Effects of vitamin E, vitamin C and polyphenols on rate of blood pressure variation: results of two randomised controlled trials

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Short running head: Antioxidants and BP variation

Word count: 7470
Number of tables: 4
Number of figures: 5
Number of supplementary digital files: 1

Key words: Blood pressure variation; vitamin E; vitamin C; polyphenols

Abstract

A high blood pressure (BP) variability, which may be an important determinant of hypertensive end-organ damage, is emerging as an important predictor of cardiovascular health. Dietary antioxidants can influence BP, but their effects on variability have yet to be investigated. We aimed to assess the effects of vitamin E, vitamin C and polyphenols on rate of daytime and nighttime ambulatory BP variation. Two randomised, double-blind, placebo-controlled trails were performed. In the first trial (vitamin E), 58 individuals with type 2 diabetes received 500 mg/d RRR-α-tocopherol, 500 mg/d mixed tocopherols or placebo for 6 weeks. In the second trial (vitamin C-polyphenols), 69 treated hypertensive individuals received 500 mg/d vitamin C, 1000 mg/d grape-seed polyphenols, both vitamin C and polyphenols, or neither (placebo) for 6 weeks. Twenty-four hour ambulatory BP and rate of measurement-to-measurement BP variation were assessed at baseline and 6 weeks. Compared with placebo, α-tocopherol, mixed tocopherols, vitamin C and polyphenols did not significantly alter daytime or nighttime rate of systolic BP, diastolic BP or pulse pressure variation ($P>0.05$). Treatment with the combination of vitamin C and polyphenols resulted in higher BP variation: nighttime rate of systolic BP variation ($P=0.022$) and pulse pressure variation ($P=0.0036$) were higher; and daytime rate of systolic BP variation was higher ($P=0.056$). Vitamin E, vitamin C or grape seed polyphenols did not significantly alter rate of BP variation. However, the increase in rate of BP variation suggests that the combination of high doses of vitamin C and polyphenols could be detrimental in treated hypertensive individuals.

Introduction

Emerging evidence suggests that variation in BP contributes to cardiovascular disease. [1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12]. The method we have used in the current study to measure rate of BP variation involves determining the slope for the change in BP between each reading over time. It provides an estimate of the rate or speed that BP changes from reading to reading during daytime and nighttime. It then provides a continuous hourly measurement of the rate of BP variation [13]. A major advantage of this method is that it increases the power to detect smaller differences compared with the use of a single summary measurement such as the standard deviation (SD). In a recently published trial we showed that this measure is more sensitive than the SD for establishing small effects on BP variation [13].

Hypertension and type 2 diabetes are associated with elevated oxidative stress [14; 15]. An increased production of free radicals in the arterial wall may reduce nitric oxide bioavailability [14] and cause endothelial dysfunction [16]. Intakes of dietary antioxidants including vitamin E, vitamin C and flavonoids have been associated with less oxidative stress [17; 18; 19], and reduced risk of cardiovascular disease [20; 21; 22; 23; 24]. However, results of intervention trials have been less consistent. High-dose vitamin E may have detrimental effects on BP [25] and cardiovascular outcomes [26]. In contrast, vitamin C [27; 28] and polyphenols [29; 30] can reduce BP, but their benefits on cardiovascular events and mortality have not been established.

The current trials were designed primarily to assess effects of vitamin E [25], and vitamin C and polyphenols [27] on BP. We found that supplementation with vitamin E resulted in significantly increased BP in individuals with type 2 diabetes [25]. We also found that while vitamin C alone reduced systolic BP, the combination of vitamin C with polyphenols significantly increased BP [27]. The BP data collected from these trials afforded the opportunity to explore for the first time the novel hypothesis that supplementation with dietary antioxidants reduces the rate of BP variation. Results of previous studies suggest that an increased intake of flavonoids, a class of water-soluble dietary antioxidants, can significantly reduce the rate of BP variation [13; 31] Therefore, the primary objective of this analysis was to assess the effects of vitamin E, vitamin C and polyphenols on the rate of BP variation.

Methods

Participants: vitamin E study

Participants were recruited from the Perth general population via newspaper advertisements between February and December 2004. A total of 58 men and women with a previous diagnosis of type 2 diabetes, via oral glucose tolerance test or prescribed oral hypoglycaemic therapy were randomised to the trial. The trial was conducted from the University of Western Australia School of Medicine and Pharmacology located at Royal Perth Hospital in Western Australia. At least 3 weeks prior to study entry, and throughout the trial, participants ceased taking any dietary supplements. Usual medication was taken as prescribed for the duration of the trial. Exclusion criteria included: body mass index greater than 35 kg/m^2; use of insulin; prior or current use of vitamin E/tocopherol supplements; type 1 diabetes; previous coronary or cerebrovascular event within the previous 6 months; current smoking; premenopausal women; regular use of nitrate medication; use of non-steroidal anti-inflammatory medication; use of the oral contraceptive; elevated serum creatinine (>110 mmol/l men or >100 mmol/l women) or alcohol intake >40 g/day men or >30 g/day women. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Western Australia Human Research Ethics Committee. Written informed consent was obtained from all subjects. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12605000093684).

Participants: vitamin C-polyphenols study

Participants were recruited from the Perth general population via newspaper advertisements between February 2002 and May 2003. A total of 74 men and women with a previous physician diagnosis of hypertension and taking one or more antihypertensive drugs for at least 3 months were randomised to the trial. The trial was conducted from the University of Western Australia School of Medicine and Pharmacology located at Royal Perth Hospital in Western Australia. All participants had a mean 24-h ambulatory systolic BP of > 125 mm Hg, and at least one additional cardiovascular disease risk factor. Additional risk factors included previous coronary or cerebrovascular event > 6 months prior to recruitment, hyperlipidaemia (total cholesterol > 6 mmol/l), use of lipid-lowering therapy, or smoking > 5 cigarettes/day. At least 3 weeks prior to study entry, and throughout the trial, participants ceased taking any dietary supplements. Usual medication was taken as prescribed for the duration of the trial. Exclusion criteria included: body mass index greater than 35 kg/m^2; previous coronary or cerebrovascular event within the past 6

months; heart failure or unstable disease; premenopausal women; use of nitrate medication; use of oral contraceptive; diagnosed diabetes mellitus; fasting glucose > 7 mmol/l, or elevated serum creatinine (men > 110 mmol/l or women > 100 mmol/l). In addition, throughout the trial participants were asked to limit tea and coffee intake to 3 cups/day, and cease all red wine and commercial fruit juice for the duration of the study in order to limit background polyphenol intake. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Western Australia Human Research Ethics Committee. Written informed consent was obtained from all subjects. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12613000514707).

Design: vitamin E study

Following a 3-week washout period, participants were allocated by the study coordinator to a study treatment via permutated block randomization, using computer-generated random numbers (generated by a biostatistician who was not involved in the conduct of the study) sealed in opaque envelopes. All study personnel and participants were blinded to treatment assignment for the duration of the study. The chief investigator held the code for the capsules in a sealed envelope which was not broken until the end of the trial. Participants were assigned to receive either: (i) 500 mg/day RRR-α-tocopherol; (ii) 500 mg/day mixed tocopherols (60% γ-, 25% δ- and 15% α-tocopherol); or (iii) 500 mg/day placebo (soybean oil stripped of tocopherols) for 6 weeks in a double-blind fashion. Capsules were taken twice each day with food as 250 mg capsules. This dose of vitamin E was chosen to reflect that used in previous intervention studies of α-tocopherol. Outcome measures were performed at the end of the washout (baseline) and at the end of the 6 week intervention (post).

