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Consumption of chokeberry (*Aronia mitschurinii*) products modestly lowered blood pressure and reduced low-grade inflammation in patients with mildly elevated blood pressure

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ABSTRACT

Previous studies suggest that consumption of chokeberries may improve cardiovascular disease risk factor profiles. We hypothesized that chokeberries (*Aronia mitschurinii*) have beneficial effects on blood pressure, low-grade inflammation, serum lipids, serum glucose, and platelet aggregation in patients with untreated mild hypertension. A total of 38 participants were enrolled into a 16-week single blinded crossover trial. The participants were randomized to use cold-pressed 100% chokeberry juice (300 mL/d) and oven-dried chokeberry powder (3 g/d), or matched placebo products in random order for 8 weeks each with no washout period. The daily portion of chokeberry products was prepared from approximately 336 g of fresh chokeberries. Urinary excretion of various polyphenols and their metabolites increased during the chokeberry period, indicating good compliance. Chokeberries decreased daytime blood pressure and low-grade inflammation. The daytime ambulatory diastolic blood pressure decreased (–1.64 mm Hg, $P = .02$), and the true awake ambulatory systolic (–2.71 mm Hg, $P = .077$) and diastolic (–1.62 mm Hg, $P = .057$) blood pressure tended to decrease. The concentrations of interleukin (IL) 10 and tumor necrosis factor α decreased (–1.9 pg/mL [$P = .008$] and –0.67 pg/mL [$P = .007$], respectively) and tended to decrease for IL-4 and IL-5 (–4.5 pg/mL [$P = .084$] and –0.06 pg/mL [$P = .059$], respectively). No changes in serum lipids, lipoproteins, glucose, and in vitro platelet aggregation were

Abbreviations: Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; γGT, γ-glutamyl transferase; GM-CSF, granulocyte macrophage colony-stimulating factor; HDL, high-density lipoprotein; HPLC, high-performance liquid chromatography; hsCRP, high-sensitive C-reactive protein; IL, interleukin; NO, nitric oxide; SBP, systolic blood pressure; TNF-α, tumor necrosis factor α.

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noted with the chokeberry intervention. These findings suggest that inclusion of chokeberry products in the diet of participants with mildly elevated blood pressure has minor beneficial effects on cardiovascular health.

1. Introduction

Different types of polyphenol-rich foods (cocoa, berries, tea) have been shown to reduce blood pressure (BP) and risk factors levels of cardiovascular disease (CVD), mainly in patients at risk. The beneficial effects of berries may be mediated by the antioxidant and anti-inflammatory properties of polyphenols. Chokeberries (*Aronia melanocarpa* and *A mitschurinii*) are one of the richest sources of polyphenols such as anthocyanins, flavonols, proanthocyanidins, and phenolic acids [1,2]. However, because of an astringent taste, they are not as widely consumed as many other berries.

In animal studies, chokeberries and their extracts have reduced total cholesterol [3,4], reduced BP [5,6], decreased weight gain, and modulated insulin, adipogenic, and inflammatory pathways [7]. In vitro studies suggest that chokeberry extracts induce relaxation of coronary arteries [8]. Intake of chokeberry products has been shown to improve lipid profile and flow-mediated dilatation of mildly hypercholesterolemic men [9,10], to decrease BP, to improve lipid profile and oxidative stress in patients with metabolic syndrome [11], and, furthermore, to improve cardiovascular risk profile in statin-treated patients [12].

Because including chokeberries in the diet may benefit cardiovascular health, we hypothesized that consumption of chokeberry (*A mitschurinii*) exerts beneficial effects on the cardiovascular risk profile in patients with prehypertension or pharmacologically untreated mild hypertension. To test our hypothesis, we conducted a randomized, crossover intervention study where untreated participants with mildly elevated BP were randomized to use chokeberry products (juice mixed with powder) or their matched placebo products in random order for 8 weeks. Our primary aim was to investigate the effect of chokeberries on BP. Additional outcomes were serum lipids, lipoproteins, glucose, cytokines, and platelet aggregation.

2. Methods and materials

2.1. Study population

Study participants were recruited through health care centers and by newspaper advertisements. The volunteers were asked to fill out a preliminary information form. Based on this information, potentially eligible participants were invited to a screening visit, which included measurements of height, weight, BP, electrocardiogram, fasting serum lipids, glucose, γ-glutamyl transferase (γGT) enzyme, and blood count. The inclusion criteria were a systolic BP (SBP) of 130 to 159 mm Hg or a diastolic BP (DBP) of 85 to 99 mm Hg, an age of 40 to 70 years, no regular use of medications (except menopausal hormone replacement therapy) or dietary supplements, no diagnosed endocrine disease, no chronic intestinal inflammatory or malabsorption disorders, a nonsmoker, no severe obesity (body mass index <35 kg/m^2), not pregnant or lactating, and not a vegetarian.

