Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol[1–3]

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ABSTRACT

Background: Berries are a particularly rich source of polyphenols. They also contain other bioactive substances, such as vitamin C. Previous studies indicated that the consumption of polyphenol-rich foods (eg, cocoa, tea, and red wine) may induce beneficial changes in pathways related to cardiovascular health. Whether the consumption of berries has similar effects is unknown.

Objective: We aimed to investigate the effects of berry consumption on hemostatic function, serum lipids, and blood pressure (BP).

Design: Middle-aged unmedicated subjects ($n = 72$) with cardiovascular risk factors consumed moderate amounts of berry or control products for 8 wk in a single-blind, randomized, placebo-controlled intervention trial.

Results: Berry consumption inhibited platelet function as measured with a platelet function analyzer (using collagen and ADP as platelet activator) [changes: 11% and −1.4% in the berry and control groups, respectively; $P = 0.018$, analysis of covariance (ANCOVA)]. Plasma biomarkers of platelet activation, coagulation, and fibrinolysis did not change during the intervention. Serum HDL-cholesterol concentrations increased significantly more (P = 0.006, ANCOVA) in the berry than in the control group (5.2% and 0.6%, respectively), but total cholesterol and triacylglycerol remained unchanged. Systolic BP decreased significantly ($P = 0.050$, ANCOVA); the decrease mostly occurred in subjects with high baseline BP (7.3 mm Hg in highest tertile; $P = 0.024$, ANCOVA). Polyphenol and vitamin C concentrations in plasma increased, whereas other nutritional biomarkers (ie, folate, tocopherols, sodium, and potassium) were unaffected.

Conclusion: The consumption of moderate amounts of berries resulted in favorable changes in platelet function, HDL cholesterol, and BP. The results indicate that regular consumption of berries may play a role in the prevention of cardiovascular disease. *Am J Clin Nutr* 2008;87:323–31.

KEY WORDS Berries, polyphenols, hemostasis, blood pressure, intervention study

INTRODUCTION

Numerous epidemiologic studies have indicated that high intakes of fruit and vegetables reduce the risk of cardiovascular disease (CVD). However, the role of different micronutrients and phytochemicals in this protection is far from clear. Polyphenols, the largest—and quantitatively the most important—group of dietary phytochemicals, may explain part of the effect. Animal studies as well as human studies, although limited in number, have shown beneficial changes in biological phenomena related to cardiovascular health after the consumption of polyphenol-rich foods such as cocoa, red wine, and tea (1, 2).

Polyphenols such as flavonols, phenolic acids, anthocyanins, and procyanidins are found in particularly high concentration in various berries (3). Berries also contain other bioactive substances, such as vitamin C, folate, potassium, and soluble fiber. In contrast with some of the other dietary sources of polyphenols, berries contain no fat, ethanol, or caffeine-related substances. Because of their high polyphenol content and the fact that their profile of bioactive components differs from that of more typically investigated sources, berries are, as a group, an interesting model food for an investigation of the health effects of polyphenols (as long as the potential effect of other berry components is kept in mind). By adding moderate amounts of berries to the diet, it may be possible to considerably increase the intake of polyphenols. The intake of nutrients such as vitamin C and folate may not similarly increase with berry consumption, because they are obtained from various commonly consumed foods.

The consumption of berries could affect pathways related to cardiovascular health by several different mechanisms. Antioxidation is one possible mechanism, because some of the dietary antioxidants that are most potent in vitro—ie, polyphenols and vitamin C—are obtained from berries. However, other mechanisms could also be involved. The range of polyphenol bioactivities observed in vitro is wide (4). Although pure polyphenols have rarely been investigated in vivo, supplementation studies with polyphenol-rich foods or extracts indicate that the compounds may exert effects in vivo as well. For instance, the ingestion of dealcoholized red wine, grape juice, or polyphenol extracts reduced blood pressure (5–7) and inhibited platelet aggregation (8, 9) in laboratory animals. Beneficial effects have been observed in humans after the ingestion of cocoa or chocolate [platelet aggregation and activation (10), HDL cholesterol

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(11, 12), blood pressure (13, 14), and endothelial function (15)], the consumption of purple grape juice [platelet aggregation and activation (16, 17)], the ingestion of onion [platelet aggregation and activation (18)], and the consumption of black tea [endothelial function (19)].

In this study, we tested the effects of relatively long-term consumption of various berries on well-established risk factors of CVD, such as blood pressure and serum lipids. We also investigated the effects of berry consumption on hemostatic function. Particular focus was on platelet function as measured with the use of a platelet function analyzer, because short closing times in that system may be a novel risk factor for acute coronary syndromes (20–22) and may be modifiable by the consumption of polyphenol-rich foods (2, 23).

