Central hemodynamics and arterial function
van den Bogaard, B.

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Chapter 8a

Effect 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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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 three-period cross-over trial we assigned 42 healthy individuals (age 62±4.5 years, 32 males) with office blood pressure of 130-159/85-99 mmHg and low added cardiovascular risk to a random treatment sequence of dairy drinks containing (1) placebo, (2) flavanol-rich cocoa with natural dose consisting of 106 mg theobromine or (3) theobromine enriched flavanol-rich cocoa with 979 mg theobromine. Treatment duration was three weeks with a two week wash-out. The primary outcome was the difference in 24-hour ambulatory systolic blood pressure between placebo and active treatment after three 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±standard error 3.2±1.1 mmHg higher 24-hour ambulatory systolic blood pressure compared to placebo (p<0.01). In contrast, two hours after theobromine enriched cocoa, laboratory peripheral systolic blood pressure was not different from placebo, while central systolic blood pressure was 4.3±1.4 mmHg lower (p<0.01). Natural dose theobromine cocoa did not significantly change either 24-hour ambulatory or central systolic blood pressure compared to placebo. In conclusion, theobromine enriched cocoa significantly increased 24-hour ambulatory systolic blood pressure, while lowering central systolic blood pressure.
INTRODUCTION

The consumption of foods and beverages rich in flavanols has been associated with a decreased risk of cardiovascular morbidity and mortality.\textsuperscript{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 mmHg for systolic BP (SBP) and 2.5 mmHg for diastolic BP (DBP).\textsuperscript{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.\textsuperscript{5-7} Besides 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.\textsuperscript{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.\textsuperscript{9} The possible beneficial actions of cocoa on BP have largely been attributed to flavanols.\textsuperscript{10} Flavanols and their metabolites may reduce BP by angiotensin-converting-enzyme (ACE) inhibition,\textsuperscript{11} nicotinamide adenine dinucleotide phosphate-oxidase activity inhibition\textsuperscript{12} and stimulating the release of nitric oxide.\textsuperscript{10, 13} Additionally, theobromine, which is invariably present in cocoa in high concentrations, could also contribute to the antihypertensive effect of cocoa.\textsuperscript{14, 15} Theobromine is thought to have vasodilating properties by inhibition of phosphodiesterase.\textsuperscript{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 high-normal blood pressure or stage I 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 as this group has no immediate indication for BP lowering therapy and will benefit most from a 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 supplemental data and figure S1 in chapter 8b). Both studies were conducted at the Academic Medical Center, Amsterdam. The studies were approved by the institutional review board and all participants gave written informed consent.
Study participants

We included 42 healthy, male or post-menopausal female volunteers aged 40-70 years with high normal BP (130-139/85-89 mmHg) or stage 1 hypertension (140-159/90-99 mmHg) with low added risk of cardiovascular disease and not taking BP affecting medication. After pre-screening with a structured telephone interview, eligible participants were invited for the first of two 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, 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/week, reported lactose intolerance, medically prescribed diet or slimming, oral medication affecting BP or when they had more than two 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 (LDL-c) >3.0 mmol/l or high density lipoprotein cholesterol (HDL-c) <1.0 mmol/l for men and <1.2 mmol/l for women or triglycerides >1.7 mmol/l; fasting glucose 5.6-6.9 mmol/l; waist circumference >102 cm for men and >88 cm for women; 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 three-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 (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 three weeks with a two week wash-out. Participants were instructed to consume 1 test drink of 200 ml daily in fasting state in the morning. Participants were allowed to have breakfast one hour after consumption of the test product. Test product allocation and order of treatment was 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 below 4 cups, (2) the intake of chocolate was restricted to milk chocolate only and (3) on the day prior to 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 supplemental data (please see supplemental data table S1 in chapter 8b).
Figure 1 Flow of participants through the study
* Possible treatment sequences were PNT, PTN, NPT, NTP, TPN and TNP (P=placebo, N=NTC and T=TEC). † 1 subject dropped out during the first treatment period, 1 during the third treatment period. Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa.

