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Beneficial effect of a polyphenol-rich diet on cardiovascular risk: a randomised control trial

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ABSTRACT

Objectives

There is previous epidemiological evidence that intake of polyphenol-rich foods has been associated with reduced cardiovascular disease risk. We aimed to investigate the effect of increasing dietary polyphenol intake on microvascular function in hypertensive participants.

Methods

All participants completed a 4-week run-in phase, consuming <2 portions of fruit and vegetables (F&V) daily and avoiding berries and dark chocolate. Subjects were then randomized to continue with the low polyphenol diet for 8 weeks or to consume a high polyphenol diet of 6 portions fruit and vegetables (including 1 portion of berries/day and 50g of dark chocolate). Endothelium-dependent (acetylcholine, ACh) and independent (sodium nitroprusside, SNP) vasodilator responses were assessed by venous occlusion plethysmography. Compliance with the intervention was measured using food diaries and biochemical markers.

Results

Final analysis of the primary endpoint was conducted on 92 participants. Between group comparison of change in maximum % response to ACh revealed a significant improvement in the high polyphenol group (p=0.02). There was a significantly larger increase in vitamin C, carotenoids and epicatechin in the high polyphenol group (between group difference p<0.001; p<0.001; p=0.008 respectively).

Conclusions

This study has shown that increasing the polyphenol content of the diet via consumption of fruit and vegetables, berries and dark chocolate results in a

significant improvement in an established marker of cardiovascular risk in hypertensive participants.

KEY QUESTIONS

What is already known about this subject?

Previous epidemiological and intervention study evidence suggests that increasing fruit and vegetable consumption improves cardiovascular disease risk. More specifically, observational evidence to date indicates that polyphenol-rich foods, in particular berries and dark chocolate, may influence cardiovascular disease risk. However, there are few polyphenol-specific dietary intervention studies of sufficiently robust design that assess the effect of polyphenol-rich foods on microvascular function.

What does this study add?

Our group conducted a randomised control trial and found that increasing intake of polyphenol-rich foods, in the form of fruit and vegetables (including berries) and dark chocolate, for 8 weeks resulted in a significant improvement in endothelium-dependent vasodilation in hypertensive participants.

How might this impact on clinical practice?

This study suggests that a well-tolerated polyphenol-rich diet through the simple addition of berries and dark chocolate could have a positive effect on microvascular function and cardiovascular risk.

Previous epidemiological and intervention study evidence suggests that increasing fruit and vegetable (F&V) consumption improves cardiovascular disease (CVD) risk (1). A previous dietary intervention by McCall and colleagues in hypertensive subjects (Fruit and Vegetable Randomised Intervention Trial, FAVRIT) demonstrated a 6% improvement in endothelium-dependent vasodilation, as a measure of vascular function, for every portion/day increase in fruit or vegetables (2). Post-hoc analysis of the FAVRIT data indicated that berry consumption was the class of fruit most associated with an improvement in microvascular function. It was hypothesised that this could be attributed to berries high polyphenol content. Observational evidence to date indicates that polyphenol-rich foods, in particular berries (3,4) and dark chocolate (5-7), may influence CVD risk. However, there are few polyphenol-specific dietary intervention studies of sufficiently robust design that assess the effect of polyphenol-rich foods on microvascular function.

The aim of the Polyphenol Intervention Trial (PPhIT) was to investigate if increasing overall polyphenol dietary intake (through the consumption of six portions of F&V/day including one portion of berries, and 50g of dark chocolate) would affect microvascular function, measured by forearm blood flow (FBF) responses to an endothelium-dependent vasodilator, and also have a beneficial effect on a range of other markers of CVD risk, such as systolic blood pressure and lipid profile, in patients with hypertension.

The FBF measure of microvascular function was chosen as the primary endpoint, not only due to its use in previous dietary interventions, including the FAVRIT study (2), but also because responses to endothelium- dependent vasodilators such as acetylcholine (ACh) have previously been shown to be reduced in individuals with hypertension (8,9). FBF responses to ACh have also been independently

associated with cardiovascular morbidity in hypertensive patients (10) and, in those with established CVD, improved FBF responses were significantly correlated with improved survival after acute coronary syndrome (11).

