Dose-related effects of flavanol-rich cocoa on blood pressure

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Short Title: Cocoa and blood pressure

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Abstract

Background

Consumption of flavanol containing cocoa products has been shown to lower blood pressure (BP), but the minimum dose required to reduce BP is not known. This study aimed to examine the effect of three different doses of cocoa flavanols (CF) on 24-hour mean arterial blood pressure.

Method

Twenty four hour ambulatory blood pressure (24-ABP) monitoring was performed in 32 men and 20 postmenopausal women with untreated mild hypertension (seated clinic BP >130/85 and <160/100 mmHg). Participants were randomised and instructed to consume daily a reconstituted cocoa beverage containing 33, 372, 712 or 1052 mg/d of CF for 6 weeks in a double-blind, parallel comparison. Seated clinic BP and 24 hr ABP were measured at 0, 3 and 6 weeks.

Results

Seated clinic BP did not change during the study period. There were significant reductions in 24-hour systolic (5.3 ± 5.1 mmHg; $p = 0.001$), diastolic (3 ± 3.2 mmHg; $p = 0.002$) and mean arterial blood pressure (3.8 ± 3.2 mmHg; $p = 0.0004$) at the 1052 mg/d CF only. No reduction in BP was seen at any other dose.

Conclusion

No evidence of dose response was seen in this experiment. The highest dose of 1052 mg CF/d was found to significantly lower BP. These results support previous evidence for CF to lower BP, however more research is needed to establish the most effective dose and food matrix.

Introduction

Several intervention studies have reported beneficial effects of consuming cocoa containing products on blood pressure (BP) [1-5] and a recent meta-analysis confirmed this effect by demonstrating that the consumption of cocoa-rich foods for seven or more days has the capacity to lower resting BP in normotensive and mildly hypertensive adults [6]. This effect of cocoa products has been widely attributed to its content of flavanols and procyanidins (oligomeric and polymeric flavanols), which for the purpose of this paper will be included in the term “cocoa flavanols” (CF) [7, 8].

The recent meta-analysis [6] included five studies which compared the antihypertensive effects of CF-rich chocolate with chocolate containing few or no CF. The amount of chocolate consumed daily ranged from 46 to 105g and provided intakes of CF ranging from 246 to 500 mg/day. There was a mean BP reduction of 4.7 / 2.8 mmHg (systolic/diastolic). In a subsequent study that was not included in the meta-analysis, Taubert et al [9] showed that consuming as little as 30 mg of CF per day in 6g of dark chocolate for 18 weeks was sufficient to lower systolic blood pressure (SBP) by 2.9 mmHg and diastolic blood pressure (DBP) by 1.9 mmHg. A more recent meta analysis by Hooper et al [10] also reported a net effect in favour in cocoa in the order of 6mmHg systolic and 3mmHg diastolic. Although the potential antihypertensive effect of chocolate consumption is interesting, the high sugar and fat content of chocolate undermines its potential as a health enhancing food [11, 12].

Concern about delivering CF in a high sugar and high fat chocolate food matrix has led to investigations of the potential antihypertensive and other cardiometabolic health benefits of relatively low fat drinks enriched with CF. In a study which examined the acute effect of consuming a flavanol-rich cocoa beverage, Schroeter et al [13] demonstrated an increase in flow mediated dilatation of the brachial artery (FMD). Four studies have been conducted examining the longer-term effects of consuming flavanol-rich cocoa beverages on cardiovascular health in various populations. In the first of these daily consumption of 446mg of cocoa flavanols by post-

menopausal women for 6 weeks produced a significant improvement in arterial function but no change in BP compared to placebo [14]. In another study individuals with type 2 diabetes took 963mg /day for a period of 30 days and also experienced improvements in endothelial function but not BP [15]. In a third study in which overweight or obese individuals consumed 902mg/day of flavanols for 12 weeks, there were improvements in endothelial function with modest yet significant reductions in BP (MAP reduced by 1.2 mmHg) [16]. The fourth study provided approximately 900mg/day of CF to individuals with essential hypertension for 2 weeks [17]. Despite the similarity of this protocol to that by which Grassi et al [4] showed BP reductions with chocolate, and despite providing almost double the daily dose of CF, there was no change in BP although there was some improvement in endothelial function. The lack of BP change in three of these studies and the relatively small change in the other following chronic consumption of large doses of CF are inconsistent with the results seen in the meta-analysis [6] and subsequent study [9] with flavanol rich chocolate consumption. These findings suggest that the dose of CF alone does not determine the change in BP, however a number of differences in the design of these studies makes direct comparisons difficult. The studies that have been published to date examined different patient populations (healthy and hypertensive adults in chocolate based protocols and hypercholesterolaemic, overweight/obese and type 2 diabetic in non-chocolate protocols), and the type 2 diabetes study was confounded by participant medications. A further complication with comparison is a lack of consistency in the technique of BP assessment. Most studies have used seated clinic BP assessments with only three studies to date utilising the preferred technique of ambulatory blood pressure (ABP) monitoring [3, 4, 18]. These three studies used the same protocol of a 15 day crossover study with dark chocolate (delivering approximately 500mg of CF) or white chocolate (CF free) in three separate subject groups including essential hypertensives and have reported the largest reductions in BP to date . With these factors in mind, a single study evaluating the potential BP lowering effects of CF in a homogenous patient population using the preferred method of ABP assessment and delivering the CF in a consistent food matrix was warranted.

Therefore, the primary aim of the present study was to determine the dose-response effect of CF delivered using a flavanol-rich cocoa beverage on 24-hour ambulatory mean arterial pressure (MAP) in an untreated borderline/mild hypertensive population. Secondary outcomes include clinic BP assessment and additional measures of 24-hour ABP.