The sample size was calculated with the assumption that a change of 3 mm Hg [13] in the primary outcome variable (BP) can be detected with intraindividual variation of 4 SDs [14], at a significance level of .05 and a power of 0.80 (n = 30). The calculations were performed with the following program: http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html (Schoenfeld DA). A total of 66 participants were screened and all eligible participants (24 women and 14 men with a mean age of 55.8 years [range, 41-69 years]) approved to participate in the study. A signed informed consent form was obtained from each participant at the screening visit. The Ethics Committee of the Hospital District of Southwestern Finland approved the study protocol.

2.2. Study design

The study was a randomized, single-blinded crossover intervention study. It consisted of two 8-week periods with no washout period between. The participants were randomly assigned (by sex and age) to use either chokeberry (*A mitschurinii*) or matched placebo juice (300 mL/d), and powder products (3 g/d) in random order for 8 weeks (Figure). The intervention was performed at the Institute for Health and Welfare, Turku, Finland.

2.3. Dietary parameters

The chokeberry treatment consisted of cold-pressed 100% chokeberry juice (300 mL/d prepared from 318 g of chokeberries) and oven-dried chokeberry powder (3 g/d corresponding to 18 g fresh chokeberries). The chokeberry juice was prepared from chokeberries crushed and macerated with pectinase for 2 hours at 40°C, after which the juice was extracted with a belt press (Kiantama Ltd, Suomussalmi, Finland). The chokeberry powder was produced by drying chokeberries in a convection oven at 50°C for 48 hours and then ground to fine powder (Finnish Berry Powders Ltd, Ähtäri, Finland). The reason for choosing chokeberry juice mixed with powder instead of whole berries was that the astringent taste of whole berries could have affected the compliance. Because the effective dose of chokeberries is not known, we estimated the highest dose that we believed could be consumed with high compliance at home. The polyphenol content of the chokeberry products is shown in Table 1.

The placebo juice consisted of water, sugar syrup (12 g/300 mL juice), and table sugar (7 g/300 mL juice). The placebo powder contained wheat flour (0.8 g/3 g powder), rice powder (1.7 g/3 g powder), and cane sugar (0.5 g/3 g powder). No flavors or colors were added. The energy content of the placebo products

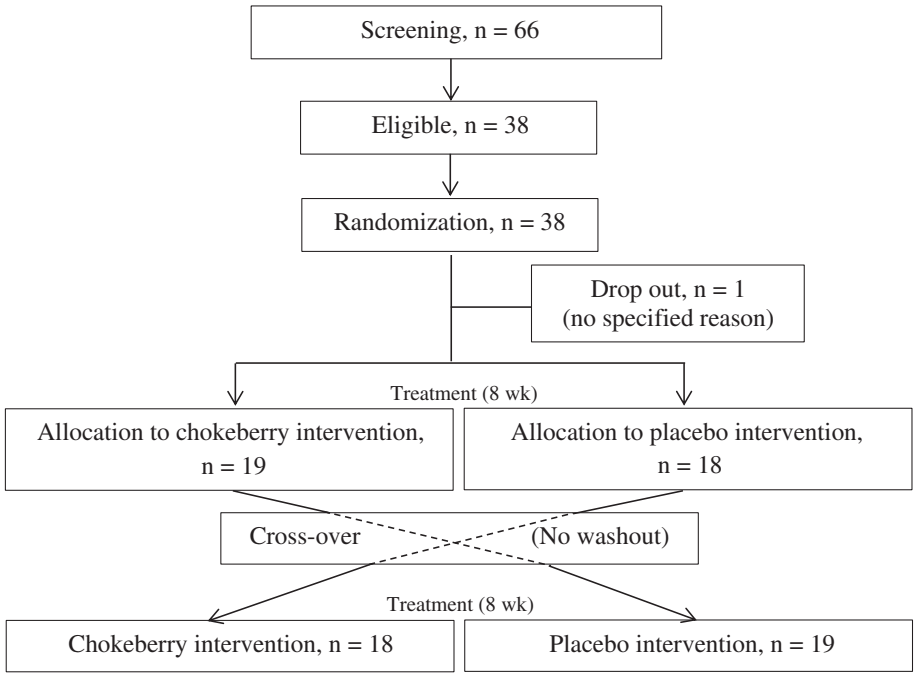

Figure – Participant recruitment, randomization, and study design.

(270 kJ/300 mL juice and 47 kJ/3 g powder) corresponded to that of the chokeberry products.

The study participants were asked to mix the dry powder into the juice before ingestion and drink 150 mL juice twice a day (at the breakfast and in the evening). Otherwise, the participants were asked to maintain their habitual diet, ordinary lifestyle, and physical activities throughout the study. The study participants kept 3-day food diaries at baseline and at the end of the two 8-week treatment periods. Food and nutrient intake was calculated by in-house software program (Finessi) using the Finnish food composition (Fineli Nutrition) database (National Institute for Health and Welfare, 2010).

2.4. *Anthropometric and BP measurements*

All measurements were performed by trained nurses at baseline before the start of the intervention and at the end of each 8-week. treatment period. Weight was measured with light clothing without shoes. A nurse measured resting (auscultatory) BP with a calibrated mercury sphygmomanometer from the right arm after sitting for 5 minutes in the measuring room with the cuff around the right upper arm. The mean of 2 measurements done at 1- to 2-minute intervals was used for statistical analyses.