Participants with hypertension were chosen because they represent a common cardiovascular risk cohort in whom modification of diet is feasible. There are several studies linking hypertension with reduced response to endothelium- dependent vasodilators (8,9,12). In addition, the current ESC guidelines for management of hypertension endorse increasing F&V intake to five portions per day, based on studies of a Mediterranean diet (13), making it a relevant population in which to conduct a randomised controlled dietary intervention trial (14).

METHODS

Overview of study and endpoints

The study used a twelve-week randomized controlled, single-blinded dietary intervention design. The study commenced with a four-week 'run-in phase' for all participants, during which they were asked to consume two portions or less of F&V, and to exclude berries and dark chocolate (low polyphenol diet). At the end of this period, subjects were randomized to continue with the above low polyphenol diet for a further eight week 'intervention period' or to consume a high polyphenol diet of six portions F&V (including one portion of berries per day) and 50g of dark chocolate per day (**Figure 1**).

The primary endpoint was between group change in maximum FBF response to the endothelium-dependent vasodilator, ACh. Secondary endpoints included between group change in self-reported polyphenol-rich food intake, between group change in biochemical markers of nutritional status, and between group change in systolic blood pressure and lipid profile. The study had ethical approval from the Office of

Research Ethics Committee Northern Ireland (ref 10/NIR03/39) and was registered at ClinicalTrials.gov (ref NCT01319786).

Inclusion Criteria

Participants aged 40-65 years, with documented grade I (140-159/90-99mmHg) or grade II (160-179/100-109mmHg) hypertension, were eligible (15). Potential participants had brachial blood pressure (BP) measured after ten minutes rest using an Omron M5-1 automatic BP monitor (Omron Healthcare, Hoofdorp, Netherlands) from the dominant arm. Three consecutive measurements were recorded and a summary BP calculated from the 2nd and 3rd readings.

Exclusion Criteria

Exclusion criteria included diabetes mellitus, acute coronary syndrome or transient ischaemic attack within three months, pregnancy or lactation, fasting triglyceride concentration >4mmol/l, alcohol consumption (>28 units/week for men and >21 units/week for women), oral anticoagulant therapy or antioxidant supplements, dietary restrictions that would limit ability to comply with the study diets and body mass index >35kg/m^2 with an impalpable brachial artery.

Randomisation

Participants' baseline habitual polyphenol dietary content was measured at the screening visit using a modified version of the food frequency questionnaire developed for the European Prospective Investigation of Cancer study (16). Eligible participants were stratified into high or low polyphenol consumers and then block randomized using a random-number generator (www.randomization.com, block size n=6). The investigator performing FBF assessment (RN) and staff conducting laboratory analysis were blinded to diet allocation.

Dietary intervention

Participants received individual nutrition consultations with a nutrition researcher (CR), as well as literature regarding portion sizes based on the United Kingdom Food Standard Agency guidelines (www.eatwell.gov.uk). All participants in the high polyphenol diet had a self-selected weekly delivery of F&V and dark chocolate (Lindt® 70% cocoa) free of charge to their homes from a local supermarket. Each participant was also contacted by telephone at weekly intervals. At each study visit, weight, hip and waist circumference were measured and questionnaires completed to quantify any change in level of physical activity.

Measurement of compliance

Dietary compliance was assessed using four-day food diaries on four occasions, two unannounced 24-hour recalls and daily study food records completed by participants. Biochemical assessment of nutritional status included measurement of plasma vitamin C and carotenoids. To specifically monitor for dark chocolate consumption, urinary epicatechins were measured, as these represent one of the major polyphenols in cocoa (17).

