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ORIGINAL CONTRIBUTION

Impact of the quantity and flavonoid content of fruits and vegetables on markers of intake in adults with an increased risk of cardiovascular disease: the FLAVURS trial

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Abstract
Purpose Limited robust randomised controlled trials investigating fruit and vegetable (F&V) intake in people at risk of cardiovascular disease (CVD) exist. We aimed to design and validate a dietary strategy of increasing flavonoid-rich versus flavonoid-poor F&V consumption on nutrient biomarker profile.
Methods A parallel, randomised, controlled, dose–response dietary intervention study. Participants with a CVD relative risk of 1.5 assessed by risk scores were randomly assigned to one of the 3 groups: habitual (control, CT), high-flavonoid (HF) or low-flavonoid (LF) diets. While the CT group ($n = 57$) consumed their habitual diet throughout, the HF ($n = 58$) and LF ($n = 59$) groups sequentially increased their daily F&V intake by an additional 2, 4 and 6 portions for 6-week periods during the 18-week study.
Results Compliance to target numbers and types of F&V was broadly met and verified by dietary records, and plasma and urinary biomarkers. Mean (±SEM) number of F&V portions/day consumed by the HF and LF groups at baseline (3.8 ± 0.3 and 3.4 ± 0.3), 6 weeks (6.3 ± 0.4 and 5.8 ± 0.3), 12 weeks (7.0 ± 0.3 and 6.8 ± 0.3) and 18 weeks (7.6 ± 0.4 and 8.1 ± 0.4), respectively, was similar at baseline yet higher than the CT group ($3.9 \pm 0.3, 4.3 \pm 0.3$, 4.6 ± 0.4, 4.5 ± 0.3) ($P = 0.015$). There was a dose-dependent increase in dietary and urinary flavonoids in the HF group, with no change in other groups ($P = 0.0001$). Significantly higher dietary intakes of folate ($P = 0.035$), non-starch polysaccharides ($P = 0.001$), vitamin C ($P = 0.0001$) and carotenoids ($P = 0.0001$) were observed in both intervention groups compared with CT, which were broadly supported by nutrient biomarker analysis.
Conclusions The success of improving nutrient profile by active encouragement of F&V intake in an intervention study implies the need for a more hands-on public health approach.

Keywords Fruits and vegetables · Flavonoids · Biomarkers · Dose response

Abbreviations

CVD	Cardiovascular disease
CT	Control
HF	High-flavonoid
LF	Low-flavonoid

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NSP Non-starch polysaccharide
F&V Fruits and vegetables

Introduction

Several epidemiological studies have indicated that the consumption of fruits and vegetables (F&V) is associated with reduced risk of cardiovascular disease (CVD) [1–3]. However, published data on the amounts and composition of F&V which contribute to risk reduction are inconsistent, as are specific dietary recommendations for F&V intakes worldwide. Within Europe, national recommendations range from generic advice to increasing F&V consumption with no specific guidelines on the number of servings to be consumed (Portugal) to recommendations to consume anywhere from 2 (Austria) to 5 (United Kingdom, UK) and 9 (Greece) portions per day [4]. In addition, there is inconsistency in the recommendations for the specific number and type of either fruits or vegetables between different countries. For example, the Netherlands recommend 2 portions of fruits and 2 portions of vegetables daily, whilst most other countries make no specification for the relative proportion of either [4]. F&V contain a wide range of potentially cardioprotective components including fibre, folate, nitrate, antioxidant vitamins and a large number of non-nutrient phytochemicals, including carotenoids and phenolic compounds such as flavonoids. Benefits with respect to lipid, haemostatic, inflammatory and vascular-related (blood pressure and vascular reactivity) biomarkers have been observed, with increased intakes of many of the above-mentioned isolated plant components [5–8]. However, there is a paucity of information regarding the relative biopotency of these components and their impact on CVD risk biomarkers when consumed as foods, rather than as isolated compounds. There is accumulating and relatively consistent evidence from intervention studies to suggest that dietary flavonoids may contribute, in large part, to the CVD benefits of increased F&V intake [9–12], amongst other bioactive phytochemicals. However, to date, this hypothesis has not been tested in an adequately powered, dose–response, randomly controlled trial (RCT).

FLAVURS (FLAvonoids and Vascular function at the University of Reading Study) is the first large-scale RCT which primarily aimed to investigate the effects of HF versus LF F&V on vascular function and other risk factors for CVD in free-living individuals at above average risk of CVD, in a dose–response manner (data not presented in this publication). Its secondary aim is to provide a detailed dietary intake data of dietary flavonoids in a UK population. The success of any randomly controlled dietary intervention trial is determined by the achievement of target dietary intakes and compliance of the participants. This paper describes and evaluates the effectiveness of the dietary strategy designed for the implementation of the target quantity of HF and LF F&V in a free-living population participating in the FLAVURS study. The major tenet of the strategy was to implement the dietary change without major disruption to other habitual dietary practices and to match other potentially bioactive components of F&V (excluding flavonoids) between the two dietary intervention groups.

Materials and methods

Subjects

Male and female participants, aged 30–70 years, were recruited from the population in and around Reading, UK through newspaper and poster advertisements, email and radio. General practitioners' databases were searched by clinical staff using specific criteria based on the FLAVURS screening tool (described below) to identify potential participants, who were informed that the purpose of the study was to determine the impact of different doses and type of F&V on CVD risk. Individuals who fulfilled the screening criteria and were willing to participate in FLAVURS were recruited in two cohorts, December 2007 to March 2008 and November 2008 to January 2009. Participants initially underwent telephone screening using a health and lifestyle questionnaire and a minimum of two random 24 h dietary recalls to assess their suitability for the study and their usual daily intake of F&V, respectively. Only individuals with a habitual F&V intake less than the population average of 4.4 portions/day [13] were recruited onto the study. Anthropometric measurements and biochemical tests were conducted in a screening visit. A scoring tool adapted from the Framingham risk score system [14] was used for recruitment to identify those individuals who were at above average risk of developing CVD, defined as a relative risk (RR) of >1.5 based on scoring a minimum of 2 points in one or more of the following criteria: (1) total plasma cholesterol (TC) [15] 6.2–7.2 mmol/L (2 points), 5.2–6.1 mmol/L (1 point); (2) high-density lipoprotein (HDL) cholesterol [16] in men ≤0.9 mmol/L (2 points), 1.0–1.1 (1 point) and women <0.9–1.1 mmol/L (2 points), 1.2–1.3 mmol/L (1 points); (3) blood pressure (BP) [17] either systolic BP (SBP) ≥140 mmHg (2 points), 130–139 mmHg (1 point) or diastolic BP (DBP) ≥90 mmHg (2 points); (4) smoking status [18] ≥10 per day (2 points); (5) obesity/adiposity [19] either body mass index (BMI) > 30 kg/m^2 (2 points), 25–30 kg/m^2 (1 point) for Asian populations >27.5 kg/m^2 (2 points), 23–27.5 kg/m^2