Design: vitamin C-polyphenols study

Following a 3-week washout period, participants were allocated to a study treatment via block randomization, using computer-generated random numbers (generated by a biostatistician who was not involved in the conduct of the study) sealed in opaque envelopes. All study personnel and participants were blinded to treatment assignment for the duration of the study. The chief investigator held the code for the capsules in a sealed envelope which was not broken until the end of the trial. Volunteers were assigned to receive either (i) 500 mg/day vitamin C and matched grape-seed polyphenol placebo, (ii) 1000 mg/day grape-seed polyphenols and matched vitamin C placebo, (iii) 500 mg/day vitamin C and 1000 mg/day grape-seed polyphenols, or (iv) matched

placebo tablets for both grape-seed polyphenols and vitamin C, for 6 weeks in a double-blind fashion. Tablets were taken twice daily at meal times as 250 mg and 500 mg of vitamin C and polyphenols, respectively. The vitamin C, polyphenols and placebo tablets were visually identical. This dose of vitamin C was chosen to reflect that used in previous intervention studies [28]. The dose of polyphenols was chosen to substantially increase total polyphenol intake. All tablets were supplied by Taractechnologies (Nurioopta, South Australia, Australia). The polyphenols in each tablet were 20.5% polymeric compounds, with a mean degree of polymerization of 2.7, and the rest was made up of monomers, dimers, and trimers. Gallic acid was 0.05 wt %[32]. Outcome measures were performed at the end of the washout (baseline) and at the end of the 6 week intervention (post).

Blood pressure and its rate of variation

During both studies, BP was assessed as 24 h ambulatory BP with BP and heart rate measured every 20 min during the day time and every 30 min at night time [25; 27]. Ambulatory BP was assessed by a trained researcher who fitted a Spacelabs monitor (Spacelabs Medical Inc. Redmond, WA, USA) and explained its use to the participants. The monitor was fitted to the non-dominant arm approximately 2.5 cm above the antecubital fossa. Participants were instructed to continue their usual daily activities and to avoid any vigorous exercise. Measurements showing an error code or those with a pulse pressure of less than 20 mm Hg were excluded from the analysis. Blood pressure traces were considered complete if more than 80% of the recordings were valid.

Within-visit rate of variation of systolic and diastolic BP, pulse pressure and heart rate were calculated for day time (08:00–20:00) and night time (22:00-06:00) periods from the 24 h ambulatory BP traces [13]. The 24 h rate of BP variation was not considered for analysis because BP usually dips overnight with sleeping and rises rapidly in the morning on waking. The periods with the steepest fall (20:00-22:00) and rise (06:00-08:00) in BP were excluded from the analysis. The within-visit rate of measurement-to-measurement BP and heart rate variation was calculated using the slope of the change in systolic BP, diastolic BP, pulse pressure and heart rate between each reading over time [13]. Ambulatory BP variability was measured as the SD of BP measurements over 24 h as the weighted 24-h SD according Bilo et al [33]. Measures of BP SD provide only a single summary measure rather than continuous hourly measurement over 24 h as is the case with the rate of BP variation. For this reason, the power to detect differences is reduced for these measures.

Body weight, biochemistry and compliance

Body weight was recorded with participants wearing light clothing and no footwear. Height was measured at baseline using a wall-mounted stadiometer. Fasting lipids and glucose were measured in plasma samples using routine laboratory methods in the PathWest Laboratory at Royal Perth Hospital, Western Australia. Compliance was assessed via post-intervention capsule/tablet counts and analysis of serum α- and γ-tocopherol levels, plasma vitamin C and urinary polyphenol metabolites, including 3-hydroxyphenylpropionic acid and 4-O-methylgallic acid, via high-performance liquid chromatography and gas chromatography-mass spectrometry [25; 27].

Statistical analysis

The sample size for each of the two studies was calculated using BP as the primary outcome. The sample size in each study provided >80% power to detect a 5 mm Hg difference in systolic blood pressure [25; 27]. The BP data collected and the sample size for each study also provided sufficient power to explore effects on rate of BP variation. An effect size of 15% or more was regarded as potentially clinically relevant. Hypertensive subjects have a higher rate of daytime and nighttime systolic BP variation of approximately 15% [6]. Using previously published data [13] we estimated that a group size of 16 or more would provide at least 80% power to detect a 4 mm Hg/h difference (~15%) in daytime rate of systolic BP variation (based on 12 hourly BP measurements, a SD of 10 mm Hg, and within-subject within and between visit correlations of 0.2), and a 3 mm Hg/h difference (~15%) in nighttime rate of systolic BP variation (based on 8 hourly BP measurements, a SD of 8 mm Hg, and within-subject within and between visit correlations of 0.3).

The primary analysis was per-protocol. This population was defined as participants who completed the intervention. Descriptive statistics are presented as mean and SD. Categorical variables are summarized by number in each category. A type-1 error rate of $P<0.05$ was the level of significance used for all hypothesis testing. Log transformation was performed on variables not normally distributed, as assessed using normal probability plots. At baseline, characteristics of participants in each group were compared using the independent-samples t-test and the chi-squared test for categorical variables. The between-group differences are presented as least squares means and 95% confidence intervals.

Outcome variables were analysed using linear mixed models in STATA. The STATA "xtmixed" and "margins" commands were used to determine baseline-adjusted between-group differences at 6 months. All analyses were by group (rather than by main effects) because we previously observed a significant interaction between vitamin C and polyphenols that affected BP [27]. Subject was

included as a random factor in each model with either a random intercept only or random intercept and random slope for hour according to a comparison of model fit which was assessed using a likelihood ratio test. Fixed effects included visit (baseline or post), treatment group, hour and treatment group X hour. The overall effect of treatment was established using the global significance test for the treatment group term. The baseline-adjusted difference between each active treatment group and placebo were also assessed for significance and reported individually. The models included post hoc adjustment for multiple comparisons using Tukey's adjustment and differences between groups were also adjusted for potential confounding factors, which were considered as covariates in separate models.

Results

Baseline characteristics

Vitamin E

Fifty-eight participants were randomised to the vitamin E trial and 55 participants completed the trial (**Figure 1**). Baseline characteristics of the participants according to treatment group are presented in **Table 1**. The mean compliance estimated using capsule counts was 97% and was not different between groups. Serum α-tocopherol concentrations increased substantially following treatment with α-tocopherol and serum γ-tocopherol concentrations increased substantially following treatment with mixed tocopherols [25; 34]. The type and dose of antihypertensive medication used was unchanged during the trial.

Vitamin C-polyphenols

Seventy-four participants were randomised to the vitamin C-polyphenols trial and 69 participants completed the trial (**Figure 2**). Baseline characteristics of the participants according to treatment group are presented in **Table 2**. The mean compliance estimated using tablet counts was 96% and was not different between groups. Plasma vitamin C concentrations increased substantially following treatment with vitamin C and urinary excretion of a polyphenol metabolite (3-hydroxyphenylpropionic acid) increased substantially following treatment with polyphenols [27]. The type and dose of antihypertensive medication used was unchanged during the trial.

Rate of blood pressure variation

Vitamin E

As reported previously, treatment with α-tocopherol or mixed tocopherols resulted in significantly higher BP (~2 to 7 mm Hg) relative to placebo in individuals with type 2 diabetes [25]. The mean daytime and night time rate of BP variation at baseline and the end of 6 weeks intervention (post) for each treatment are presented **Table 3**. Compared with placebo, treatment with α-tocopherol or mixed tocopherols did not significantly alter daytime or nighttime rate of BP variation (**Figure 3**). Adjustment for BP at the same time points did not alter interpretation of the findings. Rate of heart rate variation was not significantly altered (data not presented).