SUBJECTS AND METHODS

Subjects

Seventy-two male and female volunteers were recruited through municipal health centers and by newspaper advertisements. To be included in the study, the subjects had to have ≥1 of the following conditions: mild hypertension [140–159 mm Hg systolic blood pressure (SBP) or 90–99 mm Hg diastolic blood pressure (DBP)], elevated blood glucose (6.1–8.0 mmol/L), elevated serum total cholesterol (6.5–8.0 mmol/L) or triacylglycerol (2.0–3.9 mmol/L), and low HDL cholesterol (<1 mmol/L). Exclusion criteria were regular use of medications (except hormone replacement therapy) or dietary supplements, smoking, intestinal disorders, obesity [body mass index (in kg/m^2) ≥ 35], and vegetarianism.

The main outcome of the study was the closing time (CT) on the platelet function analyzer when ADP and collagen are used as platelet activators (CADP-CT). Power calculations were based on previous results on such CTs in healthy persons (24). The calculations showed that the allocation of 28 subjects to each group would provide a study power of 95% (significance level of 5%) for a 10% difference between groups.

All subjects completed the study. One subject in the berry group was excluded from the analyses because of inflammation, pain attacks, and use of forbidden medications (antiinflammatory agents and antibiotics).

All subjects gave written informed consent. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Study design

The study was a single-blind, randomized, placebo-controlled, 8-wk dietary intervention trial with 2 treatment groups (berry group and control group). The participants were randomly assigned to the 2 groups after stratification by sex. The main visits to the study site were at baseline and after 8 wk (for cardiovascular measurements and sample collection). Additional visits were at 2 and 5 wk; these visits were for compliance checks, meetings with a study nurse, and sample collection for nutritional biomarker analyses. The intervention was conducted during the fall of 2005.

Diets

The berry group consumed 2 portions of berries daily (the recommendation was 1 portion after lunch and 1 portion after dinner). Every other day, whole bilberries (100 g) and a nectar containing 50 g crushed lingonberries were consumed. Black currant or strawberry purée (100 g, containing 80% black currants) and cold-pressed chokeberry and raspberry juice (0.7 dL juice, containing 80% chokeberry) were consumed on the alternating days. The subjects kept the products in their home freezers and were instructed to take them out of the freezer just before consuming. Warming in a warm water bath was allowed. The addition of water to dilute the juice was also allowed. The average sucrose content of the products was 7%. The control group consumed 1 of 4 different control products each day. The products were 2 dL sugar-water, 100 g sweet semolina porridge, 100 g sweet rice porridge, and 40 g marmalade sweets. The aim was to control for the increased energy intake in the berry group. The products were kept in the freezer and melted in a water bath or a microwave the same day that they were consumed. The food products used in the study were obtained from Pakkasmarja Oy (Suonenjoki, Finland), Berry Know-How Centre (Suonenjoki, Finland), Kaskein Marja Oy (Taavetti, Finland), Immilän Marjatila (Nastola, Finland), and local supermarkets.

The subjects were instructed to maintain their normal dietary and lifestyle habits but to refrain from consuming berries from sources other than the study products. The subjects kept a diary in which consumption of the study foods was recorded daily. They used the same diary to record occurrences such as gastrointestinal symptoms and possible use of medications. The diaries were checked by a study nurse at 2, 5, and 8 wk. Complete 3-d dietary records were collected at baseline (during the week before the baseline visit) and during week 8 of the intervention. Food and nutrient intakes were calculated by using DIET 32 software (version 1.4.4; Aivo Finland Oy, Turku, Finland), which is based on Fineli, the Finnish food composition database (2005; National Public Health Institute, Helsinki, Finland).

Polyphenol analyses of berry products

Total phenolic acids in the berry products were quantified by using HPLC after alkaline and acid hydrolysis (25). Soluble flavan-3-ols (catechins and procyanidins) were quantified according to the degree of polymerization with the use of HPLC (26). Flavonols and flavanones were quantified as aglycones (27), and ellagitannins were quantified as ellagic acid by using HPLC after acid hydrolysis (28). Anthocyanins were quantified as cyanidin-3-glucoside according to a modification of the method of Gao and Mazza (29).

Sample collection and preparation

Blood was taken between 0730 and 0930 by an experienced technician. After minimum stasis, the blood samples were collected from the antecubital vein with the use of 20-gauge needles into evacuated tubes containing sodium citrate (3.2%), K_3EDTA (0.18%), lithium heparin, or no anticoagulant. Plasmas were separated within 30 min by centrifugation for 20 min at 2500 × *g* and 15 °C. Citrated plasma (2 mL) was carefully pipetted from the middle part of the plasma column (9-mL blood collection tubes were used). After that, the citrated plasmas were rapidly frozen by using dry ice and ethanol. Serum samples were centrifuged (12 min, 1000 × *g*, room temperature) within 1 h after blood collection. All samples were stored at −70 °C. Collection of 24-h urine was done twice during the study (the day before the baseline visit and the last day of the intervention).

Blood pressure measurements

Blood pressure was measured by a trained nurse using a calibrated mercury sphygmomanometer and appropriate cuff sizes on the sitting subject's right arm after a 10-min rest. The last 5 min of rest was spent in the measuring room with the cuff around the right upper arm. SBP and DBP were defined according to phase I and phase V Korotkoff sounds, respectively. Blood pressure was calculated as the mean of 3 measurements performed at 2-min intervals.

