismutase (SOD) and plasma levels of nitric oxide and C-reactive protein were measured in duplicate at baseline, 4 weeks, and 8 weeks using the following commercially available kits: Superoxide Dismutase Assay Kit, Nitrate/Nitrite Colorimetric Assay Kit, and C-Reactive Protein (human) EIA Kit according to the manufacturer's (Cayman Chemicals) instructions.

Blood Pressure and Arterial Function

Cardiovascular measurements were performed at baseline, 4 weeks, and 8 weeks on the same day as blood collection and in the supine position in a quiet, temperature-controlled room (23°C±1°C) after an overnight fast and avoidance of alcohol and caffeine for at least 24 hours. Brachial blood pressure, mean arterial pressure, carotid-femoral pulse wave velocity (cfPWV), and brachial-ankle pulse wave velocity (baPWV) were measured using an automatic device (VP-2000; Omron Healthcare). Appropriate-size blood pressure cuffs were wrapped around both arms (brachial artery) and ankles (posterior tibial artery). Electrocardiogram electrodes were placed on the forearms, and a heart sound microphone was placed on the chest. Participants rested for at least 20 minutes before data collection. Transit time was automatically determined from the time delay between the feet of the pulse waves related to the R-wave of the electrocardiogram. The distance from the carotid and femoral artery was measured with a nonelastic tape measure as a straight line, whereas the distance from the brachial to tibial arteries was calculated automatically according to the participant's height.[32] Pulse wave velocity was calculated as distance divided by transit time.[32] Two measurements were collected and averaged at each time point. Heart rate was determined from the electrocardiogram.

Statistical Analyses

An initial sample size of 24 participants per group with an attrition rate of 17% was able to produce a sample size of approximately 20 participants with >80% power to detect a significant difference ($P<0.05$). The sample size was calculated using a study conducted by Basu and colleagues,[29] which indicated that an intake of 50 g freeze-dried blueberry powder was effective in lowering SBP and diastolic blood pressure (DBP) (−6% and −4%, respectively) compared with controls (−1.5% and −1.2%).

Statistical analysis was performed using analysis of variance methods with PROC MIXED in PC SAS (version 9.1, 2006, SAS Institute). Descriptive statistics were calculated for all variables including means, standard deviations, medians, minima, and maxima. Distributions of outcome variables were examined graphically for asymmetry and for outliers. When a lack of symmetry was noted, the variable was transformed before analysis. Baseline values of serum, anthropometric, and dietary variables, which were normally distributed between the two experimental groups, were compared using two-sample *t* tests. The main

and interaction effects of the intervention (freeze-dried blueberry powder or placebo) and time (baseline, 4 weeks, and 8 weeks) on primary outcome variables (blood pressure) and secondary outcome variables (pulse wave velocity and blood biomarkers) were evaluated. A split plot model of two (group)×three (time) repeated measures analysis of variance was used for statistical analysis both within and between treatment groups. The mean changes in outcome variables during the intervention periods were compared by analyzing interaction effects of intervention and time, using the SLICE option (to analyze simple effects) in a least square means statement. Data are reported as least square mean±standard deviation. In all statistical comparisons, differences with $P<0.05$ were considered significant. Differences with $P<0.01$ were noted.

RESULTS

Baseline Characteristics and Anthropometric Measurements

A flowchart of the study enrollment is presented in the Figure. A total of 48 women who met the inclusion and exclusion criteria were randomly assigned to receive either 22 g freeze-dried blueberry powder (25 participants) or 22 g placebo powder (23 participants) daily for 8 weeks. The overall attrition rate for the 8-week intervention study was 17% (20% for the treatment group and 13% for the control group). Common reasons for not finishing the study included noncompliance with the study protocol, claims of medical and health-related issues such as gastrointestinal complaints, and personal reasons such as lack of time (see the Figure). Reported challenges to intake of the treatment regimens included difficulty mixing powders with water, taste preferences, volume, and taste fatigue. The 40 participants who completed the study were compliant with their treatments as indicated in their daily dosing diaries.

Baseline characteristic data for participants who completed the study are presented in Table 2. There were no statistically significant differences between groups for baseline characteristics including age, height, weight, BMI, and waist circumference. The mean body weights, BMI, and waist circumferences for baseline, 4 weeks, and 8 weeks are presented in Table 2. There were no significant changes in either group at any time point.