Compared with placebo, treatment with α-tocopherol or mixed tocopherols did not significantly alter the weighted 24 h systolic or diastolic BP SD (see supplementary data, Figure S1).

Vitamin C-polyphenols

As reported previously, relative to placebo, treatment with vitamin C alone resulted in lower systolic BP (~2 mm Hg), but the combination of vitamin C and polyphenols resulted in higher systolic and diastolic BP (~3 to 5 mm Hg) in treated hypertensive individuals [27]. The mean daytime and nighttime rate of BP variation at baseline and the end of 6 weeks intervention (post) for each treatment are presented **Table 4**. Compared with placebo, treatment with vitamin C alone or polyphenols alone did not significantly alter daytime or nighttime rate BP variation. However, treatment with the combination of vitamin C and polyphenols resulted in higher rate of BP variation: nighttime rate of systolic BP (P=0.022) and pulse pressure (P=0.0036) variation was significantly higher; and there was a borderline increase in daytime rate of systolic BP variation (P=0.056) (**Figure 4**). Adjustment for BP levels did not alter interpretation of the findings. Rate of heart rate variation was not significantly altered (data not presented). There was a diurnal pattern of rate of BP variation, with the nadir between 02:00 and 04:00, a rapid increase between 06:00 and 08:00, and a peak between 08:00 and 11:00. The pattern of rate of systolic BP variation over 24 hours at baseline and the end of 6 weeks for placebo and the combination of vitamin C and polyphenols is presented **Figure 5**.

Compared with placebo, treatment with vitamin C alone or polyphenols alone or the combination of vitamin C and polyphenols did not significantly alter the weighted 24 h systolic or diastolic BP SD (see supplementary data, Figure S2).

Discussion

Two randomised, double blind, placebo-controlled trials were conducted to investigate the effects of supplementation with major dietary antioxidants on BP. In the present analysis the effects of vitamin E, vitamin C and polyphenols on rate of daytime and nighttime ambulatory BP variation were explored. We previously reported that supplementation with both α-tocopherol and mixed tocopherols resulted in a significantly higher BP in these study participants with type 2 diabetes [25]. Despite the substantial increases in BP, we found no evidence for an effect of either α-tocopherol and mixed tocopherols on rate of BP variation in the present analysis. We have also previously reported that while vitamin C alone reduced systolic BP, the combination of vitamin C with polyphenols resulted in significantly increased BP in these study participants with treated hypertension [27]. The present analysis did not provide any evidence for an effect of either vitamin C or polyphenols alone to alter rate of BP variation or other measures of BP variability, but demonstrated that the combination of vitamin C with polyphenols increased BP variability. Adjustment for BP did not alter interpretation of the results.

High BP is a major risk factor for cardiovascular and total mortality [35]. Blood pressure level, measured in the office/clinic, home or ambulatory setting is the primary indicator of individual risk [1]. However, other measures derived from the measurement of BP, including nighttime BP, day-to night BP dip and measures of within and between day BP variability, may also contribute to risk. All of these measures are believed to be linked to risk by worsening hypertensive end-organ damage. However, it has yet to be established that an intervention to alter blood pressure variability, or other measures derived from measurement of BP, can reduce the risk of cardiovascular disease. Therefore, the importance of measurement of blood pressure variability, in addition to BP level, for prediction of individual risk is not clear.

There is increasing evidence that measures of BP variation provide an independent risk factor for cardiovascular disease [3; 8; 9]. In large prospective studies with event and mortality outcomes, BP variation is most often assessed as the BP variability measured using the within- or between-visit SD. Results of recent studies demonstrate that effects on both BP and BP variability determine the ultimate benefits of antihypertensive medication on cardiovascular risk [10]. In addition, BP variability may be as important as BP in determining hypertensive end-organ damage [1; 2].

The method we have used to measure BP variation involves determining the slope for the change in BP between each reading over time. It provides an estimate of the rate or speed that BP changes between readings during daytime and nighttime. This provides a continuous hourly measurement of

the rate of BP variation [13], rather than a single value within-visit SD. The calculation of hourly measurements of rate of BP variation over 24 h is a major advantage of this method because it increases the power to detect smaller differences compared with the use of a single summary measurement of variability such as the SD. Furthermore, the SD does not adequately capture the measurement-to-measurement variability. Highly variable reading-to-reading changes can have the same SD as a gradual change over several hours. Rate of BP variation has been positively associated with hypertension, carotid atherosclerosis [6] and left ventricular mass [7]. It has also been associated with end-organ damage in hypertensive patients [5], and adverse outcomes in acute stroke [12]. The rate of BP variation has yet to be related to risk of cardiovascular events or death. These studies are needed in order to establish whether rate of BP variation is a predictor of cardiovascular disease outcomes.

Hypertension is the leading risk factor for cardiovascular and total mortality. It affects one quarter of world's population, and is projected to affect one third of world's population within 20 years (~1.5 billion people) [35]. Changing diet and lifestyle are the initial primary means of addressing hypertension. Several diet and lifestyle factors are proven to lower blood pressure. These include engaging in regular moderate physical activity [36], maintaining a healthy body weight or weight loss [37], limiting alcohol consumption [38], reducing salt (sodium) intake [39], and consuming a diet rich in plant foods [40; 41]. Intakes of the major dietary antioxidants, including vitamin E, vitamin C and polyphenols are increased in plant food-rich diets. There is evidence that these dietary constituents contribute to lower blood pressure. [28; 29; 30; 42], by reducing oxidative stress[17; 18; 19], enhancing nitric oxide status and improving endothelial function[43; 44; 45]. However, the benefit of supplementation with these constituents is less clear.

High-dose supplementation with vitamin E may increase BP [25] and risk of cardiovascular disease [26]. Despite a substantial increase in BP with vitamin E supplementation, we found no evidence for an effect on the rate of BP variation. We estimated that a sample size of 16 per group would provide at least 80% power to detect a 15% difference between groups. A post-hoc power calculation based on the observed SD and within subject and between visit correlations indicated that the study had at least 80% power to detect a 16% difference in daytime rate of systolic BP variation and an 18% difference in nighttime rate of systolic BP variation. Observed differences in daytime rate of systolic BP variation were less than 5% and observed differences in nighttime rate of systolic BP variation were approximately 15%. Therefore, or results do not rule out a smaller effect of less than 16% and 18% in day-time and night-time rate of systolic BP variation respectively.

Our result indicates that mechanisms involved in regulation of BP and BP variation may differ. The differential effects of antihypertensive medications that lower BP on BP variation are consistent with this suggestion. Calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and β-blockers reduce BP. However, while calcium-channel blockers may reduce BP variability, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and β-blockers may increase BP variability [11]. Several potential mechanisms for detrimental effects on BP in this study were previously investigated. However, the investigations did not provide evidence for effects on mechanisms linked to elevated BP, including increased vasoconstriction, increased inflammation and increased oxidative stress [25; 34; 46].

The combination of vitamin C and polyphenols resulted in higher BP [27], higher rate of BP variation and higher estimates of BP variability. The magnitude of differences in rate of BP variation during daytime and nighttime were similar, but were significant only for systolic BP and pulse pressure at nighttime. These results suggest that supplementation with vitamin C and polyphenols taken together results in detrimental effects on BP and BP variability. Several potential mechanisms for these detrimental effects on BP in this study were previously investigated. The investigations explored effects on markers of oxidative stress, vasoactive fatty acid metabolites and markers of inflammation. These factors were not significantly altered during the study, and therefore do not provide evidence for effects on these mechanisms [27]. The possibility that the combination of vitamin C and grape-seed polyphenols may be interfering with the metabolism of antihypertensive drugs has not been ruled out.

