Effects of Low Habitual Cocoa Intake on Blood Pressure and Bioactive Nitric Oxide

A Randomized Controlled Trial

Dirk Taubert, MD, PhD
Renate Roesen, PhD
Clara Lehmann, MD
Norma Jung, MD
Edgar Schömig, MD

Context Regular intake of cocoa-containing foods is linked to lower cardiovascular mortality in observational studies. Short-term interventions of at most 2 weeks indicate that high doses of cocoa can improve endothelial function and reduce blood pressure (BP) due to the action of the cocoa polyphenols, but the clinical effect of low habitual cocoa intake on BP and the underlying BP-lowering mechanisms are unclear.

Objective To determine effects of low doses of polyphenol-rich dark chocolate on BP.

Design, Setting, and Participants Randomized, controlled, investigator-blinded, parallel-group trial involving 44 adults aged 56 through 73 years (24 women, 20 men) with untreated upper-range prehypertension or stage 1 hypertension without concomitant risk factors. The trial was conducted at a primary care clinic in Germany between January 2005 and December 2006.

Intervention Participants were randomly assigned to receive for 18 weeks either 6.3 g (30 kcal) per day of dark chocolate containing 30 mg of polyphenols or matching polyphenol-free white chocolate.

Main Outcome Measures Primary outcome measure was the change in BP after 18 weeks. Secondary outcome measures were changes in plasma markers of vasodilative nitric oxide (*S*-nitrosoglutathione) and oxidative stress (8-isoprostane), and bioavailability of cocoa polyphenols.

Results From baseline to 18 weeks, dark chocolate intake reduced mean (SD) systolic BP by –2.9 (1.6) mm Hg ($P < .001$) and diastolic BP by –1.9 (1.0) mm Hg ($P < .001$) without changes in body weight, plasma levels of lipids, glucose, and 8-isoprostane. Hypertension prevalence declined from 86% to 68%. The BP decrease was accompanied by a sustained increase of *S*-nitrosoglutathione by 0.23 (0.12) nmol/L ($P < .001$), and a dark chocolate dose resulted in the appearance of cocoa phenols in plasma. White chocolate intake caused no changes in BP or plasma biomarkers.

Conclusions Data in this relatively small sample of otherwise healthy individuals with above-optimal BP indicate that inclusion of small amounts of polyphenol-rich dark chocolate as part of a usual diet efficiently reduced BP and improved formation of vasodilative nitric oxide.

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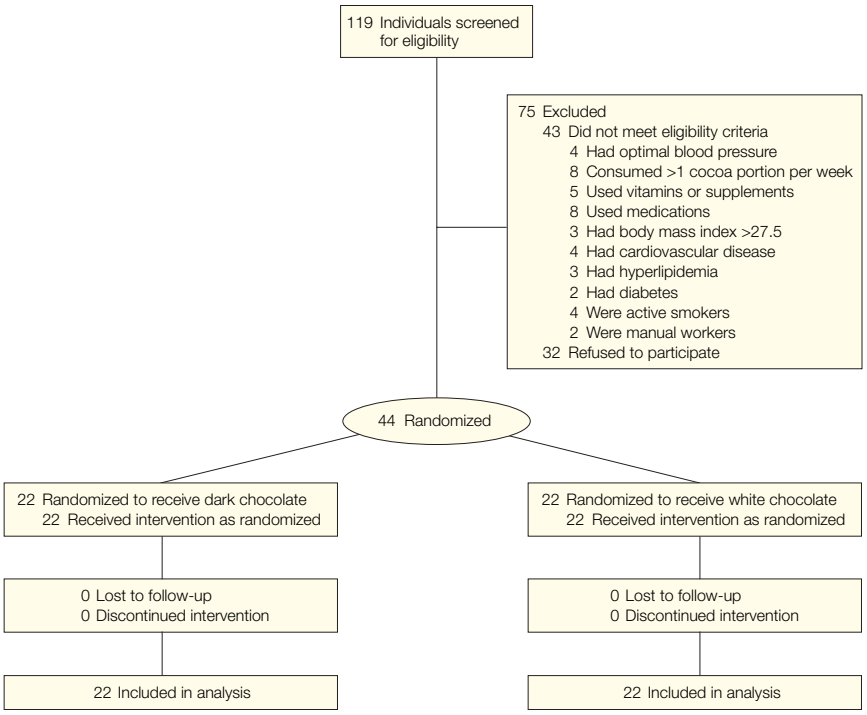
DIETS RICH IN FRUITS AND VEGetables are among the recommended lifestyle modifications to lower blood pressure (BP) and to reduce cardiovascular disease risk, specifically in prehypertension or stage 1 hypertension.[1,2] Although a variety of factors may contribute to the beneficial effects of plant foods, much attention has been given to the plant polyphenols.[3-5] Apart from fruits and vegetables, cocoa products contribute to a major proportion of total phenol intake in Western countries,[6,7] but they are not implemented in current dietary treatment advice. Cocoa liquor is especially rich in a specific subclass of polyphenols, the flavanols, that have been suggested to mediate the favorable effects of cocoa products on cardiovascular health.[8] Consumption of flavanol-rich cocoa was found to lower BP and improve endothelial function in human intervention studies. However, these effects were observed with high doses of cocoa above the habitual intake (equivalent to at least 100 g of chocolate per day) and only in the setting of short-term interventions with a maximum follow-up of 2 weeks[9-12] or in single-dose assessments.[13,14] Hence, available clinical evidence is preliminary and insufficient to recommend cocoa as an efficient antihypertensive dietary treatment option as summarized in a recent meta-analysis.[15] A particular concern is that the potential BP reduction contrib-

Author Affiliations: Departments of Pharmacology (Drs Taubert, Roesen, and Schömig) and Internal Medicine (Drs Lehmann and Jung), University Hospital of Cologne, Cologne, Germany.
Corresponding Author: Dirk Taubert, MD, PhD, Department of Pharmacology, University Hospital of Cologne, Gleueler Strasse 24, D-50931 Cologne, Germany (dirk.taubert@medizin.uni-koeln.de).

Figure 1. Flow of Participants Through the Phases of the Study



Cardiovascular disease included myocardial infarction or coronary heart disease. Body mass index is calculated as weight in kilograms divided by height in meters squared.

uted by the flavanols could be offset by the high sugar, fat, and calorie intake with the cocoa products. Furthermore, the mechanism of the BP reduction remains elusive. One of the suggested pathways is that the cocoa flavanols can enhance the formation of endothelial nitric oxide leading to vasodilation and thereby lowering BP.[8] However, no previous trial has evaluated the relation of plasma phenols, bioactive nitric oxide and BP.

The aim of this randomized controlled study was to assess the clinical efficacy of low habitual amounts of cocoa for BP reduction and to substantiate the hypothesis that cocoa phenol–stimulated nitric oxide synthesis is causative for BP lowering.