Biomarker analyses

Platelet function in citrated whole blood was analyzed with the Platelet Function Analyzer (PFA-100; Dade-Behring, Marburg, Germany) according to the manufacturer's instructions. The method mimics the in vivo hemostatic conditions during plug formation by measuring the time (ie, CT) needed for a membrane coated with either collagen and ADP (CADP-CT) or collagen and epinephrine (CEPI-CT) to occlude under high shear flow. Analyses were performed within 1 h after collection of a blood sample. A 10.1% CV was determined for the system before analyses.

The automated assay for von Willenbrand factor ristocetin cofactor activity was performed on a Behring Coagulation System coagulation analyzer using Behring Coagulation von Willebrand reagent (Dade Behring Inc, Marburg, Germany), which contains lyophilized platelets, ristocetin, and EDTA (CV 9%). The von Willenbrand factor antigen test was performed on a STA Compact coagulation analyzer using a STA Liatest vWF immunoassay (Diagnostica Stago, Asnieres sur Seine, France) (CV: 2.8%). Fibrinogen was analyzed by a liquid-phase immunoprecipitation assay (Turbox; Orion Diagnostica, Espoo, Finland) (CV: 6.2%). Other hemostatic markers were analyzed by using commercial enzyme-linked immunosorbent assays as instructed by the manufacturers: prothrombin fragment 1 + 2 (F1 + 2) (Enzygnost, Dade Behring); D-dimer and glycoprotein V (Asserachrom; Diagnostica Stago); and sICAM-1, P-selectin, and CD40L (R&D Systems Inc, Minneapolis, MN) (CVs: <7.4% for all). Serum total cholesterol and triacylglycerol were determined enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) (CVs: 1.9% and 3.4%). HDL cholesterol was assayed by a direct method (Roche Diagnostics GmbH, Mannheim, Germany) (CV: 2.1%). Plasma quercetin was analyzed by HPLC with electrochemical detection (30) and other polyphenols by a modification of a previously described gas chromatographic–mass spectrometry method (31) (CVs: <10% for all polyphenols). Vitamin C concentrations were analyzed by HPLC with electrochemical detection as described previously (32) (CV: 7.3%). Serum folate was analyzed by a radioassay based on specific folate binding (MP Biomedicals, Orangeburg, NY) (CV: 7.5%). Urinary sodium and potassium concentrations were analyzed with ion-selective electrodes (Kone Microlyte ion-selective analyzer; Kone Corp, Espoo, Finland) (CVs: 1.7% and 1.0%).

Statistical analyses

Statistical analyses were performed by using SPSS for WINDOWS software (version 15.0; SPSS Inc, Chicago, IL). Normal distributions were tested with the Shapiro-Wilk *W* test. Many biomarkers deviated from normality, and they were therefore logarithmically transformed for statistical analyses. Nontransformed data are presented as means ± SDs or means and 95% CIs. Transformed data are presented as geometric means and 95% CIs. $P \leq 0.050$ was considered significant.

Differences between treatment groups at baseline were tested with Student's *t* test for independent samples. One-factor analysis of covariance (ANCOVA) with the 8-wk value as dependent variable or repeated-measures ANCOVA with time as repeating factor—and with the use of a baseline value as covariate in both cases—was used to compare the significance of the effects of berry products and of the control products. Before the final analyses were conducted, interactions between the dependent variable and the covariate were checked by using a custom model, which included group and covariate as main effects and the group × covariate interaction. When there was no interaction, a full factorial model was chosen. If an interaction was found, the interaction term was included in the final model. For SBP and DBP, a significant group × covariate interaction was observed. To further study the effects of the intervention on these variables, the data were divided into tertiles according to baseline blood pressure values. After that, a 2-factor analysis of variance (with change from baseline as dependent variable and group and tertile as independent factors) was conducted. There was a significant group × tertile interaction for SBP, and therefore a one-factor ANCOVA (with 8-wk value as dependent variable and baseline value as covariate) was performed separately for each tertile of SBP. Spearman's rank correlation coefficients were calculated to investigate associations between changes in CADP-CT, HDL cholesterol, and blood pressure.

RESULTS

Baseline values

The baseline characteristics of the subjects are shown in **Table 1**. The calculated intakes of energy and selected nutrients at baseline are shown in **Table 2**. The means of the 2 groups at baseline did not differ significantly for any variable.

Effects of intervention on dietary intake and body weight

The intervention had no effect on the calculated intake of energy and nutrients from the background diet (data not shown). When the study products were included in the analyses, only vitamin C intake was significantly ($P = 0.001$) higher in the berry group than in the control group (Table 2).