Method

Participants

Male and post-menopausal female adults with high-normal BP or untreated mild hypertension (SBP 130-160 mmHg or DBP 85-100 mmHg) were recruited by public advertisement. Participants were excluded if they had a known diagnosis of cardiovascular disease (or a history of cardiovascular or cerebrovascular incidents); Diabetes (Type 1 or Type 2) or were taking prescribed anti-diabetic medication; renal failure; were taking BP lowering medication or supplements that may influence BP (i.e. fish oil, liquorice, polyphenols) in the preceding 3 months, or were likely to do so during the study period; an intolerance to alkaloids (caffeine, theobromine) or dairy; were currently smoking or using nicotine replacement therapy; or had any other medical condition which may have influenced the outcome of the study. The study was approved by the Human Research Ethics Committee of the University of South Australia. Each participant provided written and informed consent prior to participation. Recruitment was carried out between February and August of 2007 and the intervention was progressively conducted from April to October of 2007.

Eligibility screening

Potential participants were initially screened for eligibility by completing a health and lifestyle questionnaire and undertaking a seated clinic BP assessment. Participants with a seated BP between SBP 130-160 mmHg or DBP 85-100 mmHg were enrolled in the study. Enrolled participants returned to the clinic approximately one week later to have their BP measured under the same conditions as the initial screening to confirm the presence of high-normal BP or mild hypertension. If they requalified (i.e. SBP 130-160 mmHg or DBP 85-100 mmHg), they commenced the study protocol. If their BP fell outside these limits they were invited to return for an additional qualifying BP assessment. Participants with screening BP consistently above thresholds for BP (i.e. diastolic > 100 mmHg; systolic > 160 mmHg) were referred to their GP and not entered into the study.

Protocol outline

Participants were block-matched by the minimisation convention [19] on BP, gender, age and BMI into four treatment groups which were randomised to consume reconstituted cocoa beverages containing 33, 372, 712 or 1052 mg/d of CF for 6 weeks in a double-blind, parallel comparison. Randomisation of groups was undertaken independently of group minimisation procedure by separate staff members of the research centre not otherwise involved with the trial. Trial investigators remained blinded to treatment allocation until after the completion of data analysis. Participants then had body weight assessed and underwent seated clinic BP and 24 hour ABP assessments at baseline and were then required to consume a cocoa drink daily for the next 6 weeks. Seated clinic BP and 24 hour ABP assessments were repeated after 3 and 6 weeks (Table 2).

Cocoa supplements

The cocoa drinks were prepared by participants mixing the contents of three cocoa beverage sachets with 300 mL of water each morning and drinking 30 minutes prior to breakfast. The cocoa flavanol composition of the cocoa beverages are provided in Table 1. Sachets (dry powder – 58g net) were matched for micro and macro nutrients including total energy (mean ~ 910 kJ); saturated fat (mean ~ 1.4g); sugar (approx. mean ~ 17.5g); caffeine (mean ~ 43mg); and theobromine (mean ~ 430mg).

On all testing days (at 3 and six weeks), volunteers were instructed not to consume their cocoa beverage until after testing was completed to eliminate any acute effects of cocoa consumption. All empty sachets were returned at 3 and 6 weeks to monitor compliance. Participants were instructed to report any signs or symptoms of ill health immediately to the researchers and any action that was taken (e.g. medication) to identify any potential adverse effect of the intervention. In addition to this participants were specifically asked at each visit whether any signs or symptoms of ill health had been experienced. All reported events were documented by the researchers in each participant's case report form.

Blood pressure monitoring

Seated clinic BP was assessed after sitting quietly for at least 5 min using an automated oscillometric BP monitor (HDI/Pulsewave CR-2000 Cardiovascular Profiler, Hypertension Diagnostics Inc, Eagan, MN) in accordance with the procedures outlined by the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (VII): US Dept of Health and Human Services [20]. Four consecutive BP readings were taken at 1 min intervals by a single observer. The first reading was discarded and an average of the remaining measurements was taken to determine eligibility for study entry.

For assessment of 24 hour Ambulatory Blood Pressure (ABP), volunteers were fitted with a SpaceLabs ambulatory BP monitor (Model 90217, SpaceLabs Medical, Florida, USA) for 24 hours. An appropriately sized cuff was placed firmly around the upper non-dominant arm, centred over the brachial artery, with the monitor worn on a waist strap; ABP measurements were recorded at 15 minute intervals excepting from 11 pm – 7am (30 minute intervals). The cuff and monitor was only removed briefly for bathing and remained in place at all other times during the 24 hour recording period. Assessments at baseline, 3 and 6 weeks were carried out on the same day of the week using the same monitor, cuff size and arm. Participants were required to maintain an activity diary during each 24 hour ABP period to enable BP values to be related to activities. 24 hour ABP recordings were used to determine daytime (7am – 11pm), night-time (11pm -7am) and 24-hour averages for MAP, SBP, DBP and heart rate (HR).

Diet and lifestyle requirements

Volunteers were asked to maintain their normal physical activity patterns and were provided with a list of dietary exclusions to ensure a low-flavanol diet for 1 week prior to and during the study

period. Given the energy content of the beverages (~900 kJ), volunteers were advised on appropriate diet substitutions to avoid increasing total energy intake. To monitor compliance with this request body weight was measured at each clinic visit.

Statistical analysis

It was estimated that a sample size of 12 subjects per arm would have at least 80% power to detect a statistically significant difference in reduction in MAP between the four treatment groups (allowing for multiple comparisons) using an ANOVA at the 0.05 significance level. The estimates were based upon the assumption of a linear dose response effect achieving a 5mmHg change in 24 hour MAP with the highest dose and a standard deviation of 4mmHg as indicated by unpublished pilot data. These estimates are supported by the previously reported ABP results with CF consumption (3, 4, 18).

Baseline characteristics were compared between groups by one-way analysis of variance (ANOVA). Comparison of changes in ABP between treatment groups (i.e. CF dose) across time (i.e. 3 and 6 week assessments) was made using repeated measures analysis of covariance (ANCOVA) using baseline ABP as the covariate. Where ANCOVA showed a statistically significant main effect, post-hoc comparisons (Tukey's HSD) were conducted to identify differences between means. To optimise the analysis of dose effects on ABP, a nested ANOVA design was used to examine changes in ABP from baseline by 3 and 6 weeks with time (i.e. weeks 3 and 6) nested in dose. Comparison of changes in HR between treatment groups across time was made using repeated measures ANOVA. Statistical significance was set at $p<0.05$. All data are presented as mean ± SEM.

