Title

POLYPHENOL-RICH JUICES REDUCE BLOOD PRESSURE MEASURES IN A RANDOMIZED CONTROLLED TRIAL IN HIGH NORMAL AND HYPERTENSIVE VOLUNTEERS

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Abstract

Fruits and berries may lower blood pressure, most probably due to the high content of polyphenols. We tested whether consumption of two polyphenol-rich juices could lower blood pressure. In a randomized, double-blinded, placebo-controlled trial of 12 weeks, 134 healthy individuals, 50-70 years, with high normal range blood pressure (130/85-139/89 mmHg, 72 subjects) or stage 1/2 hypertension (140/90-179/109 mmHg, 62 subjects), were included. They consumed 500 mL/day of one of either: (i) a commercial available polyphenol-rich juice based on red grapes, cherries, chokeberries and bilberries; (ii) a juice similar to (i) but enriched with polyphenol rich extracts from blackcurrant press-residue, or (iii) a placebo juice (polyphenol contents 245.5, 305.2 and 76 mg/100 g, respectively). Resting blood pressure was measured three times, with a one minute interval, at baseline and after 6 and 12 weeks of intervention. The systolic blood pressure was significantly reduced over time (6 and 12 weeks, respectively) in the pooled juice group as compared to the placebo group in the first of the three measurements, both for the whole study group (6.9 and 3.4 mmHg, p=0.01) and even more pronounced in the hypertensive subjects when analysed separately (7.3 and 6.8, p=0.04). The variation of the blood pressure measurements was significantly reduced in the pooled juice group as compared to the placebo (1.4 mmHg and 1.7 mmHg, p=0.03). In conclusion, our findings suggest that polyphenol-rich berry juice may contribute to a blood pressure and blood pressure variability lowering effect, being more pronounced in hypertensive than in normotensive subjects.

Introduction

Intake of fruit and vegetables are associated with reduced risk of cardiovascular diseases (CVD)[1, 2]. Fruit and vegetables contain various polyphenols which have been suggested to contribute to this protective effect[3, 4].

Polyphenols constitute a large family of natural compounds widely found in plant foods. Their main function in plants is to provide protection from various sorts of stress and cellular damage. Each polyphenol molecule comprises two or more phenol units. The number and structure of these phenol units make each polyphenol compound unique with regards to their bioavailability. Moreover, due their individual bioactivities, absorption[5, 6], metabolism and cellular accumulation, as well as specific interaction with various signalling molecules, enzymes and transcription factors, may vary[7]. It is therefore likely that polyphenols from different fruits and berries will vary in their potential to exert effects on outcome measures in intervention studies. It has been shown that polyphenols have favourable effects on platelet

aggregation[8-10], blood pressure (BP)[8, 9, 11] and blood lipid composition[12, 13], factors that are associated with CVD. Some studies have identified specific polyphenols with the ability to reduce BP, such as quercetin[14]. However, whole foods seem to be more effective than supplements in the prevention of CVD[15], possibly because whole foods provide a greater variety of polyphenols. In addition, reportedly combination of several different polyphenols may exert synergistic effects[16]. How polyphenols can relax vascular tone is not known, but modulation of the balance between nitric oxide and endothelin, for example via improved antioxidative status, might be involved [17, 18].

It is well established that hypertension is a strong predictor for cardiovascular morbidity and mortality[19, 20], but also fluctuations and variability in BP correlated with disease progression. Rothwell *et al* [21] showed that both visit-to-visit variability and maximum systolic blood pressure (SBP) are both strong predictors for strokes, independent of mean SBP. In their review Parati and colleagues reported that variability of short term BP (within 24 h) is closely associated with the development, progression and severity of cardiac, vascular and renal organ damage independently of mean BP[22].

Healthy foods taken in a liquid form can easily be added to a habitual diet. However, the effects on BP of polyphenol-rich juices have not been evaluated. Hence, we hypothesized that intake of such juices would lower BP and/or lead to a more favourable profile of risk factors for CVD in apparently healthy subjects. In this 12-week randomized placebo-controlled intervention study we have tested the effect of a polyphenol-rich juice (MANA Blue) based on red grapes, cherries, chokeberries and bilberries, and a juice (Optijuice) where MANA Blue has been added polyphenol rich extracts from blackcurrant press-residue. Following a strict procedure, three measurements of SBP and diastolic blood pressure (DBP) were recorded at each visit and changes in (i) the first BP of three measurements (BP1); (ii) the mean of BP measurements number two and three (BPmean); and (iii) blood pressure variability (BPV), another predictor of cardiovascular incidents[21, 23],were analysed. In addition, lipids and other blood parameters associated with CVD were determined.