TABLE 1
Baseline characteristics of subjects[1]

	Berry group ($n = 35$)	Control group ($n = 36$)	P[2]
Females/males (n)	23/12	23/13	
Age (y)	57.5 ± 6.3[3]	58.4 ± 5.6	NS
BMI (kg/m^2)	26.0 ± 2.9	26.4 ± 3.8	NS
Systolic blood pressure (mm Hg)	128 ± 16.8	131 ± 16.4	NS
Diastolic blood pressure (mm Hg)	80 ± 8.5	82 ± 8.3	NS
Serum cholesterol (mmol/L)	6.3 ± 0.97	6.3 ± 0.96	NS
Serum HDL cholesterol (mmol/L)	1.5 ± 0.40	1.7 ± 0.54	NS
Serum triacylglycerol (mmol/L)	1.6 ± 0.60	1.6 ± 0.85	NS
Serum glucose (mmol/L)	5.4 ± 0.54	5.5 ± 0.54	NS
Hypertension (%)	47	47	
Elevated serum glucose (%)	33	14	
Abnormal lipid values (%)	78	64	

[1] Inclusion criteria are described in the Subjects section.
[2] Difference between groups by Student's *t* test for independent samples.
[3] $\bar{X}$ ± SD (all such values).

TABLE 2
Mean daily intake of selected nutrients[1]

	Berry group (n = 35)		Control group (n = 36)		
	Baseline	8 wk	Baseline	8 wk	P^2
Energy (MJ)	6.90 ± 2.1[3]	7.3 ± 2.1	7.8 ± 2.4	8.1 ± 2.4	NS
Carbohydrates (g)	193.1 ± 61.2	211.1 ± 61.2	215.2 ± 66.7	224.3 ± 66.7	NS
Protein (g)	73.8 ± 22.6	65.9 ± 18.6	80.8 ± 23.0	83.4 ± 29.4	NS
Fat (g)	64.1 ± 22.3	64.7 ± 26.3	67.0 ± 32.6	71.1 ± 26.6	NS
Saturated fat (g)	22.6 ± 9.2	24.5 ± 12.0	23.3 ± 11.3	25.5 ± 10.4	NS
Monounsaturated fat (g)	20.2 ± 8.1	20.3 ± 9.1	21.1 ± 10.2	21.3 ± 7.4	NS
Polyunsaturated fat (g)	9.8 ± 4.7	9.1 ± 5.0	10.9 ± 7.6	10.4 ± 5.1	NS
Soluble fiber (g)	4.63 ± 1.76	5.35 ± 1.76	5.37 ± 2.08	5.41 ± 2.08	NS
Vitamin C (mg)	107.2 ± 50.6	135.6 ± 36.6	112.3 ± 69.9	88.0 ± 49.5	0.001
Folate (μg)	254.1 ± 76.7	238.8 ± 75.7	280.9 ± 73.4	281.0 ± 96.9	NS
α-Tocopherol (mg)	10.64 ± 6.48	12.84 ± 6.48	10.85 ± 5.17	10.90 ± 5.17	NS
Calcium (mg)	1050 ± 358	976 ± 323	1139 ± 473	1138 ± 547	NS
Carotenoids (mg)	7.21 ± 3.94	6.95 ± 4.33	7.82 ± 6.07	7.08 ± 4.89	NS
Sodium (g)	2.65 ± 0.93	2.66 ± 0.84	2.92 ± 1.05	3.15 ± 1.15	NS
Potassium (g)	3.66 ± 1.07	3.55 ± 0.88	4.02 ± 1.12	4.10 ± 1.33	NS

[1] Intake was estimated from 3-d food records. Calculations were based on Fineli (National Public Health Institute, Helsinki, Finland), the Finnish food composition database. Intakes from the berry products and the control products were included. There were no significant differences between the 2 groups at baseline for any variable.
[2] One-factor ANCOVA with baseline value as covariate.
[3] $\bar{x}$ ± SD (all such values).

The mean daily intakes of polyphenols and nutrients from the berry products are shown in **Table 3**. According to the chemical analyses, the intake of polyphenols from the berry products was 837 mg/d. The calculated intake of vitamin C from the berry products was 61.5 mg.

The intervention had no significant effect on the body weights of the subjects. The body weights at baseline and at weeks 2, 5, and 8 were 74.2 ± 9.6, 74.9 ± 10.0, 75.0 ± 9.8, and 75.0 ± 9.9 kg and 75.6 ± 15.9, 76.4 ± 16.5, 76.1 ± 16.1, and 76.2 ± 16.3 kg in the berry and control groups, respectively.

Effects of berry consumption on nutritional biomarkers

The plasma concentrations of polyphenols increased in the berry group significantly more than in the control group ($P \leq$

TABLE 3
Mean daily intake of nutrients and polyphenols from the berry products consumed during the intervention[1]

	Bilberries	Lingonberries	Black currant and strawberry purée	Chokeberry and raspberry juice	Σ
Mean consumption/d (g)	50	25	50	35	160
Total polyphenols (mg)	358	138	133	208	837
Anthocyanins (mg)[2]	275	24	96	120	515
Procyanidins, P1–P3 (mg)[3]	8.5	48	2.0	2.5	61
Procyanidins, P4–P10 (mg)[3]	18	41	2.2	3.0	64.2
Procyanidins, PP (mg)[3]	28	18	16	53	115
Ellagitannins (mg)[4]	0	0	6.1	5.3	11.4
Phenolic acids (mg)[5]	25.2	5.0	8.8	23.5	62.5
Flavonols (mg)[5]	3.2	2.2	2.2	0.37	7.97
Flavanones (mg)[5]	0	0	0	0.18	0.18
Vitamin C (mg)	7.0	1.7	50.2	2.6	61.5
Vitamin E (mg)	0.88	0.35	0.90	0.06	2.17
Folate (μg)	5.3	0.5	6.2	2.4	14.4
Carotenoids (mg)	0.14	0.07	0.21	0.29	0.71
Soluble fiber (g)	0.3	0.1	0.8	0	1.2
Potassium (mg)	51.2	18.6	144.6	22.4	236.5