Results

Participant flow is provided in Figure 1. In total, 53 volunteers completed the study. One volunteer was excluded due to mild persistent (>3 day) gastric symptoms that may have been related to the test beverage. No other adverse events related to the study were reported. Five volunteers withdrew during the study due to personal circumstances unrelated to the study. One volunteer was excluded from the analysis due to non-compliance to the study protocol, leaving 52 volunteers, or n=12-14 volunteers per arm. Trial withdrawals and exclusions did slightly impact upon the success of the minimisation process on the matching of groups for the stated baseline parameters as can be seen in Table 1. However, no significant between group differences were seen for the blocking parameters including age (p=0.45), BMI (p=0.28), and BP (Systolic p=0.86; Diastolic p=0.45). Compliance with consumption of cocoa supplements was greater than 98% in all groups. Body mass did not change during the intervention (p = 0.48 for treatment x time).

SBP, DBP, MAP or HR for 24-hour, night, day, and seated clinic measurements for each treatment group are provided in Tables 2-5. There were no dose x time interactions for seated clinic BP, but a significant time effect for SBP (p=0.02) and MAP p=0.01) was evident. However, there were significant dose x time effects for 24 hour MAP (p=0.047) and overnight HR (p = 0.041) assessed by ABP monitoring. ANCOVA of the change from baseline (week 0) to weeks 3 and 6 (with baseline values as a covariate) revealed a significant effect of dose on 24-hour ambulatory SBP (p=0.019), DBP (p=0.017) and MAP (p=0.008) and night ambulatory SBP (p=0.0006), DBP (p=0.005) and HR (p=0.043).

Nested analysis (time nested in dose) revealed significant dose effects for 24 hour MAP (p=0.0004), SBP (0.001) and DBP (p=0.002); night time MAP (p=0.01; Fig 3) and SBP (p=0.003) and day time MAP (p=0.02; Fig 3) and SBP (p=0.02). Post hoc analysis showed that for the 1052 mg flavanol

dose, the reductions in for 24 hour MAP ($p<0.001$), SBP ($p<0.02$) and DBP ($p<0.04$) were significantly different from all other doses (refer Figure 2). There were no significant effects on HR. Figure 3 displays a consistent reduction in day and night ABP to that seen over the full 24 hours, with significant effects observed at a CF level of 1052 mg/d.

Discussion

The principal finding of the current study was that the regular consumption of flavanol-rich reconstituted cocoa beverages reduced BP in untreated patients with borderline/mild hypertension. This antihypertensive effect was only evident at the highest dose of 1052 mg flavanols/day, with doses up to and including 712 mg/day failing to provide any significant reduction in BP in this study. This is the first study to directly compare the efficacy of differing dose levels of isolated CF. The doses used were based upon the range of CF levels that have been previously reported to lower BP. These findings suggest that BP can be lowered through the regular consumption of flavanol-rich cocoa beverages, though higher levels of CF may be required than has been observed with consumption of chocolate based CF rich products. Although this study provided the highest dose of CF to date the lack of effect on BP with lower doses is supported by previous studies using a non-chocolate food matrix. Whilst there was no evidence of a dose response effect over the doses tested in this trial, it may be the case that the highest dose in this study is on the lower end of a dose response curve with this type of CF product. Additionally, the maximum potential effect on BP is yet to be determined.

Four previous studies have investigated the longer-term effects of consuming flavanol-rich cocoa beverages on BP [14-16] using beverages providing daily flavanol doses ranging from 446 – 964 mg. Of these studies only one demonstrated a reduction in BP and this was a study which provided one of the highest doses of flavanols (902 mg/day). The finding of this study is in agreement with the outcome of the current study which demonstrated that a high daily flavanol dose is required in order to achieve a reduction in BP. However, these findings are in contrast with those which have examined the BP lowering effects of cocoa flavanols delivered in a chocolate food matrix where antihypertensive effects have been demonstrated with flavanol doses which were reported to be significantly lower than the levels used in the studies with cocoa beverages. A recent meta-analysis [9] which examined the antihypertensive effects of flavanol-rich chocolate found that daily

consumption of chocolate providing relatively modest doses of cocoa flavanols and procyanidins ranging from 246 mg to 500 mg provided significant reductions in BP. Subsequently, Taubert et al [9] showed that consuming as little as 30 mg of cocoa polyphenols per day in dark chocolate for 18 weeks was sufficient to lower systolic blood pressure (SBP) by 2.9 mmHg and diastolic blood pressure (DBP) by 1.9 mmHg. This latter flavanol dose is similar to the lowest flavanol dose provided in the current study where no effect on BP was observed. Thus, it appears there may be a discrepancy in the effective dose of flavanols when delivered in a chocolate matrix compared to a beverage mix. A previous bioavailability study showed that there was no difference in the acute increases in plasma or urinary concentrations of flavanols following consumption of chocolate or dry cocoa containing the same quantities of flavanols [21], and similar bioavailability of cocoa flavanols from chocolate and other sources is also supported by comparisons from other studies [1, 13, 21]. There has been some debate over the potential for the addition of milk protein to cocoa products to reduce flavanol bioavailability [22] and this would potentially impact on efficacy when consumed in milk drinks. However, other recent studies have not supported any effect of milk on reducing flavanol bioavailability [22-24].

A noteworthy protocol consideration when comparing the studies using flavanol-rich chocolate and flavanol-rich beverages are the degree of placebo control and blinding. Studies using chocolate have typically used a flavanol-poor white chocolate placebo which limits subject blinding and is not matched for all potentially bioactive nutrients including methylxanthines (3-4, 9). The use of flavanol-rich cocoa beverages allows close matching of placebo beverage nutrient composition as well as close matching for colour and flavour to facilitate a double blinded protocol (13-16). Muniyuppa et al (17) propose that the discrepancy between the results seen with dark chocolate and flavanol-rich cocoa beverages may be entirely due to these factors. The robust placebo control and blinding used in the present study combined with ABP monitoring provides a superior assessment of the anti-hypertensive potential of CF to those conducted previously.