Subjects and methods

Study Beverages

Three different beverages were used in the study: Placebo, MANA Blue and Optijuice. Table 1 shows the nutrient and chemical characteristics of the beverages whereas the supporting Table S1 shows details and changes in content over time. MANA Blue (MANA Blue, Grape,

bilberry and chokeberries juice, Tine SA, Oslo, Norway) is a commercially available product containing red grape (*Vitis vinifera*, 67.7%), chokeberries (*Aronia melanocarpa*, 14.5%), cherry (*Prunus cerasium*, 12%), and bilberry (*Vaccinium myrtillus*, 5.8%), while the two other drinks were specifically made by Tine SA for the current study. Optijuice was made of MANA Blue (85%) added polyphenol rich extract from blackcurrant press-residue (15%), previous optimized for biological activity *in vitro*[24]. Optijuice contained more total polyphenols than MANA Blue, but was lower in hydroxyciannamic acids, as this compound was lower in the blackcurrant press-residue than in MANA Blue. A placebo drink was developed with comparable amounts of energy, carbohydrates, potassium and colour to mimic the intervention juices. It contained Maltodextrin (6.2 g), sugar (6.2 g), potassium chloride (280 mg), blueberry flavor (3504156, 25 mg), grape flavor (6103834, 20 mg), citric acid (0.01 mg, to pH4) and dye (E122 and E25/azorubin/brilliant black, 5 mg), all per 100 g beverage. Subjects were provided with sufficient volume for intake of 500 mL daily for 12 weeks. The study beverages were supplied by TINE SA (Oslo, Norway) in identical white containers, each containing 1000 mL of Optijuice, MANA Blue or placebo.

Beverage Compounds

The total content of polyphenols was determined with the Folin-Ciocalteu's method and determined as gallic acid equivalents in mg per 100 g of sample as previously described[24]. The pH differential absorbance method was used to determine the content of total monomeric anthocyanins, calculated as cyanidin-3-glucoside equivalents in mg per 100 g of sample[24]. Individual polyphenol compounds were analysed on an Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector and a MSD XCT ion trap mass spectrometer as previously described[25]. The polyphenols were quantified using: cyanidin-3-glucoside, at 520 nm, for anthocyanins; rutin, at 360 nm, for flavonols; and chlorogenic acid, at 320 nm, for hydroxycinnamic acids. All results are expressed as mg per 100 g of sample (Table S1). The ferric-reducing antioxidant power (FRAP), was assayed according to Benzie and Strain[26].

Study Subjects

The volunteers were recruited by postal mail by 10 000 invitation letters to men and women, between 50 and 70 years living in Oslo, Norway, and listed in the National Population Registry, as well as by about 400 letters distributed to the lunch areas in public transport companies. The invitation letter did not ask for BP level, but for exclusion criteria including the use of regular BP lowering medication, the presence of type 1 and 2 diabetes, smoking, or

a body mass index (BMI) above 35 kg/m^2. About 9% (n=921) subjects replied to the first invitation. Of these, 737 were found eligible to be invited for a screening visit. At the screening visit (n=627), additional exclusion criteria, such as allergy to grape, cherries, blueberries/bilberries, blackcurrant or chokeberries, changes of +/-4 kg in body weight within the last 12 weeks before start of the study, use of supplement for weight reduction, or of polyphenol-rich supplements and participation in other clinical trials or other planned activities (vacation, hospital admission etc.), were recorded. At the same time, the volunteers' BP was screened to be within the high normal range (130/85 - 139/89 mmHg) or stage 1-2 hypertension (140/90 - 179/109 mmHg), which was the main inclusion criteria. All subjects signed a written consent to participate. During the baseline visit (n=207), subjects who did not meet the BP criteria were further excluded from the study (n=54). Persons initiating BP-lowering medication during the study, not following the drinking regimen (at least 80% compliance), not showing up on all visits, or incorrect BP measurements according to the procedure, were excluded also from the analyses (Figure 1).

Study Ethics

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 Regional Committees for Medical and Health Research Ethics, Health Region South East, Norway, and written informed consent was obtained from each subject. The study is registered at Clinicaltrials.gov (NCT01568983).

