The Journal of Nutrition
Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Blueberries Decrease Cardiovascular Risk Factors in Obese Men and Women with Metabolic Syndrome[1–3]

Arpita Basu,[4]* Mei Du,[6] Misti J. Leyva,[5] Karah Sanchez,[4] Nancy M. Betts,[4] Mingyuan Wu,[6] Christopher E. Aston,[5] and Timothy J. Lyons[5,6]

[4]Nutritional Sciences, Oklahoma State University, Stillwater, OK 74078; [5]General Clinical Research Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73117; and [6]Harold Hamm Oklahoma Diabetes Center and Section of Endocrinology and Diabetes, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Abstract

Among all fruits, berries have shown substantial cardio-protective benefits due to their high polyphenol content. However, investigation of their efficacy in improving features of metabolic syndrome and related cardiovascular risk factors in obesity is limited. We examined the effects of blueberry supplementation on features of metabolic syndrome, lipid peroxidation, and inflammation in obese men and women. Forty-eight participants with metabolic syndrome [4 males and 44 females; BMI: 37.8 ± 2.3 kg/m^2; age: 50.0 ± 3.0 y (mean ± SE)] consumed freeze-dried blueberry beverage (50 g freeze-dried blueberries, ~350 g fresh blueberries) or equivalent amounts of fluids (controls, 960 mL water) daily for 8 wk in a randomized controlled trial. Anthropometric and blood pressure measurements, assessment of dietary intakes, and fasting blood draws were conducted at screening and at wk 4 and 8 of the study. The decreases in systolic and diastolic blood pressures were greater in the blueberry-supplemented group (−6 and −4%, respectively) than in controls (−1.5 and −1.2%) ($P < 0.05$), whereas the serum glucose concentration and lipid profiles were not affected. The decreases in plasma oxidized LDL and serum malondialdehyde and hydroxynonenal concentrations were greater in the blueberry group (−28 and −17%, respectively) than in the control group (−9 and −9%) ($P < 0.01$). Our study shows blueberries may improve selected features of metabolic syndrome and related cardiovascular risk factors at dietary achievable doses. J. Nutr. 140: 1582–1587, 2010.

Introduction

Nutritional epidemiology provides some evidence regarding the cardio-protective effects of foods high in polyphenols, such as berries, tea, soy, and cocoa products (1–4). Blueberries are particularly high in polyphenolic flavonoids in addition to containing significant amounts of micronutrients and fiber (5–7). American blueberries include the lowbush or wild blueberry (*Vaccinium angustifolium* Aiton) and the highbush or cultivated blueberry (*Vaccinium corymbosum* L.), both of which have superior ranking among fruits and vegetables for their antioxidant capacity, mainly due to their high anthocyanin content (93–235 mg/100 g berries) (6,7). In a comprehensive analysis of the antioxidant potential of commonly consumed polyphenol-rich beverages in the United States, blueberry juice was ranked among the top 4 contributors of dietary antioxidants after pomegranate juice, red wine, and concord grape juice (8). Berries have been commercialized as fresh or frozen whole fruits, freeze-dried berries, puree, juice, or wine (9–11). Although most processing methods cause a significant decrease in the anthocyanin content (10–12), freeze-drying has been reported to cause the least reduction in total polyphenol content of berries (11).

Several mechanistic studies provide evidence of antioxidative (13,14), antiinflammatory (14,15), antihypertensive (16,17), antidiabetic (18,19), antiobesity (20), and antihyperlipidemic (20,21) effects of blueberries, providing possible rationale for cardio-protective mechanisms. Based on these mechanistic studies, consuming blueberries might favorably alter individual components of metabolic syndrome, a rapidly escalating public health problem in the US (22). Metabolic syndrome has been characterized by abdominal adiposity, dyslipidemia (high triglycerides, low HDL cholesterol), hypertension, impaired glucose tolerance, elevated oxidative stress, inflammation, and increased risks for type 2 diabetes and atherosclerotic cardiovascular disease (CVD)[7] (22–24). Berry supplementation using

[1] Supported by the US Highbush Blueberry Council and by the University of Oklahoma Health Sciences Center General Clinical Research Center grant M01-RR14467, National Center for Research Resources, NIH.
[2] Author disclosures: A. Basu, M. Du, M. J. Leyva, K. Sanchez, N. M. Betts, M. Wu, C. E. Aston, and T. J. Lyons, no conflicts of interest.
[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: arpita.basu@okstate.edu.

[7] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; HbA_{1C}, hemoglobin A_{1C}; HNE, hydroxynonenal; IL-6, interleukin-6; MDA, malondialdehyde; MPO, myeloperoxidase; ox-LDL, oxidized LDL; RD, registered dietitian; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.