Assessment of vascular function

Participants attended for assessments of microvascular function pre- and post- the eight-week intervention period. Room temperature was maintained at 22-24°C. All testing was performed by a single investigator (RN) who remained blinded to intervention phase dietary allocations throughout the study and during statistical analysis. Participants attended between 7am-11am, having fasted for at least 12 hours and refrained from ingestion of caffeine, alcohol, nicotine or long-acting nitrate preparations.

Venous occlusion plethysmography was used to determine FBF during intra-arterial infusions of ACh and SNP, endothelium-dependent and -independent vasodilators, respectively. Each FBF study was conducted according to an established standard operating procedure within the facility (2). After infiltration with 1% lidocaine, a 27-gauge sterile needle (Cooper's Needle Works, Birmingham, UK) was inserted into the non-dominant brachial artery using an aseptic technique. At baseline and during vasodilator administration, plethysmographic measurements were made with electrically calibrated mercury-in-Silastic strain gauges (PMS Instruments, Maidenhead, UK) in both the infused and noninfused limbs, to control for unexpected stimuli (18). Each strain gauge was attached to a Hokanson EC6 plethysmograph (PMS Instruments), which in turn was connected to a dedicated computer on which the Hokanson NIVP3 Dual Channel software (Version 5.40, PMS Instruments) was installed. Initially saline was infused through the needle via an epidural catheter at a rate of 1 mL/min for at least 30 minutes, followed by incremental intra-arterial infusions of acetylcholine (50, 100, and 200 nmol/min) and sodium nitroprusside (5, 10, and 20 nmol/min). Each concentration was infused for 5 minutes before five forearm blood flow readings were made during 7-second periods of venous occlusion separated by 20-second intervals.

Mean inflow curve gradients for each concentration of both infusions were calculated using NIVP3 software. This was performed for infused and control arms to yield flow rates in ml/100ml forearm volume (i.e. % change). Each participant's responses were summarised by curves of % increase in infused:non-infused ratio against vasodilator dose. The summary measure used was maximum vasodilator response observed (10).

Laboratory analysis

Plasma vitamin C was measured on a FLUOstar Optima plate reader (BMG Labtech,

Ortenberg, Germany) adapted from the method by Vuilleumier & Keck (19). Serum concentrations of six carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin) were measured by reverse phase high performance liquid chromatography (HPLC) as described by Craft (20). Fasting serum lipid profiles (total cholesterol, high density lipoprotein (HDL) and triglycerides) were assessed using standard enzymatic colorimetric assays on an automated Cobas® 8000 Modular system biochemical analyser (Roche Diagnostics Ltd, West Sussex, UK). Low-density lipoprotein (LDL) cholesterol was calculated using a standard Friedewald formula (21). Urine collected from the volunteers between evening meal and midnight the evening before each study visit was analysed, including an enzymatic hydrolysis step, to quantify total epicatechin content using an Agilent Technologies 1100 series HPLC (Agilent Technologies, Stockport, UK) directly linked to a Waters Micromass Quattro Ultima Platinum API triple quadrupole mass spectrometer (Waters, Dublin, Ireland).

Statistical analysis and power calculation

Results are expressed as mean ± standard deviation for normally distributed continuous variables. Skewed variables were log- transformed and summarized as geometric mean and interquartile range (IQR), where appropriate. The principal analysis for each outcome variable was a between group comparison of change using independent sample t tests or chi squared test. These analyses were confirmed using analysis of covariance (ANCOVA) using post intervention values as the outcome variable and adjusting for pre intervention values. Within group comparisons were performed using paired samples t tests. Univariate linear regression analysis was also used to further examine vascular function results. All statistical analyses were performed using SPSS version 20 (SPSS. Inc, Chicago, IL).

The power calculation was based on data collected as part of the FAVRIT study (2). To detect a 33% difference (the difference detected in FAVRIT) between groups in this variable with 90% power using a 2-tailed test at the 5% significance level would require 50 participants per group. Participants who were found to have an elevated highly sensitive C-reactive protein (hsCRP) >10mg/l at any point in the study, indicating acute inflammation, were excluded from final analysis, in line with American Heart Association guidelines (22).