Study Design

This study was a double-blind, placebo controlled trial and was conducted between December 2011 and June 2012. At baseline, subjects were randomly assigned to a study group consuming 500 mL daily of (i) placebo; (ii) Optijuice; or (iii) MANA Blue for 12 weeks. The subjects were instructed to record the consumed beverages in a provided diary. They were also asked to refrain from other juice products (except juices made of apples and oranges), and from antioxidant supplements (like vitamin C) prior to study start and during the course of the study. Apart from this, the subjects were encouraged to maintain their habitual diet, physical activity, and lifestyle while enrolled in the study.

All subjects made 4 visits (screening, baseline, 6 week visit and 12 week visit) during the study. On the measurement days, the subjects had been fasting from 12 AM the day before. For the last visit, the subjects were asked to drink the last glass of study beverage between 8

and 10 PM the night before. All visits were between 8 and 10 AM to avoid diurnal fluctuations.

Blood Pressure Measurements

Fasting SBP and DBP measurements were performed blinded by trained personnel. Three measurements at 1-minute intervals were recorded after 10 minutes of rest in a waiting room followed by another 5 minutes in an investigation room where the subject sat in a resting chair with the cuff mounted and the arm at the armrest. Validated oscillometric devices (Carescape V100, GE Healthcare, Oslo, Norway) with suitable cuffs were used for the measurements. In the analyses we used the first measure (BP1), the mean (BPmean) of measure number two and three, and the standard deviation (SD) of all three measurements (BPV). Normotensive and hypertensive subjects were defined as below and above a SBP of 140 mmHg, respectively.

Laboratory Analyses

Fasting blood samples were collected at baseline and after 12 weeks. Venous blood samples were collected in vaccutainers and kept at room temperature or at 4°C until processing. Serum and plasma were obtained by centrifugation at 1500 *g* for 10 minutes at 8°C, aliquoted and frozen at -80°C. The following analyses were performed on a Maxmat PL (Maxmat, Montpellier, France): uric acid (RM URAC0200V), creatinine (RM CREP0270V), cholesterol (RM CHOL0400V), direct LDL cholesterol (RM LDLC0080V), direct HDL cholesterol (RM HDLC0120V), glucose (RM GLUP0400V), triglycerides (RM TRIG0400V), alanine transaminase (ALAT-GPT, RM ALAT0252V), aspartate transaminase (ASAT-GOT, RM ASAT0252V), (all Maxmat procedures and products, manufacturers assay numbers in brackets), phospholipids (1001140, Spinreact, Girona, Spain), non-essential fatty acids (D07940, Dialab, Wiener Neudorf, Austria), total antioxidant status (NX 2332, Randox, Crumlin, Nothers Ireland, UK) and D-roms test (MC 003, Diacron, Grosseto, Italy). In addition, the following haematological analyses were performed at Oslo University Hospital using standard procedures: Haemoglobin, haematocrit, platelet count, leukocyte count including a differential count and D-dimer.

Measurement of Body Composition

Weight, fat free mass, fat mass, total body water, and basal metabolic rate were determined using a bio-impedance analyser (Tanita TBF-300, Tanita Corp., Tokyo, Japan) at the first and last visit (baseline and week 12).

Statistical Analyses

We assumed a SD of the reduction of 11 mmHg, and based on an ANOVA test we found that a total of 210 persons would be needed to detect a difference in BP of 5 mmHG with a power of 80% and a significance level of 0.05. After screening process, 207 subjects were eligible for the study.

Changes in BP were analysed using the "mixed" command for linear mixed models in IBM SPSS (SPSS Inc., software version 16.0.1) treating time as categorical parameter, including a random intercept in the model and the following parameterization: $\beta_0$time+$\beta_1$treatment+β_2 (time x treatment). BP estimates were based on the mixed model, and p-values were generated from the SPSS test of fixed effects for the interaction term (time x treatment) from the mixed model, as is the estimated difference in change between intervention and placebo groups at different time points.

Variability of BP was calculated as SD of the three measurements at each visit and further analysed by a mixed model as described above. The residuals of the SD showed a normal distribution. Baseline statistics in Table 2 are presented as crude means with SD. Differences between groups at baseline were determined by ANOVA (Analyses of Variance) as were differences in the biochemical data. A comparison of systolic BP1 (SBP1) with systolic BPmean (SBPmean) was done by paired t-test. A $p \leq 0.05$ was considered significant.

Subgroup analyses, as described above, were performed on hypertensive subjects (140-179 mmHg) and normotensive subjects (124-139 mmHg) based on SBP1 or SBPmean at baseline.