© 2010 American Society for Nutrition.

Manuscript received March 26, 2010. Initial review completed April 8, 2010. Revision accepted June 29, 2010.
First published online July 21, 2010; doi:10.3945/jn.110.124701.

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022

chokeberries, cranberries, or a combination of berries has been shown to improve features of metabolic syndrome such as dyslipidemia, hypertension, or impaired fasting glucose in participants with existing cardiovascular risk factors (25–27). However, there is a paucity of clinical data on the cardiovascular health benefits of blueberries per se.

Thus, this study was designed to test the hypothesis that blueberry supplementation, in the form of a freeze-dried beverage, will improve features of metabolic syndrome and decrease biomarkers of lipid and lipoprotein oxidation and inflammation in study participants compared with a control group consuming equivalent amounts of fluids, in a randomized controlled trial.

Materials and Methods

Participants. Sixty-six obese men and women with metabolic syndrome (mean age, 50.0 ± 3.0 y) were enrolled in this randomized controlled study. They were screened for the qualifying criteria: 3 of 5 features of metabolic syndrome as defined by the National Cholesterol Education Program, Adult Treatment Panel (22). Recruitment and interventions were conducted at the General Clinical Research Center at the University of Oklahoma Health Sciences Center, and at the Nutritional Sciences Clinical Assessment Unit at Oklahoma State University. Participants were recruited through flyers and campus e-mail advertisements at both sites. Each potential recruit received an initial telephone screening prior to the screening visit. They were excluded if they were younger than 21 y of age; taking medications for any chronic disease, including hypoglycemic, hypolipidemic, antiinflammatory, or steroidal medications; or had liver, renal, or thyroid disorders or anemia. Potential recruits also were excluded if they were consuming antioxidants or fish oil supplements on a regular basis, were current smokers, consuming alcohol on a regular basis (except social drinking ~1–2 drinks/wk), or were pregnant or lactating. This intervention study was approved by the Institutional Review Board at the University of Oklahoma Health Sciences Center and at Oklahoma State University. All participants provided written informed consent.

Intervention. Freeze-dried blueberries provided by the US Highbush Blueberry Council (Folsom, CA) were a blend of 2 blueberry cultivars, Tifblue and Rubel, in a 1:7 ratio of freeze-dried:fresh berries, with no additives (**Table 1**). Participants received a daily dose of 50 g freeze-dried blueberries that were reconstituted in 480 mL water and vanilla extract or Splenda was added based on the preference of the participants. The participants were asked to consume 1 cup (240 mL) in the morning and the second in the evening at least 6–8 h apart. Because the beverage made with reconstituted freeze-dried blueberries was thick and sticky in consistency, participants were also asked to rinse out each cup with an additional cup of water, thus leading to the consumption of ~960 mL fluids/d in the blueberry group. The control group was asked to consume 960 mL water to match the fluid intake of the blueberry group and was provided with containers to measure out the prescribed amount of water.

Study design. This was a single-blinded controlled study in which participants were randomized to the blueberry or control group for 8 wk. Those in the blueberry group made 3 visits/wk to their study site (Monday, Wednesday, and Friday) to ensure compliance by consuming the first cup in the morning under observation by the research staff. Participants were instructed to keep the drink under refrigeration, to avoid exposing it to direct heat or light, and to avoid consuming it with any other snack or with lunch or dinner, because other foods might interfere with the absorption of the blueberry polyphenols. Participants were asked to return any unconsumed blueberry drink. Controls were provided with containers to measure 4 cups water to be consumed on a daily basis. All participants returned for follow-up visits at 2, 4, 6, and 8 wk of the study. The research staff were instructed not to discuss diet or weight issues with participants consuming blueberries to avoid potential confounding factors that might arise as a result of frequent visits of the blueberry group (compared with the biweekly visits of the control group). Participants received monetary compensation during their follow-up visits. They were asked to refrain from consuming any other source of berries or related products derived from berries such as juices, jams, and desserts. They were also asked to refrain from consuming green tea, cocoa, and soy products while participating in the study, because these were the most commonly consumed flavonoid-rich foods by the enrolled participants as identified by a screening FFQ specific for flavonoids. Participants maintained their usual diet, physical activity, and lifestyle while on the study and were also asked to record their food intakes. Body weight, height, waist circumference, and systolic and diastolic blood pressures were measured by trained personnel and blood draws were performed by registered nurses at screening and wk 4 and 8 of the study. All laboratory staff were unaware of treatment groups.