Twenty-four–hour ambulatory BP was recorded from the nondominant arm with a validated device (Diasys Integra; Novacor Sa, Rueil-Malmaison, France). It was programmed to take readings every 15 minutes during daytime (7:00 AM–9:59 PM) and every 30 minutes during nighttime (10:00 PM–6:59 AM). The participants were asked to record when going to bed and waking up. This information was used to define the true "asleep BP" and "awake BP."

Table 1 – Mean daily intake of polyphenols from the chokeberry products

	Juice	Powder	Total
Polyphenols (mg)	1947	247	2194
Flavonoids	55	3.3	58
Quercetin	52	2.8	54
Anthocyanins	966	58	1024
Cyanidin-3-galactoside	612	35	647
Cyanidin-3-glucoside	28	1.4	30
Cyanidin-3-arabinoside	290	20	310
Cyanidin-3-xyloside	25	1.2	26
Proanthocyanidins	576	169	745
Phenolic acids	351	16	367
Neochlorogenic acid	202	1.1	203
Chlorogenic acid	148	6.6	154

2.5. *Sample collection and preparation*

Blood and 24-hour urine samples were collected at baseline (week 0) and at the end of each 8-week treatment period. Blood was drawn from the antecubital vein after a minimum stasis by an experienced technician after a 10-hour overnight fast. Serum and plasma were separated, aliquoted, and frozen at –70°C until analysis. Urine was frozen in smaller aliquots at –70°C until analysis.

2.6. *Biochemical analyses*

Flavonols of the chokeberry products were analyzed as aglycones by high-performance liquid chromatography (HPLC) after acid hydrolysis [15]. Soluble flavan-3-ols (catechins and proanthocyanidins) were extracted with a mixture of acetone-methanol-water, purified using solid-phase extraction columns, and quantified with normal-phase

HPLC according to the degree of polymerization. Bound proanthocyanidins (berry powder) were hydrolyzed from the extract residue by thioacidolysis, and the flavan-3-ols (terminal units) and flavan-3-ol-thioethers (extender units) thus obtained were quantified with reversed-phase HPLC [16]. Anthocyanins and phenolic acids were extracted with 4% acetic acid in 65% aqueous methanol and determined by reversed-phase HPLC using gradient elution [17]. The analyses were performed at the Natural Resources Institute Finland.

Polyphenol concentrations in 24-hour urine were analyzed at the Institute for Health and Welfare, Helsinki, Finland, by a similar method as previously used [18]. In brief, polyphenol conjugates were first hydrolyzed by incubating them overnight with a β-glucuronidase and sulfatase mixture. After that, polyphenols were extracted with ethyl acetate, dried down under nitrogen, and then silylated using *N*-methyl-*N*-trimethylsilyltrifluoroacetamide. Deuterated ferulic acid and dihydroferulic acid standards were added to the vials before analyzing polyphenols by gas chromatography–mass spectrometry using a DB-35MS column from J&W Scientific (Folsom, CA, USA). The deuterated standards were synthesized at the University of Helsinki, Finland.

Serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose, high-sensitive C-reactive protein (hsCRP), γGT, and urine creatinine were analyzed with an AU400-analyzer (Olympus, Tokyo, Japan) using system reagents (Olympus, Dublin, Ireland) and apolipoproteins A-1 (Apo A-1) and B (Apo B) with the same instrument using Apo A-1 and Apo B reagents (Orion Diagnostica, Espoo, Finland). Urine sodium and potassium were analyzed with a flame photometer (IL943; Instrumentation Laboratories, Milan, Italy) according to the instructions of the manufacturer.

Platelet aggregation was analyzed in citrated whole blood (3.2% citrate) with a Platelet Function Analyzer (PFA100; Dade Behring, Marburg, Germany). Activation of platelets was achieved by collagen/adenosine diphosphate (CADP) and collagen/epinephrine (CEPI) stimuli, and the clot formation time was measured. A complete blood count was measured in K_2EDTA blood on a pocH-100i hematology analyzer (Sysmex, Kobe, Japan). The aggregation and blood count analyses were performed within 1 hour after sampling.

Serum cytokines (interleukin [IL] 4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, granulocyte macrophage colony-stimulating factor [GM-CSF], tumor necrosis factor α [TNF-α]) were measured with multiplex sandwich immunoassay (Milliplex High Sensitivity Human Cytokine Immunoassay; Millipore, Billerica, MA, USA) on a Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA, USA) according to the instructions of the manufacturer. The interassay coefficient of variation of different cytokines varied between 5% and 16%. Samples with concentrations below the measurement range of the assay were given the value of the lower limit of the measuring range. Thirty-three percent of the IL-4 results, 35% of IL-5, 16% of IL-10, 13% of IL-13, and 20% of GM-CSF were less than the measuring range. All serum markers, platelet aggregation, and urine sodium and potassium were analyzed at the Institute for Health and Welfare, Turku, Finland.