Results

Participant Flow

Nine hundred and five subjects (that is 9% of the invited cohort) positively responded to the invitation letters. Of these, 737 persons were eligible after self-reporting and invited for screening. 627 persons attended the screening of BP and the interview. After the screening procedure, 420 subjects did not fulfil the inclusion criteria or for other reasons were excluded from the study. At baseline another 54 subjects had BP below the eligibility criteria and were therefore not included. During the study, 19 subjects dropped out, leaving 134 subjects that completed the intervention (Figure 1). At the end of the study, four datasets were excluded from the analyses according to the exclusion criteria. Hence, the study group for analyses

consisted of 130 subjects, with 43 in the placebo group, 41 in the Optijuice group and 46 in the MANA Blue group.

Baseline Characteristics of Subjects

At baseline, the mean SBP1 and DBP1 for all subjects were 143 and 81 mmHg, respectively, and the corresponding mean values of SBPmean and DBPmean were 141 and 82 mmHg. Neither the BP values nor the anthropometric measures were significantly different among the three study groups (Table 2).

Effects on Blood Pressure in the Polyphenol-Rich Juice Groups

At baseline we observed that in the whole study group (n=130) SBP1 was on average 2.5 mmHg higher ($p<0.001$) than the SBPmean and therefore these two measures were analysed separately.

SBP1 was significantly reduced in both the Optijuice and MANA Blue intervention groups at 6 weeks ($p=0.01$ for both), but not after 12 weeks, compared to the placebo group (Table 3). There were no significant differences between the SBP1 time curves ($p=0.07$) when analysing the (time x treatment)-interaction over the full study period (12 weeks). Changes in DBP1 in the intervention groups were not different from placebo, neither for single time points nor for the complete time curve.

Since both intervention juices are very rich in polyphenols, we pooled the Optijuice and MANA Blue groups in the analysis to increase the statistical power. The SBP1 time curves for the pooled intervention group and placebo group were significantly different ($p=0.01$). The (time x treatment)-interaction revealed that after 6 weeks SBP1 were reduced by 6.9 mmHg in the pooled group as compared to the placebo ($p<0.001$), while this effect was not seen after 12 weeks (Table 3). No effects were observed for DBP1.

We did not observe any significant differences between the groups when time curves for SBPmean or DBPmean were investigated (Table S2), neither for all three groups separated nor if the two juice groups were pooled.

Larger Effect of Polyphenol-Rich Juice on Blood Pressure in Hypertensive Subjects as Compared to Normotensive Subjects

Sub-analyses of the interventions on hypertensive subjects (SBP in the range of 140-179 mmHg) based on SBP1 at baseline showed that the SBP1 time curves were not significantly different for the treatment groups (Table 4). In the pooled juice group, however, the SBP1 time curve was significantly different from the placebo (p=0.05). This difference is explained by a significantly higher reduction in the pooled group after both 6 weeks (p=0.03) and 12 weeks (p=0.04) than the placebo group. DBP1 was not affected by the juice interventions (data not shown).

Changes of BP in normotensive subjects (range of 124-139 mmHg based on SBP1 at baseline) after the intervention are presented in Table 4. In the pooled analysis of Optijuice and MANA Blue groups, we observed significant differences for the SBP1 time curve as compared to the placebo (p=0.02). However, this significant difference seems to be due to a net increase in SBP1 in the placebo group after 6 weeks (5.5 mmHg) rather than a reduction in the juice groups. No effects were seen for DBP1 (data not shown).

No effects of the interventions in hypertensive or normotensive subjects, based on SBPmean at baseline, were observed in the SBPmean measures (Table S3) or DBPmean measures (data not shown).

Effects of Polyphenol-Rich Juice on Standard Deviation as a Measure of the Variance of Three Blood Pressure Measurements

BP variance is a relevant measure in CVD development[22]. We observed that the SD of the three measurements of SBP at each visit was reduced in the pooled juice group by 1.4 mmHg (6 weeks) and 1.7 mmHg (12 weeks). Compared to the placebo group this gave a significant reduction (p=0.03) (Table 5). The reduction was more pronounced in hypertensive subjects (2.03 mmHg at 6 weeks, 2.83 mmHg at 12 weeks, p=0.01). In normotensive subjects a significant difference between placebo and pooled groups was not observed (Table 5).