[1] Intake calculations for polyphenols were based on chemical analyses of berry products. Other intakes were estimated by using Fineli (National Public Health Institute, Helsinki, Finland), the Finnish food composition database.
[2] As cyanidin-3-glucoside.
[3] Labels P1–P3 and P4–P10 indicate the degrees of polymerization of procyanidins; PP indicates polymeric procyanidins with degree of polymerization >10.
[4] As ellagic acid.
[5] As aglycones.

TABLE 4
Nutritional biomarkers at baseline and during the 8-wk intervention[1]

	Berry group (n = 35)				Control group (n = 36)				
	Baseline	2 wk	5 wk	8 wk	Baseline	2 wk	5 wk	8 wk	P^2
Quercetin (nmol/L)	21.1 (19.9, 37.4)[3]	38.9 (36.3, 60.8)	31.8 (30.6, 51.7)	34.4 (31.7, 59.1)	28.7 (25.7, 48.4)	21.9 (21.7, 36.9)	23.2 (22.8, 37.0)	24.2 (23.9, 37.2)	0.006
Caffeic acid (nmol/L)	64.2 (62.3, 95.2)	122.1 (111.9, 187.9)	134.7 (118.7, 223.4)	104.6 (100.9, 154.7)	73.7 (70.4, 117.2)	86.4 (79.4, 121.4)	70.1 (66.2, 103.0)	67.9 (63.9, 103.7)	<0.001
Protocatechuic acid (nmol/L)	108.3 (101.5, 126.8)	134.8 (126.9, 153.1)	132.7 (122.8, 157.9)	130.6 (122.1, 148.8)	110.4 (103.5, 125.0)	114.6 (107.7, 134.0)	103.1 (98.1, 117.4)	102.6 (96.2, 118.5)	0.002
p-Coumaric acid (nmol/L)	12.7 (11.9, 20.3)	18.9 (17.6, 30.7)	18.8 (15.6, 34.9)	15.7 (14.9, 24.3)	17.2 (15.9, 26.0)	14.4 (13.5, 23.1)	13.1 (8.4, 33.7)	11.0 (10.5, 15.5)	<0.001
Vanillic acid (nmol/L)	68.4 (63.0, 91.9)	95.1 (87.2, 123.2)	89.3 (81.9, 121.1)	82.3 (74.9, 118.7)	78.9 (72.3, 106.4)	84.0 (78.1, 103.4)	72.1 (65.2, 89.7)	72.2 (66.8, 90.4)	0.005
Vitamin C (μmol/L)	59.4 (55.7, 68.8)	68.0 (64.3, 75.2)	69.7 (65.9, 77.6)	70.7 (67.0, 77.6)	63.0 (59.4, 75.1)	59.4 (57.0, 72.0)	55.2 (53.0, 69.7)	54.2 (52.2, 69.9)	0.001
Folate (nmol/L)	11.4 (10.5, 14.5)	10.1 (9.3, 12.6)	9.3 (8.6, 11.3)	10.1 (9.5, 12.5)	9.2 (9.0, 12.6)	8.7 (8.5, 11.4)	8.9 (8.3, 12.1)	8.9 (8.7, 12.3)	NS
α-Tocopherol (μmol/L)	24.1 (22.5, 28.9)	25.1 (23.44, 30.0)	25.3 (23.6, 29.7)	24.1 (22.5, 28.3)	26.9 (25.3, 30.7)	27.4 (25.8, 33.0)	27.6 (24.1, 37.5)	26.5 (24.9, 32.2)	NS
γ-Tocopherol (μmol/L)	2.45 (2.3, 3.3)	2.18 (2.1, 3.1)	2.59 (2.4, 3.6)	2.38 (2.1, 3.9)	2.54 (2.5, 3.6)	2.74 (2.6, 3.9)	2.93 (2.7, 4.9)	2.47 (2.4, 3.3)	NS
Potassium (mmol)	66.2 (61.3, 79.8)	NC	NC	72.8 (68.7, 85.9)	73.2 (70.0, 84.2)	NC	NC	74.7 (70.5, 86.3)	NS
Sodium (mmol)	116.5 (109.3, 140.4)	NC	NC	133.8 (126.2, 158.0)	130.9 (121.2, 168.7)	NC	NC	143.8 (134.8, 169.0)	NS

[1] NC, sample not collected at the time point. Concentrations are given in fasting plasma or serum, except sodium and potassium, for which the amount in 24-h urine is given. There were no significant differences between the 2 groups at baseline for any variable.
[2] Repeated-measures ANCOVA, with baseline value as covariate, for biomarkers measured in plasma or serum. One-factor ANCOVA, with baseline value as covariate, for 24-h urinary sodium and potassium.
[3] Geometric $\bar{x}$; 95% CIs in parentheses (all such values).