The mechanism by which CF reduce BP has been largely attributed to their capacity to improve endothelial dilatory function [8, 25]. A number of studies have demonstrated that the regular intake of cocoa flavanols can improve endothelium-mediated vascular dilatory function, with these improvements being associated with an increased bioavailability of nitric oxide (NO) [2, 4, 25, 26, 28]. Recently Taubert et al [9] established a direct relationship which suggested that the BP lowering effects of cocoa flavanols were mediated by improved NO availability when they found a statistically significant correlation between the magnitude of reduction in BP and the magnitude of increase in S-nitrosoglutathione (a marker of NO availability) following 18weeks of consuming chocolate containing cocoa flavanols. However, some studies have demonstrated improvements in vascular dilatory function independently of changes in BP, suggesting that other mechanisms might also contribute to the antihypertensive effect. Two recent studies [14, 15] reported a sustained (non-acute) improvement in markers of endothelium-mediated dilatory function without concurrent changes to BP, while a third study [16] conducted in our laboratory showed improvement in both parameters but only a modest reduction was seen in BP while endothelium-mediated dilatory function improved by approximately 40%.

The lack of a demonstrable effect of cocoa consumption on seated clinic BP in the present study may also reflect the relative lack of sensitivity of this technique compared with ABP monitoring. Seated clinic BP reduced with time independently of flavanol dose, reflecting a habituation effect (regression to the mean) which most likely masked any treatment effect on BP. An habituation effect was not evident with ABP monitoring. In a previous study, we were able to demonstrate a statistically significant reduction in BP with clinic assessments [16], but the BP assessment protocol in that study was different from that used in the current study with clinic BP measured while participants were supine after a longer pre-assessment rest period. Additionally for the

previous study patients with elevated BP were not specifically recruited which may have reduced the likelihood of a 'white coat' hypertensive effect masking any effect of the cocoa supplement.

A key concern with the consumption of bioactive nutrients for health benefits is the question of potential adverse effects of the other ingredients. In the case of cocoa flavanols this concern is particularly associated with the high energy and saturated fat content of chocolate. According to HPLC analysis of flavanol content of various food types, to achieve the effective dose of flavanol seen in this study (1052mg) would require the daily consumption of 210g of standard dark chocolate or approximately double this amount of milk chocolate [29]. Given that 210 grams of dark chocolate provides in the order of 4700Kj and 35g of saturated fat a net health effect may be questionable [30]. This is in contrast to the 914kJ and 1.4g of saturated fat provided by the beverage used in this trial. However, depending upon the underlying causes of the previously discussed discrepancy between the effect of chocolate and flavanal rich cocoa beverages on BP, this comparison may not prove valid. It should be noted that the intention of this trial was not to evaluate the use of a flavanol rich beverage as a potential food supplement, but rather to determine the effect of various doses of cocoa flavanols on BP.

Ideally the lowest dose would deliver 0mg of flavanol; however, this would be not possible with our cocoa based product without significantly altering the flavour and or consistency of the drink, thereby compromising the treatment blinding. Although Taubert et al [9] reported an antihypertensive effect of 30mg CF/day, this has only been seen with a white vs. dark chocolate protocol and not with a well matched control so interpretation of these data are subject to the limitations listed above. Moreover others have failed to show effects on BP of considerably higher doses of isolated CF [14, 15]. Other concerns regarding the 33mg dose results in the present study relate to the lower age and BMI and greater proportion of males in this group. While care was taken to avoid this in the blocking process, with the relatively small subject numbers per treatment arm

small numbers of participant attrition can disrupt this balance. It could be argued that this may reduce the potential responsiveness of the control group, however the lack of BP reduction seen at the two intermediate doses suggests this has not influenced the overall results. Additionally, the differences seen in BMI and age were not statistically significant and the most important variable (BP) remained well matched across the four groups. The final limitation was the lack of explicit assessment of the effectiveness of the blinding protocol. The close matching of the cocoa products implies successful blinding, however these results could have been strengthened by assessing this.

In summary, this study provides the most robust assessment of the effects of cocoa flavanols on blood pressure to date. A significant antihypertensive effect of daily cocoa flavanol intake was observed at the highest dose of 1052mg flavanols/day with no BP lowering effect seen at the lower doses. This study supports the potential for cocoa flavanols to lower BP, but further research is required to determine the extent of antihypertensive benefit that can be achieved with different dietary sources and doses of flavanols in various cardiovascular pathologies.

Acknowlegements

The authors would like to acknowledge the contribution of all the study participants and MARS inc for the supply of the test product and financial support (by way of an Australian Government Food Innovation Grant) to conduct study.

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Table 1: Flavanol composition (monomers and polymers/oligomers) of daily beverage for each dose group.

Group No.	**1**	**2**	**3**	**4**
Total flavanol dose	**33**	**372**	**712**	**1052**
Epicatechin	0	69	138	208
Catechin	12	28	43	58
Other flavanols (polymeric/oligomeric)	20	275	530	785
Macronutrient				
Weight (g)	58	58	58	58
Energy (kJ)	904.5	907.9	911.2	914.6
Total fat (g)	2.6	2.6	2.6	2.6
Sat fat (g)	1.4	1.4	1.4	1.4
Total CHO (g)	31.0	31.2	31.5	31.8
Sugars (g)	17.3	17.4	17.6	17.7
Protein (g)	17.1	17.1	17.1	17.1
Caffeine (mg)	46.6	44.7	42.7	40.8
Theobromine (mg)	402.2	421.6	441.1	460.5

Table 2: Baseline characteristics. **(**mean ± SEM). M/F = ratio male to female; BMI = body mass index.