TABLE 1 Composition of freeze-dried blueberries[1]

Nutrients/antioxidant activity	*unit/50 g*
Energy,[2] *kcal*	174.0
Protein, *g*	1.7
Carbohydrates, *g*	42.3
Total sugars, *g*	30.0
Dietary fiber, *g*	9.3
Vitamin C, *mg*	86.0
Calcium, *mg*	15.0
Iron, *mg*	0.5
Potassium, *mg*	204.0
Sodium, *mg*	8.0
Phenolic components, *mg*	1624
Anthocyanins, *mg*	742
Oxygen radical absorbance capacity, *mmol TE*	17.8

[1] Source: U.S. Highbush Blueberry Council (Folsom, CA). Fresh weight replacement: 1 to 7 (freeze-dried to fresh).
[2] 1 kcal = 4.184 kJ.

Anthropometrics and blood pressure. Participants removed shoes and items in dress pockets and were weighed on a flat, uncarpeted surface with the SECA 644 Multifunctional Hand Rail Scale (SECA) and recorded to the nearest 0.1 kg. Height was measured without shoes using the Accustat Genentech Stadiometer and recorded to the nearest 0.1 cm. Systolic and diastolic blood pressures were measured in millimeters Hg using Spot Vital Signs Device (Welch Allyn). Participants were asked to lie down and relax for ~8–10 min, following which 3 blood pressure measurements were recorded at intervals of 5 min. Waist circumference was measured at the superior iliac crest using the Gulick II Tape Measure (Vital Signs). All measurements were conducted in fasting participants at screen and at wk 4 and 8 of the study.

Dietary analyses. Study participants were required to record daily food intake for 3 d at 3 time points throughout the study: screen and wk 4 and 8. Each time, the registered dietitian (RD) carefully instructed the participants on accurate food record completion using visual tools such as food models and common household measuring utensils. Instructions were also given on careful recording of details needed for recipes. Three day averages of micro- and macronutrient intakes were analyzed using Nutritionist Pro (version 3.2, 2007, Axxya Systems). All data entry was performed by RD trained and certified in using the software. All dietary data entry was verified by a second RD as a measure of quality control. If a participant ate a food that was not in the database, a food with very similar nutrient composition was chosen. Nutrient information was also obtained through food labels or recipes from participants, online sources, or at grocery stores.

Clinical analyses. Blood samples were collected immediately after each draw and serum was transported to the University of Oklahoma Medical Center Laboratory for analyses of fasting glucose, insulin, lipid profile (total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol),

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022

and other blood variables, including safety variables (hemoglobin, platelets, white blood cells, liver enzymes, creatinine, blood urea nitrogen, electrolytes, albumin, total protein, and thyroid-stimulating hormone), using automated diagnostic equipment (Abbott Architect Instruments) following standard protocols at the University of Oklahoma Medical Center. Serum hemoglobin A_{1C} (HbA_{1C}) was analyzed using a DCA 2000+ (Bayer). Insulin resistance was evaluated by homeostasis model assessment calculated as [fasting insulin (mU/L) × fasting glucose (mmol/L)]/22.5.

For assays to determine serum malondialdehyde (MDA) and hydroxynonenal (HNE) and plasma oxidized-LDL, myeloperoxidase (MPO), adiponectin, interleukin-6 (IL-6), high sensitivity C-reactive protein (CRP), soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule levels, serum and EDTA-plasma samples were collected, separated by centrifugation at 1800 × *g* for 10 min at 4°C, and stored at −80°C for subsequent analyses. NMR-determined lipoprotein subclass profile was performed in first-thaw plasma specimens using a 400-MHz proton NMR analyzer at LipoScience as described previously (28).

Biomarkers of oxidative stress. Plasma concentrations of MPO and ox-LDL were measured in duplicate with commercially available ELISA kits: Mercodia MPO ELISA and Mercodia Oxidized LDL Competitive ELISA (Mercodia) according to the manufacturer's instructions. Lipid peroxidation was measured in serum as combined MDA and HNE using a colorimetric assay according to the manufacturer's protocol (LPO-586, Oxis Health Products). The mean intra-assay CV for MPO, ox-LDL, and MDA and HNE were 4.8, 5.2, and 3.6%, respectively.

Biomarkers of inflammation. Plasma concentrations of CRP, adiponectin, IL-6, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) were determined using commercially available ELISA kits: Human CRP Quantikine ELISA, Human Total Adiponectin Quantikine ELISA, Human IL-6 Quantikine ELISA, Human sICAM-1 Quantikine ELISA, and Human sVCAM-1 Quantikine ELISA (R&D Systems) according to the manufacturer's protocols. The minimum detectable levels were 15.6, 62.5, 9.4, 15.6, and 15.6 ng/L for each assay, respectively. The inter-assay CV were 6.2, 3.6, 3.1, 3.5, and 7.6%, respectively.

