Effects on Peripheral and Central Blood Pressure of Cocoa With Natural or High-Dose Theobromine

A Randomized, Double-Blind Crossover Trial

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Abstract—Flavanol-rich cocoa products have been reported to lower blood pressure. It has been suggested that theobromine is partially responsible for this effect. We tested whether consumption of flavanol-rich cocoa drinks with natural or added theobromine could lower peripheral and central blood pressure. In a double-blind, placebo-controlled 3-period crossover trial we assigned 42 healthy individuals (age 62±4.5 years; 32 men) with office blood pressure of 130 to 159 mm Hg/85 to 99 mm Hg and low added cardiovascular risk to a random treatment sequence of dairy drinks containing placebo, flavanol-rich cocoa with natural dose consisting of 106 mg of theobromine, or theobromine-enriched flavanol-rich cocoa with 979 mg of theobromine. Treatment duration was 3 weeks with a 2-week washout. The primary outcome was the difference in 24-hour ambulatory systolic blood pressure between placebo and active treatment after 3 weeks. The difference in central systolic blood pressure between placebo and active treatment was a secondary outcome. Treatment with theobromine-enriched cocoa resulted in a mean±SE of 3.2±1.1 mm Hg higher 24-hour ambulatory systolic blood pressure compared with placebo ($P<0.01$). In contrast, 2 hours after theobromine-enriched cocoa, laboratory peripheral systolic blood pressure was not different from placebo, whereas central systolic blood pressure was 4.3±1.4 mm Hg lower ($P=0.001$). Natural dose theobromine cocoa did not significantly change either 24-hour ambulatory or central systolic blood pressure compared with placebo. In conclusion, theobromine-enriched cocoa significantly increased 24-hour ambulatory systolic blood pressure while lowering central systolic blood pressure. **(*Hypertension*. 2010;56:839-846.)**

Key Words: cocoa ■ theobromine ■ blood pressure ■ hemodynamics ■ aortic pressure waveform

The consumption of foods and beverages rich in flavanols has been associated with a decreased risk of cardiovascular morbidity and mortality.[1–3] In Western society, a large proportion of flavanol intake is through cocoa and cocoa-containing products. One of the mechanism by which cocoa could exert its presumed beneficial effects on cardiovascular disease is by lowering blood pressure (BP). There is, however, discussion about the BP-lowering potential of cocoa. A recent meta-analysis of intervention studies looking at the BP-lowering effect of flavanol-rich cocoa found a significant reduction of 4.5 mm Hg for systolic BP (SBP) and 2.5 mm Hg for diastolic BP (DBP).[4] However, most of the clinical trials in the analysis lacked adequate control treatment, and studies that included a proper control group all showed a neutral effect on DBP and SBP.[5–7] Other than a possible effect on peripheral (brachial) BP, cocoa intake may improve central hemodynamics. Central BP is thought to be an important determinant of hypertensive organ damage and might be superior to peripheral BP in predicting cardiovascular disease.[8] In a cross-sectional study in healthy individuals, increasing amounts of cocoa consumption were associated with less aortic stiffness, decreased wave reflection, and lower central SBP, whereas peripheral BP was not significantly different.[9] The possible beneficial actions of cocoa on BP have largely been attributed to flavanols.[10] Flavanols and their metabolites may reduce BP by angiotensin-converting enzyme inhibition,[11] nicotinamide adenine dinucleotide phosphate-oxidase activity inhibition,[12] and stimulating the release of nitric oxide (NO).[10,13] Additionally, theobromine, which is invariably present in cocoa in high concentrations, could also contribute to the antihypertensive effect of cocoa.[14,15] Theobromine is thought to have vasodilating properties by inhibition of phosphodiesterase.[16]

In the present study, we examined the effects of flavanol-rich cocoa drinks with natural dose or added theobromine versus placebo on peripheral and central BP in subjects with

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This trial has been registered at www.trialregister.nl (identifier NTR1453).

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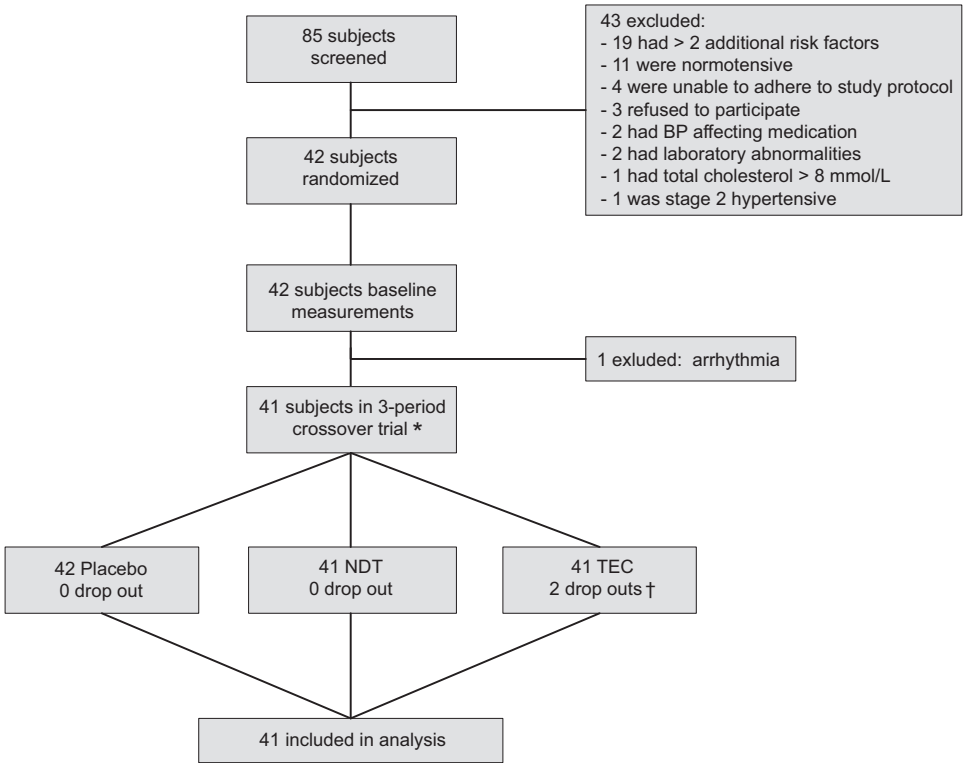

Figure 1. Flow of participants through the study. *Possible treatment sequences were PNT, PTN, NPT, NTP, TPN, and TNP (P indicates placebo; N, NTC; and T, TEC). †One subject dropped out during the first treatment period and 1 during the third treatment period.

high-normal BP or stage 1 hypertension and low-added risk for cardiovascular disease.