METHODS

Study Participants

The study was conducted between January 2005 and December 2006. Participants were unpaid volunteers recruited from a primary health care unit in Duisburg, Germany. Volunteers of both sexes, between 55 and 75 years of age, in good general health except for upper-range prehypertension (BP between 130/85 and 139/89 mm Hg) or stage 1 hypertension (BP between 140/90 and 160/100 mm Hg), not taking antihypertensive medications or nutritional supplements, and with normal plasma lipid and plasma glucose levels were eligible. Included participants were nonmanual workers or pensioners of higher socioeconomic status (household income ≥€20 000/y [US $26 500/y]), selected to aid consistency of energy expenditure. Individuals were excluded if they had cardiovascular disease; diabetes mellitus; hyperlipidemia; gastrointestinal tract disease; hepatic and renal disorders; pulmonary disease; coagulopathy; cancer; psychiatric disorders; alcohol or drug dependence; seizure disorders; history of organ transplantation; surgery within the last 12 months; positive test results for human immunodeficiency virus; hepatitis B or C; a body mass index (calculated weight in kilograms divided by height in meters squared) of more than 27.5 or less than 18.5; actively smoked tobacco within the last 5 years or had regularly taken medications or had taken any medication within the last 2 weeks before entry; used vitamin, mineral, or polyphenol supplements or food supplemented with biologically active compounds; or were regular consumers of chocolate or other cocoa products of more than 1 serving per week.

One hundred nineteen volunteers responded to an invitation to participate in an 18-week study to "assess the relation of dietary habits and health status" and were screened by evaluation of medical history, physical examination, laboratory parameters, and assessment of the individuals' habitual diet using a validated standardized semiquantitative food-frequency questionnaire.[16,17] Forty-three individuals did not meet eligibility criteria. After disclosing the planned chocolate intervention, 32 declined to participate. The remaining 44 (24 women, 20 men) were allocated to treatments with dark or white chocolate; all of them completed the study and were included in the per-protocol analysis (FIGURE 1).

The study was approved by the ethics committee of the Medical Faculty of the University of Cologne. All participants gave written informed consent.

Study Design

Eligible participants were assigned to the dark or white chocolate treatment groups by permuted randomization in sex-stratified blocks of 4 persons each, sequentially allocated to dark chocolate (2) and white chocolate (2) using a computer-generated random number sequence. To conceal allocation from investigators, instructed trained staff at a separate site not involved with the trial generated and maintained the randomization list and prepared the chocolate. Chocolate doses for each patient were wrapped in aluminum foil and provided in dated, sequentially numbered, sealed, nontransparent bags that transferred no information about the content.

All clinical investigations, dietary assessments, laboratory tests, data collection, and data analysis were performed by physicians and trained staff who were blinded to group assignment. Participants received no information about their examination data and the exact objective of the study until trial completion. It was impossible to blind the participants to the intervention because no polyphenol-free and polyphenol-rich chocolate of identical appearance was commercially available. Also, removal of the polyphenols was obvious due to the loss of bitter and astringent taste.[18] Participants were instructed that disclosing their group assignment to investigators would result in exclusion from the study.

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Participants were counseled to maintain their usual diet and physical activity and to abstain from other cocoa products during the study. For accurate estimation of nutrient intake during the study, participants noted their actual daily food intake in precoded food diaries with 127 food items in 14 food groups adapted from the food-frequency questionnaire using common household measures to define standard serving sizes (eg, slices, tablespoons, glasses).[19] Relative validity of the food diary was determined by comparison of the nutrient and energy estimates derived from nutrient databases (eg, of the US Department of Agriculture)[20] with the results from 3-day weighed food records of 2 weekdays and 1 weekend day,[21] yielding acceptable agreement with Spearman correlation coefficients between 0.71 (dietary fiber; $P < .001$) and 0.92 (carbohydrate; $P < .001$). Baseline values of nutrient and food intake were derived from food diary data during the run-in phase. Physical activity was assessed by self-report of the number of predefined daily exercises estimated to represent 1 metabolic equivalent task,[22] eg, 30-minute walking, 15-minute bicycling, 15-minute swimming. Energy expenditure of daily living, including usual household and occupational activities, was not measured.

Table 1. Energy, Nutrient, Mineral, and Specific Component Intake With Dark Chocolate (6.3 g) and White Chocolate (5.6 g)[a]

Content Per Dose	Dark Chocolate	White Chocolate
Energy, kcal	30	30
Total fat, g	1.72	1.66
Carbohydrates, g	3.38	3.54
Protein, g	0.26	0.26
Sodium, mg	3.8	3.6
Potassium, mg	16.6	16.1
Calcium, mg	6.6	8.1
Magnesium, mg	1.9	1.4
Gallic acid, mg	1.4	BLD
Catechin, mg	1.7	BLD
Epicatechin, mg	5.1	BLD
Epicatechin-gallate, mg	0.3	BLD
Procyanidin dimer, mg	6.8	BLD
Procyanidin dimer-gallate, mg	1.8	BLD
Procyanidin trimer, mg	5.3	BLD
Procyanidin trimer-galate, mg	1.0	BLD
Procyanidin tetramer, mg	3.7	BLD
Procyanidin pentamer, mg	2.6	BLD
Flavonols, mg[b]	<0.05	BLD
Caffeine, mg	3.1	BLD
Theobromine, mg	26.4	BLD

Abbreviation: BLD, below the limit of detection.
[a] Energy and nutrient composition are calculated from data provided by the manufacturers.
[b] Includes quercetin, apigenin, myricetin, kaempferol, and isorhamnetin.

Adherence to the study protocol and the reported habitual food intake was confirmed in weekly visits by direct questioning, returning of the empty bags, and assessment of food diaries. Body weight; physical activity; plasma levels of lipids and glucose; and 24-hour urinary excretion of sodium, potassium, creatinine, and nitrogen were assessed every 6 weeks.

After a cocoa-free run-in period of 7 days and an overnight fast of 12 hours, participants were allocated to receive over 18 weeks either a 6.3-g dose (one piece of a 16-piece bar of 100 g) per day of commercially available polyphenol-rich dark chocolate (Ritter Sport Halbbitter, Alfred Ritter, Waldenbuch, Germany; containing 3.1 g of cacao, a total of 30 mg of polyphenols, and 30 kcal of energy) or a matching 5.6-g dose per day of polyphenol-free white chocolate (Milka Weisse Schokolade, Kraft Foods, Bremen, Germany; containing equal energy and similar amounts of macronutrients and electrolytes) (TABLE 1). The dark chocolate dose was selected because it was at the limit of major cocoa phenol bioavailability and because it contributed less than 2% to total daily energy and nutrient intake and thus could be added to the habitual diet without adjusting other dietary intake. Participants were instructed to ingest the chocolate 2 hours after the evening meal (usually between 8 and 10 PM). Blood pressure and plasma parameters were assessed while each participant was in the 12-hour fasting state between 8 and 10 AM after the run-in period and after 6, 12, and 18 weeks of treatment. To assess acute effects of dark and white chocolate, BP and plasma parameters were assessed in each participant at 0, 60, 120, 240, 360, and 480 minutes after the first chocolate dose following run-in and after another chocolate dose the day after completion of the 18-week treatment period.

Adverse events were monitored every 6 weeks by interview, a 12-item checklist of symptoms that included altered bowel habits, bloating, and nau-

sea; physical examination; and laboratory testing. A systolic BP of more than 170 mm Hg or a diastolic BP of more than 100 mm Hg at a single visit were considered sufficient to refer a participant for antihypertensive pharmacological treatment. After the study was finished, participants were referred to their physician for further monitoring and BP management.

BP Monitoring

Blood pressure measurements were performed in a noise-protected room of constant temperature (24°C) by trained, certified staff using a validated oscillometric device with appropriately sized cuffs (Omron HEM-722C, Omron, Mannheim, Germany).[23] The cuffs were placed on the left upper arm of each participant while in a seated position as instructed by the manufacturer. Participants rested 15 minutes before recordings. At each assessment, 3 readings, taken in intervals of 5 minutes, were averaged to obtain the BP. To further minimize the confounding influence of alerting reactions on BP, measurements were performed at a separate location outside the physician's office and not associated with usual patient care.