2.7. *Statistical analyses*

Randomization was performed with the random number generator of SAS software using each participant's age and sex as the seed for randomization. According to the random number, participants were allocated to the 2 treatment groups, odd numbers to one and even numbers to the other group. The normality of variables was tested and log transformations were applied if necessary. Normally, distributed data are presented as means (±SD) and log-transformed data were back transformed and presented as medians (quartile range 1 to quartile range 3). The analysis of variance model (SAS procedure Mixed) was fitted to test period and carryover effects. Because no period or carryover effects were found, repeated-measures analysis of variance with baseline value as covariate (SAS procedure Mixed) was used to analyze the difference of the least square means of the changes from baseline between placebo and chokeberry treatments. The difference of change is presented as means (95% confidence interval). A probability value less than .05 was considered statistically significant. The statistical analyses were performed with SAS software, version 9.3 (SAS Institute, Cary, NC, USA).

3. Results

Of the 38 participants included, 37 completed the study and 1 withdrew before the start of the treatment (Figure). According to the study diaries, compliance to the study protocol was good and no adverse effects were observed. Furthermore, the 24-hour urinary excretions of various polyphenols increased during the chokeberry period compared with the placebo period, indicating that the participants consumed the chokeberry products (Table 2). Based on the food diaries, the participants did not change their habitual diets during the study, as there were no statistically significant changes in the reported intake of selected nutrients (Table 3). The average body weight also remained unchanged during the study period (Table 4). According to the chemical analyses of the chokeberry juice and powder consumed, the total amount of polyphenols in the products was relatively high and the total mean intake of polyphenols was 2194 mg/d (Table 1). The chokeberry products also contain potassium and consequently the 24-hour urinary excretion of potassium was significantly increased (Table 4).

We observed no effect of chokeberry consumption on serum lipid, lipoproteins, glucose, γGT, or hsCRP (Table 4). Chokeberry treatment decreased daytime ambulatory DBP (–1.64 mm Hg, $P = .02$) and tended to decrease the true awake SBP (–2.71 mm Hg, $P = .077$) and DBP (–1.62 mm Hg, $P = .057$; Table 5). The treatment also tended to decrease 24-hour DBP (–1.07 mm Hg, $P = .084$) but did not lower nighttime BP (Table 5) or auscultatory resting BP (Table 4).

The baseline and after-treatment values of cytokines and platelet aggregation are shown in Table 6. Chokeberry treatment decreased the concentration of IL-10 (–1.9 pg/mL, $P = .008$) and TNF-α (–0.67 pg/mL, $P = .007$). The treatment also tended to decrease the concentrations of IL-4 (–4.5 pg/mL, $P = .084$) and IL-5 (–0.06 pg/mL, $P = .059$). Chokeberry consumption had no significant effect on the serum levels of the other cytokines included in this study. Platelet aggregation measured by the closure time on a CADP- or a CEPI-coated membrane was also unaffected by the chokeberry treatment.

Table 2 – Effects of chokeberry products on urine concentration of polyphenols

	Baseline (n = 37), Medians (Q1-Q3)	Treatment period		Difference of change	
		Placebo (n = 37), medians (Q1-Q3)	Chokeberry (n = 37), medians (Q1-Q3)	Means (95% CI)	P [b]
3-Hydroxybenzoic acid [a]	3.86 (2.32-10.09)	4.43 (2.52-7.21)	11.51 (5.00-20.09)	6.38 (0.72-12.03)	.0002
3-Hydroxyphenylacetic acid	12.4 (7.2-18.3)	13.0 (8.2-21.9)	40.2 (22.7-60.2)	32.0 (19.9-44.2)	<.0001
Dihydro-3-coumaric acid	14.2 (8.9-30.7)	13.9 (7.2-24.0)	31.5 (20.4-43.5)	15.3 (9.0-21.6)	<.0001
Vanillic acid	19.6 (10.1-29.4)	18.1 (10.5-34.1)	25.6 (14.6-46.0)	5.4 (–5.6 to 16.4)	.07
Homovanillic acid	12.6 (11.1-18.3)	13.4 (10.0-23.6)	19.7 (15.3-27.9)	6.4 (2.8-10.0)	<.0001
Protocatechuic acid	11.4 (7.1-15.5)	9.8 (6.9-14.3)	16.9 (12.7-23.4)	8.8 (6.1-11.4)	<.0001
3,4-Dihydroxyphenylacetic acid	31.0 (25.5-45.8)	32.3 (24.2-43.8)	44.3 (34.9-59.2)	14.8 (7.2-22.5)	<.0001
Dihydroferulic acid	17.0 (9.6-27.8)	11.3 (7.7-25.1)	21.6 (13.5-51.4)	13.7 (5.1-22.3)	<.0001
Gallic acid	1.93 (1.39-3.21)	1.92 (1.02-2.96)	1.50 (1.02-2.23)	–0.21 (–0.81 to 0.38)	.45
Hippuric acid	1660 (1232-2174)	1499 (1094-2135)	2540 (1898-3165)	841 (516-1166)	<.0001
Dihydrocaffeic acid	22.8 (12.7-40.1)	20.6 (10.6-36.9)	36.0 (18.6-53.8)	16.7 (5.5-28.0)	.0001
3,4-Dimethylcaffeic acid	0.92 (0.41-2.41)	0.65 (0.36-2.18)	1.32 (0.89-2.39)	0.95 (0.34-1.57)	<.0001
Isoferulic acid	6.27 (3.64-10.38)	5.05 (3.04-8.70)	7.33 (5.48-10.71)	2.85 (1.09-4.61)	<.0001
Ferulic acid	17.7 (11.8-24.5)	14.6 (9.1-22.3)	15.8 (12.0-26.1)	6.5 (1.8-11.2)	.002
Caffeic acid	20.3 (12.0-35.2)	17.2 (12.0-23.9)	25.1 (14.9-37.5)	9.1 (4.2-14.0)	<.0001
Enterolactone	1.93 (0.76-2.66)	1.67 (1.11-3.50)	1.70 (1.05-3.74)	0.06 (–1.07 to 1.19)	.91
3,4-Dihydroxyphenylivaleric acid	5.7 (3.0-19.0)	5.6 (2.7-17.9)	45.0 (16.2-63.5)	30.0 (20.4-39.7)	<.0001