Methods

Our aim was to examine the effects of cocoa test products on peripheral and central BP in persons with low added cardiovascular risk and high-normal BP or stage 1 hypertension, because this group has no immediate indication for BP-lowering therapy and will benefit most from possible BP-lowering effects of cocoa products on a population level.

To ensure a correct uptake of flavanols from the cocoa test product, we first assessed its bioavailability under similar conditions as in the efficacy study (please see the online Data Supplement at http://hyper.ahajournals.org for Figure S1). Both studies were conducted at the Academic Medical Center. The studies were approved by the institutional review board, and all of the participants gave written informed consent.

Study Participants

We included 42 healthy male or postmenopausal female volunteers aged 40 to 70 years with high normal BP (130 to 139/85 to 89 mm Hg) or stage 1 hypertension (140 to 159/90 to 99 mm Hg) with low added risk of cardiovascular disease and not taking BP affecting medication. After prescreening with a structured telephone interview, eligible participants were invited for the first of 2 screening visits. At the screening visits, medical history, physical examination, and a fasting blood sample were taken. Subjects were excluded if they had experienced a cardiovascular event (stroke, transient ischemic attack, angina, myocardial infarction, and heart failure); total cholesterol >8.0 mmol/L or lipid-lowering drugs, fasting glucose >7.0 mmol/L, or use of glucose-lowering drugs; reported alcohol consumption >28 alcohol units per week; reported lactose intolerance; medically prescribed diet or slimming; oral medication affecting BP; or when they had >2 of the following cardiovascular risk factors: age >55 years for men and >65 years for women; smoking; dyslipidemia, defined as total cholesterol >5.0 mmol/L or low-density lipoprotein cholesterol >3.0 mmol/L or high density lipoprotein cholesterol <1.0 mmol/L for men and <1.2 mmol/L for women or triglycerides >1.7 mmol/L; fasting glucose 5.6 to 6.9 mmol/L; waist circumference >102 cm for men and >88 cm for women; or family history of premature cardiovascular disease. We screened 85 persons to find 42 eligible participants. The flow of participants through the study is shown in Figure 1.

Study Design

The study was a double-blind, placebo-controlled 3-period crossover trial and was conducted between November 2008 and October 2009. After baseline measurements, subjects were assigned to a random treatment sequence of acidified milk-based drinks containing the following: (1) placebo; (2) flavanol-rich cocoa powder with natural-dose (106 mg) theobromine (NTC); or (3) theobromine-enriched flavanol-rich cocoa powder with high-dose (979 mg) theobromine (TEC). Treatment duration was 3 weeks with a 2-week washout. Participants were instructed to consume 1 test drink of 200 mL daily in a fasting state in the morning. Participants were allowed to have breakfast 1 hour after consumption of the test product. Test product allocation and order of treatment were determined by a computer-generated randomized schedule. Study outcome data were collected before the first treatment and after each treatment period, as described below. During the whole trial, subjects were instructed to maintain their habitual diet with the following restrictions: (1) the daily intake of coffee had to be <4 cups; (2) the intake of chocolate was restricted to milk chocolate only; and (3) on the day before the measurement days, consumption of cocoa products, tea, coffee, and alcohol-containing beverages was prohibited. Adverse events were monitored by interview after each treatment period. Compliance was assessed by counting empty bottles. Test products were provided in sequentially numbered sealed bottles. The different test products all had similar taste and appearance. Nutritional values of the test products are shown in the online Data Supplement (Table S1).

Hemodynamic Measurements

All of the hemodynamic measurements were performed by a single investigator (B.v.d.B.) blinded for treatment allocation. At the 2

screening visits, office BP was measured 3 times at 1-minute intervals in the sitting position at the nondominant arm after 10 minutes of rest using a validated oscillometric device (Omron 705IT, Omron Healthcare Europe BV). The mean of the last 2 measurements was used for analyses. On measurement days, participants came to the hospital in a fasted state. After drawing blood, they were asked to take the last test drink of the treatment period (except for baseline measurements), and the automatic ambulatory BP monitor (ABPM) was placed on the nondominant arm. Central hemodynamics and arterial stiffness were measured in supine position after 15 minutes of rest directly after placement of the ABPM in case of the baseline measurements or 2 hours after consumption of the test product. The ABPM (SpaceLabs 90207, SpaceLabs, Inc) was programmed to record BP every 15 minutes during the day (7:00 AM to 11:00 PM) and every 30 minutes at night (11:00 PM to 7:00 AM). Hourly averages were calculated, and the following predefined day and night periods were used: day, 9:00 AM to 9:00 PM and night 12:00 AM to 6:00 AM. The ABPM assessment was accepted when ≥70% of hourly averages were available for analysis. Measurements of central hemodynamics and pulse wave velocity (PWV), a measure of aortic stiffness, were performed using the SphygmoCor system (Atcor Medical Pty Ltd), as described previously.[17] Briefly, pressure waveforms were recorded from the radial artery of the nondominant arm using applanation tonometry with a high-fidelity micromanometer (Millar Instruments). Laboratory brachial BP was used for calibration, and the corresponding central aortic waveform was generated using a generalized transfer function. Central DBP, SBP, and augmentation index (AIx) were calculated by analysis of the central waveform. AIx was corrected for heart rate of 75 bpm. We offline calculated baseline and posttreatment averaged peripheral and central pressure waves. Carotid-femoral PWV was assessed with the same device using the foot-to-foot method. Measurements were done in duplicate, and means were used for analysis. Systemic hemodynamics were measured with the Nexfin device (BMEYE BV), which uses the Finapres method to noninvasively measure continuous finger arterial BP based on a volume-clamp method.[18] We used the third finger of the dominant arm. The device measures the mean arterial pressure by taking the true integral of the arterial pressure wave over 1 beat divided by the corresponding beat interval. Brachial BPs were reconstructed from the finger arterial pressure.[19] Stroke volume (SV) was calculated using a pulse contour method. Cardiac output (CO) was the product of SV and heart rate (HR), and systemic vascular resistance (SVR) is mean arterial pressure at heart level divided by CO. Hemodynamic parameters were assessed as the average of a 3-minute recording.