(1 point) or waist circumference in men >102 cm (2 points), 94–101 cm (1 point) and women >88 cm (2 points), 80–87 cm (1 point). Key exclusion criteria included clinically diagnosed diabetes, renal or liver disease, previous stroke or myocardial infarction, on drug treatment for lipid-lowering, hypertension or on anti-inflammatory medication, pregnant or lactating. In addition, those on a diet-reducing regime, travelled regularly for work during the period of the study or who ate out frequently and did not prepare home-cooked dishes were excluded. Figure 1 shows the flow of participants through the study.

Study power

As the primary aim of the study was to investigate vascular reactivity measured by laser Doppler imaging with iontophoresis (LDI), it was calculated that a total of 180 ($n = 60$ participants per group) were required. This would enable detection of a 15 % difference in LDI vascular reactivity equivalent to 90 flux units, with an estimated standard deviation (SD) of 225 flux units, with a $P < 0.05$ and 80 % power, and an allowance for a 15 % dropout.

Study design

The study was a parallel, randomised, controlled, sequentially increasing, dose–response intervention and was formally registered as a randomised controlled trial (ISRCTN47748735). It was given a favourable ethical opinion to proceed by the Local Research Ethics Committee of the Isle of Wight, Portsmouth and South East Hampshire (REC: 07/H0501/81) and the University of Reading's Research Ethics Committee. All participants gave informed consent before participation. Participants were randomised by a minimisation procedure [20, 21] to either a control (CT) group or one of the two intervention groups: HF or LF. This procedure enabled the three groups to be closely matched for gender ratio, age, BMI, smoking habits and tea/red wine consumption. Tea and red wine are two major dietary sources of flavonoids in the UK diet, and it was deemed important to consider habitual consumption and to randomise on the level of intakes of these beverages.

After a 2-week run-in period, during which participants were unaware to which group they were to be assigned and were requested to follow their usual diet, the CT group

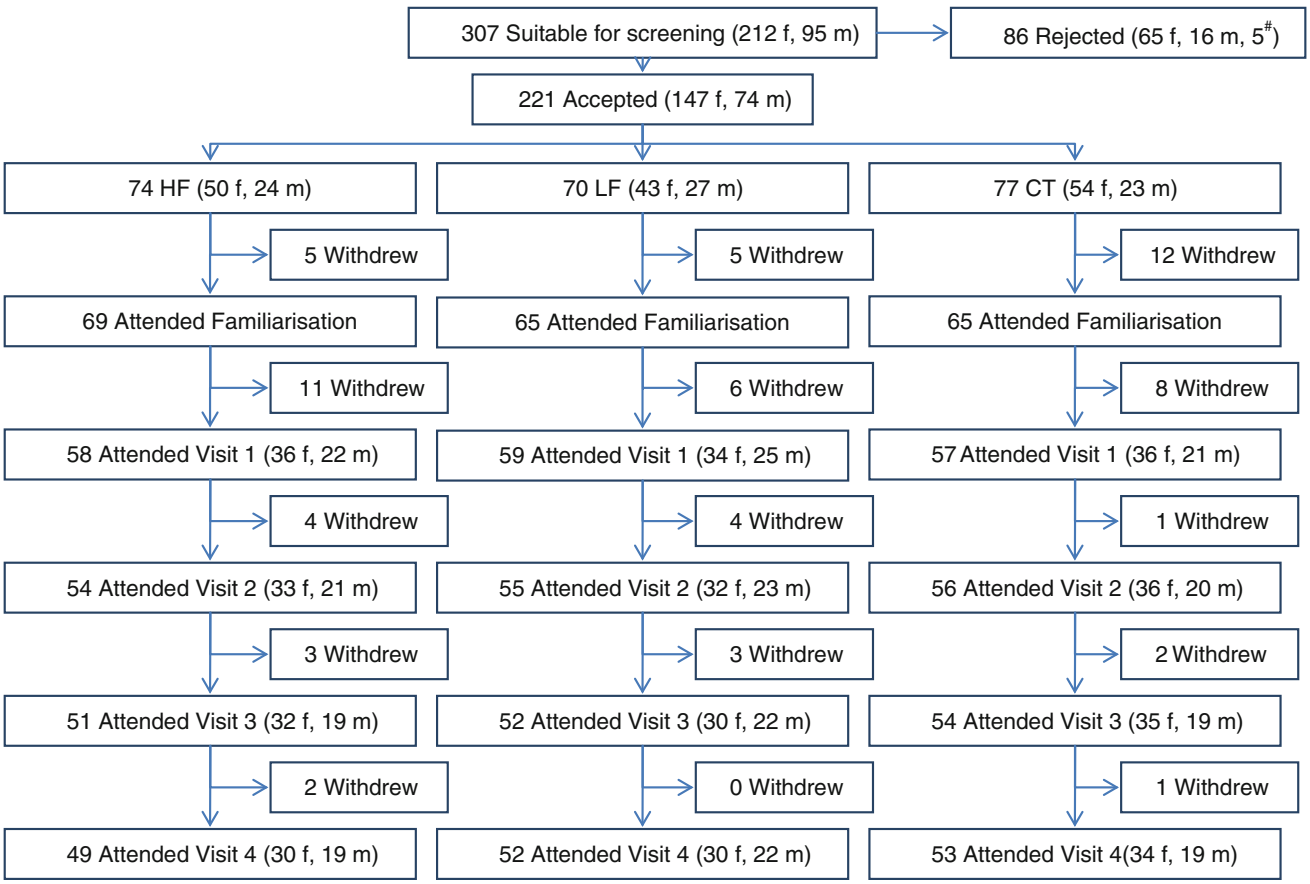

Fig. 1 Diagram to show the flow of participants through the FLAVURS study. *f* female, *m* male, # no data