Abbreviations: CI, confidence interval; Q1-Q3, quartile ranges.
The phenolic content was measured from 24-hour urine. Repeated-measures analysis of variance with baseline value as covariate was used to analyze the difference between placebo and chokeberry treatments. Data were log transformed for statistical analysis; data presented are back transformed.
[a] All concentrations are given in μmol/L.
[b] For the difference of change from baseline between chokeberry and placebo period.

4. Discussion

This randomized, placebo-controlled, crossover study was undertaken to explore the effects of consumption of chokeberry products for 8 weeks on BP and other cardiovascular risk factors in participants with untreated, mildly elevated BP. Polyphenol-rich chokeberry products had a small lowering effect on ambulatory daytime BP. However, nighttime ambulatory BP and auscultatory resting BP, which

Table 3 – Dietary intake of selected nutrients based on food diaries

	Baseline (n = 37), means ± SD	Treatment period		Difference of change	
		Placebo (n = 37), means ± SD	Chokeberry (n = 37), means ± SD	Means (95% CI)	P [a]
Energy (MJ)	7.8 ± 1.8	7.4 ± 1.9	7.3 ± 2.0	–0.12 (–0.80 to 0.57)	.7271
Protein (g)	82.9 ± 21.3	78.5 ± 22.9	78.1 ± 24.4	–0.36 (–7.56 to 6.83)	.9192
Soluble carbohydrates (g)	216 ± 54	211 ± 67	205 ± 66	–5.86 (–27.1 to 15.4)	.5788
Fat (g)	67.8 ± 24.4	62.3 ± 18.8	63.5 ± 21.3	1.26 (–6.46 to 8.99)	.7422
Saturated fat (g)	23.6 ± 10.9	22.8 ± 9.0	22.8 ± 8.6	0.08 (–2.88 to 3.04)	.9560
Monounsaturated fat (g)	22.7 ± 9.2	20.2 ± 6.5	21.3 ± 7.7	1.09 (–1.78 to 3.96)	.4468
Polyunsaturated fat (g)	11.2 ± 4.4	9.5 ± 3.2	10.3 ± 4.2	0.80 (–0.74 to 2.35)	.2997
Folate (μg)	275 ± 104	228 ± 73	232 ± 72	4.56 (–18.8 to 28.0)	.6951
Phenolic acids (mg)	630 ± 327	586 ± 297	597 ± 281	10.4 (–42.2 to 63.0)	.6917
Quercetin (mg)	3.6 ± 2.5	3.9 ± 4.0	3.6 ± 2.8	–0.23 (–1.64 to 1.17)	.7362
α-Tocopherol (mg)	9.4 ± 3.2	8.6 ± 3.3	8.8 ± 3.4	0.21 (–1.19 to 1.61)	.7616
β-Carotene (mg)	2.9 ± 2.2	2.5 ± 1.8	2.4 ± 1.9	–0.05 (–0.71 to 0.60)	.8674
Carotenoids (mg)	8.4 ± 4.4	7.0 ± 3.7	7.1 ± 3.5	0.12 (–1.11 to 1.36)	.8448
Vitamin C (mg)	126 ± 71.3	90.3 ± 50.7	102 ± 80.5	11.9 (–7.96 to 31.8)	.2319
Vitamin D (μg)	10.2 ± 6.7	8.0 ± 5.5	9.8 ± 9.8	1.80 (–1.24 to 4.83)	.2379
Potassium (g)	4.2 ± 1.1	3.8 ± 1.0	3.8 ± 1.2	0.05 (–0.25 to 0.36)	.7281
Sodium (g)	2.9 ± 0.8	2.7 ± 0.9	2.8 ± 0.9	0.03 (–0.31 to 0.37)	.8537

Abbreviations: CI, confidence interval; Q1-Q3, quartile ranges.
Repeated-measures analysis of variance with baseline value as covariate was used to analyze the difference between placebo and chokeberry treatments.
[a] For the difference of change from baseline between chokeberry and placebo period.