Group (mg flavanol/day)	**33 mg**	**372 mg**	**712 mg**	**1052 mg**
N	14	12	13	13
Age (years)	53.0 ± 6.7	56.2 ± 14.2	60.2 ± 13.7	56.8 ± 9.7
M/F	10/4	7/5	8/5	7/6
BMI (kg/m^2)	33.7 ± 16.8	28.7 ± 5.5	26.9 ± 16.2	27.9 ± 5.0

Table 3: Systolic BP (mmHg) for 24 hour, night, day and seated clinic measurement in each treatment group (mg flavanol/day) at each assessment point (mean ± SD).

Dose (mg)	BP (mmHg)	Week 0	Week 3	Week 6	Δ week 3	Δ week 6
33	24hr	133.3 ± 11.6	132.5 ± 14.6	133.9 ± 13.8	-0.8 ± -5.6	0.6 ± 7.1
N=14	Night	117.1 ± 14.2	116.9 ± 15.3	118.6 ± 10.5	-0.2 ± 6.4	1.5 ± 8.6
	Day	140.1 ± 11.2	140.0 ± 15.0	141.2 ± 15.7	-0.1 ± -7.1	1.1 ± 10.1
	Clinic	145.4 ± 9.7	142.1 ± 10.5	143.3 ± 13.1	-3.3 ± -6.0	-2.1 ± -9.0
372	24hr	133.1 ± 11.2	132.9 ± 13.3	133.4 ± 13.0	-0.2 ± -5.0	0.3 ± 6.5
N=12	Night	115.5 ± 12.3	115.6 ± 10.5	115.4 ± 13.7	0.1 ± 6.5	-0.1 ± -8.7
	Day	141.1 ± 12.3	140.8 ± 15.1	142.5 ± 14.8	-0.3 ± -6.5	1.4 ± 6.9
	Clinic	142.9 ± 10.5	142.0 ± 11.9	139.6 ± 9.0	-0.9 ± -9.0	-3.3 ± -7.9
712	24hr	127.4 ± 7.6	127.9 ± 9.0	128.9 ± 8.3	0.5 ± 6.2	1.5 ± 5.9
N=13	Night	111.0 ± 9.7	113.1 ± 10.0	114.7 ± 10.7	2.1 ± 8.7	3.7 ± 5.5
	Day	134.6 ± 7.6	134.4 ± 9.4	135.6 ± 8.0	-0.2 ± -5.5	1.0 ± 6.6
	Clinic	143.0 ± 9.0	140.2 ± 10.4	140.0 ± 9.4	-2.8 ± -6.6	-3.0 ± -9.7
1052	24hr	127.8 ± 9.4	121.0 ± 10.7	124.0 ± 10.7	-6.8 ± -5.9	-3.8 ± -5.5
N=13	Night	113.5 ± 11.8	106.8 ± 9.7	110.1 ± 7.3	-6.7 ± -8.0	-3.4 ± -8.0
	Day	133.2 ± 9.4	127.4 ± 12.1	129.8 ± 13.5	-5.8 ± -5.2	-3.4 ± -7.3
	Clinic	143.0 ± 8.0	139.8 ± 11.1	138.9 ± 13.5	-3.2 ± -3.1	-4.1 ± -12.1

Table 4: Diastolic BP (mmHg) for 24 hour, night, day and seated clinic measurement in each treatment group (mg flavanol/day) at each assessment point (mean ± SD).

Dose (mg)	BP (mmHg)	Week 0	Week 3	Week 6	Δ week 3	Δ week 6
33	24hr	82.2 ± 7.1	81.6 ± 7.9	82.8 ± 7.9	-0.6 ± -3.4	0.6 ± 4.1
N=14	Night	70.0 ± 8.6	69.7 ± 9.0	71.6 ± 6.4	-0.3 ± -5.6	1.6 ± 4.5
	Day	87.2 ± 6.4	86.7 ± 8.2	87.6 ± 9.0	-0.5 ± -4.5	0.4 ± 6.4
	Clinic	88.0 ± 6.0	87.7 ± 7.5	88.1 ± 7.9	-0.3 ± -3.7	0.1 ± 4.9
372	24hr	81.3 ± 8.7	80.6 ± 10.0	81.4 ± 10.0	-0.7 ± -2.8	0.1 ± 3.6
N=12	Night	69.3 ± 7.6	69.1 ± 9.0	69.3 ± 8.7	-0.2 ± -4.5	0.0 ± 5.8
	Day	87.0 ± 10.0	86.1 ± 11.4	87.2 ± 11.1	-0.9 ± -3.5	0.2 ± 3.6
	Clinic	86.4 ± 7.6	85.9 ± 8.0	83.9 ± 9.7	-0.5 ± -5.2	-2.5 ± -5.8
712	24hr	78.3 ± 9.4	78.2 ± 9.4	79.1 ± 10.5	-0.1 ± -3.2	0.8 ± 2.5
N=13	Night	66.9 ± 8.7	67.8 ± 10.5	69.6 ± 11.5	0.9 ± 5.4	2.7 ± 5.4
	Day	83.2 ± 10.1	82.6 ± 9.7	83.6 ± 10.5	-0.6 ± -3.2	0.4 ± 3.2
	Clinic	85.9 ± 9.4	84.4 ± 11.5	84.2 ± 10.1	-1.5 ± -5.8	-1.7 ± -5.0
1052	24hr	76.3 ± 9.0	72.5 ± 9.4	74.2 ± 8.7	-3.8 ± -3.6	-2.1 ± -3.2
N=13	Night	66.0 ± 8.3	63.3 ± 8.3	65.0 ± 6.9	-2.7 ± -4.7	-1.0 ± -4.3
	Day	80.0 ± 9.4	76.5 ± 10.1	78.3 ± 9.4	-3.5 ± -3.2	-1.7 ± -4.7
	Clinic	83.2 ± 9.4	81.2 ± 10.8	81.2 ± 8.7	-2.0 ± -6.1	-2.0 ± -3.2

Table 5: Mean arterial pressure (mmHg) for 24 hour, night, day and seated clinic measurement in each treatment group (mg flavanol/day) at each assessment point (mean ± SD).