continued on their habitual diet throughout the 18-week study, while the HF and LF groups sequentially increased their daily F&V intake by an additional 2 portions per day every 6 weeks of the study to a maximum of 6 extra portions per day by week 18, with HF and LF F&V, respectively (Supplementary Figure 1). Following consumption of a low-fat, low-flavonoid standard meal the night before and a 12-h fast, participants attended a clinical visit at the Hugh Sinclair Unit of Human Nutrition on weeks 0, 6, 12 and 18, during which fasting blood samples were taken and biochemical measures of CVD risk were determined (data not reported in this manuscript). During the entire duration of the study, participants were instructed to minimise changes in other health and lifestyle behaviours and to maintain consistent weight. They were also asked to report any use of newly prescribed medication or changes to current medication immediately, as this could have impacted on their CVD risk profile. While the HF and LF groups were provided with the intervention F&V for 18 weeks, the CT group was given shopping vouchers on weeks 6 and 18 as an incentive to remain on the study. All participants were reimbursed for their time and transport costs incurred during the study.

Dietary intervention

The run-in period provided the opportunity to monitor participants, to confirm their habitual dietary intake (through diet recalls) allowing us to exclude non-compliant participants (e.g. those with a higher than average UK F&V intake) and to identify those difficult to contact. The run-in period also allowed accurate baseline measurements of the participants to be taken at week 0.

At week 0, participants were given individualised dietary advice and a handbook detailing information on the study and the diets to which they were randomly assigned. Throughout the study, participants were in close contact with the study dietician, who provided dietary advice and support at each visit and whenever required during the intervening periods. The individualised handbook contained lists of HF or LF F&V. A portion of fruit or vegetable is equivalent to 80 g (40 g for dried foods) in weight as defined by the United Kingdom Food Standards Agency guidelines (http://www.eatwell.gov.uk/healthydiet/nutritionessentials/fruitandveg/). The handbook contained information on how 80 g of specific F&V could be defined by household measures (e.g. cups, tablespoons) or number of F&V items (e.g. grapes). Composite foods that contained F&V as component parts, such as soups and fruit strudels, were also included. In this study, LF F&V were defined as those containing a total flavonoid content of <5 mg/100 g, while HF were defined as those containing a total flavonoid content of ≥15 mg/100 g [22], see Table 1. To reduce the burden and cost of purchasing large amounts of additional F&V, and most importantly to improve compliance, the prescribed F&V were supplied to the participants. A 3-week cycle menu for each intervention diet was developed and used as a basis for creating standardised lists of F&V to be sent to participants' homes each week. This allowed for the supply of a variety of foods and made ordering and packing of the F&V easier for the researchers. To maintain freshness of the F&V provided, a pre-requisite for entering the study was that participants had adequate refrigerator or cold room storage space for the F&V

Table 1 List of high- (≥15 mg/100 g) and low-flavonoid (<5 mg/100 g) fruit and vegetables offered to the participants who were randomly assigned to the HF and LF dietary groups, respectively

High-flavonoid fruit & vegetables (≥15 mg/100 g)	Low-flavonoid fruit & vegetables (<5 mg/100 g)
Fruit	*Fruit*
Apple all varieties	Apricot, canned
Orange/tangerines, all types	Avocados
Grapefruit, pink and red, white	Kiwi
Grapes, black or red only	Mangoes
Plum, purple or red	Peaches, canned
Strawberries	Pineapple, fresh or canned
Raspberries, red or black	Pears, canned (without skin)
Cherries, sweet or sour	Grapes, white or green
Blueberries	Chickpeas
Blackberries	Kidney beans
	Pinto beans
Vegetables	*Vegetables*
Lettuce, red leaf	Green beans/string beans/snow peas/mange-tout
Cabbage, red	Broccoli
Kale	Brussels sprouts
Onions, red	Cabbage, white or savoy
Peppers, sweet green or yellow	Carrots
Radishes	Cauliflower
Eggplant	Corn, sweet
Spinach	Cucumber
Watercress	Leeks
Cherry tomatoes	Mushrooms
Onions, white or spring	Parsnips
Asparagus	Peas
	Rhubarb
	Tomato or plum, red, ripe

Flavonoid content of intervention foods derived from USDA database for the flavonoid content of selected foods, release 2.1, Jan 2007 and flavonoid analysis of foods based on method described by Nielsen and Sandstrom [24]

provided. Individual participants' dislikes for certain F&V were taken into consideration and suitable substitutions were offered from the F&V in the list. In addition to fresh, canned and frozen, a variety of composite products containing F&V were also made available to participants in the final 6 weeks of the study, to increase their choice of intervention foods and to improve compliance when 6 additional portions were required. These products included dried fruits, fruit juices, fruit smoothies, soups, pasta sauces and fruit crumbles. These composite products made up less than 10 % of the study foods provided, and advice was given to replace similar habitual foods consumed by the participants with the study foods. Information on the portion size of each product equivalent to one portion of F&V was also provided.

Participants were encouraged to consume a variety of F&V or composite foods from the list each week and have equal proportions of F&V. Advice on storing and cooking the F&V and ideas on incorporating them into meals and snacks at home, at the workplace or when eating out, together with recipes using the supplied F&V, were also included in the handbook. The participants were encouraged to weigh the F&V to assess accurately a portion and, where necessary, kitchen scales were loaned to participants. If weighing of the F&V was not practical, household measures were used to assess portion sizes.