Statistical analyses. For all measures, descriptive statistics were calculated and graphs drawn to look for outliers; no data points were determined to be outliers. Differences between blueberry and control groups at baseline were assessed using Student's *t* tests.

Changes in measurements over the 8-wk study period were assessed by calculating the difference between the pre- and postintervention measurements in each group. For each variable, significance was assessed by comparing the change over the 8-wk study period between blueberry and control groups using Student's *t* tests. Target sample size was calculated to include 25 participants/group to detect significant differences in certain dependent variables with 80% power. Multiple hypothesis testing was not accounted for and all statistical tests were 2-tailed with significance level set at 0.05. SPSS for Windows (version 15.0, SPSS, 2006) was used for the statistical calculations.

Results

In this randomized controlled trial, 66 men and women with metabolic syndrome were enrolled upon qualification. Among 34 enrolled participants in the blueberry group, 9 withdrew on account of side effects, including nausea, vomiting, constipation, and diarrhea following blueberry intervention during the first week of the study, leading to a 27% drop-out rate in the blueberry group. Among 32 participants enrolled in the control group, 1 was excluded on account of smoking, 6 withdrew due to personal reasons (not disclosed), and 2 withdrew due to time constraints, leading to a 28% drop-out rate in the control group. The temporary gastrointestinal discomfort was also reported by participants who completed the 8-wk blueberry treatment, which did not persist beyond the first week of the intervention. Thus, 48 participants actually completed the 8-wk study of whom 25 were in the blueberry group and 23 were controls. Baseline characteristics, including age, BMI, and safety variables, did not differ between the blueberry and control groups (**Table 2**). For the 25 participants in the blueberry group, compliance was high, with 96.5% of the blueberry drink consumed and 100% compliance visits/wk. The controls reported 100% compliance in terms of water intake and biweekly visits.

The decreases in systolic and diastolic blood pressures were greater in the blueberry-supplemented group (−6 and −4%, respectively) than in controls (−1.5 and −1.2%) ($P = 0.003$ and $P = 0.04$). Changes in body weight, waist circumference, HbA_{1C}, insulin resistance, and serum glucose concentration and lipid profile did not differ between the groups. Blood pressure outcomes remained significantly different when data were analyzed without participants on stable antihypertensive medications in the blueberry and control groups. Those in the blueberry group had a significantly lower baseline serum LDL-cholesterol concentration than controls and no significant changes were noted between groups (**Table 3**). NMR-based lipid particle concentrations were not significantly affected by blueberry intervention (**Supplemental Table 1**).

The decreases in oxidized LDL (ox-LDL) and combined MDA and HNE concentrations were greater in the blueberry-supplemented group (−28 and −17%, respectively) than in controls (−9 and −9%) ($P = 0.009$ and $P = 0.005$). Changes in plasma CRP, IL-6, MPO, adhesion molecules (sICAM-1, sVCAM-1), and adiponectin concentrations did not differ between the groups (**Table 4**).

Changes in dietary intakes did not differ between the blueberry and control groups (**Supplemental Table 2**).

Discussion

An emerging body of evidence indicates the role of blueberries as cardio-protective agents, although few human studies have been reported to support this claim. Our test dose of 50 g freeze-dried blueberries, equivalent to ~350 g or 2.3 cups fresh blueberries,

TABLE 2 Baseline characteristics and serum biomarkers of participants with metabolic syndrome completing 8-wk supplementation with freeze-dried blueberries or control treatment[1]

	Blueberry	Control
n	25	23
Age, *y*	51.5 ± 3.0	48.0 ± 3.3
BMI, kg/m^2	38.1 ± 1.5	37.5 ± 3.0
M/F *n/n*	2/23	2/21
Aspartate aminotransferase, *U/L*	25.3 ± 1.3	25.7 ± 3.0
Alanine aminotransferase, *U/L*	33.1 ± 2.6	34.0 ± 4.7
Blood urea nitrogen, *mmol/L*	4.6 ± 0.2	4.2 ± 0.4
Creatinine, $\mu mol/L$	70.7 ± 8.8	80.0 ± 18.0
Albumin, *g/L*	41.0 ± 1.0	35.0 ± 2.0
Hemoglobin, *g/L*	138.0 ± 2.0	143.0 ± 2.0
White blood cells, $n \times 10^{-9}$	7.0 ± 0.4	6.8 ± 0.5
Antihypertensive medication users, %	22.0	21.0
Multivitamin users, %	12.0	10.0

[1] Data are means ± SE.