Laboratory Analyses

Baseline glucose and lipids were measured using standard clinical analytic equipment. Plasma renin activity (PRA) was determined by quantifying angiotensin I generation during incubation of plasma as described previously.[20]

Study Outcomes

The primary outcome was the difference in 24-hour ambulatory SBP between placebo and active cocoa products after 3 weeks of treatment. Secondary outcomes were differences between placebo and active treatment in 24-hour ambulatory DBP, central BP, and systemic hemodynamics after 3 weeks of treatment.

Sample Size and Statistical Analysis

On a population level, a reduction of 2 mm Hg in DBP or 3 to 4 mm Hg in SBP would result in at least a 15% lower mortality from stroke and a 9% lower mortality from ischemic heart disease.[21] We, therefore, considered a difference in SBP of 4 mm Hg clinically relevant and assumed an SD of the difference of 8.3 mm Hg for ambulatory SBP.[22] We calculated that 36 persons would be needed to detect a 4-mm Hg difference between placebo and cocoa treatment with a power of 80% and a significance level of 0.05. To account for withdrawal and failed measurements, we randomized 42 subjects. Baseline data are expressed as mean plus SD for continuous variables and as n (%) for categorical variables. Primary and secondary outcome data were analyzed using linear mixed models with compound symmetry repeated covariance type with treatment as a fixed effect and correction for baseline measurements, age, sex, and body mass index and expressed as means plus SE and 95% CI. Least-square differences were used for pairwise comparisons. A $P<0.05$ was considered significant. Data were analyzed using SPSS software version 16.0.1 (SPSS Inc).

Table 1. Baseline Characteristics of Participants

Parameters	Mean	SD
n	42	
Age, y	62	4.5
Male, n (%)	32 (76)	
Office SBP, mm Hg	142	14.0
Office DBP, mm Hg	84	7.9
Height, cm	177	8.1
Weight, kg	82	9.0
BMI, kg/m^2	25.9	2.4
Fasting glucose, mmol/L	4.9	0.6
TC, mmol/L	5.77	0.77
LDL-C, mmol/L	3.72	0.66
HDL-C, mmol/L	1.55	0.42
Triglycerides, mmol/L	1.06	0.41
Smoking, n (%)	1 (2)	

Data are mean with SD unless otherwise specified. BMI indicates body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Role of the Funding Source

This investigator initiated study was sponsored by Unilever. The investigators carried out the study and were responsible for data retrieval and management. The investigators performed the data analysis and prepared the article. The contractual agreement between the Academic Medical Center and Unilever allowed the sponsor to review and comment on the article, but the investigators remained responsible for its contents and decision to submit the results for publication.

Results

Baseline Characteristics

The study group consisted of 42 persons (76% men) with a mean age of 62 years and office SBP and DBP of 142/84 mm Hg. Baseline characteristics are shown in Table 1.

Study Outcomes

We tested for time, treatment order, and carryover effects, none of which were present. We performed all of the analyses with correction for baseline parameters and in a second model additionally for age, sex, and body mass index. Because the differences between the 2 models were small, we report here the fully corrected model.

Ambulatory BP

Table 2 shows the primary study outcomes. Except for a 1.2-mm Hg higher 24-hour mean DBP in the NTC group, there were no significant differences between placebo and NTC treatment in ambulatory SBP or DBP for all of the predefined time periods. In the group receiving TEC, mean 24-hour ambulatory SBP and DBP were 3.2±1.1/1.3±0.6-

Table 2. 24-Hour Ambulatory BPs After Intake of Test Product

Parameter	Placebo	NTC	TEC	Placebo vs NTC, *P*	Placebo vs TEC, *P*
SBP 24 h, mm Hg	123.1 (120.9 to 125.4)	125.4 (122.3 to 126.7)	126.3 (124.1 to 128.5)	0.22	<0.01
SBP day, mm Hg	128.6 (126.0 to 131.1)	130.0 (127.4 to 132.6)	132.3 (129.7 to 134.8)	0.27	<0.01
SBP night, mm Hg	111.8 (109.0 to 114.7)	113.5 (110.7 to 116.3)	114.4 (111.6 to 117.2)	0.24	0.07
DBP 24 h, mm Hg	76.0 (74.6 to 78.6)	77.2 (75.8 to 78.6)	77.3 (75.9 to 78.7)	0.05	0.04
DBP day, mm Hg	79.8 (78.3 to 81.4)	81.0 (79.5 to 82.6)	81.7 (80.1 to 83.2)	0.13	0.02
DBP night, mm Hg	68.1 (66.2 to 70.0)	69.3 (67.3 to 71.2)	68.8 (66.9 to 70.7)	0.22	0.48
HR 24 h, bpm	66.8 (64.8 to 68.7)	67.2 (65.3 to 69.1)	70.8 (68.9 to 72.7)	0.55	<0.001
HR day, bpm	71.0 (68.4 to 73.7)	71.8 (69.1 to 74.4)	76.0 (73.4 to 78.6)	0.46	<0.001
HR night, bpm	60.6 (58.7 to 62.5)	60.8 (58.9 to 62.7)	63.4 (61.5 to 65.3)	0.79	0.001