RESULTS

Summary of recruitment

The CONSORT recruitment summary is illustrated in **Figure 2.** Overall, 155 individuals attended for screening. Of these, 51 (33%) did not meet the inclusion criteria or were unable to participate. In total, 104 participants commenced the study; two withdrew during the wash out period due to family or work reasons. At randomization, 102 patients (51 per group) were allocated to either a high or low polyphenol diet. There were three dropouts during the intervention phase: two from the high polyphenol group due to personal reasons and a change in antihypertensive therapy respectively, and one from the low polyphenol group due to personal illness. Therefore, 99 participants completed the study between January 2010 and April 2013.

Of these, six had a recorded serum hsCRP>10mg/l at one or more time points during the study and were excluded from further analyses. Of the remaining 93 participants, there were missing post-intervention plasma samples required for vitamin C analysis for one participant, one patient did not return a complete set of food diaries, and post-intervention FBF studies had to be abandoned in another patient due to computer hardware issues on that visit. A full data set was therefore available on a

total of 90 participants. Baseline results prior to entry to washout period are not shown.

Baseline characteristics

Baseline pre-intervention characteristics recorded after the 4-week run-in period are summarized in **Table 1**. There were no significant differences between the two groups, with the exception of total cholesterol and low-density lipoprotein (LDL), which were both higher in the high polyphenol group (p=0.020 and p=0.038 respectively).

Changes in measure of compliance

Results for self-reported polyphenol intake and micronutrients are summarized in **Table 2**. Between group change showed a significant increase in self-reported polyphenol intake in the high polyphenol group compared with the low polyphenol group (p<0.001 for all). There was also a significant between-group difference in change in micronutrient status: concentrations of urinary epicatechin (p<0.001), plasma vitamin C (p<0.001) and all serum carotenoids (with the exception of lycopene (p=0.098)) with a significantly larger increase in the high polyphenol group compared to the low polyphenol group.

Changes in microvascular function

Comparison between the two dietary groups showed maximum % response to ACh (infused:non-infused arm ratios) was significantly improved in the high polyphenol group (p=0.02) relative to the control group (**Table 3**). There was no significant difference in the between group change in response to SNP (p=0.17). Dose response curves were plotted for mean saline and infused:non-infused ratios for each ACh concentration per group pre- and post-intervention (**Figure 3**) to illustrate

the improvement in high polyphenol FBF post-intervention. Further analysis of microvascular function is included in **Appendix 1.**

Changes in markers of cardiovascular risk

There were no significant changes in BMI within (low polyphenol group 29.8 kg/m^2 pre- and 29.8 kg/m^2 –post (p=0.842), high polyphenol group 31.6 kg/m^2 and 31.6 kg/m^2 –post (p=0.961)) or between groups (p=0.897) during the intervention (**Table 3**). There was a trend towards a larger decrease in systolic blood pressure (SBP) in the high polyphenol group compared to the low polyphenol group; this was of borderline statistical significance (p=0.059). Within-group analysis found that SBP decreased significantly within both the low polyphenol (141.7±9.3 mmHg pre- *vs* 136.9±10.6 mmHg post-, p<0.001) and high polyphenol group (140.6±8.9 mmHg pre- *vs* 131.6±12.5 mmHg post-, p=0.001) by 4.8mmHg and 9.0mmHg respectively.

There was a significant between-group difference in change in total cholesterol with the high polyphenol diet showing an improvement (0.2 mmol/L reduction) compared with an increase in concentration in the low polyphenol diet (0.1 mmol/L increase) p=0.042). There were no significant between-group differences in change in any of the remaining lipid parameters.

DISCUSSION

The PPhIT study aimed to extend a previous dietary intervention (FAVRIT) that had been shown to improve microvascular function in hypertensive subjects. This study develops those findings by showing that increasing polyphenol content with the addition of berries and dark chocolate potentially has additional benefits on microvascular function and CVD risk. The ideal combination of polyphenol-rich foods for cardiovascular health remains under debate. It was hypothesized that this diet

would provide increased benefits to cardiovascular health and, importantly, given its palatability, be easily adopted by the general public.