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022

TABLE 3 Change in anthropometrics, blood pressure, and serum glucose and lipid concentrations in participants with metabolic syndrome after 8-wk supplementation with freeze-dried blueberries or control treatment[1]

	Δ (0–8 wk)	
Variable	Blueberry	Control
n	25	23
Body weight, *kg*	−0.4 ± 0.30	0.5 ± 0.40
Waist circumference, *cm*	−0.4 ± 0.30	−0.5 ± 1.10
Systolic blood pressure, *mm Hg*	−7.8 ± 2.50*	−2.0 ± 2.80
Diastolic blood pressure, *mm Hg*	−2.5 ± 1.10*	0.7 ± 2.00
Glucose, *mmol/L*	0.1 ± 0.20	−0.1 ± 0.20
HbA_{1C}, *%*	0.1 ± 0.10	0.2 ± 0.10
Homeostasis model assessment of insulin resistance	0.7 ± 0.50	−0.2 ± 0.20
Triglycerides, *mmol/L*	0.0 ± 0.10	0.1 ± 0.20
Total cholesterol, *mmol/L*	0.2 ± 0.20	0.2 ± 0.30
HDL cholesterol, *mmol/L*	0.0 ± 0.02	0.0 ± 0.02
LDL cholesterol, *mmol/L*	0.1 ± 0.10	0.0 ± 0.20

[1] Data are means ± SE. *Different from control, $P < 0.05$.

was overall well tolerated and conforms to the daily fruit and vegetable recommendations of at least 5 servings for US adults (29). We also selected this dose to investigate the therapeutic effects of blueberries in a standard freeze-dried form on features of metabolic syndrome in men and women with low fruit intake (30).

Blueberries have been reported to exert favorable effects on features of metabolic syndrome and type 2 diabetes in animal models of obesity (19,20). Human intervention studies investigating the effects of berries on metabolic syndrome are limited. Clinical trials involving chokeberry juice supplementation showed significant improvements in fasting glucose, lipids, and HbA1c in type 2 diabetics, and, cranberry, or bilberry and black currant extract supplementations, were shown to improve dyslipidemia in type 2 diabetics, or hyperlipidemic patients, respectively (25,26,31). However, in our study, blueberry supplementation for 8 wk did not affect fasting serum glucose, insulin, lipid profiles, and body weight or waist circumference. These null effects may be due to the fact that participants with metabolic syndrome in our blueberry intervention group had normal baseline levels of glucose, insulin, and lipids (except low HDL cholesterol). Also, additional dietary adjustments and longer study duration may be needed to affect adiposity in these men and women. On the other hand, blueberry supplementation significantly decreased systolic and diastolic blood pressures in our prehypertensive participants, which conforms to the findings of previous studies on the blood pressure-lowering effects of cranberry intervention in healthy humans or mixed berry supplementation in those with CVD risk factors (27,32). Mechanistic studies explain the role of blueberries or anthocyanins in ameliorating hypertension by significantly increasing endothelial nitric oxide synthase levels in bovine and human endothelial cells (33,34), decreasing vasoconstriction via nitric oxide-mediated pathway, or decreasing renal oxidative stress and, thereby, systolic blood pressure in rodent models of human essential hypertension (16,17). Thus, our study is the first to our knowledge to report that blueberries have antihypertensive effects in people with metabolic syndrome. Because hypertension is an independent and significant CVD risk factor (35) and can be mitigated by dietary practices (36), blueberry supplementation may be a potential therapeutic dietary measure and needs further confirmation in larger controlled studies.

TABLE 4 Change in plasma biomarkers of oxidative stress and inflammation in participants with metabolic syndrome after 8-wk supplementation with freeze-dried blueberries or control treatment[1]

	Δ (0–8 wk)	
Variables	Blueberry	Control
n	25	23
CRP, *mg/L*	0.2 ± 0.50	0.4 ± 1.50
sICAM-1, *ng/L*	−0.1 ± 0.02	0.0 ± 0.03
sVCAM-1, *ng/L*	−0.1 ± 0.04	0.0 ± 0.05
IL-6, *pg/L*	0.0 ± 0.01	0.0 ± 0.01
Adiponectin, *μg/L*	0.0 ± 0.01	0.0 ± 0.01
ox-LDL, *U/L*	−30.0 ± 4.00*	−9.6 ± 9.50
MPO, *μg/L*	2.5 ± 5.00	−2.4 ± 8.50
MDA and HNE, *μmol/L*	−0.2 ± 0.03*	−0.1 ± 0.01

[1] Data are means ± SE. *Different from control, $P < 0.01$.