Data shown are means (95% CI) calculated with linear mixed model with correction for baseline values, age, sex, and body mass index.

mm Hg higher compared with placebo ($P<0.01/P=0.04$). The increase in ambulatory SBP and DBP was significant for the daytime ($P<0.01$ and $P=0.02$) but not for the nighttime period ($P=0.07$ and $P=0.48$). The mean 24-hour increase in HR was 4.0 bpm ($P<0.001$) after TEC treatment, whereas NTC had no effect. Figure 2 shows the hourly averages of SBP and DBP after intake of the test product. The SBP increment in the TEC group was present during the day, with a peak 2 to 3 hours after intake.

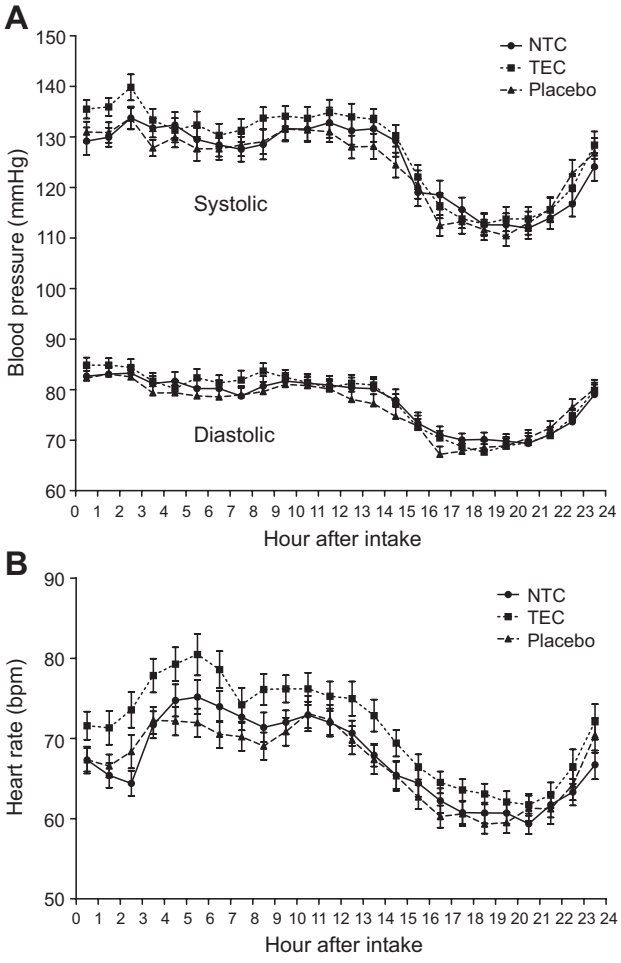

Figure 2. A, 24-Hour SBP and DBP profiles after intake of test product. Data shown are mean±SE. B, 24-Hour HR profile after intake of test product. Data shown are mean±SE.

Central Hemodynamics

Central hemodynamic measurements (Table 3) were performed 2 hours after intake of the test drink, coinciding with the peak plasma levels of the flavanols. Compared with placebo, central SBP and DBP were 4.3±1.4/1.1±0.8 mm Hg lower in the TEC group ($P=0.003/P=0.19$). AIx was 6.7±1.4% lower ($P<0.001$) in the TEC group and persisted after correction for HR (5.3±1.4%; $P<0.001$). Figure 3 shows the mean peripheral and central pressure waves stratified for treatment. Although the peripheral pressure waves all show similar peak systolic pressures, the shape of the peripheral pressure wave is more concave and has a lower late systolic part. This corresponds with a reduction in wave reflection and the lower systolic peak of the central wave. To further examine the effect of TEC on peripheral and central BP, we used a model of the arterial system to calculate central pressure and flow from the peripheral pressure waves, allowing separation into forward and backward waves by waveform analysis (please see the online Data Supplement for online supplemental methods and Figure S2). In the model, the late systolic part of the forward wave and the magnitude of the backward wave of the TEC group were smaller compared with placebo. Central systolic pressure, as the resultant of the forward and backward pressures, was decreased compared with placebo. PWV was significantly higher in both active treatment groups compared with placebo (8.4±0.2 versus 8.7±0.1 versus 9.0±0.1 m/s for placebo, NTC, and TEC, respectively; $P<0.001$).

Systemic Hemodynamics

Table 3 shows systemic hemodynamics. Mean arterial pressure was not different between the treatment groups. In the TEC group, HR was higher and SV was lower compared with placebo, resulting in a similar CO between the 2 groups. None of the active treatment groups had a significant effect on SVR compared with placebo.