Table 2. Baseline Characteristics of Participants Assigned to Dark and White Chocolate[a]

Characteristics	Mean (SD)		*P* Value[b]
	Dark Chocolate (n = 22)	White Chocolate (n = 22)	
Age, y	63.4 (4.7)	63.7 (4.8)	.83
Weight, kg	71.2 (8.0)	70.7 (6.7)	.82
Body mass index	24.0 (1.6)	23.9 (1.5)	.81
Waist circumference, cm	79.2 (3.2)	78.9 (2.8)	.89
Blood pressure, mm Hg			
Systolic	147.7 (7.1)	147.5 (8.0)	.94
Women	147.3 (8.5)	146.4 (9.0)	.82
Men	148.3 (5.3)	148.9 (6.9)	.83
Diastolic	86.4 (4.1)	86.7 (3.8)	.79
Women	86.0 (4.4)	87.3 (4.3)	.49
Men	86.8 (3.8)	86.0 (3.1)	.61
Heart rate, beats/min	67.5 (5.6)	67.5 (5.3)	.98
Physical activity, exercises/d[c]	0.3 (0.1)	0.3 (0.2)	.90
Cholesterol, mg/dL			
Total	189 (14)	190 (15)	.83
LDL	120 (10)	121 (10)	.75
HDL	49 (8)	50 (9)	.91
Triglycerides, mg/dL	100 (14)	96 (15)	.86
Glucose, mg/dL	89 (6)	88 (7)	.82
S-nitrosoglutathione, nmol/L	0.33 (0.14)	0.32 (0.15)	.84
Women	0.31 (0.17)	0.33 (0.14)	.80
Men	0.35 (0.10)	0.31 (0.17)	.53
Total 8-isoprostane, pmol/L	490 (104)	489 (102)	.98
Women	490 (122)	488 (128)	.97
Men	489 (83)	489 (64)	.99
Urinary values per 24 h			
Nitrogen, g	10.6 (2.5)	10.7 (2.6)	.82
Creatinine, g	1.46 (0.36)	1.43 (0.33)	.93
Sodium, mEq	157.2 (24.4)	155.7 (23.2)	.77
Potassium, mEq	54.6 (9.6)	53.9 (8.5)	.80

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.
SI conversion factors: To convert total, LDL, and HDL cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113; glucose to mmol/L, multiply by 0.0555; creatinine to mmol, multiply by 8.84. Body mass index is calculated as weight in kilograms divided by height in meters squared.
[a] Data are normally distributed.
[b] *P* values are calculated by unpaired 2-tailed *t* test.
[c] Physical activity is expressed as the number of exercises per day corresponding to 1 metabolic equivalent task.

Laboratory Analysis

Venous blood was drawn into tubes containing EDTA, and plasma was obtained by immediate centrifugation at 3000*g* for 5 minutes at 4°C, snap frozen in liquid nitrogen, and stored at –80°C until analysis.

Total (free and esterfied) plasma 8-isoprostane (8-epi-PGF_2) was measured using a commercially available immunoassay (EIA kit No. 516351, Cayman Chemical Co, Ann Arbor, Michigan) pursuant to the manufacturer's instructions.

For measurement of *S*-nitrosoglutathione, following precipitation of proteins with acetonitrile (2:1 v/v), plasma samples were analyzed by liquid chromatography tandem mass spectrometry using positive-electrospray ionization by single-reaction monitoring of the precursor ion $[M+H]^+ \rightarrow$ product ion transition m/z 337 → 307 with thiamazole (0.5 µg/mL, m/z 115 → 57) as internal standard. The lower limit of detection was 0.1 nmol/L, intraday and interday precision were 5.2% and 7.6%, respectively.

Major cocoa polyphenols and methylxanthines were determined in the defatted chocolate extracts by liquid chromatography tandem mass spectrometry analysis using negative-electrospray ionization in single-reaction monitoring mode.[24-26] Composition per study dose of 6.3 g of dark chocolate and 5.6 g of white chocolate is provided in Table 1. Plasma concentrations of the phenols were determined in acetonitrile-precipitated samples. The lower limit of detection was 0.1 ng/mL.

Plasma concentrations of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and glucose were determined by conventional enzymatic assays. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula.

Twenty-four-hour urine collections were obtained on day 7 of the run-in and after 6, 12, and 18 weeks of the intervention period.

Pharmacokinetic plasma parameters of cocoa phenols from assessments at the beginning and at the end

of the study were obtained by fitting the individual concentration-time data by a linear 1-compartment model using WinNonlin Professional version 2.1 (Pharsight Corporation, Palo Alto, California).

Data Analysis and Sample Size Calculation

Nominal *P* values are presented. $P < .05$ is considered a statistically significant difference. Normal distribution was assessed by the D'Agostino and Pearson omnibus normality test.[27] Pairwise within-group differences were assessed using the paired *t* test and between-group differences by the unpaired *t* test. For greater statistical power between-group differences in outcome were also reported using an analysis of covariance adjustment of baseline imbalances. For multiple pairwise comparisons, *P* values were adjusted by the method of Holm.[28] Overall significance of differences comparing more than 2 measurements in the same individual was evaluated by repeated measures analysis of variance. Linear correlation between 2 variables was assessed by the Pearson test. The coefficient of correlation *r* is given.

The minimum sample size required to determine whether blood pressure was affected by the cocoa treatments was determined using the paired *t* test by imputing a standard deviation of the change in systolic BP of 2.0 mm Hg and in diastolic blood pressure of 1.5 mm Hg from our previous intervention with 100 g of dark chocolate over 2 weeks in a very similar population.[9] From studies with antihypertensive drugs,[29] a decrease in systolic BP of 1.5 mm Hg and in diastolic BP of 1.0 mm Hg was considered the limit for significant cardiovascular risk reduction. To detect this difference at a power of 0.8 with 95% confidence, a minimal sample size of 20 individuals in each group was calculated.

All analyses were performed using SPSS version 11.0 and SigmaStat version 3.0 (SPSS Inc, Chicago, Illinois).

RESULTS

Baseline Characteristics

At baseline, the groups did not differ in terms of anthropometric and hemodynamic measures; food, energy and nutrient intake; energy expenditure from physical activity; levels of plasma lipids and glucose, *S*-nitrosoglutathione, 8-isoprostane; or urinary excretion of sodium, potassium, creatinine, and total nitrogen (TABLE 2 and TABLE 3). There were also no differences in characteristics between women and between men of both groups and no within-group sex contrasts in systolic and diastolic BP, or *S*-nitrosoglutathione and 8-isoprostane levels (Table 2). The food-frequency questionnaire data revealed a very low prestudy consumption of cocoa products with a mean (SD) of 0.9 (0.5) servings per month in participants assigned to dark chocolate and 1.1 (0.6) in participants assigned to white chocolate ($P = .69$). Five participants in the dark chocolate group and 3 in the white chocolate group drank 1 to 2 cups of black tea per day, 2 participants in the dark chocolate group and 3 in the white chocolate group drank from 0.5 to 2 glasses of red wine per week.

Adherence to the Study Protocol

All participants completed the study and attended all monitoring sessions.