Blood Pressure and Arterial Stiffness

No significant differences were noted between groups at baseline (Table 3). As presented in Table 3, after 8 weeks of treatment, both SBP and DBP were significantly lower ($P<0.05$ and $P<0.01$, respectively) than baseline levels whereas there were no significant changes in the control group. Significant ($P<0.05$) group×time changes were noted for both SBP and DBP at 8 weeks. In addition, in terms of mean percent changes, there were 5.1% (range=0% to −9.9%) and 6.3% (range=−1.3% to −11.0%) reductions in mean SBP and DBP, respectively, in the blueberry group, whereas there were no reductions in mean SBP (mean=+0.7%; range=−4.9% to +6.7%) and DBP in the control group (mean=+2.6%; range=−2.5% to +7.9%) There were no differences observed in either treatment group at 4 weeks. There was a significant ($P<0.01$) reduction in baPWV from baseline at 8 weeks and there was a group×time interaction ($P<0.05$) in the blueberry group, whereas there were no changes in the control group. There was no significant effect of blueberry supplementation on mean arterial pressure, cfPWV, and heart rate at any time point.

Blood Biomarkers

Blood biomarkers are presented in Table 4. There were no significant changes in C-reactive protein levels at any time point in either treatment group. SOD levels were significantly ($P<0.01$) increased at 4 and 8 weeks compared with baseline in the blueberry and control groups. Nitric oxide levels were significantly ($P<0.01$) increased in the blueberry group at 8 weeks compared with baseline values, whereas there were no changes in the control group.

DISCUSSION

We found that daily incorporation of freeze-dried blueberry powder into the diet of postmenopausal women with pre- and stage 1-hypertension for 8 weeks improves blood pressure and arterial stiffness potentially through enhanced nitric oxide-mediated vasodilation. To our knowledge, this is the first study to evaluate the effects of blueberries on arterial function as was done in this study, as well as in this study population.

In terms of the effects of blueberries on blood pressure, the results of the present study showed 5.1% and 6.3% reductions in mean systolic SBP and DBP, respectively, in the blueberry group, whereas there were no significant decreases in the control group. These findings are in agreement with Basu and colleagues[29] who noted a 6% and 4% reduction in SBP and DBP, respectively, after 8 weeks of supplementation with 50 g freeze-dried blueberry powder in middle-aged obese men and women with metabolic syndrome. Therefore, the current findings support that regular blueberry consumption, even in a different study population, is effective in lowering blood pressure. On the contrary, the consumption of 250 g blueberries by adult smokers for 3 weeks produced no changes in blood pressure in a study by McAnulty and colleagues.[33] Several studies have shown that smokers respond differently than nonsmokers to dietary interventions,[34-36] which is a possible reason for this observation. Another likely reason for this finding is that the duration of their study was too short to observe an effect on blood pressure as indicated by no significant changes in blood pressure after 4 weeks of treatment in the current study.

It is important to note that the findings of this study do not suggest that the effectiveness of the dose of blueberry powder used in this study matches that of antihypertensive medications. This is evidenced by the fact that although significant reductions were noted in SBP and DBP at 8 weeks in the blueberry-treated group, mean SBP levels remained in the prehypertensive range at the end of the treatment period. It is possible that higher doses and/or longer intervention time may result in further reductions in SBP to that of the normal range. However, the changes in blood pressure noted in this study are of clinical significance because they demonstrate that blood pressure can be favorably altered by the addition of a single dietary component (eg, blueberries).

Pulse wave velocity is a noninvasive method for assessing arterial stiffness and has been shown to predict future cardiovascular events.[31] Epidemiologic[37,38] and clinical