The F&V in the HF and LF lists were specifically chosen to maximise differences in dietary flavonoid content. As a result, the two diets contained different quantities of other potentially bioactive components such as non-starch polysaccharide (NSP) and folate, which were generally higher in the LF F&V; and carotenoids and vitamin C, which were on average present in greater quantities in the HF F&V. To minimise differences in the intakes of these potentially bioactive nutrients and phytochemicals between intervention groups, specific advice was given to the HF and LF groups regarding the choice and frequency of consumption of certain foods from the lists. For example, while participants in the LF group were encouraged to eat kiwi fruit at least 3 times per week to maintain the vitamin C content in their diet, those in the HF group were asked to limit the quantity of vitamin C-rich fruits consumed per day. An example of the daily contribution of nutrients and phytochemicals from the supplied F&V for 2, 4 and 6 additional portions of F&V is shown in Table 1.

Measures of compliance and dietary assessment

Participants in the intervention groups indicated the type and number of additional portions of F&V they consumed each day on record forms. A minimum of two random structured 24 h dietary recalls were conducted by trained nutritionists and dieticians over the telephone during the run-in period and during each 6-week intervention period. Portion sizes were confirmed at the study visits using a standard portion size atlas [23]. While the record forms only monitored compliance to the additional F&V consumed, the random 24 h recalls helped to determine the participant's overall F&V consumption and general dietary intake at all stages of the study.

To measure participants' adherence to the prescribed F&V, the number of portions consumed was calculated from the record forms and recalls and compared to target intakes. The dietary recalls were further analysed using the nutrient analysis software, DietPlan 6 (Forestfield Software Ltd, Horsham, West Sussex, UK), to assess the nutrient composition of the diets. As the flavonoid composition of foods was not available in the UK food database (McCance and Widdowson, 6th edition), flavonoid values from the USDA Flavonoid Database (2007; release 2.1) were incorporated into the UK food composition database using the DietPlan 6 software. In addition, 'in house' flavonoid chemical analysis of a number of F&V and composite foods was performed. The flavonoid content of these foods was also added to the database. It is recognised that various food processing or preparation methods would alter the flavonoid content of the original raw food [24, 25]. However, data for these foods are often unavailable. In order to achieve the best estimate of the representative intake of flavonoids from the dietary recalls, food items that differed in processing or preparation, such as frozen or boiled, were thus assigned similar flavonoid content to that of their raw food or closest processing counterparts. For composite foods not analysed for flavonoid values, such as pizza, soups, salads, fruit smoothies and fruit juices, individual ingredients by weight were determined and the total flavonoid content of each composite food was estimated by the summation of the flavonoid values of its individual ingredients. Where there was a lack of information on flavonoid-poor foods, they were assigned a zero value for their flavonoid content.

Biomarkers of F&V intake

To validate findings from the dietary recalls, the following biomarkers were measured: plasma vitamin C, carotenoids and folate, and urinary flavonoids and potassium. Urinary sodium was also determined, as this is an important biomarker of dietary sodium, which was important to monitor as changes in intake could potentially impact on the CVD outcome measures of vascular reactivity and blood pressure. Plasma carotenoids were measured using the method of Thurnham et al. [26] using a Hewlett Packard 1,050 high pressure liquid chromatograph (HPLC) with a Nucleosil 100-5C18, 25 cm × 4.6 cm column (Hichrom Ltd, Reading, Berks, UK) with a flow rate of 1.5 mL/min and the

detector set to monitor at 292 nm for α- and γ-tocopherol; 450 nm for lutein, β-cryptoxanthin, α-carotene and β-carotene; and 472 nm for lycopene. For plasma vitamin C and uric acid, plasma samples were treated with metaphosphoric acid and stored at −80 °C prior to analysis by HPLC with UV detection [27]. Plasma folate was measured using the method of O'Broin and Kelleher [28]. This method is based on the growth response of chloramphenicol resistant *Lactobacillus rhamnosus* 10463 (NCIMB, Bucksburn, Aberdeen, UK) in plasma samples, assessed with turbidity absorbance at 600 nm after a 24-h incubation period. Folate concentrations in plasma samples were calculated from a calibration curve of folic acid standards in the range 0–30 μmol/L.

Urinary potassium and sodium were determined by atomic absorption spectrophotometry (AAS) [29]. In brief, 24 h urine samples, acidified to pH 3.5 with HCl, were diluted with deionised water (1:1,200), after which caesium chloride was added (0.5 % W/V CS (0.04 M)). Urinary potassium was measured at 766.5 nm and sodium at 589.0 nm (AOAC Official method for the detection of minerals 985.35, 1996), using AAS instrument novAA-350 (Analytikjena with WinAAS software version 4.5.0). To detect urinary flavonoids, acidified 24 h urine samples were centrifuged at 1,700*g* for 10 min, filtered, pH adjusted to 4.9–5.1 and then subjected to enzyme hydrolysis using a β-glucuronidase and arylsulfatase enzyme mixture [30]. This was followed by solid phase extraction [31] and then analysed using HPLC (Agilent technologies, 1200 series) with a Nova-Pak® C18 4 μm (4.6 × 250 mm) cartridge Waters column with guard cartridge and a flow rate of 0.7 mL/min. The ferric reducing antioxidant power (FRAP) (a measure of oxidative status) of plasma was determined using the method of Benzie and Strain [32], adapted for use with 96-well microtitre plates [33]. A Genios plate reader (Tecan Ltd., Dorset, UK) was used to measure the absorbance at 593 nm.