Hemodynamic measurements
All hemodynamic measurements were performed by a single investigator (BvdB) blinded for treatment allocation. At the two screening visits office BP was measured 3 times at 1-minute intervals in sitting position at the non-dominant arm after 10 minutes rest using a validated oscillometric device (Omron 705IT, Omron Healthcare Europe BV, Hoofddorp, the Netherlands). The mean of the last 2 measurements was used for analyses. On measurement days, participants came to the hospital in 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 non-dominant arm. Central hemodynamics and arterial stiffness were measured in supine position after 15 minutes rest directly after
placement of the ABPM in case of the baseline measurements or two hours after consumption of the test product. The ABPM (Spacelabs 90207, Spacelabs Inc., Redmond, Washington, USA) was programmed to record BP every 15 min during the day (07:00 – 23:00 h) and every 30 min at night (23:00 – 07:00 h). Hourly averages were calculated and the following predefined day and night periods were used: day 09:00-21:00 and night 0:00-6:00. The ABPM assessment was accepted when at least 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, West Ryde, Australia) as described previously.17 Briefly, pressure waveforms were recorded from the radial artery of the non-dominant arm using applanation tonometry with a high-fidelity micromanometer (Millar Instruments, Texas, USA). 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 beats per minute (AIx@hr75). We off-line calculated baseline and post-treatment 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, Amsterdam, the Netherlands), which uses the Finapres method to non-invasively measure continuous finger arterial blood pressure based on a volume-clamp method.18 We used the third finger of the dominant arm. The device measures the mean arterial pressure (MAP) by taking the true integral of the arterial pressure wave over 1 beat divided by the corresponding beat interval. Brachial blood pressures 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 SVR is MAP at heart level divided by CO. Hemodynamic parameters were assessed as the average of a three minute recording.

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

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

On a population level a reduction of 2 mmHg in DBP or 3-4 mmHg in SBP would result in at least a 15% lower mortality from stroke and a 9% lower mortality from ischemic heart disease. We therefore considered a difference in SBP of 4 mmHg clinically relevant and assumed a standard deviation of the difference of 8.3 mmHg for ambulatory SBP. We calculated that 36 persons would be needed to detect a 4 mmHg 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 randomised 42 subjects. Baseline data are expressed as mean plus standard deviation (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, gender and BMI and expressed as means plus standard error (SE) and 95% confidence interval (CI). Least square differences were used for pair wise comparisons. A p-value <0.05 was considered significant. Data were analysed using SPSS software version 16.0.1 (SPSS Inc., Chicago, Illinois, USA).

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 manuscript. The contractual agreement between the AMC, Amsterdam and Unilever allowed the sponsor to review and comment on the manuscript, 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% male) with a mean age of 62 years and office SBP and DBP of 142 / 84 mmHg. Baseline characteristics are shown in table 1.

Study outcomes

We tested for time, treatment order and carry-over effects, none of which were present. We performed all analyses with correction for baseline parameters and in a second model additionally for age, gender and BMI. Because the differences between the two models were small, we here report the fully corrected model.

Ambulatory blood pressure Table 2 shows the primary study outcomes. Except for a 1.2 mmHg higher 24-hour mean DBP in the NTC group, there were no significant differences between
### Table 1 Baseline Characteristics of Participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Age yrs</td>
<td>62</td>
<td>4.5</td>
</tr>
<tr>
<td>Male no (%)</td>
<td>32 (76%)</td>
<td></td>
</tr>
<tr>
<td>Office SBP mmHg</td>
<td>142</td>
<td>14.0</td>
</tr>
<tr>
<td>Office DBP mmHg</td>
<td>84</td>
<td>7.9</td>
</tr>
<tr>
<td>Height cm</td>
<td>177</td>
<td>8.1</td>
</tr>
<tr>
<td>Weight kg</td>
<td>82</td>
<td>9.0</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Fasting glucose mmol/L</td>
<td>4.9</td>
<td>0.6</td>
</tr>
<tr>
<td>TC mmol/L</td>
<td>5.77</td>
<td>0.77</td>
</tr>
<tr>
<td>LDL-c mmol/L</td>
<td>3.72</td>
<td>0.66</td>
</tr>
<tr>
<td>HDL-c mmol/L</td>
<td>1.55</td>
<td>0.42</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.06</td>
<td>0.41</td>
</tr>
<tr>
<td>Smoking no (%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means with standard deviation (SD) or otherwise specified. Abbreviations: SBP=systolic blood pressure, DBP=diastolic blood pressure, BMI=body mass index, TC=total cholesterol; LDL-c=low density lipoprotein cholesterol; HDL-c=high density lipoprotein cholesterol.

### Table 2 24 hour Ambulatory Blood Pressures after Intake of Test Product

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

Data shown are means (95% confidence interval) calculated with linear mixed model with correction for baseline values, age, gender and body mass index. Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa, SBP=systolic blood pressure, DBP=diastolic blood pressure, HR=heart rate.
Peripheral and central blood pressure after cocoa placebo and NTC treatment in ambulatory SBP or DBP for all predefined time periods. In the group receiving TEC, mean 24-hour ambulatory SBP and DBP were 3.2±1.1 /1.3±0.6 mmHg higher compared to placebo. \((p<0.01/p=0.04)\) The increase in ambulatory SBP and DBP was significant for the day-time \((p<0.01 \text{ and } p=0.02)\), but not for the night-time period \((p=0.07 \text{ and } p=0.48)\). 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

**Figure 2a** 24-hour Systolic and Diastolic Blood Pressure Profiles after Intake of Test Product
Data shown are means ±SE. Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa.