Biomarkers of lipid and lipoprotein oxidation such as MDA and ox-LDL levels are elevated in population with abdominal adiposity and metabolic syndrome and have also been associated with coronary artery disease (24,37,38). In our 8-wk study, decreases in plasma ox-LDL and serum MDA and HNE levels were significantly greater in the blueberry-supplemented group than in controls. Our findings are similar to the previous intervention studies reporting the effects of blueberries, cranberry juice, or freeze-dried strawberries in lowering lipid hydroperoxides in smokers, ox-LDL in healthy volunteers, or MDA and HNE in women with metabolic syndrome, respectively (39–41). The antioxidant effects of blueberries have also been reported by studies using cellular and animal models of oxidative stress (13,14), thus providing mechanistic evidence that needs to be strengthened by larger controlled clinical trials. However, in our study, blueberries did not affect plasma MPO, an independent predictor of CVD and a significant contributor to oxidative stress (42). Thus, further investigation is needed to define the effects of berry polyphenols on MPO in participants with metabolic risk factors.

Biomarkers of inflammation such as CRP, IL-6, and adhesion molecules ICAM-1 and VCAM-1 are significantly elevated in metabolic syndrome and positively associated with CVD (43,44). On the other hand, adiponectin, an antiinflammatory cytokine, is significantly decreased in metabolic syndrome and inversely related to CVD (45). The antiinflammatory effects of berries have been suggested by limited epidemiological observations. The Women's Health Study showed a borderline significant risk reduction of elevated CRP (≥3 mg/L) among women consuming higher amounts of strawberries [≥2 servings/wk (150 g/wk)], whereas blueberry intake had no significant association with risks of CVD, including CRP levels (46). Analyses of NHANES data (1999–2002) also revealed a significant inverse association between serum CRP and anthocyanin intakes among U.S. adults (47). In our study, changes in plasma levels of adhesion molecules, CRP, IL-6, and adiponectin did not differ between the blueberry and control groups. In a recently reported study, Curtis et al. (48) showed similar null effects of elderberry anthocyanin extracts on inflammatory biomarkers such as CRP and IL-6 in healthy postmenopausal women in a 12-wk study. Thus, longer study duration or a higher dose of berry polyphenols may be needed to lower inflammatory

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022

biomarkers. Berry extracts or anthocyanin treatment has been shown to reduce inflammation-related parameters in animal and cellular models (14,15). Thus, further investigation is needed to define the antiinflammatory effects of berries or anthocyanins in cases of obesity and metabolic syndrome.

Certain limitations of our study include a cohort comprised primarily of women, the side effects and drop-outs following blueberry intervention, and the absence of a dose-response treatment. The gastrointestinal side effects were anticipated as a result of additional fiber intake in the form of a concentrated berry powder, especially in our participants with habitual low fiber and fruit intakes (Supplemental Table 2) (30). Though it led to a 27% drop-out rate in the blueberry arm, those who completed the entire 8-wk study also experienced this temporary gastrointestinal discomfort during the first week, which was later alleviated, and participants reported high compliance to the blueberry beverage. For future studies, administration of reconstituted freeze-dried blueberries in 3 or 4 doses throughout the day, or using 2 cups conventional frozen blueberries, as well as dietary adjustments for total fiber intake may have improved tolerability while exerting similar health benefits. Also, we did not detect parent anthocyanins or metabolites in serum samples as a measure of compliance, mainly because blood draws were conducted in a 10- to 12-h fasting state, which allows complete clearance and excretion of anthocyanins (49). Finally, our control group was consuming plain water to match the fluid intake of the intervention group, whereas a fiber- and energy-matched control beverage may lead to better elucidation of the role of polyphenols in the observed health effects of berries.

In conclusion, our study findings suggest a cardio-protective role of dietary achievable doses of blueberries in men and women with metabolic syndrome, which includes a significant decrease in systolic and diastolic blood pressures and plasma ox-LDL and lipid peroxidation. Our clinical data are supported by previously reported mechanistic studies and limited human intervention studies using single or mixed berries or anthocyanin extracts. However, our findings specifically show the cardio-protective effects of blueberries in improving features of metabolic syndrome. These results warrant further investigation and provide some evidence for including blueberries as part of healthy dietary practices.

Acknowledgments

A.B., C.E.A., and T.J.L. designed the intervention study; M.D., M.J.L., K.S., M.W., and A.B. conducted research and laboratory measurements; C.E.A. and N.M.B. analyzed data; and A.B., C.E.A., and T.J.L. wrote the paper. All authors read and approved the final manuscript.