Table 3. Baseline Energy and Nutrient Intake and Consumption of Key Food Items of Participants Assigned to Dark and White Chocolate[a]

	Mean (SD)		
	Dark Chocolate (n = 22)	White Chocolate (n = 22)	*P* Value[b]
Daily energy and nutrient intake			
Total energy, kcal/d	1992 (50)	1993 (62)	.89
Fat, % of total energy	33.8 (1.7)	33.3 (1.9)	.85
Saturated	14.5 (0.9)	14.3 (0.7)	.81
Monounsaturated	10.9 (0.8)	11.2 (0.7)	.78
Polyunsaturated	8.4 (0.6)	7.8 (0.8)	.64
Carbohydrates, % of total energy[c]	48.2 (2.1)	48.4 (1.9)	.93
Alcohol, % of total energy[c]	2.4 (0.9)	2.2 (1.1)	.71
Protein, % of total energy[c]	15.6 (1.1)	16.1 (1.4)	.58
Total dietary nitrogen, g[d]	13.1 (1.4)	13.5 (1.6)	.62
Fiber, g per 1000 kcal	10.0 (1.3)	10.5 (1.3)	.88
Cholesterol, mg per 1000 kcal	148.6 (11.7)	147.3 (12.6)	.76
Food groups, servings/d			
Fruits and fruit juices	2.6 (0.9)	2.4 (0.6)	.67
Vegetables	2.1 (0.5)	2.1 (0.5)	.91
Grains, refined and whole	4.2 (1.1)	3.8 (1.2)	.60
French fries, potatoes	0.7 (0.3)	0.8 (0.2)	.82
Low-fat dairy products	0.8 (0.3)	0.9 (0.5)	.68
High-fat dairy products	1.6 (0.8)	1.4 (1.0)	.58
Fats and oils	3.4 (0.5)	3.3 (0.7)	.72
Meat[e]	2.2 (0.7)	2.4 (0.7)	.88
Poultry	0.9 (0.6)	0.8 (0.4)	.65
Fish and seafood	0.2 (0.2)	0.3 (0.2)	.67
Eggs	0.2 (0.1)	0.2 (0.1)	.96
Sweets and desserts	2.6 (0.7)	2.5 (1.0)	.69
Coffee or tea	2.4 (0.8)	2.5 (0.6)	.83
Alcohol	0.9 (0.4)	0.8 (0.5)	.76

[a] Values are derived from precoded food diaries during the 7-day run-in. Data are normally distributed.
[b] *P* values are calculated by unpaired 2-tailed *t* test. $P < .05$ is considered a statistically significant difference.
[c] Energy values were calculated as follows: for protein, 4.0; for carbohydrate minus insoluble fiber 4.0; for fat, 9.0; and for alcohol, 6.9 kcal/g.
[d] Dietary nitrogen is calculated from protein in grams divided by 6.25.
[e] Includes beef, pork, lamb, veal, ham, sausage.

None of the data were lost. According to the participants' self-reports and the weekly monitoring of the returned empty bags, all chocolate portions were eaten and no other cocoa products were consumed. However, 14 of 2772 (0.5%) of the dark chocolate and 22 of 2772 (0.8%) of the white chocolate portions were consumed up to 24 hours after the scheduled time points. Participants showed strict adherence to the reported habitual frequency of food intake and physical activity. From the participants' food diaries, we calculated no changes in nutritional composition of diets from the run-in phase to the end of the intervention (TABLE 4). Due to the marginal energy, nutrient, and electrolyte contribution of the daily dark or white chocolate doses to the total diet; energy intake; body weight; plasma lipids and glucose levels; and urinary excretion of sodium, potassium, creatinine, and total nitrogen did not change during the study (Table 4 and TABLE 5).

Adverse Events

According to participants' own reports and results of physical examination and laboratory parameters, no adverse event occurred in either treatment group. None of the participants reached the predefined BP threshold that required antihypertensive drug therapy. None of the participants received any medication during the study.

Outcomes

Blood Pressure. Compared with baseline, systolic and diastolic BP declined progressively over time among participants who received dark chocolate. From baseline to 6 weeks, BP reductions were not significant, from baseline to 12 weeks systolic BP declined by a mean (SD) of –2.4 (1.4) mm Hg and diastolic BP by –1.3 (0.6) mm Hg, and from baseline to 18 weeks systolic BP decreased by –2.9 (1.6) mm Hg and diastolic BP by –1.9 (1.0) mm Hg. Systolic and diastolic BP remained unchanged throughout the treatment period among those in the white chocolate group.

At baseline, 19 of the 22 participants (86%) in the dark chocolate group

Table 4. Changes in Energy and Nutrient Intake and Consumption by Key Food Items by Participants During Dark and White Chocolate Interventions[a]

	Dark Chocolate Group, Mean (SD) Changes From Baseline by Week				White Chocolate Group, Mean (SD) Changes From Baseline by Week			
	6	12	18	*P* Value[b]	6	12	18	*P* Value[b]
Changes in daily energy and nutrient intake								
Total energy, kcal/d	9.5 (21.4)	11.9 (16.8)	7.2 (16.6)	.94	4.8 (19.1)	–2.3 (14.3)	9.6 (14.4)	.90
Fat, % of total energy	0.3 (0.4)	0.4 (0.6)	0.2 (0.4)	.83	–0.4 (0.8)	0.8 (1.0)	0.1 (0.4)	.71
Saturated	0.1 (0.2)	0.3 (0.3)	–0.1 (0.5)	.68	–0.4 (0.5)	0.4 (0.8)	0.0 (0.7)	.74
Monounsaturated	0.2 (0.4)	0.0 (0.4)	0.2 (0.3)	.84	0.1 (0.3)	0.3 (0.5)	0.2 (0.4)	.83
Polyunsaturated	–0.1 (0.3)	0.1 (0.5)	0.2 (0.4)	.76	–0.1 (0.4)	0.2 (0.6)	0.1 (0.5)	.80
Carbohydrates, % of total energy	–0.5 (0.6)	–0.4 (0.4)	–0.4 (0.4)	.91	–0.1 (0.6)	–0.4 (0.3)	–0.2 (0.6)	.89
Alcohol, % of total energy	–0.1 (0.3)	–0.2 (0.4)	0.0 (0.4)	.77	–0.2 (0.4)	0.0 (0.3)	–0.1 (0.4)	.78
Protein, % of total energy[c]	0.5 (0.7)	0.5 (0.5)	0.4 (0.5)	.79	0.5 (0.4)	-0.3 (0.6)	0.3 (0.4)	.62
Total dietary nitrogen, g[d]	0.3 (0.4)	0.3 (0.3)	0.2 (0.4)	.85	0.3 (0.3)	-0.2 (0.4)	0.2 (0.3)	.71
Fiber, g per 1000 kcal[c]	–0.4 (0.8)	0 (1.2)	0.1 (1.3)	.94	–0.1 (1.2)	0.4 (0.9)	0 (1.1)	.95
Cholesterol, mg per 1000 kcal	3.3 (5.0)	4.6 (7.8)	0.8 (4.3)	.66	–2.5 (5.4)	3.8 (6.8)	2.9 (7.1)	.60
Food groups, servings/d								
Fruits and fruit juices	–0.1 (0.5)	0.0 (0.6)	0.2 (0.8)	.68	0.0 (0.4)	0.0 (0.5)	0.1 (0.6)	.80
Vegetables	0.0 (0.3)	0.1 (0.3)	0.1 (0.3)	.72	0.0 (0.4)	–0.2 (0.4)	0.0 (0.5)	.78
Grains (refined and whole)	–0.3 (0.8)	–0.2 (0.9)	–0.1 (0.4)	.62	–0.1 (0.6)	–0.1 (0.6)	0.0 (0.4)	.83
French fries, potatoes	0.0 (0.2)	0.0 (0.1)	0.0 (0.2)	.97	0.0 (0.4)	–0.2 (0.5)	0.1 (0.4)	.75
Low-fat dairy products	–0.1 (0.3)	–0.1 (0.3)	0.0 (0.4)	.79	–0.1 (0.5)	0.0 (0.4)	0.0 (0.4)	.84
High-fat dairy products	0.1 (0.4)	0.2 (0.6)	0.1 (0.4)	.83	–0.1 (0.4)	0.0 (0.3)	1.4 (1.0)	.58
Fats and oils	0.0 (0.3)	0.0 (0.3)	–0.1 (0.2)	.88	0.0 (0.3)	-0.2 (0.5)	0.1 (0.4)	.76
Meat[e]	0.1 (0.2)	0.2 (0.3)	0.0 (0.2)	.77	–0.1 (0.3)	0.1 (0.2)	0.0 (0.2)	.79
Poultry	0.0 (0.2)	0.1 (0.3)	0.2 (0.2)	.63	0.0 (0.2)	0.1 (0.3)	0.0 (0.3)	.73
Fish and seafood	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	.92	0.0 (0.1)	0.1 (0.2)	0.0 (0.2)	.86
Eggs	0.0 (0.1)	0.0 (0.1)	0.0 (0.2)	.93	0.0 (0.2)	0.0 (0.1)	0.0 (0.1)	.95
Sweets and desserts	–0.2 (0.4)	–0.1 (0.5)	–0.2 (0.3)	.74	–0.1 (0.5)	–0.2 (0.4)	–0.2 (0.3)	.72
Coffee or tea	0.1 (0.2)	0.0 (0.2)	0.0 (0.2)	.92	0.0 (0.1)	0.0 (0.2)	0.0 (0.2)	.96
Alcohol	–0.1 (0.3)	–0.1 (0.3)	0.0 (0.2)	.87	–0.2 (0.3)	0.1 (0.4)	–0.1 (0.3)	.77