Dose (mg)	BP (mmHg)	Week 0			Week 3			Week 6			Δ week 3			Δ week 6		
33	**24hr**	98.5	±	8.2	97.8	±	9.4	98.9	±	9.4	-0.7	±	-3.4	0.4	±	4.9
	Night	85.8	±	9.4	85.3	±	10.5	87.4	±	7.1	-0.5	±	-5.2	1.6	±	4.9
N=14	**Day**	104.1	±	7.5	103.5	±	9.7	104.3	±	10.5	-0.6	±	-4.5	0.2	±	6.7
	Clinic	107.1	±	6.7	105.9	±	8.2	106.5	±	9.4	-1.2	±	-3.4	-0.6	±	-4.9
372	**24hr**	98.3	±	8.0	98.4	±	9.7	98.9	±	9.4	0.1	±	3.5	0.6	±	4.5
	Night	85.8	±	8.3	85.8	±	8.0	85.9	±	8.7	0.0	±	5.2	0.1	±	6.0
N=12	**Day**	104.0	±	8.7	104.0	±	11.4	105.1	±	10.7	0.0	±	4.8	1.1	±	5.2
	Clinic	105.3	±	6.9	104.6	±	8.7	102.5	±	8.7	-0.7	±	-5.5	-2.8	±	-4.1
712	**24hr**	95.4	±	6.9	95.2	±	7.2	96.0	±	8.3	-0.2	±	-4.0	0.6	±	3.6
	Night	82.6	±	7.6	84.1	±	8.3	85.8	±	9.7	1.5	±	6.9	3.2	±	4.7
N=13	**Day**	101.0	±	6.5	99.9	±	7.6	100.9	±	7.9	-1.1	±	-4.0	-0.1	±	-4.0
	Clinic	105.0	±	8.3	103.0	±	9.7	102.8	±	8.7	-2.0	±	-5.4	-2.2	±	-5.8
1052	**24hr***	93.8	±	6.9	89.2	±	8.7	90.8	±	7.2	-4.6	±	-4.0	-3.0	±	-3.2
	Night	82.8	±	7.9	79.2	±	7.6	80.8	±	5.8	-3.6	±	-5.4	-2.0	±	-6.1
N=13	**Day**	97.9	±	7.2	93.8	±	9.7	95.2	±	8.7	-4.1	±	-4.0	-2.7	±	-4.7
	Clinic	103.1	±	7.6	100.7	±	9.7	100.4	±	9.4	-2.4	±	-7.2	-2.7	±	-7.9

***** Significant dose x time interaction (p=0.047)

Table 6: Heart Rate (beats/min) for 24 hour, night, day and seated clinic measurement in each treatment group (mg flavanol/day) at each assessment point (mean ± SD).

Dose (mg)	BP (mmHg)	Week 0			Week 3			Week 6			Δ week 3			Δ week 6		
33	**24hr**	68.1	±	9.7	70.8	±	10.5	70.6	±	10.9	2.7	±	5.2	2.5	±	6.0
	Night	60.4	±	8.6	62.4	±	8.2	61.6	±	10.1	2.0	±	3.4	1.2	±	4.9
N=14	**Day**	71.5	±	10.9	74.2	±	12.0	74.4	±	11.2	2.7	±	6.4	2.9	±	6.7
	Clinic	64.6	±	13.1	65.0	±	13.1	63.9	±	12.3	0.4	±	4.9	-0.7	±	-6.4
372	**24hr**	72.7	±	10.4	72.5	±	9.7	73.5	±	10.4	-0.2	±	-2.4	0.8	±	5.9
	Night	64.4	±	10.7	64.3	±	11.4	64.3	±	9.7	-0.1	±	-3.8	-0.1	±	-5.9
N=12	**Day**	76.3	±	10.0	76.3	±	9.4	77.9	±	10.7	0.0	±	-3.5	1.6	±	5.2
	Clinic	72.4	±	14.2	70.0	±	10.7	69.2	±	11.1	-2.4	±	-6.6	-3.2	±	-6.6
712	**24hr**	68.6	±	8.7	70.4	±	10.5	69.7	±	8.3	1.8	±	4.0	1.1	±	3.2
	Night	61.2	±	7.9	63.1	±	10.8	61.9	±	7.2	1.9	±	4.7	0.7	±	2.5
N=13	**Day**	71.9	±	9.7	73.4	±	10.8	73.2	±	9.0	1.5	±	4.3	1.3	±	4.7
	Clinic	64.8	±	8.7	65.9	±	9.0	64.6	±	7.6	1.1	±	6.1	-0.2	±	-4.3
1052	**24hr**	74.1	±	5.4	74.3	±	9.0	76.9	±	9.4	0.2	±	7.2	2.8	±	7.2
	Night	67.3	±	6.5	66.3	±	7.6	70.8	±	7.2	-1.0	±	-5.0	3.5	±	4.3
N=13	**Day**	77.0	±	6.1	78.1	±	10.5	79.8	±	10.5	1.1	±	9.4	2.8	±	9.0
	Clinic	74.3	±	7.2	74.1	±	11.9	71.8	±	10.8	-0.2	±	-9.7	-2.5	±	-9.4