0.006 for all) (**Table 4**). Depending on the time point, the increases in the berry group were 51–84% for quercetin, 63–109% for caffeic acid, 21–24% for protocatechuic acid, 24–49% for p-coumaric acid, and 20–39% for vanillic acid. Vitamin C concentrations also increased significantly ($P = 0.001$) more in the berry group than in the control group. The increase ranged from 11% to 16%, depending on the time point. The intervention had no significant effect on plasma concentrations of folate, α-tocopherol, and γ-tocopherol or on 24-h urinary sodium and potassium excretion. Potential biomarkers of coffee and chocolate consumption—ie, paraxanthine and theobromine—also were unaffected (data not shown).

Compliance

All subjects randomly assigned to the 2 intervention groups completed the study. According to the diaries kept by the subjects, compliance was very good. The fact that plasma polyphenol and vitamin C concentrations increased in the berry group supports the interpretation.

Effect of berry consumption on blood pressure

The intervention had an overall effect on both SBP and DBP ($P = 0.050$ and 0.044, respectively) (**Table 5**). SBP decreased slightly in the berry group (1.5 mm Hg) and increased slightly in the control group (0.5 mm Hg). There was no significant change in DBP in the berry group and only a small increase (0.9 mm Hg) in the control group. There was a significant interaction between group and the baseline values (= covariate) for both SBP and DBP in one-factor ANCOVA ($P < 0.001$ for both), which indicates a subgroup effect. In the subgroup analyses performed for SBP, the difference between groups was significant in the highest tertile only (**Figure 1**). In the highest tertile, the mean decreases in SBP were 7.3 and 0.2 mm Hg in the berry and control groups, respectively ($P = 0.024$). Changes in SBP did not correlate with changes in CADP-CT or HDL cholesterol.

Effect of berry consumption on plasma lipids

Total serum cholesterol and triacylglycerol were unaffected by the intervention (Table 5). Serum HDL-cholesterol concentrations increased significantly more in the berry group than in the control group (5.2% and 0.6%, respectively; $P = 0.006$). There was a correlation between the change in CADP-CT and HDL cholesterol values in the berry group ($r = 0.40$, $P = 0.017$) but not in the control group ($r = 0.09$, $P = 0.611$).

Effect of berry consumption on platelet function and hemostatic biomarkers

Berry consumption significantly ($P = 0.018$) inhibited platelet function, as measured by CADP-CT (Table 5). CTs in this system were prolonged by 11.0% in the berry group and shortened by 1.4% in the control group. The intervention had no significant effect on CEPI-CT values or plasma biomarkers of platelet activation, coagulation, or fibrinolysis (Table 5).

DISCUSSION

In this study, consumption of berries for 2 mo reduced blood pressure, increased HDL-cholesterol concentrations, and prolonged PFA-100 CTs (CADP-CT), which indicated that some of the constituents of berries, alone or in combination with the others, exert hemostatic and vascular effects in vivo. Compliance with the study protocol appeared to be excellent in the intervention. The fact that the amount of berries consumed was moderate (100 g berries + 1 small glass of berry drink/d) and the fact that

TABLE 5
Blood pressure (BP) and biomarkers of lipid metabolism and hemostasis at baseline and after consumption of berries or control products for 8 wk[1]