The primary endpoint of the PPhIT study was between group change in maximum FBF response to the endothelium-dependent vasodilator, ACh. Between group comparison showed that those subjects who received a high polyphenol diet had a significantly greater improvement in maximum % response to ACh, compared to the low polyphenol group (p=0.02). This suggests that increasing consumption of polyphenol-rich foods improves endothelium-dependent forearm blood flow response in hypertensive volunteers.

Therefore, the present findings indicate that increasing polyphenol intake, and specifically including berries, alongside consumption of a moderate portion of dark chocolate, could result in a significant improvement in endothelium-dependent responses. The available prognostic association data (10) suggest that improving endothelium-dependent FBF with a diet rich in polyphenols could potentially modify CVD risk. An effect due to displacement of other foods from participants' diets cannot, however, be excluded.

There was a significant between group change in reported fruit and vegetable intake. Given that self-reported data is prone to reporting bias, independent confirmation was provided by biomarkers, including assessment of plasma vitamin C and a panel of serum carotenoids. This indicated good compliance with the dietary intervention. The epicatechin content of the dark chocolate provided to participants was not measured during this study. However, previous studies have measured epicatechin levels in the brand of dark chocolate used in this intervention. Using this data, the amount of dark chocolate provided would have delivered a dose of 35.7mg epicatechin/day (23). Compliance with dark chocolate was demonstrated by a

significant increase in evening meal to midnight urinary epicatechin levels detected on between group testing. Urinary epicatechin use has been found to be superior to plasma epicatechin measurement as peak levels are detected 6-12h post-consumption (24,25). The addition of dark chocolate to the regimen may have increased the palatability of the diet. Participant feedback showed a 90% approval rating for the overall diet, indicating that it was well tolerated (26).

There was a trend towards greater lowering in SBP within the high polyphenol group compared to the low polyphenol group (mean change: -9.1±12.4mmHg vs -4.8±8.7mmHg respectively, p=0.059) on between group analysis. Both SBP and DBP decreased significantly on within group analysis. However, the decrease in blood pressure in both active and control groups may represent 'regression to the mean' (27), a phenomenon has been described in previous BP studies (2). Few polyphenol dietary intervention trials have assessed SBP. Previous calculations estimate that 115 participants would be required per group to detect a 5mmHg change in SBP with 90% power; therefore this study may have been underpowered to detect a difference in SBP between groups, and a type II error is possible (2).

Between group testing demonstrated a significantly greater reduction in total cholesterol (p=0.042) and a trend for greater decrease in LDL (p=0.063) in the high polyphenol group compared to the low polyphenol group. To put this into a clinical context, the change post-intervention in LDL in high polyphenol group in the PPhIT study equated to a 4.3% decrease in LDL concentrations, which, based on published data, would equate to a reduction in CVD mortality of approximately 4% (28).

In contrast to the PPhIT study, most polyphenol studies have used single food interventions. Combining different polyphenol-rich foods may provide a cumulative increase in benefit due to the synergistic effect of several bioactive compounds.

Potential mechanisms for the beneficial effects of polyphenols include: their antioxidant and vasodilator functions, their ability to alter the expression of endothelial nitric oxide synthase, and their ability to induce production of other endogenous vasodilators. However, many other cardiovascular benefits may exist, as described in recent reviews (29,30). The participants in the PPhIT study were free-living and allowed to make their own choices of type of F&V to be consumed. They also had hypertension, and other cardiovascular risk factors. The results therefore are more relevant to 'real-life' than studies involving in-patient admission or supervised meals.