Indirect evidence suggests that dietary polyphenols may contribute to a lower rate of BP variation. A component of black tea solids, which are rich in polyphenols, was found to reduce the rate of systolic BP variation during nighttime by up to 16% [13], and a supplement containing polyphenols found in chocolate and soy was found to reduce rate of pulse pressure variation during daytime by approximately 20% [31]. The observed differences between polyphenols alone and placebo in rate of BP variation in the current study were generally less than 5%. Post-hoc analysis of power based on the observed SD and within subject and between visit correlations indicated that the study had at least 80% power to detect a 15% difference in daytime rate of systolic BP variation and a 19% difference in nighttime rate of systolic BP variation. Therefore, we cannot rule out smaller benefits of polyphenols.

There is evidence that polyphenols derived from tea [29; 43], chocolate [47; 48] and soy [49; 50] can enhance endothelial function and lower blood pressure. Although the present analysis does not support a role for the polyphenols to reduce BP variation, the structure of the polyphenols may

influence bioactivity. Grape seed polyphenols are primarily polymeric proanthocyanidins which are metabolized to smaller molecular weight phenolic acids, with unknown bioactivity, in the large intestine [32]. There is stronger evidence that the monomeric flavonoids found in tea, chocolate and soy can be absorbed and have direct effects on vascular function [51] by enhancing nitric oxide status [44].

Therefore, we have shown that vitamin E, vitamin C and polyphenols did not significantly alter daytime or nighttime rate of BP variation. However, treatment with the combination of vitamin C and polyphenols resulted in higher BP variation. While mechanisms responsible are not known, the results do suggest that the combination of high doses of vitamin C and polyphenols could be detrimental in treated hypertensive individuals.

Acknowledgements

Cognis Nutrition and Health, Cardinal health and Taractechnologies provided the supplements.

Financial support

Funding for the study was provided by the National Health and Medical Research Council of Australia, grant numbers 139067 and 254568. JMH and GAH were supported by National Health and Medical Research Council Fellowships. NCW was supported by a Medical Research Foundation/University of Western Australia Postdoctoral Fellowship..

Conflict of interest

None

Authorship

JMH, KDC, IBP, JHYW, LJB, NCW designed the research, JMH, KDC, IBP, CPB, JHYW, LJB, NCW performed the research, JMH, RJW, CPB, EVL, GAH, NCW analysed the data, JMH, RJW conducted the statistical analyses and JMH, KDC, RJW, IBP, CPB, JHYW, LJB, EVL, GAH, NCW wrote the manuscript. All authors read and approved the final manuscript.

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Table 1 Baseline characteristics of participants in the vitamin E study according to treatment group

	α-Tocopherol		Mixed tocopherols		Placebo	
	mean	SD	Mean	SD	mean	SD
n	18		19		18	
Male/female	13/5		12/7		16/2	
Age (y)	64	7	58±	4	62±	7
BMI (kg/m^2)	29.2	2.0	27.7	2.8	27.6	4.3
Daytime systolic BP (mm Hg)	133.7	13.3	135.9	9.8	129.9	14.1
Daytime diastolic BP (mm Hg)	79.3	7.1	82.7	8.7	77.5	8.1
Nighttime systolic BP (mm Hg)	120.6	14.5	120.4	11.5	112.3	10.2
Nighttime diastolic BP (mm Hg)	68.2	8.5	69.9	8.6	63.7	7.8
Hypertension n (%)	10 (56)		13 (68)		12 (67)	
Antihypertensive medication n (%)	10 (56)		9 (47)		9 (50)	
Current smoker n (%)	0 (0)		0 (0)		0 (0)	
Alcohol drinker n (%)	13 (72)		17 (89)		17 (94)	

Results are mean and SD or n (%). BP, blood pressure; BMI, body mass index. α-Tocopherol, 500 mg/day of RRR-α-tocopherol; Mixed tocopherols, 500 mg/day mixed tocopherols (60% γ-, 25% δ- and 15% α-tocopherol); Placebo, 500 mg/day placebo (soybean oil stripped of tocopherols). Hypertension, use of antihypertensive medication or 24 hour ambulatory systolic BP>125 mm Hg. Current smoker, smoking more than 5 cigarettes per day. Alcohol drinker, consumes at least one standard drink of alcohol (10 g) per week. There were no significant differences between groups in baseline characteristics.

Table 2 Baseline characteristics of participants in the vitamin C-polyphenols study according to treatment group

	Vitamin C		Polyphenols		Vitamin C + Polyphenols		Placebo	
	mean	SD	Mean	SD	mean	SD	mean	SD
n	19		16		16		18	
Male/female	12/7		12/4		10/6		14/4	
Age (y)	60	6	61	6	62±	7	64±	8
BMI (kg/m^2)	28.7	3.6	27.7	3.4	28.6	2.6	29.3	4.3
Daytime systolic BP (mm Hg)	137.9	12.7	138.2	14.8	143.5	11.4	136.4	11.6
Daytime diastolic BP (mm Hg)	84.8	9.5	83.3	10.9	83.5	11.3	81.0	8.7
Nighttime systolic BP (mm Hg)	125.6	13.7	124.9	13.5	130.4	17.1	127.9	11.5
Nighttime diastolic BP (mm Hg)	74.5	10.2	72.7	11.0	72.7	14.4	72.8	9.3
Hypertension n (%)	19 (100)		16 (100)		16 (100)		18 (100)	
Antihypertensive medication n (%)	19 (100)		16 (100)		16 (100)		18 (100)	
Current smoker n (%)	1 (5)		2 (13)		1 (6)		0 (0)	
Alcohol drinker n (%)	14 (74)		8 (50)		15 (94)		11 (61)	

Results are mean and SD or n (%). BP, blood pressure; BMI, body mass index. . Vitamin C, 500 mg/day vitamin C. Polyphenols, 1000 mg/day grape-seed polyphenols. Placebo, matched vitamin C placebo or grape-seed polyphenols placebo. Hypertension, use of antihypertensive medication or 24 hour ambulatory systolic BP>125 mm Hg. Current smoker, smoking more than 5 cigarettes per day. Alcohol drinker, consumes at least one standard drink of alcohol (10 g) per week. The percent of alcohol drinkers differed across the groups (P=0.045), but otherewise there were no significant differences between groups in baseline characteristics.

Table 3 The mean and SD of daytime and night time rate of blood pressure (BP) variation at baseline and the end of 6 weeks intervention (post) for α-tocopherol, mixed tocopherol and placebo treatments.

	α-Tocopherol				Mixed Tocopherols				Placebo			
	baseline		post		baseline		post		baseline		post	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Daytime rate of BP variation												
Systolic (mm Hg/h)	26.7	10.5	26.5	11.0	27.0	11.1	26.2	9.0	23.3	8.7	25.2	8.8
Diastolic (mm Hg/h)	18.1	6.5	18.7	6.1	19.0	7.2	16.8	7.1	17.5	7.4	18.0	6.4
Pulse pressure (mm Hg/h)	26.6	9.5	22.9	8.5	24.6	9.3	24.7	7.9	21.5	8.4	22.1	8.6
Heart rate variation (bpm/h)	18.9	10.1	18.2	9.5	18.9	11.1	19.6	10.0	20.1	12.1	17.9	9.7
Nighttime rate of BP variation												
Systolic (mm Hg/h)	19.3	7.5	20.7	8.8	17.9	8.2	18.5	8.5	18.6	6.3	18.9	6.6
Diastolic (mm Hg/h)	15.1	6.7	16.8	6.8	14.1	5.7	14.6	6.6	15.1	5.6	14.8	6.3
Pulse pressure (mm Hg/h)	15.0	7.2	15.3	7.7	14.7	7.9	13.3	6.4	14.2	5.8	13.9	6.6
Heart rate variation (bpm/h)	8.9	6.1	9.9	5.6	9.1	5.6	9.3	5.6	10.2	7.2	11.5	9.2

Results are mean and SD. BP, blood pressure. α-Tocopherol, 500 mg/day of RRR-α-tocopherol; Mixed tocopherols, 500 mg/day mixed tocopherols (60% γ-, 25% δ- and 15% α-tocopherol); Placebo, 500 mg/day placebo (soybean oil stripped of tocopherols).