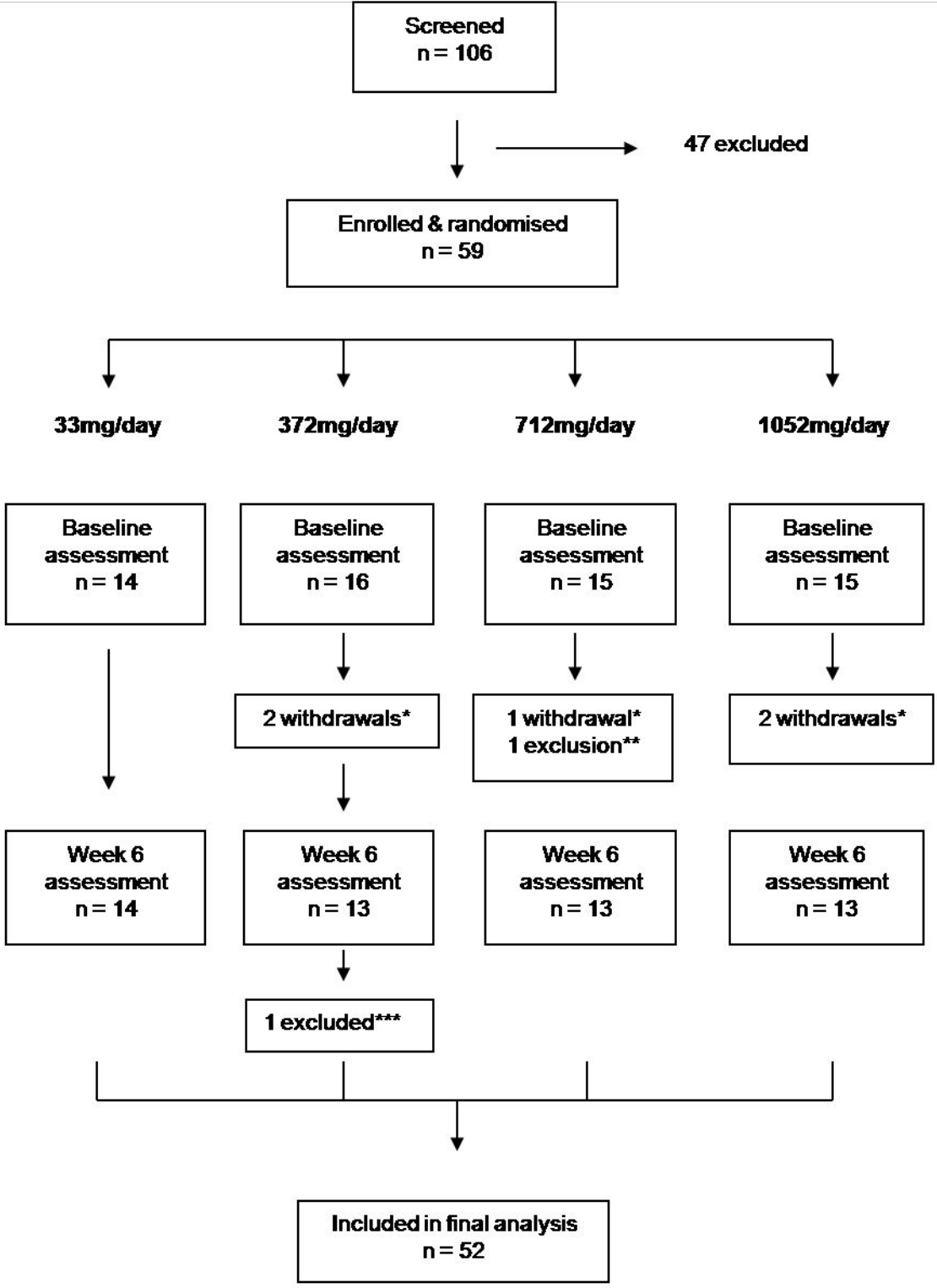
Table 7: Summary of study

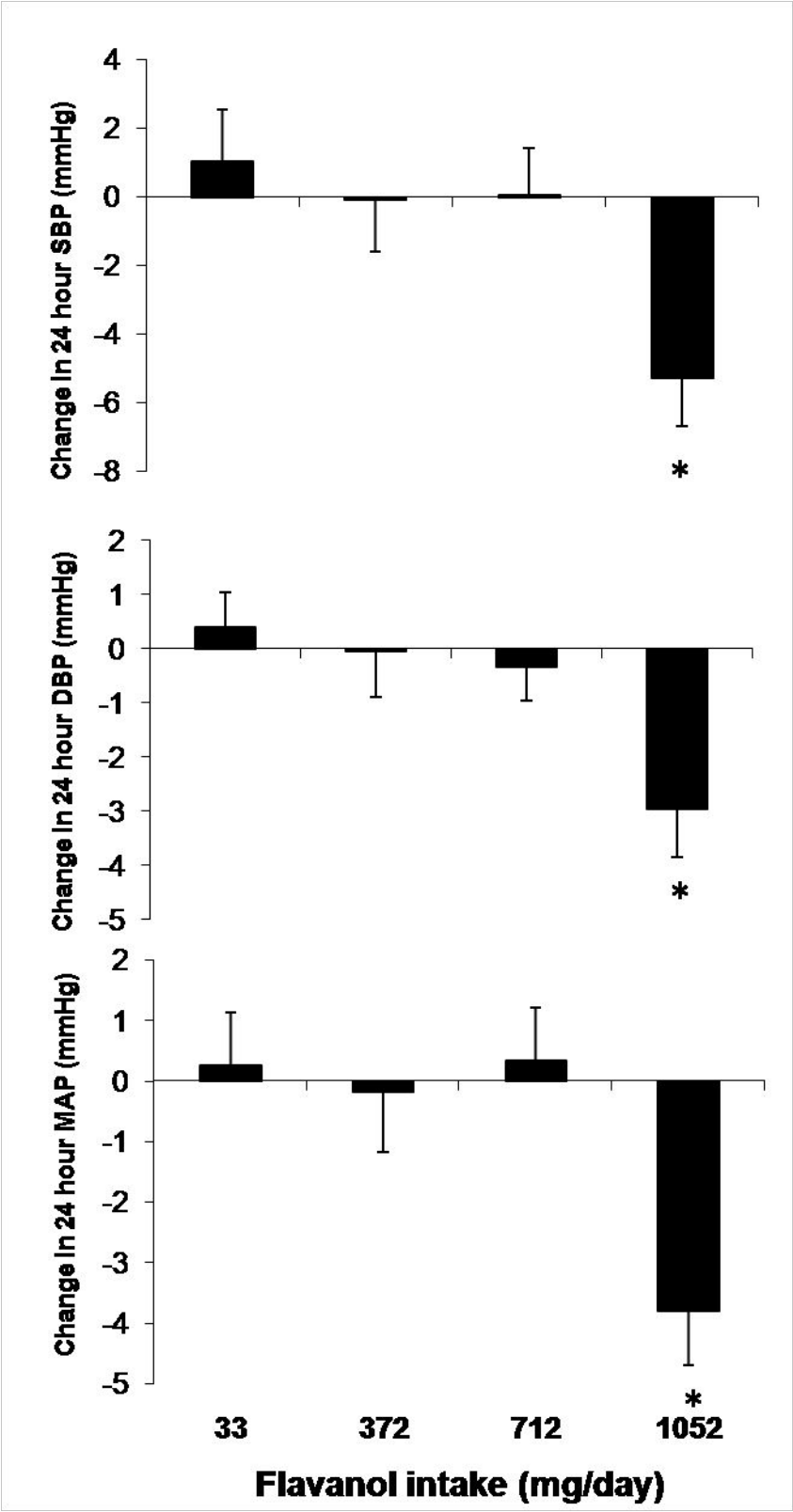
What is known about this topic?	What this study adds
• **Consumption of cocoa rich chocolate has been shown to lower blood pressure (BP)** A meta-analysis of several intervention trials has demonstrated a BP lowering effect of cocoa rich dark chocolate compared to cocoa free white chocolate. • **Cocoa flavanols (CF)improve endothelial function** Isolated CF and cocoa solids have been shown to enhance endothelial vasodilatation in the same manner as cocoa rich chocolate. • **Lack evidence for effect of CF on BP** Chronic consumption of isolated cocoa solids delivering CF doses in excess of those provided in chocolate have not lowered BP.	• **Robust evaluation of effects of isolated cocoa solids on BP** Dose response evaluation of CF on ambulatory BP in borderline/mild hypertensives. • **The study identifies a threshold dose for cocoa flavanols** Only the highest dose of CF (1052mg/day) lowered BP. This would account for lack of effect on BP with sub-threshold doses of CF in previous studies.