Plasma Renin Activity

PRA was not different after the 2 cocoa treatments compared with placebo. PRA was 0.87±0.11 pmol of angiotensin I per milliliter per hour (95% CI: 0.64 to 1.09 pmol of angiotensin I per milliliter per hour) for placebo, 0.64±0.11 pmol of

Table 3. Central and Systemic Hemodynamics 2 Hours After Intake of Test Product

Parameter	Placebo	NTC	TEC	Placebo vs NTC, *P*	Placebo vs TEC, *P*
Peripheral SBP, mm Hg	137.4 (133.9 to 140.9)	138.7 (135.1 to 142.1)	137.6 (134.1 to 141.2)	0.39	0.88
Peripheral DBP, mm Hg	81.6 (79.5 to 83.7)	81.6 (79.5 to 83.7)	80.3 (78.2 to 82.4)	1.00	0.14
Central SBP, mm Hg	128.9 (125.2 to 132.5)	129.5 (125.9 to 133.2)	123.7 (120.0 to 127.4)	0.66	0.001
Central DBP, mm Hg	82.5 (80.4 to 84.6)	82.6 (80.5 to 84.7)	81.1 (79.0 to 83.3)	0.89	0.14
AIx, %	27.0 (24.7 to 29.4)	27.6 (25.3 to 29.9)	20.4 (17.9 to 22.8)	0.70	<0.001
AIx@hr75, %	19.4 (17.0 to 21.7)	19.9 (17.6 to 22.2)	14.1 (11.8 to 16.5)	0.71	<0.001
PWV, m/s	8.2 (7.9 to 8.6)	8.5 (8.2 to 8.9)	8.8 (8.4 to 9.1)	0.04	<0.001
MAP, mm Hg	99.9 (96.6 to 103.2)	101.9 (98.6 to 105.2)	99.8 (96.5 to 103.1)	0.18	0.96
SV, mL	82.1 (79.0 to 85.2)	82.3 (79.2 to 85.4)	78.9 (75.8 to 82.0)	0.87	0.02
HR, bpm	59.0 (56.6 to 61.4)	59.0 (56.6 to 61.4)	61.6 (59.2 to 64.0)	0.96	0.001
CO, L/min	5.0 (4.7 to 5.3)	5.0 (4.7 to 5.3)	5.0 (4.7 to 5.3)	0.90	0.82
SVR, dyn · s/cm^5	1713 (1595 to 1832)	1739 (1620 to 1857)	1687 (1568 to 1806)	0.59	0.57

Data shown are mean (95% CI) calculated with linear mixed model with correction for baseline values, age, sex, and body mass index. AIx@hr75 indicates augmentation index corrected for heart rate of 75 bpm; MAP, nexfin mean arterial pressure.

angiotensin I per milliliter per hour (95% CI: 0.41 to 0.86 pmol of angiotensin I per milliliter per hour) for NTC, and 0.77±0.11 pmol of angiotensin I per milliliter per hour (95% CI: 0.53 to 1.00 pmol of angiotensin I per milliliter per hour) for TEC.

Compliance, Withdrawal, and Adverse Events

The overall compliance rate was >99% for all of the treatment groups. Three (7%) of 42 participants dropped out of the study. Two subjects withdrew because they experienced adverse events after consumption of the test product: 1 case of nausea and 1 case of headache. These adverse events occurred in the TEC treatment group and resolved immediately after cessation of the test product. One participant was withdrawn from the study at baseline because sinus arrhythmia prohibited correct hemodynamic measurements. With TEC treatment, 10 subjects reported a laxative effect compared with 2 in the placebo and 2 in the NTC group. No serious adverse events were reported.

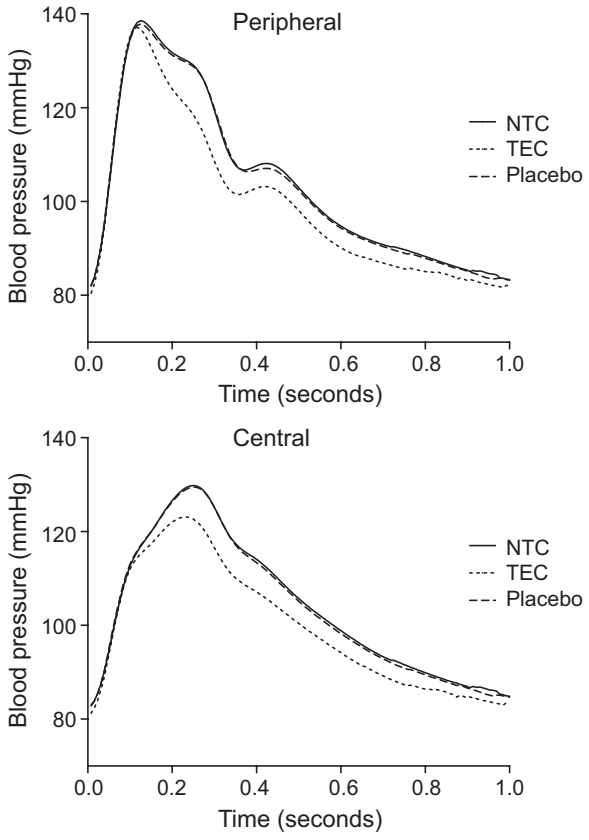

Figure 3. Peripheral and central pressures waves 2 hours after intake of test product.

Discussion

In this study, we show that flavanol-rich cocoa drinks enriched with theobromine significantly increased 24-hour ambulatory SBP compared with placebo. In contrast, 2 hours after theobromine-enriched cocoa, laboratory peripheral SBP was not different from placebo, whereas central SBP was lower. Treatment with flavanol-rich cocoa drinks with natural theobromine content did not significantly change either ambulatory or central SBP compared with placebo in this group of middle-aged individuals with high-normal BP or grade I hypertension and at low added risk for cardiovascular disease.

Normal Dose Theobromine Cocoa

The lack of a peripheral BP-lowering effect observed in our study is in contrast with a meta-analysis that examined the BP-lowering effect of cocoa.[4] The majority of the trials included in this meta-analysis, however, used white chocolate as a control, and only 3 studies used a double-blind design with adequate control treatment.[5–7] This was confirmed by a summary of all open-label and double-blind cocoa studies showing that the BP-lowering benefits of cocoa were confined to open label trials only.[23] Contrary to this is a more recent double-blind study, not implemented in the latter summary, showing a significant 4.2-mm Hg decrease in SBP after 30 days of treatment in 16 patients with previous coronary artery disease.[24] In our study we were able to detect a difference of 2.6 mm Hg in ambulatory SBP between groups but found no effect in the NTC group; together with

the findings of previous randomized double-blind trials, we, therefore, think that the BP-lowering effect of cocoa is undetermined. An alternative explanation might be the differences in the test products. The majority of the positive open label studies, but not all, used chocolate bars, whereas the negative, double-blind studies used cocoa drinks. Possibly the chocolate matrix is essential for the BP-lowering effect, either by effects of substances in chocolate other than flavanols or by a synergistic effect between flavanols and these substances.