Literature Cited

1. Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr. 2007; 85:895–909.
2. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y, Tsuji I. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA. 2006;296:1255–65.
3. Liu L, Nettleton JA, Bertoni AG, Bluemke DA, Lima JA, Szklo M. Dietary pattern, the metabolic syndrome, and left ventricular mass and systolic function: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2009;90:362–8.
4. Janszky I, Mukamal KJ, Ljung R, Ahnve S, Ahlbom A, Hallqvist J. Chocolate consumption and mortality following a first acute myocardial infarction: the Stockholm Heart Epidemiology Program. J Intern Med. 2009;266:248–57.
5. U.S. Department of Agriculture, Agricultural Research Service. 2009. USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl.
6. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, et al. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J Agric Food Chem. 1998;46:2686–93.
7. Neto CC. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. Mol Nutr Food Res. 2007;51:652–64.
8. Seeram NP, Aviram M, Zhang Y, Henning SM, Feng L, Dreher M, Heber D. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J Agric Food Chem. 2008;56:1415–22.
9. Brownmiller C, Howard LR, Prior RL. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. J Food Sci. 2008;73:H72–9.
10. Klopotek Y, Otto K, Böhm V. Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. J Agric Food Chem. 2005;53:5640–6.
11. Wojdyło A, Figiel A, Oszmiański J. Effect of drying methods with the application of vacuum microwaves on the bioactive compounds, color, and antioxidant activity of strawberry fruits. J Agric Food Chem. 2009;57:1337–43.
12. Srivastava A, Akoh CC, Yi W, Fischer J, Krewer G. Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. J Agric Food Chem. 2007;55: 2705–13.
13. Sellappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J Agric Food Chem. 2002;50:2432–8.
14. Ahmet I, Spangler E, Shukitt-Hale B, Juhaszova M, Sollott SJ, Joseph JA, Ingram DK, Talan M. Blueberry-enriched diet protects rat heart from ischemic damage. PLoS ONE. 2009;4:e5954.
15. Lau FC, Bielinski DF, Joseph JA. Inhibitory effects of blueberry extract on the production of inflammatory mediators in lipopolysaccharide-activated BV2 microglia. J Neurosci Res. 2007;85:1010–7.
16. Shaughnessy KS, Boswall IA, Scanlan AP, Gottschall-Pass KT, Sweeney MI. Diets containing blueberry extract lower blood pressure in spontaneously hypertensive stroke-prone rats. Nutr Res. 2009;29:130–8.
17. Kalea AZ, Clark K, Schuschke DA, Klimis-Zacas DJ. Vascular reactivity is affected by dietary consumption of wild blueberries in the Sprague-Dawley rat. J Med Food. 2009;12:21–8.
18. Martineau LC, Couture A, Spoor D, Benhaddou-Andaloussi A, Harris C, Meddah B, Leduc C, Burt A, Vuong T, et al. Anti-diabetic properties of the Canadian lowbush blueberry *Vaccinium angustifolium* Ait. Phytomedicine. 2006;13:612–23.
19. DeFuria J, Bennett G, Strissel KJ, Perfield JW II, Milbury PE, Greenberg AS, Obin MS. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. J Nutr. 2009;139:1510–6.
20. Prior RL, Wu X, Gu L, Hager T, Hager A, Wilkes S, Howard L. Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet. Mol Nutr Food Res. 2009;53: 1406–18.
21. Kahlon TS, Smith GE. In vitro binding of bile acids by blueberries (*Vaccinium* spp.), plums (*Prunus* spp.), prunes (*Prunus* spp.), strawberries (*Fragaria X ananassa*), cherries (*Malpighia punicifolia*), cranberries (*Vaccinium macrocarpon*) and apples (*Malus sylvestris*). Food Chem. 2007;100:1182–7.
22. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009; 120:1640–5.
23. Haffner SM. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. Am J Cardiol. 2006;97:S3–11.
24. Holvoet P, Lee DH, Steffes M, Gross M, Jacobs DR Jr. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA. 2008;299:2287–93.