A concerted effort was made to keep other lifestyle variables constant during the study, reflected by static BMI and activity levels. However, in free-living individuals it is impossible to control for all variables in lifestyle. Due to budget and time constraints the PPhIT study was unable to use a factorial design to allow examination of the specific effect of berries and dark chocolate over and above increasing fruit and vegetable intake alone. As such this study cannot conclusively state whether polyphenols or other bioactive components within these foods, such as carotenoids or vitamin C, working in isolation or synergistically, have led to the improvement in the primary endpoint. Future work could include comparison between low and high fruit and vegetable versus high polyphenol diets to definitively test the hypothesis that a greater improvement in FBF responses to ACh can be achieved with specific inclusion of polyphenol-rich foods, compared to a fruit and vegetable-rich diet alone.

CONCLUSIONS

Increasing intake of polyphenol-rich foods, in the form of fruit and vegetables, berries and dark chocolate, for 8 weeks resulted in a significant improvement in endothelium-dependent vasodilation in hypertensive participants. This study suggests that a well-tolerated polyphenol-rich diet could have a positive effect on microvascular function and cardiovascular risk.

TABLES

Table 1- Pre-eight week intervention period participant characteristics according to diet allocation.

	Low polyphenol (n=47)	High polyphenol (n=46)	Between group comparison P-value
Age(years)[*]	55.6 (6.8)	54.0 (6.7)	0.235
Males(%)[§]	28 (59.6)	22 (47.8)	0.301
Body Mass Index(kg/m²)[*]	29.8 (5.0)	31.6 (6.5)	0.148
Systolic blood pressure(mmHg)[*]	141.7 (9.3)	140.6 (8.9)	0.589
Diastolic blood pressure(mmHg)[*]	85.5 (6.9)	84.5 (7.6)	0.460
Current smoker(%)[§]	4 (8.5)	6 (13.0)	0.523
Former smoker(%)[§]	24 (51.1)	18 (39.1)	0.300
Anti-hypertensive therapy(%)[§]	37 (78.7)	36 (78.3)	0.999
Lipid-lowering therapy(%)[§]	21 (44.7)	19 (41.3)	0.835
Total cholesterol(mmol/L)[*]	5.2 (1.2)	5.8 (1.3)	**0.020**
HDL(mmol/L)[*]	1.4 (0.4)	1.4 (0.4)	0.568
LDL(mmol/L)[*]	4.2 (1.3)	4.7 (1.4)	**0.038**
Triglycerides(mmol/L)[†]	1.6 (1.0,2.3)	1.6 (1.1,2.1)	0.796
Total cholesterol/HDL ratio[*]	3.9 (1.3)	4.2 (1.2)	0.267
Max response ACh (infused:noninfused)(%)[*‡]	123.9 (99.6)	109.1 (84.7)	0.447
Max response SNP (infused:noninfused)(%)[*‡]	126.3(120.4)	107.7(70.1)	0.369

HDL- high-density lipoprotein; **LDL**- low-density lipoprotein; **ACh**- acetylcholine; **SNP**- sodium nitroprusside.

*Normally distributed data summarized as mean (SD). Difference between groups assessed using independent samples t-test.

†Skewed data was logarithmically transformed and presented as geometric mean (IQ range). Difference between groups assessed using independent samples t-test.

§Categorical variables are summarized as n (%). Difference between groups assessed using Chi-square test.

‡based on 92 subjects due to missing data for one participant in low polyphenol group.

Table 2- Pre- and post-intervention period results of self reported dietary intake and micronutrient concentrations by diet allocation

	Low polyphenol (n=47)		Change	Within group P-value	High polyphenol (n=46)		Change	Within group P-value	Between group P-value
	Pre	Post			Pre	Post			
F&V intake[*‡] (portions/day)	1.38(0.80)	1.25(0.57)	-0.13 (-0.35,0.10)	0.252	1.40 (0.71)	6.82 (2.12)	5.42 (4.71,6.14)	**<0.001**	**<0.001**
Berries (portions/day)[§‡]	0	0	0	N/A	0	1.00 (0.80,1.25)	1.00 (0.77,1.25)	**<0.001**	**<0.001**
Dark Chocolate (g/day)[§‡]	0	0	0	N/A	0	50.0 (37.5,50.0)	50.0 (37.5,50.0)	**<0.001**	**<0.001**
Epicatechin (nmol/mol crt)[*]	0.12(0.20)	0.10 (0.12)	-0.02 (-0.08,0.06)	0.629	0.10(0.30)	0.30(0.29)	0.19 (0.07,0.31)	**0.002**	**<0.001**
Vitamin C (μmol/l)[†‡]	25.1 (18.3,52.3)	19.6 (11.8,48.9)	0.79 (0.63, 0.96)	**0.021**	28.4 (18.6,57.5)	51.7 (43.9,68.6)	1.82 (1.34, 7.25)	**<0.001**	**<0.001**