[a] Values are derived from precoded food diaries. All change values are normally distributed.
[b] *P* values of overall differences between treatment periods are calculated by 1-way repeated measures analysis of variance. $P < .05$ is considered a statistically significant difference.
[c] Energy values were calculated as follows: for protein, 4.0; for carbohydrate minus insoluble fiber, 4.0; for fat, 9.0; and for alcohol, 6.9 kcal/g.
[d] Dietary nitrogen is calculated from protein in grams divided by 6.25.
[e] Includes beef, pork, lamb, veal, ham, and sausage.

had stage 1 hypertension and 3 (14%), upper-range prehypertension. In the white chocolate group, 18 (82%) had stage 1 hypertension and 4 (18%), upper-range prehypertension. After 18 weeks, all members of the dark chocolate group had lower systolic or diastolic BP and 4 individuals (18%) had changed classification from hypertension to upper-range prehypertension (mean BP reduction, –3.3/–2.3 mm Hg), corresponding to a 21% relative risk reduction of the hypertensive state. However, none of the participants achieved lower-range prehypertension (<130/85 mm Hg) or an optimal BP (<120/80 mm Hg). After 18 weeks' consuming white chocolate, none had changed BP classification.

In the dark chocolate group, the magnitude of BP reductions was higher in individuals with higher baseline BP. The changes in systolic and diastolic BP from baseline to 18 weeks were linearly correlated with systolic and diastolic baseline BP, respectively (r=–0.61, P=.003 for systolic BP; r=–0.71, P<.001 for diastolic BP). In the white chocolate group, no significant correlations were observed (FIGURE 2).

***S*-Nitrosoglutathione.** Compared with baseline, after dark chocolate intake, the 12-hour fasting plasma levels of *S*-nitrosoglutathione increased progressively over time while levels were unchanged for white chocolate intake (Table 4). In the dark chocolate group individual changes in *S*-nitrosoglutathione were strongly inversely correlated with changes in systolic BP (r=–0.64, P=.001 after 12 weeks; r=–0.80, P<.001 after 18 weeks of treatment) and in diastolic BP (r=–0.72, P<.001 after 12 weeks; r=–0.67; P<.001 after 18 weeks).

8-Isoprostane. Levels of total plasma 8-isoprostane—a reliable and specific biomarker of oxidative stress[30,31]—were not affected by dark or white chocolate intake.

Plasma Phenols. No cocoa phenols were detected in any of the 12-hour fasting plasma samples of dark and white chocolate groups.

Between-Group Changes

FIGURE 3 shows mean (95% confidence interval [CI]) between-group differences in primary and secondary outcomes after 6, 12, and 18 weeks of treatment.

Compared with the white chocolate group, those in the dark chocolate group experienced progressively lowered systolic and diastolic BP over time. This was accompanied by an increase in plasma levels of *S*-nitrosoglutathione but was not associated with differences in the oxidative stress marker 8-isoprostane (Figure 3). Analysis of covariance–adjusted outcome differences pro-

Table 5. Changes in Characteristics of Participants During Dark and White Chocolate Interventions[a]

Characteristics	Dark Chocolate Group, Mean (SD) Change From Baseline by Week				White Chocolate Group Mean (SD) Change From Baseline by Week			
	6	12	18	*P* Value[b]	6	12	18	*P* Value[b]
Weight, kg	–0.08 (1.24)	0.09 (1.18)	0.13 (1.02)	.84	0.12 (1.31)	–0.10 (1.15)	0.14 (1.13)	.80
Body mass index	–0.02 (0.40)	0.03 (0.39)	0.04 (0.36)	.87	0.04 (0.43)	–0.03 (0.38)	0.05 (0.38)	.78
Blood pressure, mm Hg								
Systolic	–0.6 (1.6)[c]	–2.4 (1.4)[d]	–2.9 (1.6)[d]	<.001	–0.1 (1.7)	0.4 (1.9)	0.1 (1.6)	.71
Diastolic	–0.3 (1.1)[e]	–1.3 (0.6)[d]	–1.9 (1.0)[d]	<.001	0.1 (1.9)	0.3 (1.7)	0.0 (1.8)	.84
Heart rate, beats/min	0.2 (2.4)	–0.3 (2.1)	0.1 (2.2)	.71	0.1 (2.0)	0.1 (2.2)	–0.1 (2.0)	.93
Physical activity, exercises/d	0.0 (0.1)	0.0 (0.1)	0.0 (0.2)	.95	–0.1 (0.2)	0.0 (0.2)	0.0 (0.2)	.77
Cholesterol, mg/dL								
Total	–2.7 (2.3)	–2.7 (1.5)	1.2 (3.1)	.82	2.6 (3.0)	0.8 (3.8)	–2.3 (3.1)	.73
LDL	–2.3 (2.0)	–1.9 (2.2)	1.2 (2.3)	.78	2.0 (4.2)	–1.1 (3.1)	–1.5 (2.8)	.69
HDL	1.7 (1.5)	–0.8 (1.7)	1.3 (2.2)	.85	0.4 (4.1)	–3.5 (2.3)	1.8 (1.5)	.68
Triglycerides, mg/dL	–2.6 (6.2)	1.8 (9.6)	–4.4 (7.1)	.71	3.5 (5.3)	–7.0 (6.3)	–1.8 (8.8)	.79
Glucose, mg/dL	0.5 (1.1)	–0.6 (1.6)	–2.2 (2.5)	.46	–0.3 (1.1)	–0.4 (2.0)	0.9 (1.5)	.88
S-nitrosoglutathione, nmol/L	0.02 (0.09)[f]	0.19 (0.11)[d]	0.23 (0.12)[d]	<.001	–0.01 (0.09)	0.00 (0.12)	0.01 (0.12)	.85
Total 8-isoprostane, pmol/L	1 (10)	1 (11)	0 (9)	.97	–2.0 (11)	–1 (10)	1 (12)	.74
Urinary values per 24 h								
Nitrogen, g	0.2 (0.7)	0.1 (0.8)	0.2 (0.7)	.92	–0.1 (0.6)	0.3 (1.0)	0.3 (0.7)	.74
Creatinine, g	0.02 (0.05)	–0.01 (0.06)	–0.01 (0.05)	.87	–0.04 (0.08)	–0.03 (0.06)	0.01 (0.07)	.75
Sodium, mEq	2.6 (8.4)	–0.3 (7.6)	1.7 (6.5)	.74	-1.8 (7.8)	–3.3 (8.2)	–0.9 (7.7)	.80
Potassium, mEq	1.4 (5.3)	1.5 (5.5)	–0.6 (4.8)	.66	1.1 (5.6)	1.9 (6.2)	1.2 (5.0)	.83