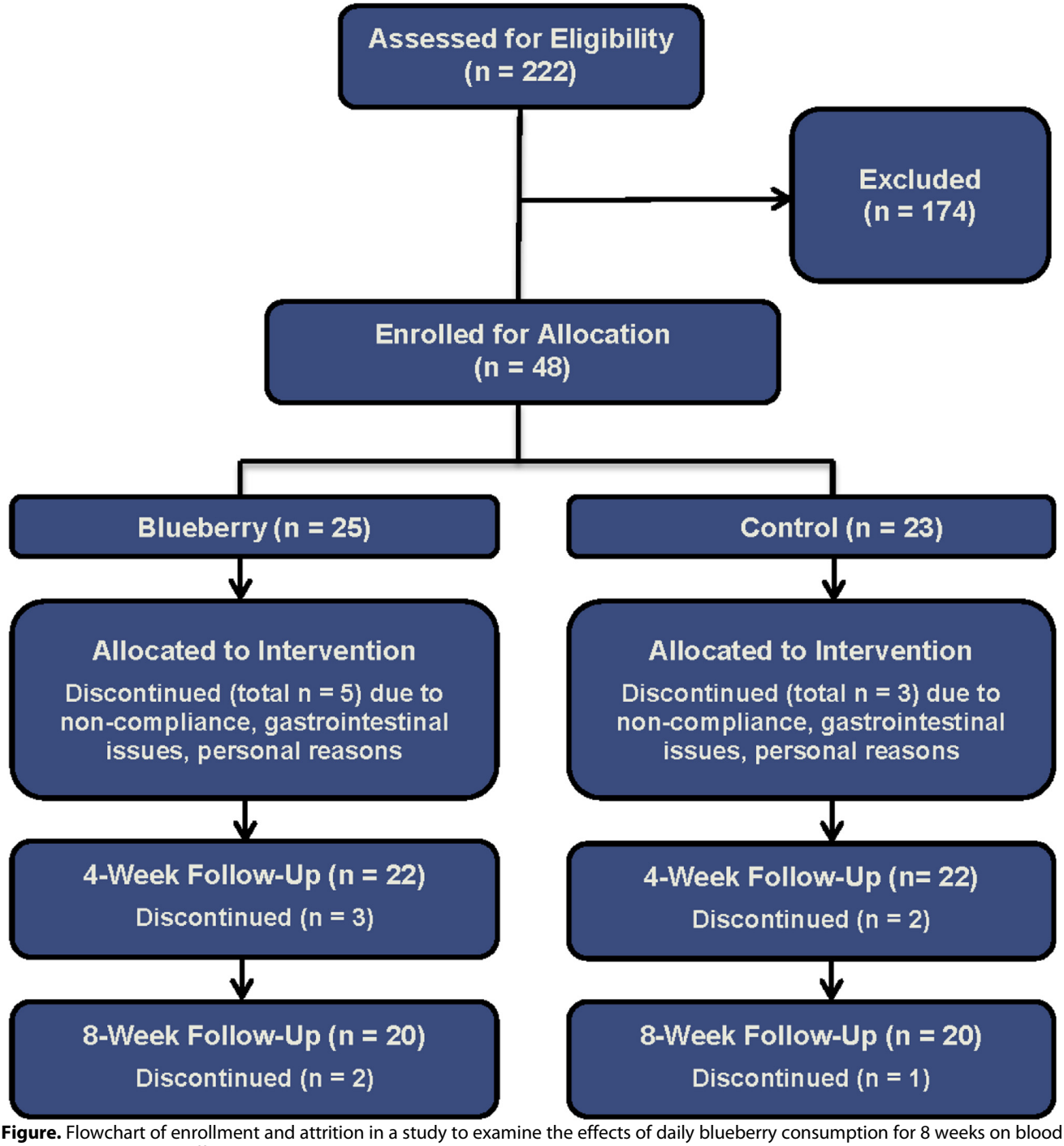

Figure. Flowchart of enrollment and attrition in a study to examine the effects of daily blueberry consumption for 8 weeks on blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension.

intervention studies[39-41] have demonstrated that flavonoids and flavonoid-rich foods are associated with improvements in pulse wave velocity, and therefore, arterial stiffness. In the current study, baPWV, which is a composite measure of central (aortic) and peripheral arterial stiffness, was significantly reduced after 8 weeks in the blueberry-treated group, whereas there were no changes in the control group. baPWV has been shown to be highly associated with cfPWV, considered the gold standard measure of aortic stiffness, and as such is an emerging index of central arterial stiffness.[32,42,43] However, no changes were noted in cfPWV in either group. We have previously reported that dietary interventions, including a hypocaloric diet[44] and supplementation with watermelon[45] led to reduced baPWV but had no effect on aortic pulse wave velocity. These findings, along with the results of the current study, suggest that peripheral arteries may be more responsive to dietary interventions than central arteries.