	Berry group ($n = 35$)		Control group ($n = 36$)		
	Baseline	8 wk	Baseline	8 wk	P[2]
Systolic BP (mm Hg)[3]	127.8 (122.0, 133.6)	126.3 (121.5, 131.1)	131.1 (125.6, 136.7)	131.7 (125.8, 137.5)	0.050
Diastolic BP (mm Hg)[3]	79.6 (76.6, 82.6)	79.6 (76.7, 82.5)	82.07 (79.9, 84.9)	83.04 (79.8, 86.3)	0.044
Cholesterol (mmol/L)[3]	6.25 (5.92, 6.59)	6.20 (5.91, 6.49)	6.34 (6.01, 6.66)	6.27 (5.96, 6.59)	0.228
HDL cholesterol (mmol/L)[3]	1.55 (1.41, 1.68)	1.63 (1.47, 1.79)	1.66 (1.48, 1.84)	1.67 (1.50, 1.84)	0.006
Triacylglycerol (mmol/L)[4]	1.45 (1.35, 1.76)	1.40 (1.30, 1.68)	1.47 (1.35, 1.96)	1.53 (1.40, 2.0)	0.118
CADP-CT (s)[4]	76.3 (70.9, 87.8)	84.7 (79.1, 95.1)	83.1 (77.8, 93.7)	81.9 (77.6, 89.8)	0.018
CEPI-CT (s)[4]	120.7 (111.8, 138.8)	117.8 (109.4, 134.6)	124.5 (114.5, 149.1)	128.2 (117.6, 153.7)	0.213
vWF:Ag[4]	125.6 (117.6, 148.8)	121.1 (113.3, 143.7)	132.4 (119.7, 170.2)	125.9 (115.6, 154.3)	0.860
vWF:RCo[4]	110.3 (104.2, 129.7)	104.7 (98.6, 122.2)	112.2 (106.1, 130.6)	106.8 (100.7, 123.8)	0.876
F1+2[4]	126.9 (120, 169.7)	149.5 (129.1, 243.8)	112.0 (112.9, 173.2)	134.0 (125.9, 187.9)	0.779
Glycoprotein V[4]	44.5 (41.8, 50.5)	44.05 (41.7, 48.5)	45.89 (42.9, 52.3)	42.64 (40.4, 46.9)	0.316
CD40L[4]	93.4 (107.7, 170.5)	107.3 (76.1, 277.9)	156.5 (149.7, 239.9)	127.7 (111.9, 259.7)	0.797
P-selectin[4]	32.8 (30.7, 38.7)	35.9 (33.6, 42.3)	36.6 (33.9, 43.3)	38.1 (35.6, 43.9)	0.392
Fibrinogen[4]	3.21 (3.04, 3.50)	3.33 (3.12, 3.76)	3.27 (3.13, 3.50)	3.14 (3.00, 3.41)	0.108
D-Dimer[4]	405.3 (376.1, 583.3)	387.2 (356.9, 552.1)	465.3 (423.5, 638.3)	410.8 (372.7, 568.2)	0.594
slCAM-1[4]	216.2 (202.7, 247.0)	216.9 (203.6, 247.6)	232.8 (219.1, 265.4)	229.5 (216.4, 255.9)	0.965

[1] CADP-CT and CEPI-CT, closing time in platelet function analyzer with collagen and ADP or collagen and epinephrine as platelet activator; vWF:Ag, von Willebrand factor antigen; vWF:RCo, von Willebrand factor ristocetin cofactor activity; F1+2, prothrombin activation fragment 1+2; sICAM-1, soluble cell adhesion molecule-1.

[2] One-factor ANCOVA with the baseline value as covariate.

[3] Values are $\bar{x}$; 95% CIs in parentheses.

[4] Values are geometric $\bar{x}$; 95% CIs in parentheses.

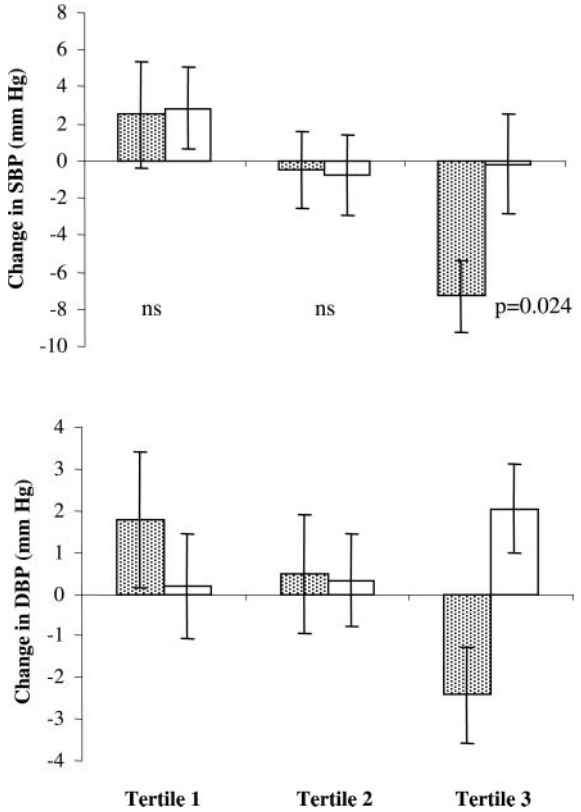

FIGURE 1. Mean ± SEM changes in systolic (SBP) and diastolic (DBP) blood pressure in the berry (▨) and control (□) groups during the intervention according to tertile of baseline value. The values were normally distributed. The overall difference between groups, analyzed by one-factor ANCOVA with baseline value as covariate, was significant (*see* Table 5). The covariate × group interaction was significant ($P < 0.0001$ for both SBP and DBP), which indicates a subgroup effect. Therefore, the data were divided into tertiles according to baseline blood pressure value; the ranges were 102.0–121.3, 122.0–132.7, and 133.3–184.7 mm Hg for SBP, and 62.7–77.3, 78.0–84.7, and 86.7–99.3 mm Hg for DBP in tertiles 1, 2, and 3, respectively. After that, a 2-factor ANOVA, with change from baseline as the dependent variable and group and tertile as independent factors, was performed for SBP and DBP. A significant ($P = 0.050$) group × tertile interaction was observed for SBP but not for DBP. Therefore, a one-factor ANCOVA with baseline value as the covariate was performed separately for each tertile of SBP (but not DBP). The groups differed significantly ($P = 0.024$) in the highest tertile of SBP.