SI conversion factors: To convert total, LDL, and HDL cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113; glucose to mmol/L, multiply by 0.0555; creatinine to mmol, multiply by 8.84.

[a] All change values are normally distributed.

[b] *P* values of overall differences between treatment periods are calculated by 1-way repeated measures of analysis of variance. $P < .05$ indicates a significant difference between treatment periods. In the case of an overall significant difference ($P < .05$), pairwise multiple comparisons are performed by paired *t* tests and calculation of Holm-adjusted *P* values with $P < .05$ indicating a significant difference vs baseline.

[c] $P = .16$ vs baseline.

[d] $P < .001$ vs baseline.

[e] $P = .21$ vs baseline.

[f] $P = .36$ vs baseline.

duced similar results; analysis of covariance–fitted differences in means between dark and white chocolate after 18 weeks were –2.9 mm Hg (95% CI, –3.9 to –2.0; $P<.001$) for systolic BP, –1.9 mm Hg (95% CI, –2.7 to –1.1; $P<.001$) for diastolic BP, 0.24 nmol/L (95% CI, 0.17-0.31; $P<.001$) for *S*-nitrosoglutathione and –0.2 pmol/L (95% CI, –6.5 to 6.1; $P=.94$) for 8-isoprostane.

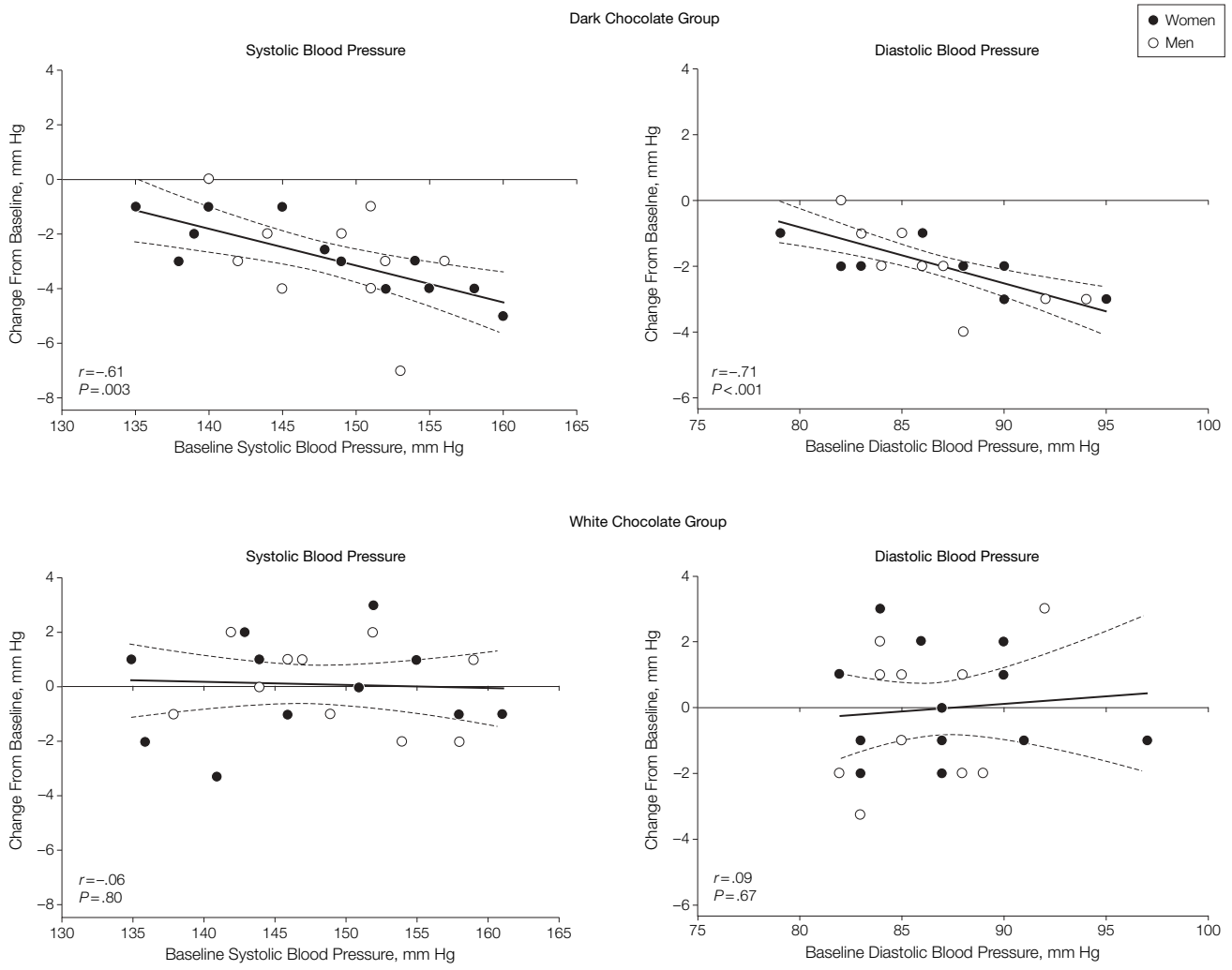
Results were not different between women and men. Compared with white chocolate intake, 18 weeks of dark chocolate intake decreased the systolic BP by a mean change score of –2.9 mm Hg (95% CI, –4.2 to –1.6) in women and –3.0 mm Hg (95% CI, –4.6 to –1.4) in men ($P=.93$). The corresponding reductions in diastolic BP were –1.9 mm Hg (95% CI, –3.0 to –0.8) in women and –1.8 mm Hg (95% CI, –3.3 to –0.3) in men ($P=.89$). Compared with white chocolate, the dark chocolate group's *S*-nitrosoglutathione plasma levels increased by 0.23 nmol/L (95% CI, 0.13-0.33) in women and by 0.25 nmol/L (95% CI, 0.13-0.37) in men ($P=.79$).

Changes in fasting plasma glucose levels did not significantly differ after 18 weeks between groups (–3.1 mg/dL; 95% CI, –7.4 to 1.4; $P=.29$), indicating that unlike short-term interventions with 100 g of dark chocolate,[10,11] the 6.3-g dark chocolate dose was insufficient to affect glucose metabolism or insulin sensitivity. (To convert plasma glucose to millimoles per liter, multiply by 0.0555).