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022

25. Simeonov SB, Botushanov NP, Karahanian EB, Pavlova MB, Husianitis HK, Troev DM. Effects of Aronia melanocarpa juice as part of the dietary regimen in patients with diabetes mellitus. Folia Med (Plovdiv). 2002;44:20–3.
26. Lee IT, Chan YC, Lin CW, Lee WJ, Sheu WH. Effect of cranberry extracts on lipid profiles in subjects with Type 2 diabetes. Diabet Med. 2008;25:1473–7.
27. Erlund I, Koli R, Alfthan G, Marniemi J, Puukka P, Mustonen P, Mattila P, Jula A. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. Am J Clin Nutr. 2008;87:323–31.
28. Lyons TJ, Jenkins AJ, Zheng D, Klein RL, Otvos JD, Yu Y, Lackland DT, McGee D, McHenry MB, et al. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. Diabet Med. 2006;23: 955–66.
29. USDA MyPyramid.gov: Inside the Pyramid; OMB Number 0584- 0535; [cited 2010 Feb 27]. Available from: http://www.mypyramid.gov/pyramid/index.html.
30. National Center for Chronic Disease Prevention and Health Promotion. Behavioral Risk Factor Surveillance System [cited 2010 Mar 25]. Available from: http://apps.nccd.cdc.gov/BRFSS/display.asp?yr=2007&cat=FV&qkey=4415&state=OK.
31. Qin Y, Xia M, Ma J, Hao Y, Liu J, Mou H, Cao L, Ling W. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. Am J Clin Nutr. 2009;90:485–92.
32. Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. Br J Nutr. 2008;99:352–9.
33. Xu JW, Ikeda K, Yamori Y. Upregulation of endothelial nitric oxide synthase by cyanidin-3-glucoside, a typical anthocyanin pigment. Hypertension. 2004;44:217–22.
34. Lazzè MC, Pizzala R, Perucca P, Cazzalini O, Savio M, Forti L, Vannini V, Bianchi L. Anthocyanidins decrease endothelin-1 production and increase endothelial nitric oxide synthase in human endothelial cells. Mol Nutr Food Res. 2006;50:44–51.
35. Wright JT Jr, Dunn JK, Cutler JA, Davis BR, Cushman WC, Ford CE, Haywood LJ, Leenen FH, Margolis KL, et al. Outcomes in hypertensive black and nonblack patients treated with chlorthalidone, amlodipine, and lisinopril. JAMA. 2005;293:1595–608.
36. Levitan EB, Wolk A, Mittleman MA. Relation of consistency with the dietary approaches to stop hypertension diet and incidence of heart failure in men aged 45 to 79 years. Am J Cardiol. 2009;104:1416–20.
37. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation. 1998;98:1487–94.
38. Armutcu F, Ataymen M, Atmaca H, Gurel A. Oxidative stress markers, C-reactive protein and heat shock protein 70 levels in subjects with metabolic syndrome. Clin Chem Lab Med. 2008;46:785–90.
39. Ruel G, Pomerleau S, Couture P, Lamarche B, Couillard C. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. Metabolism. 2005;54:856–61.
40. Basu A, Wilkinson M, Penugonda K, Simmons B, Betts NM, Lyons TJ. Freeze-dried strawberry powder improves lipid profile and lipid peroxidation in women with metabolic syndrome: baseline and post intervention effects. Nutr J. 2009;8:43.
41. McAnulty SR, McAnulty LS, Morrow JD, Khardouni D, Shooter L, Monk J, Gross S, Brown V. Effect of daily fruit ingestion on angiotensin converting enzyme activity, blood pressure, and oxidative stress in chronic smokers. Free Radic Res. 2005;39:1241–8.
42. Nicholls SJ, Hazen SL. Myeloperoxidase and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2005;25:1102–11.
43. Conen D, Rexrode KM, Creager MA, Ridker PM, Pradhan AD. Metabolic syndrome, inflammation, and risk of symptomatic peripheral artery disease in women: a prospective study. Circulation. 2009; 120:1041–7.
44. Jacobs M, van Greevenbroek MM, van der Kallen CJ, Ferreira I, Blaak EE, Feskens EJ, Jansen EH, Schalkwijk CG, Stehouwer CD. Low-grade inflammation can partly explain the association between the metabolic syndrome and either coronary artery disease or severity of peripheral arterial disease: the CODAM study. Eur J Clin Invest. 2009;39:437–44.
45. Matsushita K, Tamakoshi K, Yatsuya H, Wada K, Otsuka R, Takefuji S, Hotta Y, Kondo T, Murohara T, et al. Further inflammatory information on metabolic syndrome by adiponectin evaluation. Int J Cardiol. 2008;124:339–44.
46. Sesso HD, Gaziano JM, Jenkins DJ, Buring JE. Strawberry intake, lipids, C-reactive protein, and the risk of cardiovascular disease in women. J Am Coll Nutr. 2007;26:303–10.
47. Chun OK, Chung SJ, Claycombe KJ, Song WO. Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. J Nutr. 2008;138:753–60.
48. Curtis PJ, Kroon PA, Hollands WJ, Walls R, Jenkins G, Kay CD, Cassidy A. Cardiovascular disease risk biomarkers and liver and kidney function are not altered in postmenopausal women after ingesting an elderberry extract rich in anthocyanins for 12 weeks. J Nutr. 2009;139: 2266–71.
49. McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res. 2007;51:702–13.

Downloaded from https://academic.oup.com/jn/article/140/9/1582/4600242 by guest on 20 August 2022