Statistical analysis

A linear mixed models methodology [34] was applied using SAS software version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The random effect explained the within-subject variability across the study. A compound symmetry matrix was used as the variance–covariance matrix. The Kenward–Rogers methodology [35] to estimate the degrees of freedom in mixed models was applied. The fixed effects firstly adjusted for the baseline and cohort, followed by treatment group and then the remaining covariates. The covariates were treatment, visit, baseline BMI, age, gender, smoking and tea/red wine consumption. The Kolmogorov–Smirnov test [36] was used to assess normality, and a natural log transformation was applied to non-normal data. The predicted values versus standardised residuals were plotted to validate each model. The impact of potential outliers was assessed before the effect of removing them from the model was assessed. Significant effects were studied further, carrying out orthogonal contrasts where an orthogonal base could be found. Estimates of contrasts to assess the differences were also calculated. Some variables, for example, urinary flavonoids and plasma carotenoids, presented a significant number of zero values due to recorded values below the detection limit of the assays. To investigate the probability of detection, a generalised linear mixed model (GLMM) was used. Using a logit link, the data set was then assessed for the values that were detected. Adjustments for potential overdispersion were included. The mixed models were fitted using proc mixed and the GLMM models using proc glimmix. A P value of <0.05 was considered as significant.

Results

Habitual nutrient intake and plasma phytochemical status

A total of 174 participants (68 men, 106 women) commenced the study, with 154 (60 men, 94 women) completing all 4 visits. The flow of participants through the study is shown in Fig. 1. There was an 11.5 % attrition rate, with change in medication, personal and work-related reasons given for withdrawal. The participants were well matched for baseline characteristics (Table 2). The baseline nutrient intakes of the participants are shown in Table 4, and the data are similar to those reported for average UK population intakes [13]. The participants randomised to the HF, LF and CT groups were well matched in their macronutrient (Table 4) and micronutrient (Table 5) intake, with no significant differences observed between any groups at baseline. However, some gender differences were observed, with male participants consuming higher energy ($P = 0.002$), protein ($P = 0.004$), starch ($P = 0.001$), total sugars ($P = 0.001$), potassium ($P = 0.01$) and folate ($P = 0.015$) compared with the female participants in the study (data not shown). It was also observed that those below 50 years of age had significantly lower plasma β-cryptoxanthin ($P = 0.03$), β-carotene ($P = 0.01$) and total carotenoid ($P = 0.04$) concentrations than the older age group (data not shown).

Compliance to prescribed quantity of F&V intake

At baseline, the total number of habitually consumed F&V portions was similar in the HF (3.8 ± 0.3), LF (3.4 ± 0.3) and CT (3.9 ± 0.3) groups (mean ± SEM). A time ×

treatment interaction was observed ($P = 0.015$) for the number of portions of F&V consumed, as assessed by the 24 h dietary recalls. There was a significantly higher number of F&V portions consumed in the HF and LF group compared with the CT group at 6, 12 and 18 week ($P = 0.015$) with a dose-dependent increase in F&V portions in the HF and LF groups between visit 2–4 (Table 3).

HF and LF group participants were asked to record the number and types of the study F&V consumed daily. According to these daily record forms, both groups consumed more than the prescribed 2 extra portions of F&V at week 6 of the study (mean ± SEM, 2.6 ± 0.2 and 2.5 ± 0.2 additional F&V portions/day), achieved the prescribed 4 extra portions of F&V at 12 weeks (4.3 ± 0.2 and 4.0 ± 0.1 additional F&V portions/day) and less than the prescribed 6 extra portions of F&V at 18 weeks (5.7 ± 0.1 and 5.6 ± 0.2 additional F&V portions/day) for HF and LF, respectively. However, when the total F&V intake of the participants was examined (Table 4), it was apparent that participants were replacing between 1 and 2 portions of their habitually consumed F&V with the study F&V, particularly between 12 and 18 weeks when an additional 6 portions were required.

Table 2 Baseline characteristics mean ± SEM of participants randomised to each FLAVURS treatment group ($n = 174$)

	HF ($n = 58$)	LF ($n = 59$)	CT ($n = 57$)	P value
Males: Females	22:36	25:34	21:36	NS
Age (year)	50 ± 1	51 ± 1	52 ± 1	NS
Waist (cm)	93.4 ± 0.8	93.9 ± 0.7	92.3 ± 1.0	NS
Weight (kg)	78.4 ± 1.0	80.0 ± 1.0	77.2 ± 1.2	NS
BMI (kg/m^2)	27.6 ± 0.3	28.0 ± 0.3	27.3 ± 0.4	NS
Glucose (mmol/L)	5.6 ± 0.0	5.8 ± 0.1	5.5 ± 0.0	NS
Red wine (units) + tea (cups) consumed per week				
Low consumers (0–7 units + cups per week)	15	13	16	NS
Medium consumers (7–28 units + cups per week)	20	21	21	NS
High consumers (>28 units + cups per week)	20	20	20	NS
Number of smokers	8	8	6	NS

Participants were excluded if they had >21 units of alcohol per week

Table 3 An example of daily nutrient and phytochemical intakes provided by the fruits and vegetables (F&V) for participants in the intervention groups advised to consume an additional 2, 4 and 6 additional portions of HF and LF F&V daily

	2 Portions		4 Portions		6 Portions	
	LF	HF	LF	HF	LF	HF
Energy (kcal/day)	65	60	176	147	345	345
Energy (kJ/day)	273	251	739	617	1,450	1,450
Fat (g/day)	1	1	4	1	10	7
NSP (Engl) (g/day)	3	3	7	6	10	9
Total sugar (g/day)	9	11	21	27	39	51
Starch (g/day)	2	1	5	2	10	9
Vitamin C (mg/day)	50	52	93	127	138	194
Folate (μg/day)	50	52	94	104	158	157
Potassium (mg/day)	397	353	759	747	1,192	1,218
Carotene (μg/day)	1,149	1,161	2,991	2,923	4,131	3,810
Total flavonoids (mg/day)	3	49	6	121	7	198
Anthocyanidins (mg/day)	0	27	0	66	0	112
Flavan-3-ols (mg/day)	1	7	2	17	1	27
Flavones (mg/day)	1	0	1	1	1	1
Flavonols (mg/day)	2	7	3	19	4	33
Flavanones (mg/day)	0	8	0	19	0	23

Participants in the CT group increased their habitual F&V intake by approximately half a portion daily throughout the study period, as shown in Table 4.