Despite the lack of effect on peripheral BP in our trial, cocoa flavanols have been shown to cause NO-dependent vasodilation in the rat[25] and in humans.[10] It is conceivable that the effects of cocoa on vascular function may be counterbalanced by reflex sympathetic activation or fluid retention. However, we consider this unlikely, because we did not observe any differences in HR or changes in PRA in the NTC group.

Theobromine-Enriched Cocoa

Based on the vasodilating effects of theobromine, we and others hypothesized that theobromine could be partially responsible for the presumed BP-lowering effect of cocoa.[15] NTC and TEC only differ in theobromine dose, so differences seen between these groups are caused by theobromine or a synergistic effect with cocoa. Unexpectedly, we observed an opposite effect on peripheral and central SBP in the TEC treatment group. Although HR was significantly higher in those receiving TEC treatment, we did not observe any difference in CO or SVR between those receiving TEC treatment and placebo. Furthermore, PRA was similar among the treatment groups, suggesting no significant change in volume status. Finally, we observed a small but significant increase in PWV in the TEC treatment group compared with placebo.

Theobromine has been shown to exert an inhibitory effect on parasympathetic activity[26] and is a selective antagonist of the A1 adenosine receptor[27]: these mechanisms could explain the increase in HR without changes in CO or SVR in the TEC group. The increases in HR and PWV observed in the present study result in a forward wave that is larger in amplitude but more concave in shape (please see the online Data Supplement). The higher forward wave results in a higher peripheral peak systolic pressure. Although the proposed mechanisms may explain the increase in SBP and HR, the observed decrease in central SBP needs further explanation. The lower central SBP can be explained by a decrease in wave reflection. AIx, as a measure of wave reflection, is principally determined by HR, arterial stiffness, and reflection site.[28,29] The difference in AIx between TEC and placebo remained after correction for HR, and HR, therefore, cannot fully explain the observed effect. The increase in arterial stiffness that was observed in the TEC group would amplify rather than diminish AIx. Thus, a likely explanation for the decrease in AIx is a shift of the reflection site away from the heart. Theobromine is thought to have an endothelium-independent vasodilating effect by inhibiting the breakdown of cAMP in the arterial smooth muscle cell.[16] This vasodilation could alter the reflection site and lower the AIx and central BP while having less effect on peripheral BP. In the Conduit Artery Function Evaluation Study, calcium channel blocker/angiotensin-converting enzyme inhibitor treatment compared with β-blocker/diuretic treatment lowered peripheral BP to the same extent, whereas central BP and AIx decreased more in the calcium channel blocker/angiotensin-converting enzyme inhibitor group.[30] In line with this, our wave separation model showed a lower magnitude of the backward wave after TEC treatment consistent with decreased wave reflection as a result of vasodilation. When combined with the more concave forward wave, because of the increase in HR, this results in a lower central pressure. Differential effects on peripheral and central pressure have also been described for dobutamine, which is a positive chronotropic and a vasodilatory agent. Increasing doses of dobutamine in patients undergoing coronary angiography for the evaluation of coronary heart disease significantly increased peripheral BP while decreasing AIx and central SBP.[31]

In contrast to the 24-hour BP increase, 2 hours after intake of TEC, laboratory BP was not different compared with placebo. Although laboratory BP was not a predefined outcome measure of this study and our study was not powered to demonstrate differences in laboratory peripheral BP, it is conceivable that a small theobromine-induced, sympathetically mediated rise in BP was obscured by a larger white coat effect that is inherent to laboratory BP readings.

The treatment with TEC caused an increase in adverse events, most notably a laxative effect. Adenosine is known to inhibit the motility of the colon; adenosine antagonism leads to stimulation of colon motility and would explain the adverse events observed in our study.[32]

Limitations

There are some limitations of our study that deserve attention. The lack of a BP-lowering effect after consumption of flavanol-rich cocoa drinks with naturally occurring theobromine could be explained by the content and bioavailability of the flavanols. The test products used in our trial consisted of acidified milk drinks with cocoa powder. It has been shown previously that dissolving cocoa powder in milk does not change flavanol bioavailability.[33] Our bioavailability study (please see the online Data Supplement) confirmed the uptake of flavanols under similar conditions as in this trial. The amount of epicatechin used in our test product was 25 mg with NTC and 24 mg with TEC treatment. Epicatechin is believed to contribute to the vascular effects of cocoa by its ability to stimulate NO release from the endothelium.[34] Two short-term open label studies that have demonstrated a BP-lowering effect of cocoa products used 66 mg of epicatechin,[35,36] and a third study used 5.1 mg of epicatechin for a treatment period of 18 weeks.[37] Although another double-blind cocoa study using 174 mg of epicatechin failed to demonstrate a BP-lowering effect after 2 weeks,[6] we cannot exclude that the amount of epicatechin and the treatment period may have contributed to the lack of a BP-lowering effect observed in our study. Central hemodynamic parameters, contrary to the ambulatory BP, were measured 2 hours after intake of the test product, which limits the comparison of the 2 modalities. Finally, intake of flavanols in our study

was controlled by asking the participants not to change their diet except for refraining from the intake of dark chocolate. Subjects could have unknowingly consumed more or less flavanols during a particular treatment period. Because treatment was blinded and randomized, it is unlikely that this could have affected the outcome of the study.