Table 4 The mean and SD of daytime and night time rate of blood pressure variation at baseline and the end of 6 weeks intervention (post) for each vitamin C, polyphenols, vitamin C + plyphenols and placebo treatments

	Vitamin C				Polyphenols				Vitamin C + Polyphenols				Placebo			
	baseline		post		baseline		post		baseline		post		baseline		post	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Daytime rate of BP variation																
Systolic (mm Hg/h)	25.1	9.0	27.1	10.7	27.9	10.8	26.2	10.0	26.0	9.9	30.5	10.4	27.3	9.1	26.1	9.3
Diastolic (mm Hg/h)	19.6	7.6	20.8	8.4	18.4	6.6	18. 7	6.6	18.2	6.9	20.5	7.9	19.9	7.2	19.3	7.2
Pulse pressure (mm Hg/h)	23.2	8.8	22.8	9.0	23.3	10.2	23.0	8.9	23.2	8.4	25.8	11.6	24.4	9.6	23.3	9.1
Heart rate variation (bpm/h)	16.3	9.7	16.9	8.7	15.5	8.8	15.8	11.9	15.7	7.1	16.0	7.3	16.7	8.3	15.9	8.4
Nighttime rate of BP variation																
Systolic (mm Hg/h)	18.0	7.0	19.2	8.6	19.5	7.8	17.8±	8.1	17.8	9.4	21.7	10.6	18.0	8.7	17.5	7.0
Diastolic (mm Hg/h)	14.1	6.5	15.9	6.3	15.4	6.0	14.8	7.1	15.2	7.3	17.1	7.2	14.5	6.1	14.1	5.8
Pulse pressure (mm Hg/h)	13.6	5.6	14.2	6.3	14.0	6.0	12.7	5.6	13.6	7.2	17.3	9.0	14.6	7.7	13.3	5.7
Heart rate variation (bpm/h)	8.1	4.4	8.6	5.2	8.4	5.7	7.0	4.1	8.2	4.9	9.1	5.2	7.2	4.1	8.1	4.9

Results are mean and SD. BP, blood pressure. Vitamin C, 500 mg/day vitamin C. Polyphenols, 1000 mg/day grape-seed polyphenols. Placebo, matched vitamin C placebo or grape-seed polyphenols placebo.

Figure 1 Vitamin E study design and flow of participants (Adapted from Ward et al [25])

Figure 2 Vitamin C-polyphenols study design and flow of participants (Adapted from Ward et al [27])

Figure 3 Differences in rate of blood pressure (BP) variation during daytime and nighttime for α-tocopherol (α-Toc) and mixed tocopherols (Mixed Toc) relative to placebo. Values are mean and SEM.

Figure 4 Differences in rate of blood pressure (BP) variation during daytime and nighttime for vitamin C (VC), polyphenols (Poly) and vitamin C plus polyphenols (VC+Poly) relative to placebo. Values are mean and SEM. * $P<0.05$.

Figure 5 Diurnal pattern of rate of systolic blood pressure variation for placebo and the combination of vitamin C and polyphenols. Data are the unadjusted (raw) mean values, calculated as the 3 h moving average, for each hour over 24 hours according to treatment at baseline and 6 weeks (post). The periods with the steepest fall (20:00-22:00) and rise (06:00-08:00) in blood pressure were excluded from the analysis.