Biomarker Analyses

Blood samples for haematological and biochemical analyses were collected at baseline and at the end of study, at week 12. The mean baseline values were within the normal range for all markers (data not shown). The results showed that only ALAT was significantly different in the three groups during the time course (p<0.001), on average -0.7, -8.9 and 1.2 U/L in the

placebo, Optijuice and MANA Blue study groups, respectively. Two dataset in the Optijuice group were above normal range at baseline and reduced over 50% by the end of the study. These datasets were considered out of range and removed before analyses not to create a false positive reduction in the Optijuice group. At baseline, the average values for ALAT were 25.8, 26.8, 24.8 U/L for placebo, Optijuice and MANA Blue, respectively. At the end of the study, the average values for ALAT were 25.2, 17.9 and 26.0 U/L for placebo, Optijuice and MANA Blue, respectively.

Anthropometric Analyses

Body composition and weight were determined at the first and last visit (baseline and week 12). There were no significant differences in weight or body composition (data not shown).

Discussion

Previous epidemiological studies and some intervention studies have suggested a role for polyphenols in BP reduction[8, 9, 11, 27]. This study, which is the first placebo controlled intervention study on the effects of berry juice on BP, strongly indicates that polyphenol-rich berry juice alone can reduce BP and short time BP variation. We analysed changes of the first of three BP measurements (BP1), the mean of the two following measurements (BPmean), as well as the BPV to evaluate the effect of the polyphenol-rich juices on BP.

Our results demonstrated that BP1 was significantly reduced in the pooled polyphenol-rich juice group as compared to the placebo group. It is well known that the first recording in repeated BP measurements usually is higher than the two next[28], as observed in this study. This may be regarded as a "white coat effect"[28], that is, an observed increased BP taken at a doctor's office compared to BP measured at home or with ambulatory BP. In many studies this measurement has therefore been excluded from the analyses. Probably, BP1 is more sensitive to stress and sympathetic activation, similar to the elevated BP observed during mental or acute stress tests[29-31]. The association between stress-related elevated BP and CVD is well established[32]. Our results suggest that a possible mechanism of the beneficial effects of fruits and berries on CVD could be through reduction of the elevated BP during stressful situations and not necessarily on the resting BP, which in our study was not significantly changed during the intervention period.

Further, we observed that the BPV, determined by the SD of the three measurements at each visit, was reduced by the polyphenol-rich intervention. Akita *et al*. showed that cacao liquor

polyphenols reduced BPV in rabbits[33]. Hodgson *et al.* showed that black tea lowered the rate of BPV in human[34] although he was not able to detect the same effects by specific vitamins or grape seed intervention[35]. The present study is the first to show reduction in BPV in a clinical placebo controlled intervention trial. Reduction in BPV is likely to reduce the risk of CVD[22] as both visit-to-visit and ambulatory BPV are predictors of cardiovascular incidents[21, 23]. Possible mechanisms behind these findings may be that high BPV leads to stress on the vessel wall, which again may result in damage and initiation of CVD. We have defined BPV as the SD of the three SBP measurements at each visit. Other studies have used SD of ambulatory or visit-to-visit BP measurements[22], or even the slope of SBP from beat to beat[36]. We suggest that the variation in three SBP measurements over a time period of 3-4 minutes also may reflect a relevant pathophysiological condition similar to BPV determined by other methods.

We were surprised to observe that the reduction in SBP1 was most evident in the intervention group after 6 weeks (6.4 mmHg, pooled group) while only a 0.8 mmHg further reduction was detected between week 6 and 12. This time course could reflect the reduction of anthocyanins we observed in both juices over time. However, we did not observe any differences in effect on SBP1 between the Optijuice and the MANA Blue group at neither 6 nor 12 weeks although the Optijuice contained 5 times more anthocyanins at both time points (41.8 - 20.3 mg/100 g; and 8.6 - 4.1 mg/100 g for Optijuice and MANA Blue, respectively). That is, if the concentration in MANA Blue at starting point (8.6 mg/100 g) was sufficient for the observed effect the six first weeks, there has to be other reasons than the decrease in anthocyanin concentration for the lack of further reduction in SBP1 in the Optijuice group, still containing 20.3 mg/100 g. We therefore assume that even the lowest concentration of anthocyanins in the present juices were sufficient to exert the observed effects.

For the placebo group, the SBP1 time curve had a different shape; here there was no reduction the first 6 weeks while the most evident reduction occurred between weeks 6 and 12. This could be explained in part by seasonal variations[37] or other reasons for natural fluctuation, which also the intervention group would be susceptible to. These results underline the great importance of including placebo groups in intervention studies to obtain reliable results.