Table 4 – Effects of chokeberry products on weight, resting BP, serum lipids, glucose, CRP, γGT, and urinary electrolytes

Variables	Baseline (n = 37), means ± SD	Treatment period		Difference of change	
		Placebo (n = 37), means ± SD	Chokeberry (n = 37), means ± SD	Means (95% CI)	P [a]
Weight (kg)	76.3 ± 14.2	77.0 ± 14.2	77.0 ± 14.3	−0.003 (−0.45 to 0.44)	.9902
Body mass index (kg/m^2)	25.9 ± 3.3	26.1 ± 3.2	26.1 ± 3.2	−0.02 (−0.17 to 0.13)	.7869
Glucose (mmol/L)	5.25 ± 0.42	5.25 ± 0.43	5.28 ± 0.46	0.02 (−0.09 to 0.14)	.6836
Cholesterol (mmol/L)	5.2 ± 0.79	5.34 ± 0.86	5.31 ± 0.78	−0.03 (−0.23 to 0.17)	.7438
Triglycerides (mmol/L)	1.09 ± 0.36	1.12 ± 0.44	1.06 ± 0.40	−0.06 (−0.16 to 0.04)	.2175
HDL cholesterol (mmol/L)	1.68 ± 0.46	1.66 ± 0.47	1.63 ± 0.46	−0.03 (−0.08 to 0.02)	.2699
Apo A-1 (mmol/L)	1.55 ± 0.23	1.57 ± 0.24	1.53 ± 0.23	−0.04 (−0.08 to 0.001)	.0581
Apo B (mmol/L)	1.00 ± 0.16	1.04 ± 0.18	1.03 ± 0.16	−0.01 (−0.06 to 0.03)	.5978
Cholesterol/HDL ratio	3.25 ± 0.69	3.38 ± 0.76	3.43 ± 0.77	0.05 (−0.06 to 0.16)	.3987
Apo B/Apo A-1 ratio	0.66 ± 0.14	0.68 ± 0.16	0.69 ± 0.15	0.009 (−0.02 to 0.04)	.5188
CRP	1.57 ± 1.92	1.72 ± 1.89	1.86 ± 2.69	−0.14 (−0.58 to 0.86)	.7002
γGT (U/L)	26.2 ± 15.1	30.5 ± 22.9	29.03 ± 19.9	−1.45 (−7.29 to 4.37)	.7373
dU-potassium (mmol)	86.8 ± 22.8	82.7 ± 22.6	97.9 ± 29.4	14.3 (6.54 to 22.1)	.0007
dU-sodium (mmol)	138.5 ± 43.1	152.9 ± 70.0	140.0 ± 65.6	−15.6 (−34.9 to 3.6)	.1076
SBP (mm Hg)	133.0 ± 10.0	132.9 ± 11.7	130.6 ± 13.4	−2.30 (−5.65 to 1.06)	.1736
DPB (mm Hg)	82.9 ± 7.2	80.5 ± 7.9	81.3 ± 7.3	0.80 (−1.47 to 3.07)	.4812

Abbreviations: CI, confidence interval; dU, 24-hour urinary excretion; Q1-Q3, quartile ranges.
Repeated-measures analysis of variance with baseline value as covariate was used to analyze the difference between placebo and chokeberry treatments.
[a] For the difference of change from baseline between chokeberry and placebo period.

was measured after a 10-hour fast, remained unchanged. Our findings are in line with previous animal studies and with the proposed BP-lowering mechanisms of chokeberries. In animal studies, the BP-lowering effect of chokeberries has been short lived, peaking at 3 hours but still being noticeable after 6 hours [6]. Chokeberry compounds have been suggested to be weak angiotensin-converting enzyme inhibitors [19]. In other studies, polyphenols have been reported to reduce BP through nitric oxide (NO)–related mechanisms by activation or increased expression of endothelial NO synthase [20] rather than through reduction of angiotensin-converting enzyme activity [21]. An NO-mediated vasodilatation and BP reduction induced by polyphenols may be short lived and explain why ambulatory BP in our study tended to decrease only during the daytime. In our study, chokeberries decreased daytime ambulatory DBP but only tended to decrease daytime SBP. This finding may be only a result of chance. On the other hand, NO-mediated vasodilatation takes place in smaller