**Figure 2b** 24-hour Heart Rate Profile after Intake of Test Product
Data shown are means±SE. Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa.
product. The SBP increment in the TEC group was present during the day, with a peak 2 to 3 hours after intake.

**Central hemodynamics** Central hemodynamic measurements (table 3) were performed two hours after intake of the test drink coinciding with the peak plasma levels of the flavanols. Compared to placebo central SBP and DBP were 4.3±1.4/1.1±0.8 mmHg lower in the TEC group (p=0.003/p=0.19). Alx 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. Whereas 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 backward and forward waves by waveform analysis (please see supplemental methods and figure S2 in chapter 8b). In the model the late systolic part of the forward and the magnitude of the backward wave of the TEC group were smaller compared to placebo. Central systolic pressure, as the resultant of the forward and backward

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>NTC</th>
<th>TEC</th>
<th>Placebo vs NTC</th>
<th>Placebo vs TEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral SBP, mmHg</td>
<td>137.4 (133.9-140.9)</td>
<td>138.7 (135.1-142.1)</td>
<td>137.6 (134.1-141.2)</td>
<td>0.39</td>
<td>0.88</td>
</tr>
<tr>
<td>Peripheral DBP, mmHg</td>
<td>81.6 (79.5-83.7)</td>
<td>81.6 (79.5-83.7)</td>
<td>80.3 (78.2-82.4)</td>
<td>1.00</td>
<td>0.14</td>
</tr>
<tr>
<td>Central SBP, mmHg</td>
<td>128.9 (125.2-132.5)</td>
<td>129.5 (125.9-133.2)</td>
<td>123.7 (120.0-127.4)</td>
<td>0.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Central DBP, mmHg</td>
<td>82.5 (80.4-84.6)</td>
<td>82.6 (80.5-84.7)</td>
<td>81.1 (79.0-83.3)</td>
<td>0.89</td>
<td>0.14</td>
</tr>
<tr>
<td>Alx, %</td>
<td>27.0 (24.7-29.4)</td>
<td>27.6 (25.3-29.9)</td>
<td>20.4 (17.9-22.8)</td>
<td>0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alx@hr75, %</td>
<td>19.4 (17.0-21.7)</td>
<td>19.9 (17.6-22.2)</td>
<td>14.1 (11.8-16.5)</td>
<td>0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>8.2 (7.9-8.6)</td>
<td>8.5 (8.2-8.9)</td>
<td>8.8 (8.4-9.1)</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99.9 (96.6-103.2)</td>
<td>101.9 (98.6-105.2)</td>
<td>99.8 (96.5-103.1)</td>
<td>0.18</td>
<td>0.96</td>
</tr>
<tr>
<td>SV, ml</td>
<td>82.1 (79.0-85.2)</td>
<td>82.3 (79.2-85.4)</td>
<td>78.9 (75.8-82.0)</td>
<td>0.87</td>
<td>0.02</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>59.0 (56.6-61.4)</td>
<td>59.0 (56.6-61.4)</td>
<td>61.6 (59.2-64.0)</td>
<td>0.96</td>
<td>0.001</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.0 (4.7-5.3)</td>
<td>5.0 (4.7-5.3)</td>
<td>5.0 (4.7-5.3)</td>
<td>0.90</td>
<td>0.82</td>
</tr>
<tr>
<td>SVR, dyn.s/cm²</td>
<td>1713 (1595-1832)</td>
<td>1739 (1620-1857)</td>
<td>1687 (1568-1806)</td>
<td>0.59</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Data shown are means (95% confidence interval) calculated with linear mixed model with correction for baseline values, age, gender and body mass index. Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa, SBP=systolic blood pressure, DBP=diastolic blood pressure, Alx=augmentation index, Alx@hr75=augmentation index corrected for heart rate of 75 beats per minute, PWV=pulse wave velocity, MAP=nexfin mean arterial pressure, SV=stroke volume, HR=heart rate, CO=cardiac output, SVR=systemic vascular resistance.
Pressures, was decreased compared to placebo. PWV was significantly higher in both active treatment groups compared to placebo, 8.4±0.2 vs 8.7±0.1 vs 9.0±0.1 m/s for placebo, NTC and TEC (p<0.001).