Table 2. Characteristics of study participants assessed in an 8-wk clinical trial evaluating the effects of freeze-dried blueberry powder supplementation vs placebo on blood pressure and arterial stiffness in postmenopausal women with pre- and stage 1-hypertension

	Blueberry			Control		
Variable	Baseline	4 wk	8 wk	Baseline	4 wk	8 wk
	←mean±standard deviation[a]→					
Age (y)	59.7±4.58	—	—	57.3±4.76	—	—
Height (cm)	164±5.32	—	—	165.48±8.35	—	—
Weight (kg)	82.1±18.52	82.2±18.18	82.1±18.21	88.4±21.39	90.3±20.44	88.4±21.72
Body mass index	30.1±5.94	30.2±5.90	30.2±5.96	32.7±6.79	32.7±6.54	32.1±6.82
Waist circumference (cm)	105±16.60	99±18.01	103±14.76	98±19.83	110±38.32	101±19.17

[a]Baseline values were not significantly different between groups. There were no significant within-group changes for weight, body mass index, or waist circumference for either group.

Increased baPWV has been reported[46-48] to be predictive of the progression of pre-hypertension to hypertension. Our findings showing that blueberry consumption decreased SBP and DBP following blueberry consumption may be explained by the noted changes in baPWV. After 8 weeks, DBP moved from the prehypertensive range to the normal blood pressure range, whereas SBP remained in the prehypertensive range in both groups, despite a significant reduction in SBP in the blueberry-treated group. Hence, it can be suggested that daily blueberry consumption may be effective in preventing the progression of pre-hypertension to hypertension in postmenopausal women, which may in part be explained by improvements in arterial stiffness as indicated by reductions in baPWV. Further, these findings are of additional clinical significance because baPWV has been reported to be an independent predictor of coronary atherosclerosis in postmenopausal women.[49]

Although the exact cause of hypertension is unknown, one of the mechanisms leading to increased blood pressure and arterial stiffness is suggested to be endothelial dysfunction. It is known that oxidative stress leads to an increase in the production of superoxide anions by NADPH oxidase, which is able to react with nitric oxide to form peroxynitrate leading to a reduction in nitric oxide bioavailability and endothelial damage in the arteries.[12,13] As was mentioned earlier, it has been demonstrated[30] that improvements in flow-mediated vasodilation, indicative of enhanced endothelial function, were closely associated with increases in circulating polyphenol metabolites from blueberry consumption as well as decreases in neutrophil NADPH oxidase activity, which would enhance nitric oxide bioavailability. Although NADPH oxidase activity or flow-mediated vasodilation was not assessed in the present study, based on the results of previous studies it can be suggested that the reductions in blood pressure and arterial stiffness noted in the present study may have been due, in part, to enhanced endothelial-dependent vasodilation. In the current study, blueberry consumption increased nitric oxide levels compared with no significant changes in the control group. Although there were significant increases in SOD at 4 and 8 weeks in the blueberry group, these changes were also observed in the control group. These findings suggest that blueberry consumption per se may not influence the bioavailability of nitric oxide through the dismutation of superoxide by SOD but rather may influence nitric oxide

Table 3. The effects of 8 wk of supplementation with freeze-dried blueberry powder vs placebo on hemodynamic parameters at baseline, 4 wk, and 8 wk in postmenopausal women with pre- and stage 1-hypertension

	Blueberry			Control		
Variable	Baseline	4 wk	8 wk	Baseline	4 wk	8 wk
	←mean±standard deviation→					
Systolic blood pressure (mm Hg)	138±14	136±15	131±17*[a]	138±15	136±15	139±15
Diastolic blood pressure (mm Hg)	80±7	77±10	75±9**[a]	78±8	78±11	80±8
Mean arterial pressure (mm Hg)	99±9	97±11	95±11	98±9	97±11	97±11
Carotid-femoral pulse wave velocity (cm/sec)	1,234±201	1,269±225	1,254±214	1,233±238	1,241±216	1,256±229
Brachial-ankle pulse wave velocity (cm/sec)	1,498±179	1,466±203	1,401±122**[a]	1,470±194	1,464±174	1,477±175
Heart rate (beats/min)	65±10	66±9	66±9	66±7	66±6	65±6

[a]$P<0.05$ for groupxtime interaction.
*$P<0.05$ for within-group differences in comparison with baseline.
**$P<0.01$ for within-group differences in comparison with baseline.