Table 5 – Effects of chokeberry consumption on ambulatory blood pressure

	Baseline (n = 37), means ± SD	Treatment period		Difference of change	
		Placebo (n = 37), means ± SD	Chokeberry (n = 37), means ± SD	Means (95% CI)	P [a]
24-h					
SBP (mm Hg)	129 ± 9.8	129 ± 10.2	128 ± 10.3	−1.02 (−2.99 to 0.96)	.3026
DBP (mm Hg)	85 ± 6.6	85 ± 7.2	84 ± 6.9	−1.07 (−2.29 to 0.15)	.0838
Day (7 AM–9:59 PM)					
SBP (mm Hg)	135 ± 11.0	136 ± 10.6	134 ± 11.0	−1.98 (−4.53 to 0.57)	.1234
DBP (mm Hg)	89 ± 7.5	89 ± 7.8	87 ± 7.4	−1.64 (−3.04 to 0.24)	.0230
Night (10 PM–6:59 AM)					
SBP (mm Hg)	115 ± 9.8	112 ± 12.5	114 ± 15.0	1.47 (−2.51 to 5.46)	.4581
DBP (mm Hg)	76 ± 7.1	75 ± 7.4	75 ± 8.4	0.39 (−1.84 to 2.61)	.7256
Awake					
SBP (mm Hg)	136 ± 11.4	137 ± 11.3	134 ± 11.1	−2.71 (−5.73 to 0.32)	.0777
DBP (mm Hg)	89 ± 7.9	89 ± 8.5	88 ± 7.6	−1.62 (−3.29 to 0.05)	.0568
Sleep					
SBP (mm Hg)	113 ± 10.6	112 ± 12.9	112 ± 15.0	1.66 (−2.91 to 6.23)	.4652
DBP (mm Hg)	75 ± 7.2	75 ± 7.4	74 ± 8.3	0.01 (−2.56 to 2.58)	.9938

Abbreviations: CI, confidence interval; Q1-Q3, quartile ranges.
Repeated-measures analysis of variance with baseline value as covariate was used to analyze the difference between placebo and chokeberry treatments.
[a] For the difference of change from baseline between chokeberry and placebo period.

Table 6 – Effect of chokeberry products on inflammation markers and platelet aggregation

Inflammation markers (pg/mL)	Baseline (n = 37), medians (Q1-Q3)	Treatment period		Difference of change	
		Placebo (n = 37), medians (Q1-Q3)	Chokeberry (n = 37), medians (Q1-Q3)	Means (95% CI)	P [a]
IL-4	26.2 (3.2-155.5)	42.1 (3.2-168.4)	42.8 (3.2-141.3)	−4.5 (−9.5 to 0.5)	.0839
IL-5	0.22 (0.13-0.62)	0.35 (0.13-0.91)	0.28 (0.13-0.88)	−0.06 (−0.14 to 0.03)	.0595
IL-6	3.24 (1.84-10.96)	4.48 (1.91-11.62)	5.52 (1.67-10.80)	−0.51 (−1.11 to 0.08)	.1313
IL-7	7.92 (4.06-10.78)	8.67 (4.46-12.30)	10.01 (4.71-12.55)	−0.04 (−0.86 to 0.79)	.9000
IL-8	6.57 (5.11-11.64)	7.23 (5.54-11.69)	7.10 (5.31-10.56)	−0.24 (−0.67 to 0.20)	.4315
IL-10	6.04 (1.84-9.11)	5.84 (2.51-12.48)	4.74 (1.10-9.72)	−1.9 (−3.63 to 0.21)	.0077
IL-13	28.9 (7.75-70.64)	23.6 (8.19-70.59)	28.3 (7.15-67.41)	−1.9 (−4.69 to 0.87)	.1171
TNF-α	6.6 (4.57-8.04)	7.04 (5.04-8.42)	6.75 (4.52-8.58)	−0.67 (−1.14 to 0.21)	.0068
GM-CSF	3.76 (1.01-5.84)	2.54 (1.00-7.15)	3.12 (0.98-6.73)	−0.69 (−1.68 to 0.29)	.1328
Platelet aggregation	Means ± SD	Means ± SD	Means ± SD	Means (95% CI)	P [a]
CEPI-CT (s)	129 ± 33	132 ± 54	130 ± 35	−3 (−16 to 11)	.6949
CADP-CT (s)	82 ± 17	1 ± 15	83 ± 15	1.2 (−2.2 to 4.6)	.4860
PLT ($\times 10^9$/L)	251 ± 64	248 ± 62	248 ± 64	0.2 (−6.9 to 6.4)	.9478

Abbreviations: CT, closure time; CI, confidence interval; PLT, platelet count; Q1-Q3, quartile ranges.
Repeated-measures analysis of variance with baseline value as covariate was used to analyze the difference between placebo and chokeberry treatments. Data for inflammation markers were log transformed for statistical analysis; data presented are back transformed.
[a] For the difference of change from baseline between chokeberry and placebo period.

resistance arteries rather than in larger conduit arteries. Increased DBP reflects increased peripheral resistance and increased SBP also increased stiffness of the large arteries. Accordingly, one might expect that the BP-lowering effects of chokeberries would be seen predominantly for DBP. In line with our findings, different effects on SBP and DBP have also been reported in studies with nitrate-rich beetroot supplementation [22,23].

The participants of our study were relatively healthy and their BP values only slightly elevated. The small BP-lowering effect of chokeberries may therefore be explained by small BP-lowering margins in the first place. Moreover, the age of the participants might be important as a systematic review of studies investigating the effects of chocolate found a BP-lowering effect only in individuals younger than 50 years, suggesting that functional NO-mediated mechanisms may be needed for the vasodilatory and BP-lowering effects of polyphenols [24]. Only 30% of the participants included in our study were younger than 50 years.