Acute Effects

FIGURE 4 depicts the time course of acute effects of dark chocolate on plasma levels of cocoa phenols, BP, *S*-nitrosoglutathione, and 8-isoprostane. Ingestion of a portion of dark chocolate (6.3 g) resulted in a tran-

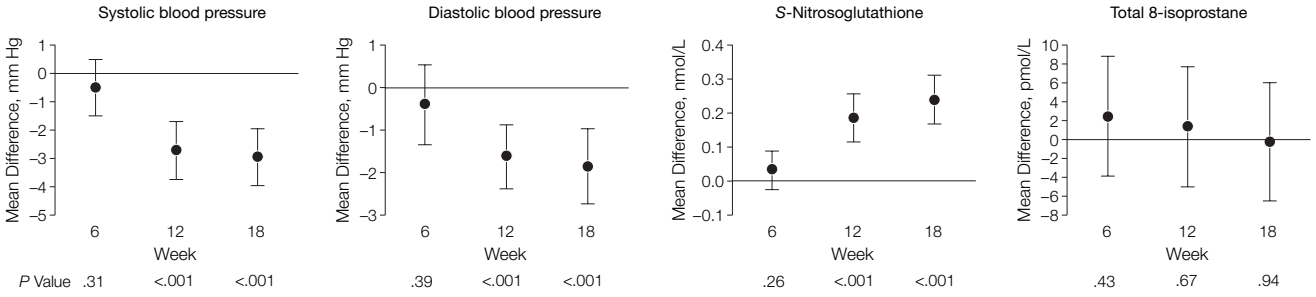
Figure 2. Changes in Blood Pressure After 18 Weeks of Dark or White Chocolate in Relation to Baseline Blood Pressure



Linear regression lines and 95% confidence intervals are depicted. Correlation coefficients *r* and nominal *P* values are calculated by the Pearson test.

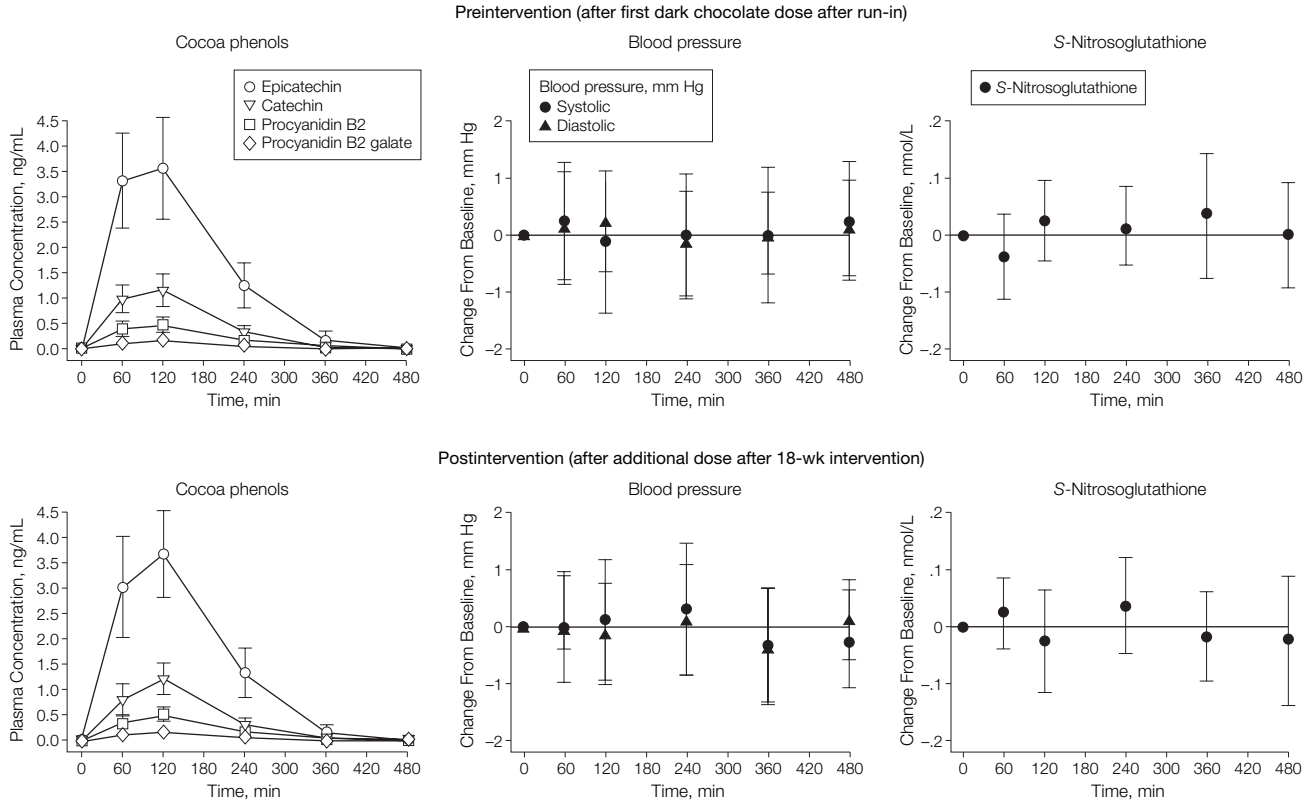
sient elevation of cocoa polyphenols in plasma at 1 to 6 hours. From the phenols that were identified in dark chocolate, only the flavanol monomers epicatechin and catechin and the dimers procyanidin B2 and procyanidin B2 gallate were recovered in plasma. No differences in pharmacokinetic parameters (TABLE 6) were observed between preintervention (first dark chocolate dose after run-in) and postintervention plasma concentrations (additional dark chocolate dose after 18 weeks of dark chocolate intake), which excluded an accumulation of plasma phenols by the once-daily dosing regimen. Compared with the corresponding baseline values, systolic and diastolic BP, *S*-nitrosoglutathione, and 8-isoprostane did not change acutely (Figure 4). After intake of a portion of

Figure 3. Between-Group Comparisons of Blood Pressure, *S*-Nitrosoglutathione, and Total 8-Isoprostane Levels After Dark and White Chocolate



Error bars indicate 95% confidence intervals of differences in mean change scores. Nominal *P* values were calculated for pairwise between-group differences in change by 2-tailed *t* test.

Figure 4. Acute Changes From Baseline in Plasma Levels of Cocoa Polyphenols, Systolic and Diastolic Blood Pressure, and *S*-Nitrosoglutathione After Intake of a 6.3-g Dose of Dark Chocolate



Symbols represent arithmetic means; error bars indicate standard deviation. Plasma levels of cocoa phenols before chocolate intake (t=0) were below the detection limit of 0.1 ng/mL.

white chocolate (5.6 g), no cocoa phenols were detected in any of the plasma samples and BP, and *S*-nitrosoglutathione and 8-isoprostane levels did not change (data available on request).

COMMENT

In this randomized controlled trial, we demonstrated that intake of low habitual amounts of dark chocolate caused progressive reductions of systolic and diastolic BP in older subjects with prehypertension or stage 1 hypertension without inducing weight gain or other adverse effects. Another important finding of our study was that the decrease in systolic and diastolic BP was associated with an increase in circulating levels of vasodilative *S*-nitrosoglutathione, suggesting a causative role of *S*-nitrosoglutathione for BP regulation.