It is of particular interest to reduce and control BP in subjects with SBP/DBP $\geq$ 140/90 mmHg. We therefore performed a sub-analysis to examine the effect of the intervention in hypertensive- and normotensive subjects, both for BP1 and BPmean. We observed that

subjects with SBP1/DPB1 ≥ 140/90 mmHg showed a significant reduction in SBP1 (7.3 and 6.8 mmHg after 6 and 12 weeks, respectively, p=0.05) when combining the two polyphenol juice groups as compared to placebo. This is in accordance with other studies showing that intervention with fruits and berries has the strongest effect on a higher starting BP[8, 9].

To date there are a few clinical trials supporting the notion that fruit and berries, through their polyphenol content, are potential BP lowering foods[8, 9, 27, 38] although this has long been suggested by epidemiological studies[4]. The mechanism behind the effects of polyphenol-rich food has not been identified and the research of which polyphenols that are most important for the biological effects is quite scarce. Therefore we believe that it is important to include a variety of polyphenol-rich fruits and berries in interventions with the purpose of studying beneficial effects of polyphenols. In line with this we included a combination of grape, cherries, bilberries, chokeberries and blackcurrant in the intervention juices. Since peels and seeds in fruits and berries are enriched with polyphenols, a large amount of the valuable polyphenols are often lost in the press-residue instead of in the juice[39]. Therefore, an extract from blackcurrant press-residue, previously optimized for biological activity[24], was introduced in one of the juice groups.

Both juices had high levels of total polyphenols and FRAP, both measures of antioxidant capacity or reducing properties (Table 1). The amounts of total polyphenols and FRAP in Optijuice, which contained the blackcurrant peel extract, were about 20% higher than in MANA Blue. The concentrations of flavonols were also somewhat higher (28%) in Optijuice, while the concentrations of total hydroxycinnamic acids were equal in the two juices, explained by the low content of hydroxyciannamic acids in blackcurrant. The main difference between the juices was the higher content of anthocyanins, the major polyphenol compounds in the juices, where Optijuice had about 5-fold higher concentration than MANA Blue. In addition, the composition of anthocyanins differed, Optijuice, naturally being especially rich in anthocyanins from blackcurrants (i.e. glucosides and rutinosides of delphinidin and cyanidin (Table S1). Despite these differences, we did not observe any differences on the effect on BP between these juices. In this study it was therefore not possible to reveal any effects of dose- or content of polyphenols. We therefore chose to pool the two groups to increase the statistical power in several of the analyses.

In the present study, subjects were instructed to refrain from other juice products, from antioxidant supplements and otherwise encouraged to maintain their habitual diet, physical

activity, and lifestyle during the study. Our main intention with this study was to investigate the effect of intake of 500 mL polyphenol rich juice in an open randomized controlled trial with free-living subjects without any other constrains. Other polyphenol rich beverages as coffee, tea and wine have shown beneficiary effects on risk factors of cardiovascular disease risk factors although not unambiguous on BP. A normal intake of these beverages or other polyphenol rich foods may have affected the BP in our study, both by itself but also by synergy with the study juices. However, since this study was placebo controlled, we suggest that the effects in the study are caused by the study juices and not by lifestyle or intake of other polyphenol rich foods.

Biochemical markers associated with polyphenol intake as well as BP changes were analysed. Of all biochemical markers analysed, only Alanin transaminase, ALAT, a liver damage marker, was significantly reduced in only the Optijuice groups, containing blackcurrant. The protective effect on liver of polyphenols in general[40] and blackcurrant in particular[41] has previously been suggested. The average values of all biochemical markers tested in the study population were within normal range. In general it is not desired to alter normal blood values by food intervention. We were therefore not surprised that the study juices did not lead to other changes in the biochemical markers tested in this study.

Conclusions

In the present study, the polyphenol-rich juice significantly reduced SBP1 in a group of middle-aged individuals. The reduction was more pronounced in hypertensive than in normotensive subjects. Further, we found that the juice also reduced BPV.

Our results suggest that a possible mechanism of the beneficial effects of fruits and berries for CVD protection could be through reduction of the stress-sensitive BP and not necessarily reduction of the resting BP. If future studies can confirm these findings, we suggest that such juice may be beneficial for subjects with high BP and may contribute to postpone introduction of hypertensive drugs.

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Conflict of interest

Rune Blomhoff has an interest in AS Vitas, Oslo, Norway. The other authors declare no competing financial interests.