Figure 1

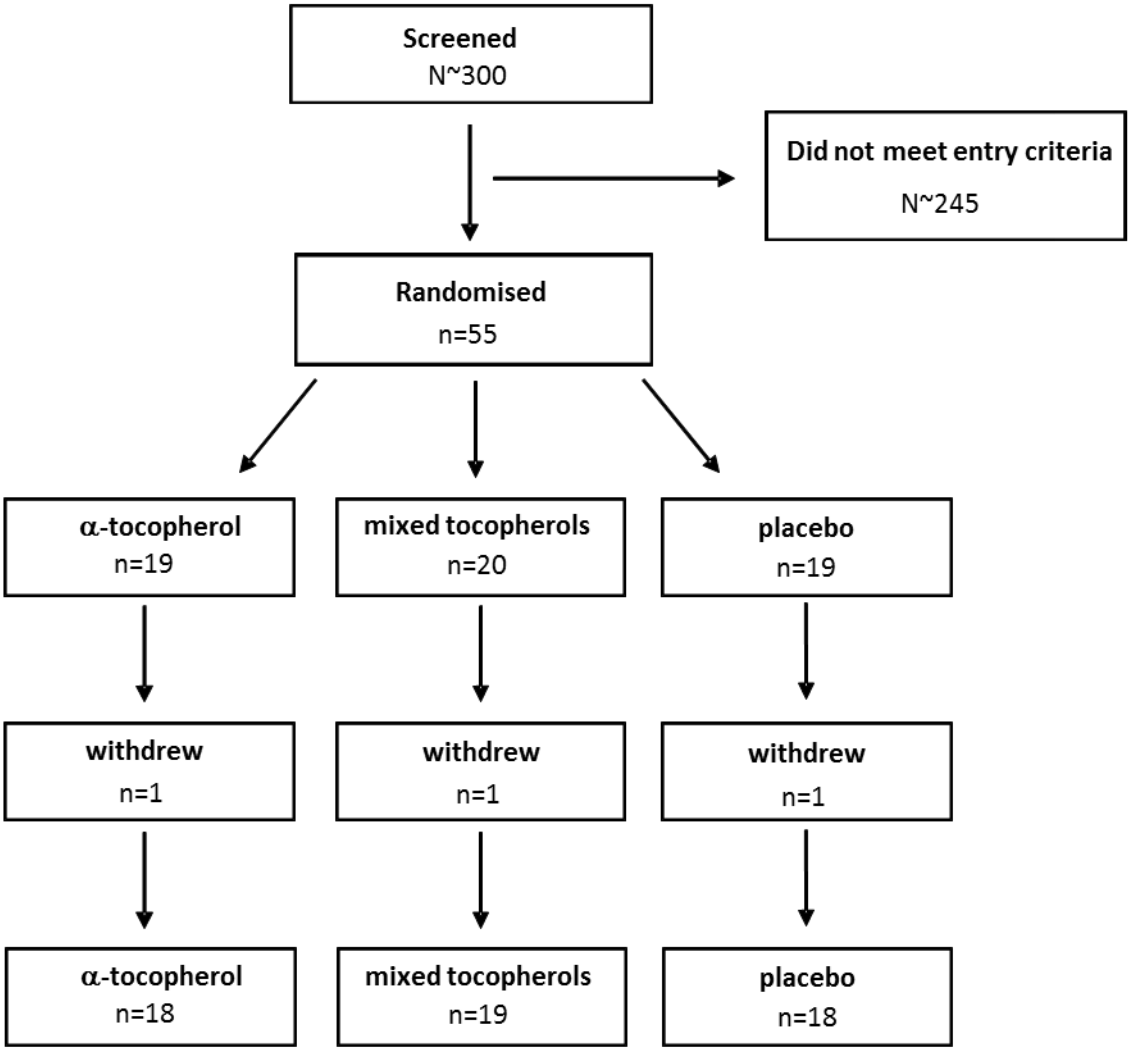
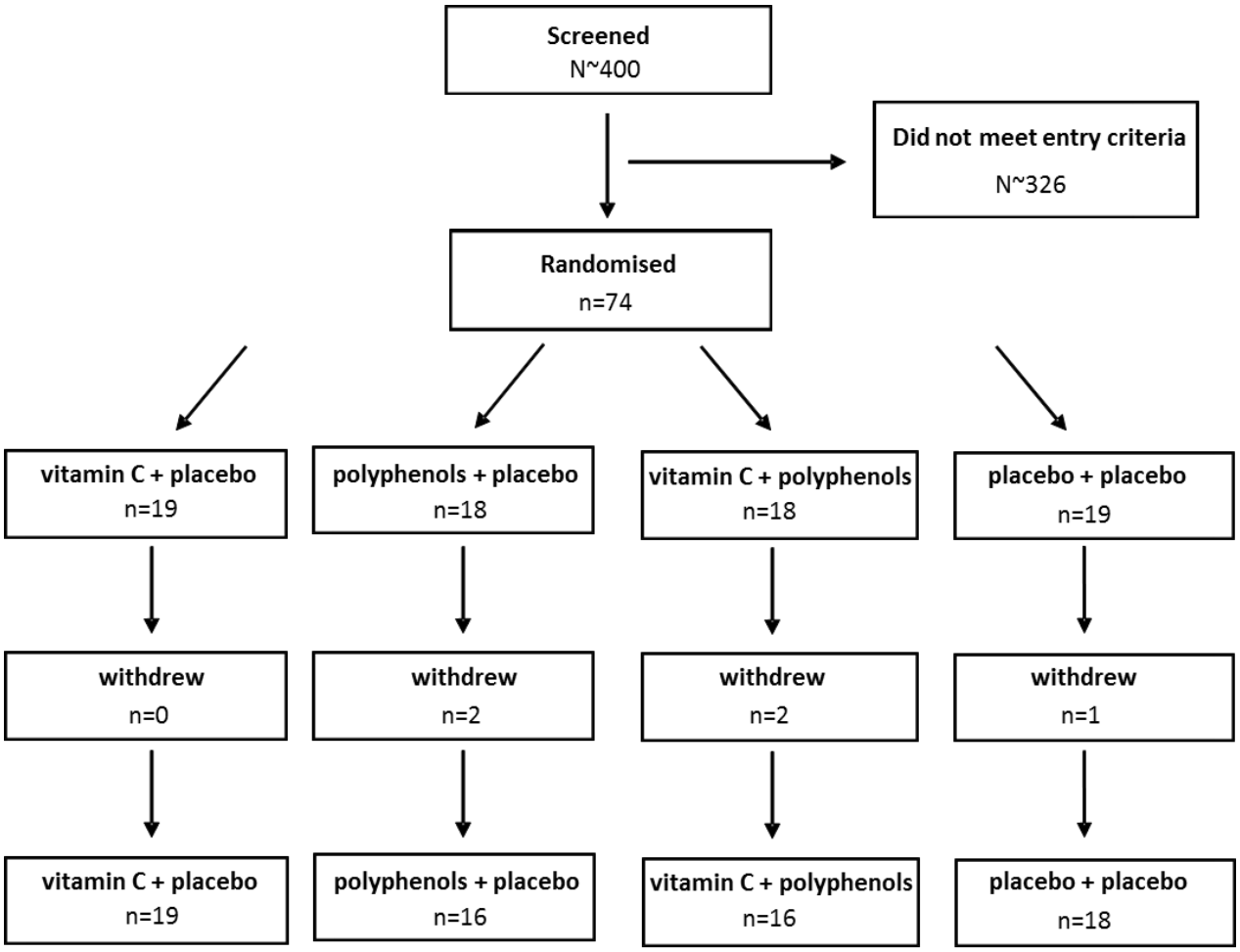

Screened
N~300
Did not meet entry criteria
N~245
Randomised
n=55
α-tocopherol
n=19
mixed tocopherols
n=20
placebo
n=19
withdrew
n=1
withdrew
n=1
withdrew
n=1
α-tocopherol
n=18
mixed tocopherols
n=19
placebo
n=18

Figure 2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Did not meet entry criteria
N~326
Randomised
n=74
vitamin C + placebo
n=19
polyphenols + placebo
n=18
vitamin C + polyphenols
n=18
placebo + placebo
n=19
withdrew
n=0
withdrew
n=2
withdrew
n=2
withdrew
n=1
vitamin C + placebo
n=19
polyphenols + placebo
n=16
vitamin C + polyphenols
n=16
placebo + placebo
n=18

Figure 3

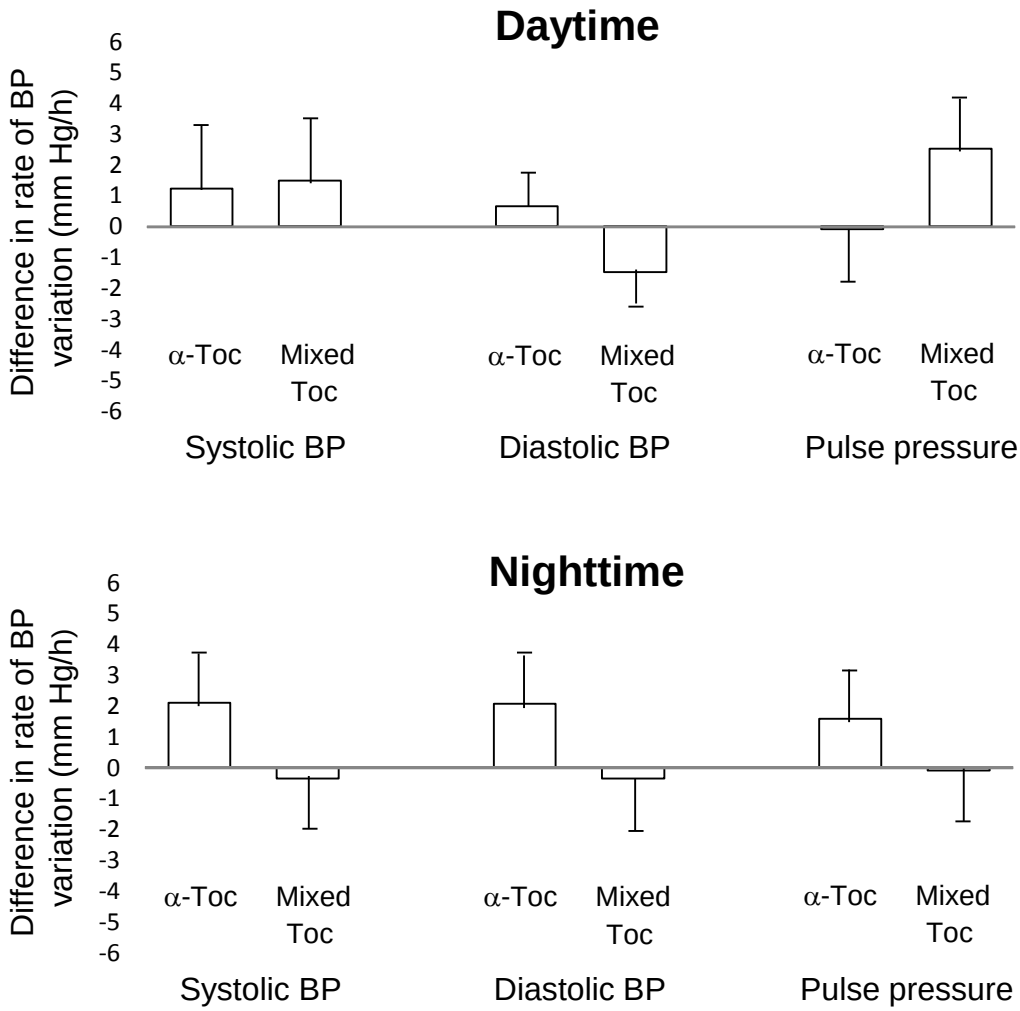

Daytime
Difference in rate of BP variation (mm Hg/h)
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
α-Toc
Mixed Toc
α-Toc
Mixed Toc
α-Toc
Mixed Toc
Systolic BP
Diastolic BP
Pulse pressure
Nighttime
Difference in rate of BP variation (mm Hg/h)
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
α-Toc
Mixed Toc
α-Toc
Mixed Toc
α-Toc
Mixed Toc
Systolic BP
Diastolic BP
Pulse pressure