**Systemic hemodynamics** Table 3 shows systemic hemodynamics. MAP was not different between the treatment groups. In the TEC group HR was higher and SV was lower compared to placebo resulting in a similar CO between the two groups. None of the active treatment groups had a significant effect on SVR compared to placebo.

**PRA** PRA was not different after the two cocoa treatments compared to placebo, PRA was 0.87±0.11 (95% CI 0.64-1.09) pmol Ang l/ml/h for placebo, 0.64±0.11 (95% CI 0.41-0.86) pmol Ang l/ml/h for NTC and 0.77±0.11 (95% CI 0.53-1.00) pmol Ang l/ml/h for TEC.
Compliance, withdrawal and adverse events

The overall compliance rate was > 99% for all treatment groups. Three out of forty-two (7%) participants dropped out of the study. Two subjects withdrew because they experienced adverse events after consumption of the test product: one case of nausea and one 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 to 2 in the placebo and 2 in the NTC group. No serious adverse events were reported.

DISCUSSION

In this study we show that flavanol-rich cocoa drinks enriched with theobromine significantly increased 24-hour ambulatory SBP compared to placebo. In contrast, two hours after theobromine enriched cocoa, laboratory peripheral systolic blood pressure was not different from placebo, while central systolic blood pressure was lower. Treatment with flavanol-rich cocoa drinks with natural theobromine content did not significantly change either ambulatory or central SBP compared to 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. The majority of the trials included in this meta-analysis, however, used white chocolate as a control and only three studies used a double blind design with adequate control treatment. 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. Contrary to this is a more recent double blind study, not implemented in the latter summary, showing a significant 4.2 mmHg decrease in SBP after 30 days of treatment in 16 patients with previous coronary artery disease. In our study we were able to detect a difference of 2.6 mmHg 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, while the negative double-blind studies used cocoa drinks. Possibly the chocolate matrix is essential for the blood pressure lowering effect, either by effects of substances in chocolate other than flavanols or by a synergistic effect between flavanols and these substances.
Peripheral and central blood pressure after cocoa

Despite the lack of effect on peripheral BP in our trial, cocoa flavanols have been shown to cause NO-dependent vasodilation in the rat\textsuperscript{25} and in humans.\textsuperscript{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 as 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.\textsuperscript{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 to placebo.

Theobromine has been shown to exert an inhibitory effect on parasympathetic activity\textsuperscript{26} and is a selective antagonist of the A1 adenosine receptor\textsuperscript{27}; these mechanisms could explain the increase in HR without changes in CO or SVR in the TEC group. The increase in HR and PWV observed in the present study results in a forward wave that is larger in amplitude, but more concave in shape (please see supplemental data). 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. Alx, as a measure of wave reflection, is principally determined by HR, arterial stiffness and reflection site.\textsuperscript{28, 29} The difference in Alx 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 Alx. Thus a likely explanation for the decrease in Alx 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.\textsuperscript{16} This vasodilation could alter the reflection site and lower the Alx and central BP, while having less effect on peripheral BP. In the Conduit Artery Function Evaluation (CAFE) study, calcium channel blocker (CCB)/ACE inhibitor treatment compared to beta-blocker/diuretic treatment lowered peripheral BP to the same extent, while central BP and Alx decreased more in the CCB/ACE inhibitor group.\textsuperscript{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, due to 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. In contrast to the 24-hour blood pressure increase, two hours after intake of TEC laboratory blood pressure was not different compared to placebo. Although laboratory blood pressure was not a predefined outcome measure of this study and our study was not powered to demonstrate differences in laboratory peripheral blood pressure, it is conceivable that a small theobromine induced sympathetically mediated rise in blood pressure was obscured by a larger white coat effect that is inherent to laboratory blood pressure 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.

**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 previously been shown that dissolving cocoa powder in milk does not change flavanols bioavailability. Our bioavailability study (please see supplemental data in chapter 8b) 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. Two short term open label studies that have demonstrated a BP lowering effect of cocoa products used 66 mg of epicatechin, and a third study used 5.1 mg of epicatechin for a treatment period of 18 weeks. Although another double-blind cocoa study using 174 mg of epicatechin failed to demonstrate a BP lowering effect after two weeks, 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 two hours after intake of the test product, which limits the comparison of the two 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. Since treatment was blinded and randomised, it is unlikely that this could have affected the outcome of the study.

**Conclusion** 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 two hours after consumption of theobromine enriched cocoa drinks.
Compared to 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 blood pressure over peripheral pressure is observed in a limited number of studies, while there is an overwhelming amount of evidence showing a decrease in mortality with peripheral blood pressure lowering. Whether the central blood pressure lowering effect could, at least in part, be responsible for the presumed beneficial actions of cocoa on cardiovascular disease remains to be determined.
REFERENCES