Impact of the dietary intervention on macronutrient and micronutrient intakes

The macronutrient intakes of the HF, LF and CT group throughout the 18-week study period are shown in Table 4. There were no significant differences in the dietary intakes of all macronutrients between intervention groups at baseline. No changes in total energy or protein intake throughout the study period were observed. A dose-dependent increase in total sugar intake was observed for the two intervention groups, which reached significance after 6 weeks ($P = 0.0001$) for both HF (22 ± 1.3 %E) and LF (20 ± 1.8 %E) (Table 4). At 12 (24 ± 1.6 %E) and 18 (25 ± 1.4 %E) week, the HF group consumed a significantly higher amount of total sugars compared with the LF (21 ± 1.0; 22 ± 1.1 %E, respectively. $P = 0.008$) and CT group (20 ± 1.5; 21 ± 1.4 %E, respectively. $P = 0.0006$).

The non-starch polysaccharide (NSP) intake for the two intervention groups was significantly higher than the CT group throughout the study period (excluding baseline) (Table 4). The NSP intake of the CT group remained constant throughout the study (14.2 ± 0.6; 14.4 ± 0.6; 15.1 ± 0.9; 14.2 ± 0.7 g/day for 0, 6, 12 and 18 weeks), whereas the NSP intake for HF (13.8 ± 0.8; 16.2 ± 0.8; 16.0 ± 0.6; 17.1 ± 0.9 g/day) and LF (13.0 ± 0.7; 16.2 ± 0.7; 17.3 ± 0.8; 18.5 ± 0.8 g/day) groups was similar during the first 12 weeks, although at 18 weeks, the LF group consumed significantly higher levels of NSP compared to the HF group ($P = 0.04$) (Table 4).

Selected micronutrient intakes of the HF, LF and CT groups throughout the 18-week study period are shown in Table 5. While the vitamin C intake of the HF (96 ± 10 mg/day), LF (88 ± 11 mg/day) and CT (103 ± 9 mg/day) groups was similar at baseline, the levels in the HF (178 ± 13; 200 ± 13; 257 ± 19 mg/day) and LF (159 ± 13; 169 ± 13; 187 ± 10 mg/day) groups at 6, 12 and 18 weeks, respectively, were significantly greater than those in the CT group (treatment × time interaction = $P = 0.0001$) which did not change over the study period (Table 5). The vitamin C intake of the HF and LF groups was similar during the first 12 weeks, but at 18 weeks, the HF group consumed significantly more vitamin C ($P = 0.002$), as illustrated in Fig. 2a. The baseline folate intakes for the HF (283 ± 18 μg/day), LF (241 ± 12 μg/day) and CT (250 ± 12 μg/day) groups were similar. The HF and LF groups had similar folate intakes throughout the study period (Table 5), which were between 7.5 and 37.6 % greater than the control group after 6 weeks (HF 317 ± 17 μg/day; LF 278 ± 14 μg/day; CT 279 ± 14 μg/day), and remained significantly different from the control group until the end of the study ($P < 0.001$) (Table 5).

The baseline dietary intakes of potassium were similar in the HF (2,972 ± 121 mg/day), LF (2,875 ± 124 mg/day) and CT (2,879 ± 82 mg/day) groups but increased in all groups during the study (time effect only $P = 0.001$). The sodium intakes at baseline were similar in all groups (HF: 2,660 ± 232 mg/day, LF: 2,721 ± 173 mg/day, CT: 2,554 ± 139 mg/day). There were significant time ($P = 0.02$) and treatment effects ($P = 0.03$) for sodium intake, although no time × treatment interaction. The sodium intakes in the HF and LF groups were not significantly different, but the values were between 2.9 and 13.2 % lower than that of the control group throughout the study ($P = 0.01$) (Table 5).

Impact of intervention on dietary phytochemicals

The baseline dietary flavonoid intakes for the HF, LF and CT groups were similar at (mean ± SEM) 593 ± 71, 554 ± 69 and 549 ± 61 mg/day, respectively. Of these, it was estimated that for each group, respectively, 36 ± 5, 29 ± 5 and 39 ± 6 mg/day were derived from habitually consumed F&V and approximately 557 ± 70 , 525 ± 69 and 510 ± 59 mg/day originated from other dietary sources including tea, coffee and red wine. For the LF and CT groups, there was no significant change in total dietary flavonoid intake throughout the intervention period. In contrast, the total flavonoid intake in the HF group was significantly greater than the other groups at 12 weeks (628 ± 68 mg/day, $P = 0.007$) and 18 weeks (705 ± 67 mg/day, $P = 0.0001$). Within the HF group, total flavonoid intake significantly increased after 12-week ($P = 0.0001$) of the study (Table 5). When the contribution of dietary flavonoid intake from F&V was excluded, no difference in flavonoid intake from all other sources was observed within or between the HF, LF and CT group over the 18-week study period (Table 5). When considering intakes of flavonoids derived solely from F&V consumption, intakes in the LF and CT groups remained constant and were not significantly different from each other during the 18-week study period. In contrast, the flavonoid intake from F&V of the HF group increased significantly in a dose-dependent manner throughout the study, with values of 65, 94 and 141 mg/day at 6, 12 and 18 weeks, respectively ($P < 0.006$) (Fig. 3a).