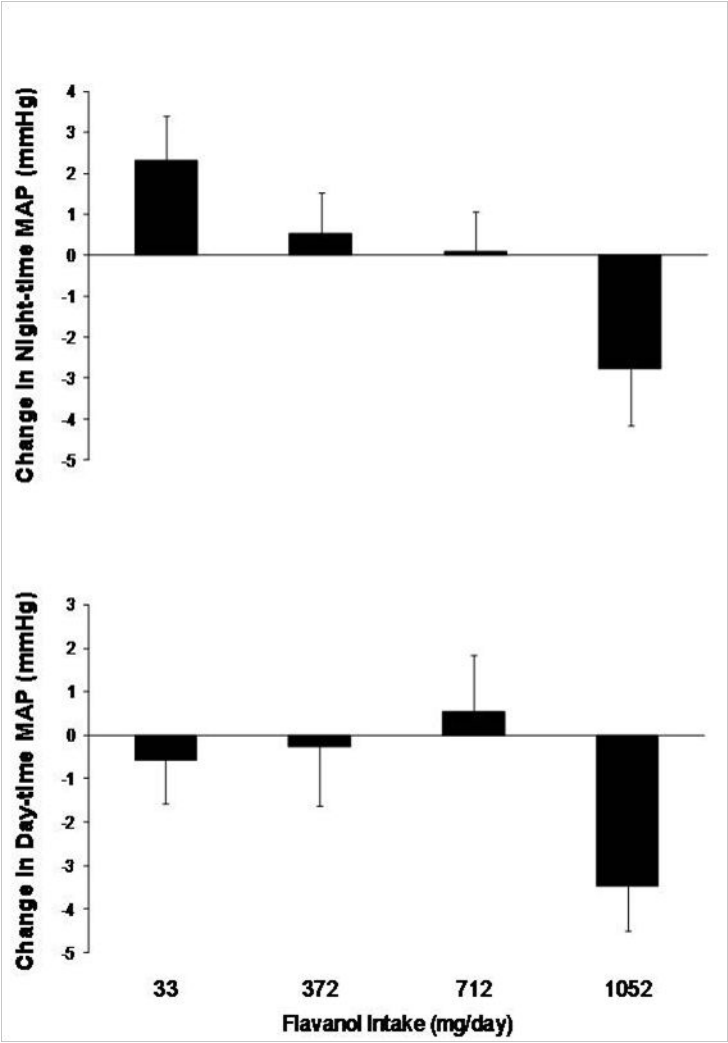
Figure 1: Consort diagram * withdrawn for personal or health reasons not related to study protocol; ** excluded due to gastric symptoms possibly related to cocoa beverage; ***excluded due to non-compliance to study protocol (deliberate weight loss during intervention period).

Figure 2: Dose-related effects of cocoa flavanols on 24 hour ambulatory blood pressure. Values represent means ± SEM of changes from baseline (average of changes to 3 and 6 weeks). *Significantly different from all other doses by nested analysis ($p<0.001$).

Figure 3: Dose-related effects of cocoa flavanols on daytime and overnight ambulatory blood pressures. Values represent means ± SEM of changes from baseline (average of changes to 3 and 6 weeks).


Screened
n = 106
47 excluded
Enrolled & randomised
n = 59
33mg/day
372mg/day
712mg/day
1052mg/day
Baseline assessment n = 14
Baseline assessment n = 16
Baseline assessment n = 15
Baseline assessment n = 15
2 withdrawals*
1 withdrawal*
1 exclusion**
2 withdrawals*
Week 6 assessment n = 14
Week 6 assessment n = 13
Week 6 assessment n = 13
Week 6 assessment n = 13
1 excluded***
Included in final analysis
n = 52


Change In 24 hour SBP (mmHg)
4
2
0
-2
-4
-6
-8
*
Change In 24 hour DBP (mmHg)
2
1
0
-1
-2
-3
-4
-5
*
Change In 24 hour MAP (mmHg)
2
1
0
-1
-2
-3
-4
-5
*
33
372
712
1052
Flavanol intake (mg/day)


Change in Night-time MAP (mmHg)
4
3
2
1
0
-1
-2
-3
-4
-5
Change in Day-time MAP (mmHg)
3
2
1
0
-1
-2
-3
-4
-5
33
372
712
1052
Flavanol Intake (mg/day)