	Low polyphenol (n=47)		Change	Within group P-value	High polyphenol (n=46)		Change	Within group P-value	Between group P-value
	Pre	Post			Pre	Post			
Lutein(μmol/l)[†]	0.16 (0.12,0.20)	0.15 (0.11,0.20)	0.98 (0.90, 1.06)	0.595	0.15 (0.11,0.19)	0.20 (0.15,0.26)	1.32 (1.22, 1.44)	**<0.001**	**<0.001**
Zeaxanthin (μmol/l)[†]	0.04 (0.03,0.05)	0.04 (0.03,0.05)	0.97 (0.91, 1.03)	0.331	0.04 (0.03,0.05)	0.05 (0.04,0.06)	1.18 (1.07, 1.30)	**0.001**	**0.001**
βcryptoxanthin (μmol/l)[†]	0.05 (0.04,0.08)	0.05 (0.03,0.08)	0.92 (0.83, 1.03)	0.134	0.05 (0.04,0.07)	0.07 (0.05,0.10)	1.29 (1.15, 1.45)	**<0.001**	**<0.001**
α-carotene (μmol/l)[†]	0.12 (0.09,0.16)	0.11 (0.08,0.14)	0.90 (0.82, 0.99)	**0.037**	0.12 (0.10,0.15)	0.14 (0.11,0.20)	1.17 (1.05, 1.30)	**0.006**	**0.001**
β-carotene (μmol/l)[†]	0.24 (0.15,0.36)	0.21 (0.14,0.32)	0.91 (0.82, 1.01)	0.064	0.26 (0.19,0.36)	0.30 (0.23,0.42)	1.16 (1.05, 1.28)	**0.006**	**0.001**

	Low polyphenol (n=47)		Change	Within group P-value	High polyphenol (n=46)		Change	Within group P-value	Between group P-value
	Pre	Post			Pre	Post			
Lycopene (μmol/l)†	0.45 (0.33,0.69)	0.43 (0.33,0.58)	0.95 (0.85, 1.06)	0.343	0.47 (0.38,0.66)	0.53 (0.41,0.69)	1.12 0.95, 1.31)	0.177	0.098
Total carotenoids (μmol/l)†	1.09 (0.84,1.47)	0.99 (0.75,1.34)	0.93 (0.86, 1.09)	0.800	1.13 (0.91,1.36)	1.32 (1.06,1.67)	1.17 (1.06, 1.29)	**0.003**	**0.001**

F&V- fruit and vegetables, **N/A**- not applicable

˙Normally distributed variables at baseline were summarised as mean (SD). Within group change assessed using paired samples t test and between group differences in change assessed using independent samples t test. Change expressed as mean difference (95%CI).

†Skewed variables were logarithmically transformed and baseline values summarised as geometric mean (IQR). Within group change assessed using paired samples t test and between group differences in change assessed using independent samples t-test. Change expressed as geometric mean (95%CI), which, due to the logarithmic transformation, represents the ratio of the post to pre value.

§Non-parametric data at baseline presented as median (IQR). Within group change assessed using Wilcoxon Signed Rank test and between group differences In change calculated using Mann-Whitney U-test. Change expressed as median difference (IQR).

‡Based on 92 patients due to incomplete food diary and insufficient Vitamin C sample in high polyphenol group.