Authorship

Torunn Elisabeth Tjelle: Design of study, recruiting subjects, test sampling from subjects, analyses and interpreting of data, statistical analyses, drafting and finalizing manuscript.
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Table 1. Nutrient and chemical characteristics of beverages (per 100 g)

Supporting table S1 shows a more detailed list of single components as well as their change over time.

	Placebo	Optijuice	MANA Blue
Energy (kJ)	207.7	221.1	224.4
Carbohydrate (mg)	12.5	12.9	13.1
Ascorbic acid (mg)	0.0	3.2	3.0
Sodium (mg)	-	0.02	0.02
Potassium (mg)	145	156	136.1
Total phenolics (mg)	76	305	246
Total monomeric anthocyanins (mg)	0.0	41.3	11.9
Phenolic compounds (mg)			
Total individual anthocyanins	0.0	41.8	8.6
Total flavonols	0.0	9.0	7.0
Total hydroxycinnamic acids	0.0	20.9	22.3
Ferric reducing antioxidant power (mmol Fe)	0.0	3.2	2.7

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Table 2. Baseline Characteristics of Participants

Data are presented as mean with standard deviation in brackets. Variation is the standard deviation of triplicate measurements of systolic blood pressure. There were no statistical differences between groups determined by ANOVA.

	All participants (n=130)		Placebo (n=43)		Optijuice (n=41)		MANA Blue (n=46)	
Males/Females	90/40		30/13		30/11		30/16	
Age	62	(6)	62	(6)	62	(6)	61	(6)
SBP1	143	(13)	141	(12)	145	(14)	143	(12)
DBP1	81	(8)	81	(9)	82	(8)	82	(8)
SBPmean	141	(10)	140	(10)	142	(11)	140	(10)
DBPmean	82	(8)	82	(8)	82	(8)	82	(8)
Variation	4.6	(3.8)	4.0	(3.6)	5.2	(2.6)	4.5	(3.3)
BMI	26	(3)	26	(3)	27	(4)	26	(3)

SBP1 and DBP1 indicate first systolic and diastolic blood pressure recording, respectively. SBPmean and DBPmean are the mean of systolic or diastolic blood pressure recording two and three, respectively. BMI, body mass index.

Table 3. Blood pressure measurements: first blood pressure measurement (BP1) in all subjects

Data shown are estimated values generated from the mixed model. P-values are also taken from the mixed model.

	Mean BP (mmHg)						Diff. placebo		Interaction (time x treatment)	
Group	Baseline	95% CI	6 weeks	95% CI	12 weeks	95% CI	6 week	12 week	p^* w6, w12	$p^\dagger$ grouped
SBP1 (mmHg)										
Placebo	140.905	(136.9,145.0)	141.5	(137.4,145.5)	137.1	(133.0,141.1)				
Optijuice	145.074	(141.0,149.2)	138.4	(134.3,142.5)	138.0	(133.9,142.1)	-7.2	-3.3	0.01,0.24	0.07‡
MANA Blue	143.894	(140.1,147.7)	137.8	(133.9,141.6)	136.5	(132.7,140.4)	-6.7	-3.5	0.01,0.19	
Pooled	144.443	(141.7,147.2)	138.1	(135.3,140.8)	137.2	(134.4,140.0)	-6.9	-3.4	<0.001,015	0.01§
DBP1 (mmHg)										
Placebo	80.4	(77.9,83.0)	78.9	(76.3,81.5)	78.4	(75.8,80.9)				
Optijuice	81.7	(79.1,84.3)	80.0	(77.4,82.6)	80.9	(78.3,83.5)	-0.2	1.3	0.85,0.30	0.75‡
MANA Blue	81.9	(79.5,84.3)	80.0	(77.6,82.4)	80.0	(77.6,82.5)	-0.4	0.2	0.77,0.85	
Pooled	81.8	(80.0,83.6)	80.0	(78.2,81.7)	80.5	(78.7,82.2)	-0.3	0.7	0.78,0.49	0.61§

SBP1, systolic blood pressure; DBP1, diastolic blood pressure; Diff. placebo, estimated differences in treatment groups from placebo; CI, Confidence intervals.

* p-value for changes from baseline to week 6 and 12, respectively, compared to the Placebo group

† p-value for the overall test of no (time x treatment)-effect, using

‡ all three treatment groups (the placebo and the two intervention groups), and using

§ the placebo and the pooled juice group.