Figure 4

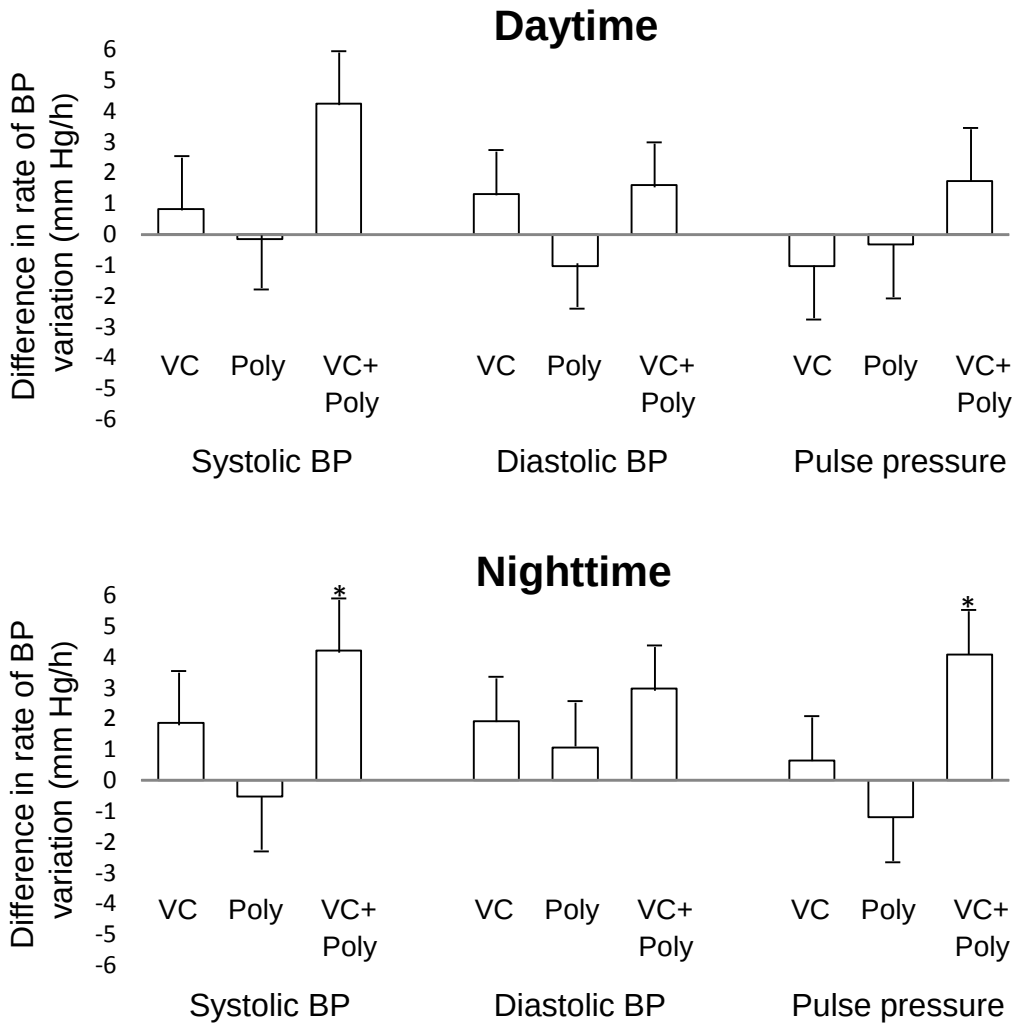
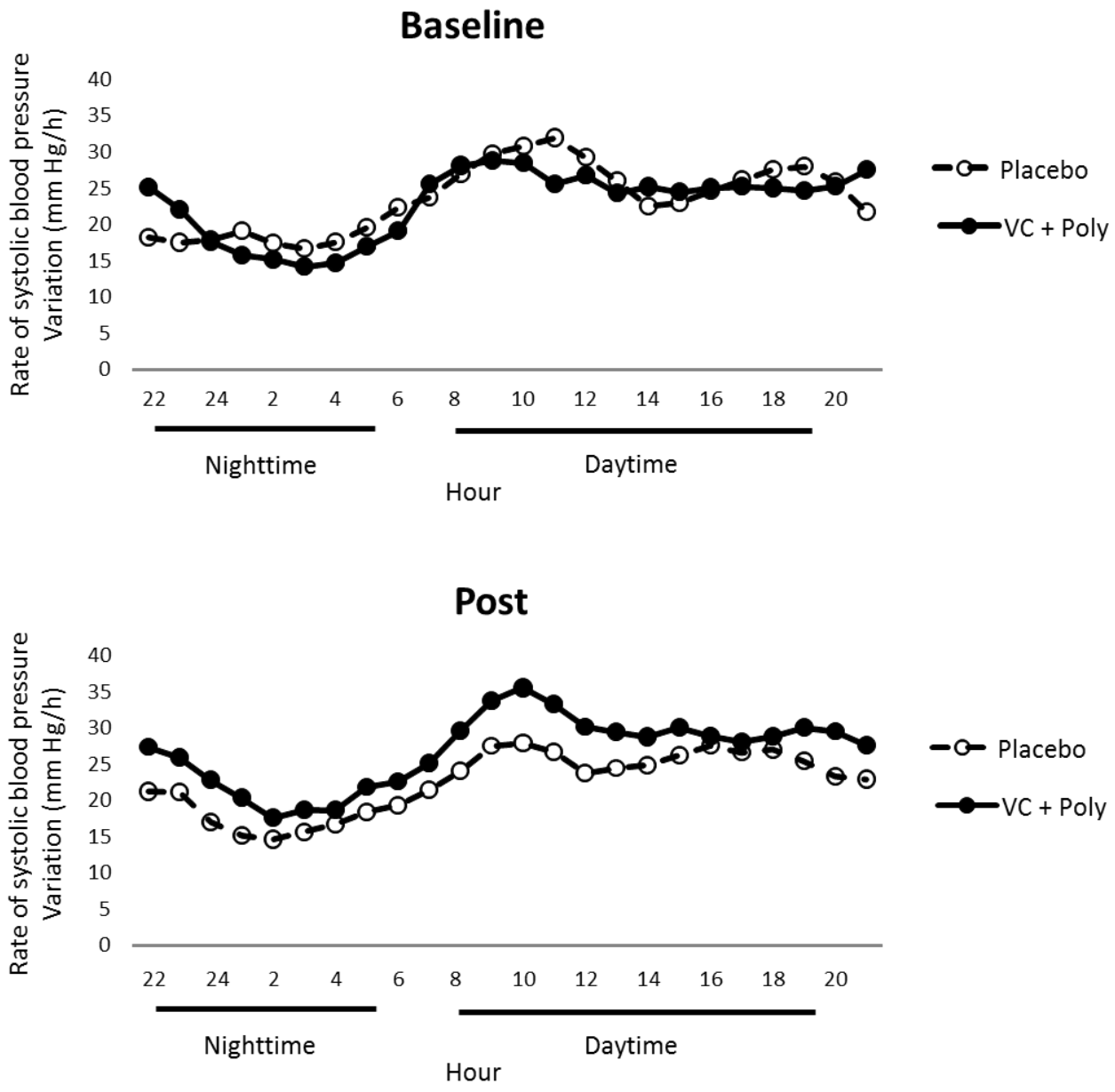