Table 3 Pre- and post-intervention period results of cardiovascular risk assessments by diet allocation

	Low polyphenol (n=47)		Change	Within group P-value	High polyphenol (n=46)		Change	Within group P-value	Between group P-value
	Pre	Post			Pre	Post			
Max response to Ach, infused:non-infused(%)[*‡]	123.9 (99.6)	136.7 (143.7)	12.8 (-34.8,60.4)	0.590	109.1 (84.7)	225.0 (180.4)	115.9 (70.1, 161.6)	**<0.001**	**0.02**
Max response to SNP, infused:non-infused(%)[*‡]	126.3 (120.4)	106.5 (87.7)	-19.7 (-59.9,18.4)	0.303	107.7 (70.1)	120.2 (92.5)	12.5 (-14.7, 39.7)	0.358	0.169
Systolic blood pressure(mmHg)[*]	141.7 (9.3)	136.9 (10.6)	-4.83 (-7.38,-2.28)	**<0.001**	140.6 (8.9)	131.6 (12.5)	-9.06 (-12.8,-5.40)	**<0.001**	**0.059**
Diastolic blood pressure(mmHg)[*]	85.5 (6.9)	82.0 (6.1)	-3.53 (-5.24,-1.82)	**<0.001**	84.5 (7.6)	79.1 (8.2)	-5.35 (-7.81,-2.88)	**<0.001**	0.227

	Low polyphenol (n=47)		Change	Within group P-value	High polyphenol (n=46)		Change	Within group P-value	Between group P-value
	Pre	Post			Pre	Post			
Body mass index(kg/m^2)*	29.8(5.0)	29.8(5.0)	0.01 (-0.10,0.12)	0.842	31.6(6.3)	31.6 (6.5)	-0.01 (-0.24,0.23)	0.961	0.897
Total cholesterol(mmol/L)*	5.2(1.2)	5.3(1.1)	0.10 (-0.08,0.28)	0.250	5.8(1.3)	5.6(1.0)	-0.21 -0.57,0.04)	0.097	**0.042**
HDL(mmol/L)*	1.4(0.4)	1.4(0.3)	0.01 (-0.03,0.05)	0.725	1.4(0.4)	1.5(0.4)	0.02 (-0.03,0.06)	0.482	0.753
LDL(mmol/L)*	4.2(1.3)	4.3(1.2)	0.08 (-0.10,0.26)	0.366	4.7(1.4)	4.5(1.0)	-0.25 (-0.57,0.06)	0.109	0.063
Triglycerides (mmol/L) †	1.6 (1.0, 2.32)	1.5 (1.1, 2.4)	0.97 (0.90, 1.05)	0.470	1.6 (1.1, 2.1)	1.6 (1.1, 2.3)	0.99 (0.89, 1.10)	0.825	0.807

ACh= acetylcholine, **SNP**= sodium nitroprusside, **95%CI**= 95% confidence intervals, **HDL**- high-density lipoprotein; **LDL**- low-density lipoprotein

*Normally distributed variables at baseline were summarised as mean (SD). Within group change assessed using paired samples t test and between group differences in change assessed using independent samples t test. Change expressed as mean difference (95%CI).

†Skewed variables were logarithmically transformed and baseline values summarised as geometric mean (IQR). Within group change assessed using paired samples t test and between group differences in change assessed using independent samples t-test. Change expressed as geometric mean (95%CI), which, due to the logarithmic transformation, represents the ratio of the post to pre value.

‡Based on 92 subjects due to missing microvascular function data on one patient in low polyphenol group.

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ABBREVIATIONS

ACh- acetylcholine

BMI- body mass index

BP- blood pressure

CVD- cardiovascular disease

F&V- fruit and vegetables

FAVRIT- fruit and vegetable randomised intervention trial

FBF- forearm blood flow

hsCRP- highly sensitive C-reactive protein

IQR- interquartile range

LDL- low density lipoprotein

PPhIT- polyphenol intervention trial

SBP- systolic blood pressure

SD- standard deviation

SNP- sodium nitroprusside

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