different good-tasting berries or berry products were used likely contributed to the high compliance. During the intervention, the subjects consumed, in addition to their habitual diets, either fresh-frozen berries and lightly processed berry products or control products. The berry products were prepared from black currants, lingonberries (similar to small cranberries), bilberries (sometimes called European blueberries), and chokeberries, as well as from small amounts of strawberries, red raspberries, and sucrose. The abovementioned are the most commonly consumed berries in the Nordic countries (except for chokeberries, which are more commonly consumed in some eastern European countries). We used a combination of different berries, instead of only one berry type, to ensure a high intake of various polyphenols and to minimize the intake of other bioactive components obtained from the individual berry types (which represented 3 different families and 5 different genera). As seen in Table 3, all berries provided polyphenols of various types. Vitamin C was mainly obtained from black currants. The calculated intakes of energy, energy-containing nutrients, vitamins (except vitamin C), and minerals did not differ significantly between the 2 groups during the intervention.

According to the intake and bioavailability data obtained in this study, polyphenols and vitamin C are the most likely berry constituents to exert effects in vivo after the consumption of berries. However, although the intake and plasma concentrations of vitamin C increased in the berry group, it is questionable whether changes of this magnitude would result in measurable changes in CVD biomarkers in persons with adequate intakes. In contrast, the estimated intake of total polyphenols in the berry group was 3 times that in the control group [assuming a median intake of ≈400 mg from the habitual diet (33, 34)]. Consequently, the plasma concentrations of polyphenols, such as quercetin, caffeic acid, protocatechuic acid, p-coumaric acid, and vanillic acid, were significantly greater in the berry group.

Current knowledge of the bioactivities and health effects of the known constituents of berries indicates that polyphenols, rather than ascorbic acid (35, 36), are responsible for the effects observed in the present study. Previous studies showed that polyphenols such as quercetin and catechins exhibit potentially beneficial changes in pathways related to cardiovascular health. However, other, less studied compounds such as the polyphenol metabolites could exhibit similar effects. It is interesting that many polyphenols and polyphenol metabolites contain an orthodiphenolic structure—ie, 2 adjacent hydroxyl groups on the aromatic B ring—which is particularly efficient in antioxidation (37). However, several mechanisms (related or unrelated to antioxidation) could mediate the vascular and hemostatic effects observed for polyphenols (1, 4). For instance, effects on hydrogen peroxide production (38), phospholipase C activation (38), calcium signaling (39), thrombin formation (39) and signaling (40), platelet activation (41, 42), and nitric oxide–mediated glycoprotein IIb/IIIa down-regulation (43) have been reported.

In the present study, berry consumption inhibited platelet function (CADP-CT), reduced blood pressure, and increased HDL concentrations. We hypothesize that the results may be partly explained by changes in nitric oxide metabolism. Similar results have been observed in animal studies investigating agents that affect nitric oxide (alone or in combination with polyphenols). In one study, administration of a nitric oxide synthase inhibitor decreased HDL cholesterol and increased total cholesterol, blood pressure, and plasma procoagulant biomarkers, whereas the administration of a nitric oxide donor resulted in opposite effects (44). In another study, supplementation with regular or dealcoholized red wine prolonged bleeding time and decreased platelet adhesion and thrombus weight, whereas the administration of a nitric oxide inhibitor prevented the effects (45). The PFA-100 was not used as a model in those studies, but other investigators (46) have shown that in vitro incubation of human blood with L-arginine, a precursor of nitric oxide, prolongs CADP-CT.

The observed changes in HDL cholesterol and blood pressure are noteworthy, particularly because the amount of berries consumed was quite moderate. Changes in these risk factors that are of the same magnitude as those observed in this study are clinically relevant. To put this into perspective, it has been reported that a 1% decrease in the cardiovascular event rate was seen for each 1% increase in HDL cholesterol (47). Therefore, a 5% increase, such as we observed, is meaningful. For blood pressure,

a net decrease of 2.1/0.9 mm Hg would reduce CVD mortality by 5–6% (48). A greater decrease was observed in the hypertensive subjects, and thus the result can be considered promising.

The inhibition of platelet function after berry consumption (as indicated by the increase in CADP-CT values) is intriguing. The PFA-100 system mimics the high-shear conditions occurring in the bloodstream during plug formation. Several studies indicated that short PFA-100 CTs may be a previously unrecognized risk factor for acute coronary syndromes (20–22). It is interesting that the measurement may be influenced by dietary polyphenols [cocoa and chocolate have been studied (10, 23)]. It should be noted that the mechanisms involved with PFA-100 measurements still are incompletely understood, although the importance of von Willebrand factor and the effect of aspirin (on CEPI-CT only) are well established (49). The consumption of berries did not have an effect on CEPI-CT or plasma biomarkers of hemostatic function. The fact that we used fasting blood samples may partly explain the lack of effect on short-lived biomarkers such as F1 + 2 and CD40L.

In conclusion, we found favorable changes in platelet function, blood pressure, and HDL cholesterol after the consumption of berries for 2 mo. The findings are important, because they may partly explain the CVD-protective role of a diet rich in fruit and vegetables. Other types of studies are now warranted to identify the compounds and mechanisms that are responsible for the observed effects.

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