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Table 4. Changes in BP1 in hypertensive and normotensive subjects

Data shown are estimated values generated from the mixed model. P-values are also taken from the mixed model.

	Mean BP (mmHg)						Diff. placebo		Interaction (time x treatment)	
Group	Baseline	95% CI	6 weeks	95% CI	12 weeks	95% CI	6 week	12 week	*p** w6, w12	*p*† grouped
SBP1 (mmHg) in Hypertensive Subjects										
Placebo (n=24)	149.3	(143.8,154.8)	145.8	(134.4,151.3)	142.5	(137.0,148.0)				
Optijuice (n=23)	154.0	(148.5,159.5)	142.8	(137.2,148.3)	140.7	(135.2,146.3)	-7.7	-6.5	0.05, 0.10	0.19‡
MANA Blue (n=25)	152.8	(147.6,158.0)	138.9	(137.0,147.4)	142.2	(133.7,144.1)	-7	-7.1	0.07, 0.06	
Pooled (n=48)	153.3	(149.6,157.1)	142.5	(138.7,146.2)	139.8	(136.0,143.5)	-7.3	-6.8	0.03, 0.04	0.05§
SBP1 (mmHg) in Normotensive Subjects										
Placebo (n=19)	130.7	(126.8,134.7)	136.2	(132.2,140.2)	130.5	(126.5,134.4)				
Optijuice (n=18)	133.7	(129.6,137.7)	132.8	(128.7,136.9)	134.4	(130.4,138.5)	-6.4	1.0	0.05, 0.74	0.08‡
MANA Blue (n=21)	132.9	(129.1,136.7)	132.2	(128.5,136.0)	133.6	(129.8,137.4)	-6.1	1.0	0.05, 0.75	
Pooled (n=39)	133.3	(130.5,136.0)	132.5	(129.7,135.2)	134.0	(131.3,136.7)	-6.2	1.0	0.02, 0.71	0.02§

Hypertensive Subjects, subjects with SBP1 in the range of 140-179 mmHg at baseline; Normotensive Subjects, subjects with SBP1 below 140 mmHg at baseline; SBP1, systolic blood pressure; DBP1, diastolic blood pressure; Diff. placebo, estimated differences in treatment groups from placebo; CI, confidence intervals.

* p-value for changes from baseline to week 6 and 12, respectively, compared to the Placebo group

† p-value for the overall test of no (time x treatment)-effect, using

‡ all three treatment groups (the placebo and the two intervention groups), and using

§ the placebo and the pooled juice group.

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Table 5: Variance of triplicate blood pressure measurements

Data shown are estimated values of standard deviation, the variance, of triplicate systolic blood pressure measurements and difference of standard deviation in intervention group from placebo (Diff. from placebo) generated from the mixed model. P-values are also taken from the mixed model.

	Variance (mmHg)						Diff. placebo		Interaction (time x treatment)	
Group	Baseline	95% CI	6 weeks	95% CI	12 weeks	95% CI	6 week	12 week	p* w6, w12	$p^{\dagger}$ grouped
All subjects										
placebo (n=43)	4.0	(3.2,4.8)	4.2	(3.4,5.0)	4.7	(3.9,5.5)				
pooled (n=87)	4.8	(4.3,5.4)	3.6	(3.1,4.2)	3.8	(3.3,4.4)	-1.4	-1.7	0.04,0.01	0.03
Hypertensive subjects										
placebo (n=23)	4.1	(2.9,5.2)	4.3	(3.2,5.5)	5.2	(4.1,6.4)				
pooled (n=46)	6.0	(5.2,6.8)	4.2	(3.5,5.0)	4.3	(3.5,5.1)	-2.0	-2.8	0.04,0.01	0.01
Normotensive subjects										
placebo (n=20)	4.0	(3.0,5.0)	4.1	(3.1,5.1)	4.2	(3.2,5.2)				
pooled (n=41)	3.4	(2.7,4.1)	2.9	(2.2,3.6)	3.3	(2.6,4.0)	-0.7	-0.4	0.46,0.62	0.75

Hypertensive subjects, mean value of SBP triplicate above 140 mmHg; Normotensive subjects, mean value of SBP triplicate below 140 mmHg; SD, standard deviation; Diff. from placebo, difference in intervention group from placebo; CI, confidence interval; SBP, systolic blood pressure.

* p-value for changes from baseline to week 6 and 12, respectively, compared to the Placebo group

† p-value for the overall test of no (time x treatment)-effect.

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