Conclusions

Flavanol-rich cocoa drinks enriched with theobromine significantly increased 24-hour ambulatory SBP in a group of middle-aged subjects with high-normal BP or grade I hypertension and low added risk of cardiovascular disease. Despite an increased peripheral SBP, central SBP was lower 2 hours after consumption of theobromine-enriched cocoa drinks. Compared with placebo we could not demonstrate any effect of the flavanol-rich cocoa product with normal theobromine content on SBP.

Perspectives

Although there are several epidemiological studies that demonstrate a lower risk of cardiovascular disease with increasing amounts of cocoa intake possibly through lowering peripheral BP, the majority of adequately controlled cocoa intervention trials have not been able to confirm this. Our results add to these findings by showing no effect of cocoa containing natural theobromine content on peripheral SBP using ABPM. We consider the differential effects of TEC on peripheral and central SBP remarkable. The possibly higher prognostic value of central BP over peripheral pressure is observed in a limited number of studies, whereas there is an overwhelming amount of evidence showing a decrease in mortality with peripheral BP lowering. Whether the central BP-lowering effect could, at least in part, be responsible for the presumed beneficial actions of cocoa on cardiovascular disease remains to be determined.

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Disclosures

R.D. is a full-time employee of Unilever. B.E.W. is a full-time employee and holds shares of BMEYE, the manufacturer of the Nexfin device.

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Effects on Peripheral and Central Blood Pressure of Cocoa With Natural or High-Dose Theobromine: A Randomized, Double-Blind Crossover Trial

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ONLINE SUPPLEMENT

EFFECTS ON PERIPHERAL AND CENTRAL BLOOD PRESSURE OF COCOA WITH NATURAL OR HIGH DOSE THEOBROMINE: A RANDOMISED DOUBLE-BLIND CROSS-OVER TRIAL

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BIOAVAILABILITY STUDY

Study design

We recruited twelve healthy male volunteers (aged 22 ± 2 yrs) by advertisement. After a three day polyphenolic poor diet (consumption of tea, wine and chocolate was not allowed) the subjects consumed in a fasting state an acidified milk based test drink with cocoa containing 500 mg of polyphenols. We draw blood before (t = 0) and ½, 1, 1½, 2, 3, 4, 6 and 8 hours after consumption of the test product for kinetic profiles by determining plasma concentrations of epicatechin (EC), (-)-catechin (C), and the microbial products of catechins 5-(3,4-dihydroxyphenyl)-γ-valerolactone (V1) and 5-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone (V2). Furthermore we collected 24-hours urine to measure accumulation of EC and C.

Laboratory analysis

Plasma catechins were measured by high performance liquid chromatography-multiple reaction monitoring-mass spectrometry (HPLC-MRM-MS), urine accumulation of catechins was determined using gas chromatography-mass spectrometry (GC-MS). Sample preparation: To a 200 µl plasma or 24-h urine sample, 20 µl 10% ascorbic acid containing 0.1% EDTA and 20 µl 1.5 M NaOAc (pH 4.8), 10 ng internal standard (taxifolin / ethylgallate), and 500 units glucuronidase was added, mixed and incubated at 37 °C for 45 min. Then 300 µl water, 10 ul 2 N HCl and 1 ml EtOAc was added and vortexed for 30 sec, followed by centrifugation at 3000 x g for 10 min. The EtOAc fraction was collected and the extraction was repeated twice. All samples were analyzed by HPLC-MRM-MS using calibration standards from 0 to 500 ng/ml. Note that methylated forms of catechins will not be detected with this preparation.

Results

Prior to consumption of the cocoa test product plasma concentrations of EC, C, V1 and V2 were virtually not detectable. Plasma concentrations of EC and C increased significantly, reaching peak values of 63 and 4.7 µg/L within one hour after consumption of the cocoa product (see supplemental figure S1). V1 and V2 increased more gradually, still rising 8 hours after test product consumption (54 and 2 µg/L at t=8 h). The measured EC concentrations are similar to the values reported in literature[1]. Forty grams of chocolate, containing 892 mg polyphenols, increased EC plasma concentrations to max 111 µg/L two hours after consumption of the chocolate. Peak values of EC in the chocolate study may have shifted due to gradual stomach emptying of the high fat and sugar product. In the present study, the rapid metabolization of the catechins was reflected in elevated urinary excretion of EC (165 mg in 24 h) and C (10 mg in 24 h) after consumption of the cocoa test product compared to a placebo product (1 and 2 mg, respectively).

WAVEFORM SEPARATION ANALYSIS

Methods

We used a model of the human total arterial system as described previously[2], which is based on the original model published by Westerhof et al.[3]. In short, the model consists of 121 segments of artery. Each segment is based on Womersley's oscillatory flow theory, and the wall material is viscoelastic[4]. The local peripheries are modelled with Windkessels[5,6]. With the model pressure and flow at any location in the arterial tree can be calculated from another location. We used radial pressure waves measured with applanation tonometry and calibrated with brachial blood pressure to derive aortic pressure and flow as calculated by the model for the mean baseline, placebo, NTC and TEC pressure waves. Backward and forward waves were separated with waveform analysis as described by Westerhof et al.[7]. Effects of higher PWV in the TEC group were not modelled.