The dietary carotenoid intake at baseline was similar for the HF (3.0 ± 0.4 μg/day), LF (2.5 ± 0.4 μg/day) and CT (2.9 ± 0.3 μg/day) groups, although in the HF and LF groups, significantly higher concentrations were observed compared with the CT group throughout the study ($P = 0.001$), with no significant difference between the two intervention groups (Table 5). There was a significant

Table 4 Summary of mean ± SEM daily macronutrient intake of the HF, LF and CT groups throughout the 18-week study period

Energy and nutrient intake per day	HF				LF			
	0 week	6 weeks	12 weeks	18 weeks	0 week	6 weeks	12 weeks	18 weeks
Energy (kcal)	1,817 ± 61	1,859 ± 88	1,789 ± 80	1,882 ± 90	1,808 ± 65	1,792 ± 71	1,789 ± 60	1,791 ± 69
Energy (MJ)	7.60 ± 0.25	7.78 ± 0.36	7.49 ± 0.33	7.87 ± 0.37	7.56 ± 0.27	7.50 ± 0.29	7.49 ± 0.25	7.49 ± 0.28
Protein (%E)	17 ± 0.8	15 ± 0.8	16 ± 0.7	16 ± 0.8	17 ± 0.9	17 ± 0.8	16 ± 0.7	16 ± 0.7
Starch (%E)	23 ± 1.0	22 ± 1.2	21 ± 1.4	18 ± 1.2	26 ± 1.3	23 ± 1.2	21 ± 1.1	22 ± 1.2
Sugars (%E)†	19 ± 1.2	22 ± 1.3^{a‡}	24 ± 1.6^{AB†}	25 ± 1.4^{AB‡}	18 ± 1.4	20 ± 1.8^{a‡}	21 ± 1.0^{A†}	22 ± 1.1^{A‡}
Fat (%E)	36 ± 1.9	38 ± 2.2	35 ± 2.0	34 ± 2.1	35 ± 1.4	36 ± 2.0	35 ± 1.9	35 ± 2.0
SFA (%E)	14 ± 0.8	15 ± 1.1	14 ± 1.0	12 ± 0.9	13 ± 0.7	13 ± 0.9	12 ± 0.7	12 ± 0.8
MUFA (%E)	12 ± 0.6	12 ± 0.7	12 ± 0.7	11 ± 0.7	11 ± 0.5	12 ± 0.8	12 ± 0.7	11 ± 0.8
PUFA (%E)	6 ± 0.4	6 ± 0.4	6 ± 0.4	6 ± 0.5	6 ± 0.3	6 ± 0.4	6 ± 0.5	6 ± 0.5
NSP (g)	13.8 ± 0.8	16.2 ± 0.8^{A†,a‡}	16.0 ± 0.6^{A‡}	17.1 ± 0.9^{AC‡}	13.0 ± 0.7	16.2 ± 0.7^{D†,a‡}	17.3 ± 0.8$^{D\ddagger,b+}$	18.5 ± 0.8^{CD‡}
F&V (portion)‡	3.8 ± 0.3	6.3 ± 0.4^{B+}	7.0 ± 0.3^{B+}	7.6 ± 0.4^{B+}	3.4 ± 0.3	5.8 ± 0.3^{D+}	6.8 ± 0.3^{D+}	8.1 ± 0.4^{D+}

Energy and nutrient intake per day	CT				*P* value
	0 week	6 weeks	12 weeks	18 weeks	Time × treatment
Energy (kcal)	1,844 ± 75	1,929 ± 70	1,859 ± 83	1,802 ± 71	NS
Energy (MJ)	7.72 ± 0.31	8.07 ± 0.29	7.78 ± 0.34	7.54 ± 0.29	NS
Protein (%E)	16 ± 0.7	16 ± 0.8	16 ± 0.8	16 ± 0.7	NS
Starch (%E)	27 ± 1.7	25 ± 1.1	25 ± 1.2	24 ± 1.4	NS
Sugars (%E)†	19 ± 1.2	20 ± 1.3	20 ± 1.5^{B†}	21 ± 1.4^{B‡}	0.02
Fat (%E)	34 ± 1.7	35 ± 1.8	36 ± 2.1	34 ± 1.9	NS
SFA (%E)	13 ± 0.8	14 ± 0.8	13 ± 0.9	12 ± 0.8	NS
MUFA (%E)	11 ± 0.6	12 ± 0.7	12 ± 0.8	12 ± 0.7	NS
PUFA (%E)	6 ± 0.4	5 ± 0.4	6 ± 0.4	6 ± 0.4	NS
NSP (g)	14.2 ± 0.6	14.4 ± 0.6^{AD†}	15.1 ± 0.9^{AD‡}	14.2 ± 0.7^{AD‡}	0.0001
F&V (portion)‡	3.9 ± 0.3	4.3 ± 0.3^{BD+}	4.6 ± 0.4^{BD+}	4.5 ± 0.3^{BD+}	0.015

HF high-flavonoid F&V group, *LF* low-flavonoid F&V group, *CT* control group, *NS* non-significant, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *NSP* non-starch polysaccharide, *F&V* fruits and vegetables, *%E %* of food energy, † refers to total sugars; ‡ F&V, no. of portions of fruits and vegetables calculated using the methodology of the National Diet and Nutrition Survey, 2010 [13]. Data are obtained from the dietary analysis of the 24 h dietary recalls (minimum 2 random recalls per time point)

$^{+}$ $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$. Superscript uppercase letters ($^{A–D}$) refer to inter-treatment comparisons: ^{A} HF significantly higher than LF; ^{B} HF significantly higher than CT; ^{C} LF significantly higher than HF; ^{D} LF significantly higher than CT. Superscript lowercase letters ($^{a–b}$) refer to intra-treatment comparisons across time: ^{a} significant difference from 0 to 6 weeks; ^{b} significant difference from 6 to 12 weeks

Table 5 Summary of mean ± SEM daily intake of selected micronutrients, carotenoids and flavonoids of the HF, LF and CT groups throughout the 18-week study period