Table 4. The effects of 8 wk of supplementation with freeze-dried blueberry powder vs placebo on blood biomarkers at baseline, 4, and 8 wk in postmenopausal women with pre- and stage 1-hypertension

	Blueberry			Control		
Variable	Baseline	4 wk	8 wk	Baseline	4 wk	8 wk
	←——————————mean±standard deviation——————————→					
C-reactive protein (mg/mL)	2.49±0.87	2.44±1.01	2.3±1.08	2.69±0.80	2.64±0.87	2.29±1.20
Superoxide dismutase (U/mL)	0.21±0.06	0.36±0.11**	0.50±0.22**	0.23±0.05	0.40±0.06**	0.49±0.15**
Nitric oxide (μM)	9.11±7.95	13.86±11.45	15.35±11.16*	9.81±7.20	9.20±5.95	10.73±5.63

*$P<0.05$ for within-group difference.
**$P<0.01$ for within-group difference.

production. In fact, it has been demonstrated that flavonoids improve endothelium-dependent vasodilation through endothelial production of nitric oxide and not through superoxide production.[50] As mentioned above, SOD levels were increased at 4 and 8 weeks compared with baseline corresponding values in both the blueberry-treated group and the control group. Unfortunately, we cannot offer a reasonable explanation for this phenomenon other than a time effect. Nonetheless, this is speculative and needs confirmation in future studies. In terms of blood pressure regulation, nitric oxide is produced from the amino acid L-arginine by endothelial nitric oxide synthase in the endothelium, the inner layer of the blood vessels. However, nitric oxide can also be produced by inflammatory cells through inducible nitric oxide synthase and neuronal nitric oxide synthase.[51] Because there were no significant increases in the inflammatory marker measured (ie, C-reactive protein) and SBP and DBP levels were reduced at 8 weeks, it can be suggested that the increase in nitric oxide levels was not related to inducible nitric oxide synthase but rather endothelial nitric oxide synthase.

Our study has several possible limitations. First, the study duration was relatively short and it is unclear whether a longer intervention period would result in greater reductions in blood pressure and arterial stiffness, as well as a reduction in cfPWV. Second, this study only assessed one dose of blueberry powder and it would be beneficial to compare different doses to identify any possible dose–response relationship. Third, because freeze-dried blueberry powder was used as the intervention and not fresh blueberries, it is difficult to assume that freeze-dried blueberries are as effective as the fresh berries and therefore future studies are needed to investigate this. However, among the various methods of processing berries, freeze-drying berries, including blueberries, has been reported to cause the least loss of key nutrients, including total polyphenols, anthocyanins, and antioxidant activity than other forms of processing.[52] In addition, it was recently reported[53] that although processing blueberries (eg, freeze-drying for use in baked products) significantly reduced anthocyanin content, it led to an increase in the content of other polyphenols, including chlorogenic acid and flavanol dimers and trimers and exerted the similar postconsumption vascular effects (eg, improved flow-mediated vasodilation). Fourth, because the sample size and power were calculated based on changes in blood pressure, it is possible that the study was not adequately powered to detect differences in other parameters of interest. Fifth, although an initial 3-day food record was obtained from participants at the beginning of the study, this was not done at the end of the study and should be considered a limitation. In addition, physical activity was not assessed throughout the duration of the study. However, study participants agreed not to change their diets or physical activity patterns for the duration of the study. Another limitation of the study is that the blueberry and placebo powders were not analyzed for nutrient composition in a double-blind fashion but rather the study was single-blind. Finally, the population used for the study was a specific population of postmenopausal women with pre- and stage 1-hypertension and, therefore, the results of the present study are not generalizable to other populations.

CONCLUSIONS

Blueberry consumption may help in reducing both SBP and DBP and improving arterial stiffness in postmenopausal women with pre- and stage 1-hypertension, in part, through increasing the production of nitric oxide and its vasodilatory effect. This suggests that regular consumption of blueberries over the long term could potentially delay the progression of hypertension and reduce cardiovascular risk in postmenopausal women. Nonetheless, freeze-dried blueberry powder was used as the intervention in the present study. We speculate that the consumption of fresh blueberries would produce similar effects as those observed in this study, although this needs confirmation. In addition, this study was 8 weeks in duration and it remains unknown whether interventions longer than 8 weeks, less frequent blueberry consumption, or lower portions would produce the same results.

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