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SUPPLEMENTAL TABLE

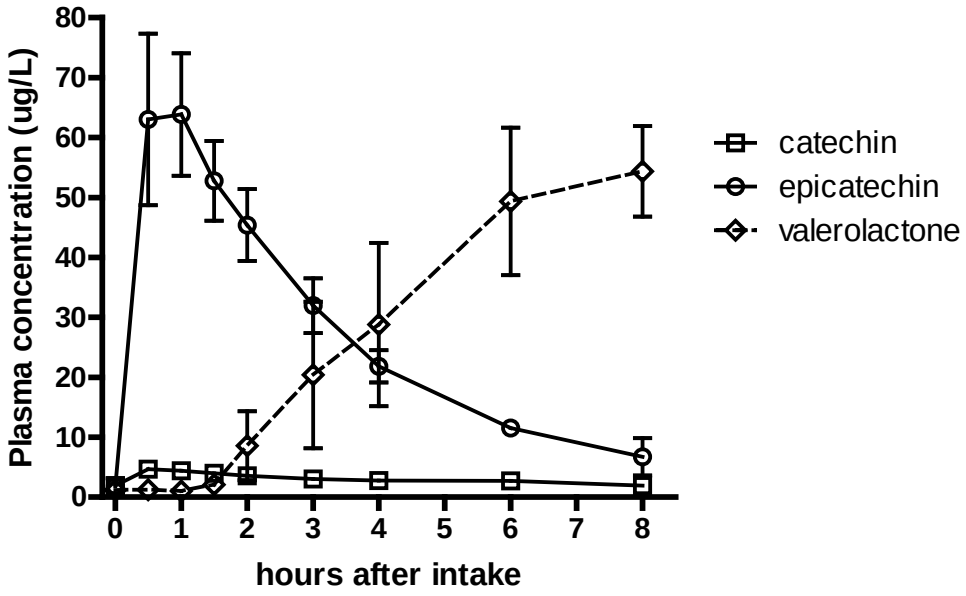
Supplemental Table S1. **Nutritional Content of the Acidified Milk Based Test Drinks per 200 gram**

Content per dose		**Placebo**	**NTC**	**TEC**
Cocoa powder*	g	0	3.6	3.6
Energy	kcal	72	84	90
Total fat	g	2.7	3.1	3.0
Carbohydrates	g	10.4	11.2	11.4
Protein	g	1.6	3.0	4.2
Total Polyphenols	mg	0	500	558
Flavanols (1-10 units)[‡] [$]	mg	0	305	340
Catechin[‡]	mg	0	13.4	13.6
Epicatechin[‡]	mg	0	25	24
Caffeine[‡]	mg	0	10.4	10.2
Theobromine[‖]	mg	0	106	979

Abbreviations: NTC = natural dose theobromine cocoa, TEC = theobromine enriched cocoa. *ActicoaTM cocoa powder (Barry Callebaut, Belgium), †Gallic-acid equivalents using the Folin-Ciocalteu method and an acidified methanol extraction; ‡Measured by HPLC, $61% of total polyphenols, || Measured by H NMR

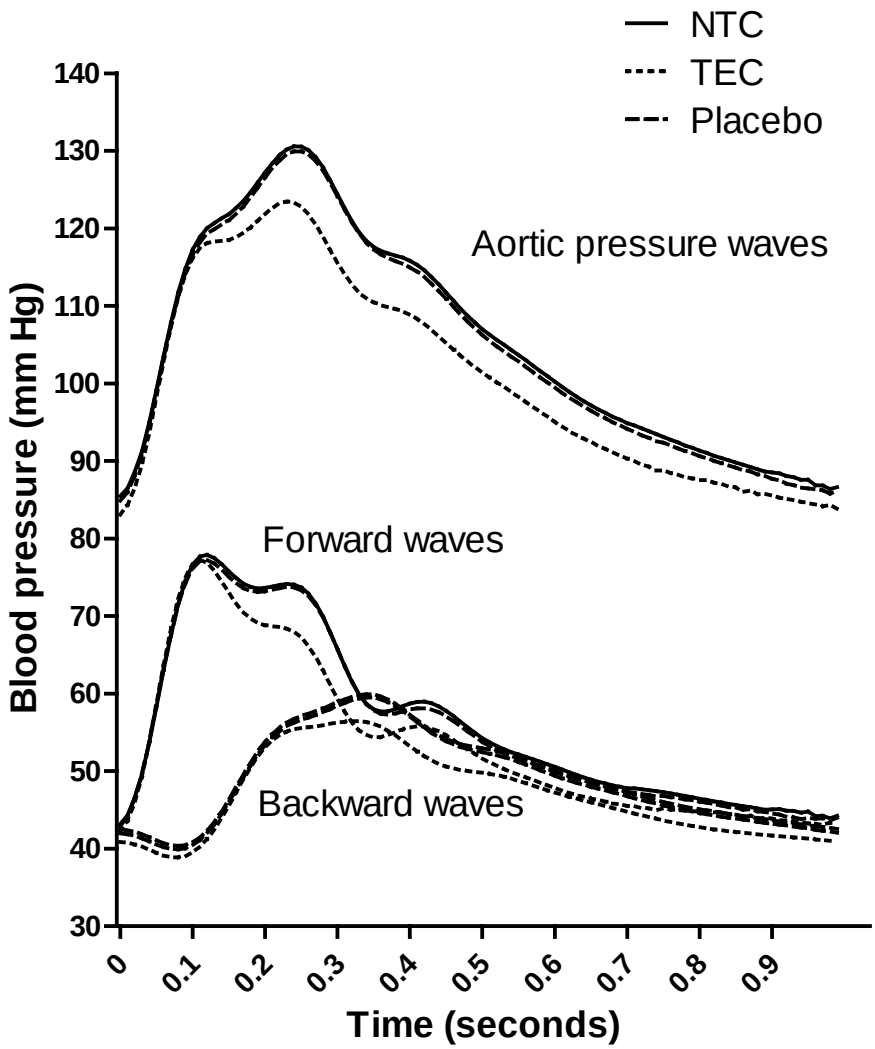
SUPPLEMENTAL FIGURES

Supplemental Figure S1



Supplemental Figure S1. **Plasma Concentrations of Catechin, Epicatechin and Valerolactone after Consumption of Acidified Milk Drinks with Cocoa Containing 500 mg Polyphenols.**

Supplemental Figure S2



Supplemental Figure S2. **Total Pressure, Backward, Forward Waves after Intake of Test Product.**
The upper panel shows the central aortic pressure wave per treatment group, The lower panel shows forward and backward waves.