In addition to polyphenols, another compound possibly contributing to the BP reduction is potassium, which is present in chokeberry juice as well. The 24-hour urinary excretion of potassium increased by 14.3 mmol corresponding to a 560-mg increase in daily potassium intake during the chokeberry period. A meta-analysis of potassium supplementation studies suggests that a corresponding increase in potassium intake would explain a half of the observed SBP and a quarter of the observed DBP decrease in daytime BP [25]. However, the effect should also have been seen for nighttime BP and for auscultatory resting BP, which was not the case. Therefore, increased potassium intake during the chokeberry supplementation is an implausible explanation for the reduced daytime BP observed in our study.

The chokeberry-enriched diet had no effect on serum lipids, lipoproteins, glucose, and hsCRP. Our participants were nondiabetic and had rather normal lipid profiles. In line with our findings, several other studies have found that chokeberries have no effects on serum total cholesterol, triglycerides, or HDL levels in normotensive and prehypertensive but otherwise healthy individuals [26,27]. Few studies have investigated the effects of chokeberry on inflammation markers in humans. Studies on mildly hypercholesterolemic men showed beneficial changes in the lipid profile [9,10] but no change in CRP levels [9] after ingestion of chokeberry juice. Similarly, CRP levels did not change in a study with individuals with metabolic syndrome [11]. In our study of mildly hypertensive individuals with normal hsCRP levels (<2 mg/L), we observed no change in CRP after chokeberry ingestion. However, small changes in some cytokines were observed. Chokeberries decreased the circulating concentration of IL-10 and TNF-α and tended to decrease IL-4 and IL-5. Proinflammatory activation of vascular endothelial cells is recognized as a key event in hypertension and other CVD. Tumor necrosis factor α impairs the ability of the endothelium to produce NO, promoting vasoconstriction. Interleukin 4, produced by T_H2 cells, is considered anti-inflammatory but has been shown to have proatherogenic properties in atherosclerotic mice and to induce production of reactive oxygen radicals and decrease NO availability in endothelial cells [28,29]. The main function of IL-10 is to limit and terminate inflammatory responses where the inhibitory effects of IL-10 on IL-1 and TNF-α production are crucial. Interleukin 10 directly affects the function of T cells and inhibits IL-2, TNF-α, and IL-5 production. Interleukin 5 has been shown to reduce atherosclerotic plaques in mice by increasing secretion of antibodies that inhibit oxidized low-density lipoprotein uptake by macrophages [30]. Our findings indicate a slight improvement of the profile toward lower risk for CVD with lower levels of proinflammatory cytokines leading in turn to lower levels of regulative anti-inflammatory cytokines.

In the present study, no effect of chokeberries on platelet function was observed. In a previous study, which included participants with more risk factors of CVD, consumption of

various berries and berry products reduced platelet function (CADP closure time) [13]. Different berries contain different amounts and type of polyphenols and other bioactive substances, which may explain why some berries affect platelet aggregation and others do not. The effect of chokeberries on aggregation can also be temporary. Sikora et al. [31] showed that 1 month of chokeberry consumption induces significant changes in platelet function but after 2 months of consumption, these changes are almost completely reversed.

The effects of chokeberries observed in this study may be mediated by the polyphenols found in urine after the chokeberry treatment. The metabolism of polyphenols is rather complicated and has been described in more detail elsewhere [32,33]. Most of the measured polyphenols are phenolic acids, which are obtained from chokeberry as such, but also as metabolites of other polyphenols present. For instance, ferulic, protocatechuic, and hippuric acid are metabolized from anthocyanins. 3,4-Dihydroxyphenylacetic acid and homovanillic acid, on the other hand, are quercetin metabolites. In our study, chokeberry consumption clearly increased 24-hour excretion of many polyphenols and polyphenol metabolites. However, the amounts excreted were not particularly high. This is because chokeberry contains mostly large-molecular-weight proanthocyanidins and anthocyanins, which are poorly absorbed. Whether the amounts of polyphenols or metabolites reaching the blood stream or tissues were high enough for beneficial health effects is unknown.

Our study was limited to a small population of relatively healthy individuals. It is possible that effects on glucose and lipid metabolism, for example, would have been seen in individuals with elevated levels of these risk factors. Furthermore, the participants consumed juice and powder, and the result applies to consumption of these rather than to fresh, whole berries.

Overall, our study showed that consumption of chokeberry juice and powder for 8 weeks had minor but favorable effects on CVD risk factors in metabolically healthy individuals with mildly elevated BP. Our results confirmed the hypothesis that an intake of chokeberry products exerts beneficial effects on BP and circulating levels of cytokines, but according to our study, it exerts no effects on biomarkers of glucose and lipid metabolism or platelet aggregation. The favorable effect seen on BP was small and short lived, suggesting that the use of chokeberries as BP-lowering treatment is limited. Although effects of this magnitude are not pharmacologically relevant, they are likely to become meaningful if these types of products are consumed in combination with other healthy foods and are incorporated into a generally healthy diet.

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