Daytime
Difference in rate of BP variation (mm Hg/h)
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
VC
Poly
VC+ Poly
VC
Poly
VC+ Poly
VC
Poly
VC+ Poly
Systolic BP
Diastolic BP
Pulse pressure
Nighttime
Difference in rate of BP variation (mm Hg/h)
*
*
VC
Poly
VC+ Poly
VC
Poly
VC+ Poly
VC
Poly
VC+ Poly
Systolic BP
Diastolic BP
Pulse pressure

Figure 5


Baseline
Rate of systolic blood pressure Variation (mm Hg/h)
40
35
30
25
20
15
10
5
0
22 24 2 4 6 8 10 12 14 16 18 20
Nighttime
Daytime
Hour
Placebo
VC + Poly
Post
Rate of systolic blood pressure Variation (mm Hg/h)
40
35
30
25
20
15
10
5
0
22 24 2 4 6 8 10 12 14 16 18 20
Nighttime
Daytime
Hour
Placebo
VC + Poly

SUPPLEMENTARY DATA

Effects of vitamin E, vitamin C and polyphenols on rate of blood pressure variation: results of two randomised controlled trials

Jonathan M Hodgson[1], Kevin D Croft[1], Richard J Woodman[2], Ian B Puddey[1], Catherine P Bondonno[1], Jason HY Wu[1,3], Lawrence J Beilin[1], Elena V Lukoshkova[4], Geoffrey A Head[5], Natalie C Ward[1]

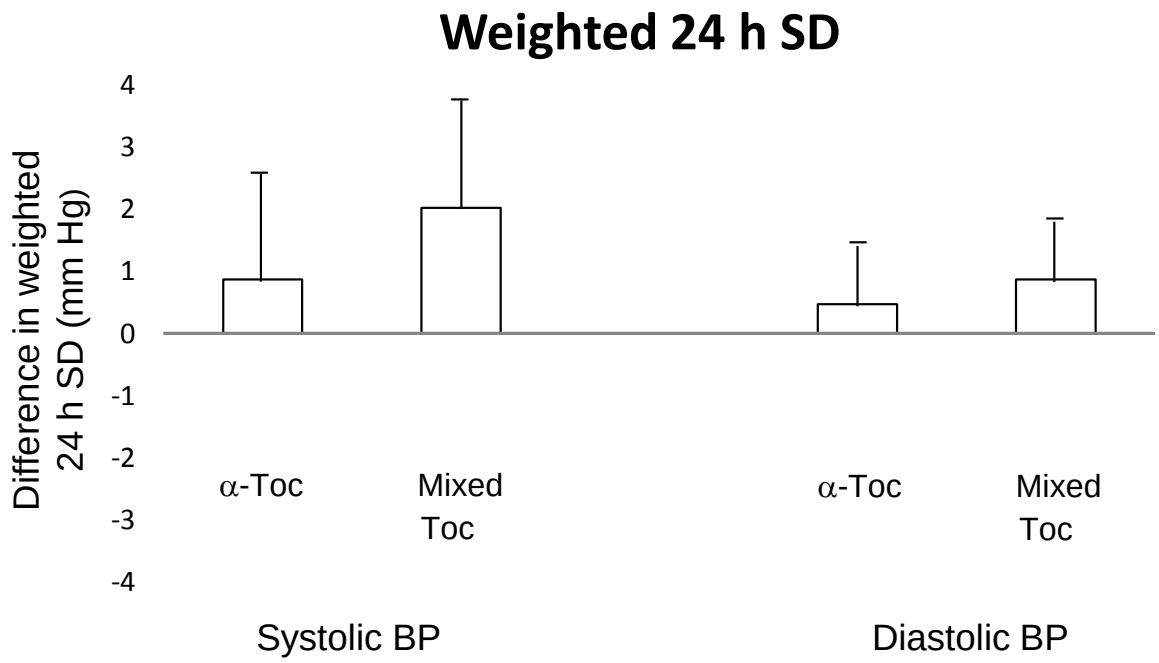


Figure S1 Differences in the weighted 24 h standard deviation (SD) of blood pressure (BP) for α-tocopherol (α-Toc) and mixed tocopherols (Mixed Toc) relative to placebo. Values are mean and SEM.

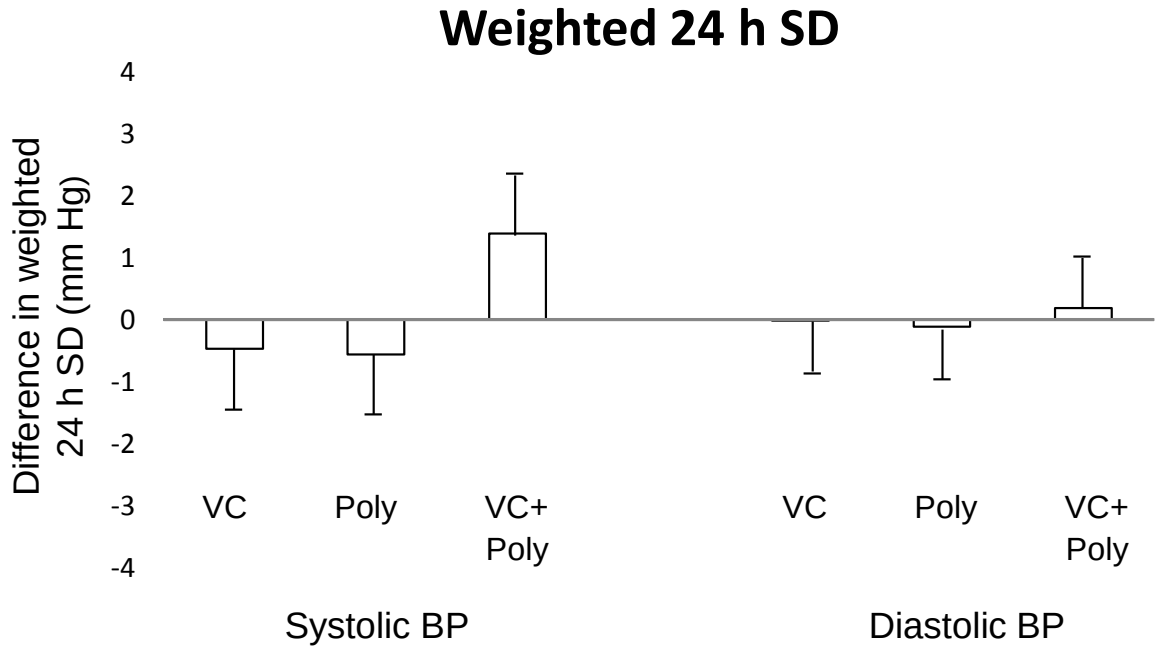


Figure S2 Differences in the weighted 24 h standard deviation (SD) of blood pressure (BP) for vitamin C (VC), polyphenols (Poly) and vitamin C plus polyphenols (VC+Poly) relative to placebo. Values are mean and SEM.