Although the magnitude of the BP reduction was small, the effects are clinically noteworthy. On a population basis, it has been estimated that a 3-mm Hg reduction in systolic BP would reduce the relative risk of stroke mortality by 8%, of coronary artery disease mortality by 5%, and of all-cause mortality by 4%.[1] Furthermore, the blood pressure reductions in our randomized trial are in the same range that were reportedly associated with habitual cocoa intake (a median of 4.2 g per day) in an epidemiological study involving elderly men.[32] However, the high degree of relative risk reduction of about 50% in cardiovascular or all-cause mortality in that study suggests that cocoa or other dietary components may be associated with additional cardioprotective effects beyond BP decrease.

The apparent mechanism by which dark chocolate lowered BP suggests a chronic increase in the production of nitric oxide in the vascular endothelium. The unstable nitric oxide reacts with thiol groups to form stable *S*-nitrosothiols[33] that have been suggested to contribute to BP regulation.[34] *S*-nitrosothiol-albumin—the main constituent of the circulating *S*-nitrosothiols pool—is thought to provide a reservoir of nitric oxide bioactivity, whereas low-mass *S*-nitrosothiols have been proposed to transduce the vasodilative and hypotensive activity.[34] Hence, our finding that individual changes in circulating levels of *S*-nitrosoglutathione—the key low-mass *S*-nitrosothiol—explained much of the variability in systolic and diastolic BP reduction provides strong support for this hypothesis.

It is likely that the cocoa flavanols in dark chocolate were responsible for the observed effects on *S*-nitrosoglutathione and BP, although no direct relation with flavanol plasma levels could be established. The short elimination half-lives of the flavanols prevented accumulation of plasma levels and thus no phenols could be detected at any of the 12-hour postdose monitoring sessions. In previous studies intake of high doses of flavanol-rich cocoa produced a transient improvement of endothelial function and an increase of bioactive nitric oxide.[13,14] However, in our study, the flavanol concentrations were probably too low to induce acute biochemical or hemodynamic effects. Still, the steadily repeated exposition of the vascular endothelium to low flavanol concentrations may have caused a chronic activation of endothelial nitric oxide synthesis. This accords with in vitro work in which stimulation of endothelial cells with polyphenols upregulated transcription of endothelial nitric oxide synthase and subsequent nitric oxide synthesis.[35] The involvement of chronic endothelial nitric oxide stimulation in response to cocoa phenols is further supported by a study of Fisher et al,[36] in which the pressor response to infusion of a nitric oxide synthase inhibitor increased after 4 days of ingestion of flavanol-rich cocoa. The similar amount of BP reduction in our study compared with previous studies using much larger chocolate doses over a short time[15] suggests that the cumulative phenol dose may determine the magnitude of transcriptional endothelial nitric oxide synthase activation and thus sustained BP reduction.

Table 6. Pharmacokinetic Parameters of Cocoa Phenols Derived From a 6.3-g Dose of Dark Chocolate on Day 1 and After 18 Weeks of Intervention[a]

	Mean (SD)		
	Day 1 of Intervention	18 Weeks of Intervention	*P* Value[b]
Epicatechin			
AUC∞, ng/mL × min	761 (210)	774 (253)	.82
C_{max}, ng/mL	3.63 (1.02)	3.58 (0.92)	.78
T_{max}, min	77 (4)	81 (6)	.70
$T_{1/2el}$, min	54 (3)	56 (5)	.79
Catechin			
AUC∞, ng/mL x min	234 (61)	228 (56)	.77
C_{max}, ng/mL	1.12 (0.31)	1.01 (0.26)	.58
T_{max}, min	78 (9)	82 (6)	.69
$T_{1/2el}$, min	54 (6)	58 (7)	.68
Procyanidin B2			
AUC∞, ng/mL x min	99 (30)	102 (32)	.90
C_{max}, ng/mL	0.45 (0.15)	0.43 (0.14)	.94
T_{max}, min	81 (8)	86 (9)	.62
$T_{1/2el}$, min	56 (6)	57 (5)	.91
Procyanidin B2 gallate			
AUC∞, ng/mL x min	33 (14)	33 (13)	.91
C_{max}, ng/mL	0.14 (0.06)	0.14 (0.06)	.98
T_{max}, min	89 (10)	85 (8)	.72
$T_{1/2el}$, min	62 (7)	59 (6)	.76

Abbreviations: AUC∞, total area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $T_{1/2el}$, elimination half-life.
[a] Values were obtained for the 22 subjects assigned to dark chocolate by fitting the individual data by a linear 1-compartment model. Data are normally distributed.
[b] *P* values are calculated by paired 2-tailed *t* test. $P < .05$ is considered a statistically significant difference.

The lack of acute and chronic effects of dark chocolate on the antioxidant plasma state in our study is consistent with previous reports[37,38] and excludes the possibility that alterations of the reduction and oxidation state could have influenced the equilibrium between thiols and nitrosothiols and thereby nitric oxide–dependent vasodilatory activity.[39]

Our study has some limitations. The sample size was relatively small. The participants were not blinded to the chocolate interventions, which is an inherent problem with dietary interventions and included the risk of expectation bias. We have not determined which of the cocoa phenols or their metabolites were responsible for the nitric oxide stimulation and BP lowering associated with the dark chocolate intake. Additional mechanisms of cocoa phenols apart from elevation of nitric oxide bioavailability (eg, angiotensin-converting enzyme inhibition)[40] or of other components in the cocoa liquor (eg, theobromine)[41] may have contributed to the BP decrease, although the strong negative correlation of *S*-nitrosoglutathione changes with BP changes does not support a substantial impact of any additional effect. In particular, there has been no indication that theobromine caused hemodynamic or cardiac alterations, despite its high content in dark chocolate.[42] The study population was very homogenous because strict eligibility criteria and patient surveillance were used to prevent confounding influences of medications, diet, other lifestyle factors, or concomitant diseases on blood pressure effects. Hence, our results may be valid only for individuals who are older and mildly hypertensive but otherwise healthy. However, short-term interventions with polyphenolrich cocoa products revealed BP-lowering effects also in younger normotensive[10,12] and hypertensive individuals.[11] Furthermore, in agreement with results from the present study, BP reductions were more pronounced in hypertensive[11] compared with normotensive participants.[10] Compared with usual follow-up periods of 6 to 8 weeks in assessments of antihypertensive drug efficacy,[43-45] the study period of 18 weeks was long enough to show the efficacy of dark chocolate to lower BP, but effects on cardiovascular end points must be evaluated in long-term randomized controlled studies with larger numbers of participants.

The most intriguing finding of this study is that small amounts of commercial cocoa confectionary convey a similar BP-lowering potential compared with comprehensive dietary modifications[46,47] that have proven efficacy to reduce cardiovascular event rate.[48,49] Whereas long-term adherence to complex behavioral changes is often low and requires continuous counseling,[50] adoption of small amounts of flavanol-rich cocoa into the habitual diet is a dietary modification that is easy to adhere to and therefore may be a promising behavioral approach to lower blood pressure in individuals with above-optimal blood pressure. Future studies should evaluate the effects of dark chocolate in other populations and evaluate long-term outcomes.

Author Contributions: Taubert had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Taubert.
Acquisition of data: Taubert, Roesen, Lehmann, Jung.
Analysis and interpretation of data: Taubert, Roesen, Lehmann, Jung, Schömig.
Drafting of the manuscript: Taubert.
Critical revision of the manuscript for important intellectual content: Taubert, Roesen, Lehmann, Jung, Schömig.
Statistical analysis: Taubert, Roesen.
Administrative, technical, or material support: Taubert, Roesen, Lehmann, Jung, Schömig.
Study supervision: Taubert, Schömig.
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