Daily micronutrient intake	HF				LF			
	0 week	6 weeks	12 weeks	18 weeks	0 week	6 weeks	12 weeks	18 weeks
Vitamin C (mg/day)	96 ± 10	178 ± 13^{Ba‡}	200 ± 13^{B‡}	257 ± 19^{Ac‡,B†}	88 ± 11	159 ± 13^{4a‡}	169 ± 13^{D‡}	187 ± 10^{D‡}
Folate (μg/day)	283 ± 18	317 ± 17^{a†}	323 ± 14^{B†}	358 ± 17^{B†}	241 ± 12	278 ± 14^{a+}	322 ± 18^{D†}	300 ± 13^{D†}
Potassium (mg/day)	2,972 ± 121	3,128 ± 133	3,340 ± 114	3,503 ± 135	2,875 ± 124	3,108 ± 117	3,293 ± 123	3,317 ± 113
Sodium (mg/day)	2,660 ± 232	2,437 ± 140	2,168 ± 141	2,193 ± 161	2,721 ± 173	2,277 ± 109	2,292 ± 137	2,530 ± 157
Flavonoids F&V (mg/day)	36 ± 5	101 ± 10^{a‡}	130 ± 11^{b†}	177 ± 13^{c‡}	29 ± 5	32 ± 4	32 ± 4	30 ± 4
Flavonoids other (mg/day)	557 ± 70	566 ± 81	498 ± 66	519 ± 67	525 ± 69	503 ± 63	469 ± 58	528 ± 74
Total flavonoids (mg/day)	593 ± 71	653 ± 81	628 ± 68^{AB†,b‡}	705 ± 67^{AB‡}	554 ± 69	528 ± 62	504 ± 60^{A†}	567 ± 77^{A‡}
Anthocyanins (mg/day)	22 ± 4	56 ± 8	66 ± 8	91 ± 10	14 ± 4	14 ± 3	11 ± 2	18 ± 4
Flavan-3-ols (mg/day)	577 ± 71	619 ± 88	509 ± 66	532 ± 63	517 ± 69	487 ± 63	485 ± 58	532 ± 76
Flavones (mg/day)	3.1 ± 1.5	1.4 ± 0.2	1.8 ± 0.2	2.1 ± 0.2	1.1 ± 0.3	1.6 ± 0.2	1.4 ± 0.1	1.9 ± 0.3
Flavonols (mg/day)	29 ± 3	43 ± 3	42 ± 3	51 ± 3	26 ± 3	26 ± 2	28 ± 3	29 ± 3
Flavanones (mg/day)	10 ± 2	16 ± 2	25 ± 2	27 ± 3	10 ± 2	10 ± 2	10 ± 2	8 ± 2
Carotenes (mg/day)	3.0 ± 0.4	5.1 ± 0.6^{B†}	4.3 ± 0.4^{B†}	5.3 ± 0.5^{B†}	2.5 ± 0.4	4.3 ± 0.5^{D†}	5.9 ± 0.6^{D†}	6.3 ± 0.6^{D†}

Daily micronutrient intake	CT				*P* value
	0 week	6 weeks	12 weeks	18 weeks	Time × treatment
Vitamin C (mg/day)	103 ± 9	98 ± 9^{BD‡}	110 ± 12^{BD‡}	115 ± 10^{B†,D‡}	0.0001
Folate (μg/day)	250 ± 12	279 ± 14	264 ± 12^{BD†}	262 ± 13^{BD†}	0.035
Potassium (mg/day)	2,879 ± 82	3,106 ± 104	3,019 ± 124	3,036 ± 96	NS
Sodium (mg/day)	2,554 ± 139	2,616 ± 137	2,625 ± 193	2,328 ± 135	NS
Flavonoids F&V (mg/day)	39 ± 6	37 ± 5	49 ± 9	44 ± 5	0.0001
Flavonoids other (mg/day)	510 ± 59	523 ± 68	553 ± 72	498 ± 69	NS
Total flavonoids (mg/day)	549 ± 61	558 ± 67	574 ± 70^{B†}	539 ± 68^{B‡}	0.006
Anthocyanins (mg/day)	21 ± 4	16 ± 4	33 ± 9	22 ± 4	0.0006
Flavan-3-ols (mg/day)	522 ± 59	549 ± 66	552 ± 71	504 ± 68	NS
Flavones (mg/day)	1.2 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	0.0008
Flavonols (mg/day)	26 ± 2	26 ± 2	29 ± 3	28 ± 3	0.0001
Flavanones (mg/day)	11 ± 3	10 ± 2	9 ± 2	12 ± 2	0.0001
Carotenes (mg/day)	2.9 ± 0.3	2.5 ± 0.3^{BD†}	2.5 ± 0.3^{BD†}	3.0 ± 0.4^{BD†}	0.0001

HF high-flavonoid F&V group, *LF* low-flavonoid F & V group, *CT* control group, *NS* non-significant, *F&V* fruits and vegetables. Data are obtained from the dietary analysis of the 24 h dietary recalls (minimum 2 random recalls per time point)

$^{+}$ $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$. Superscript uppercase letters ($^{A–D}$) refer to inter-treatment comparisons: ^{A} HF significantly higher than LF; ^{B} HF significantly higher than CT; ^{C} LF significantly higher than HF; ^{D} LF significantly higher than CT. Superscript lowercase letters ($^{a–c}$) refer to intra-treatment comparisons across time: ^{a} significant difference from 0 to 6 weeks; ^{b} significant difference from 6 to 12 weeks; ^{c} significant difference from 12 to 18 weeks

interaction between carotenoid intake and age ($P = 0.0419$), with older participants consuming a significantly higher dietary intake than younger groups throughout the study period (data not shown).

Impact of the dietary intervention on plasma and urinary biomarkers of intake

There were no significant differences in the baseline excretion of total flavonoids in the HF (mean ± SEM: 92 ± 15 μg/day), LF (91 ± 21 μg/day) and CT (57 ± 12 μg/day) groups or in the excreted flavonoids concentrations throughout the study duration in the LF and CT groups (Table 6). In contrast, the HF group excreted significantly higher total flavonoids at 6 weeks (158 ± 25 μg/day), 12 weeks (211 ± 40 μg/day) and 18 weeks (236 ± 41 μg/day) compared with the LF and CT groups (time × treatment effect, $P = 0.0001$) (Table 6; Fig. 3b). There was a dose-dependent increase in total flavonoids excreted daily by the HF group such that the concentration excreted per day by the HF group was between 16 and 84 % greater than the LF and CT groups. It was also observed that those who drank ≤1 cup of tea or units of red wine daily had a significantly lower (between 5.3 and 78 %) urinary excretion of total flavonoids than those consuming ≥2 cups tea or units of red wine daily (data not shown). There was a

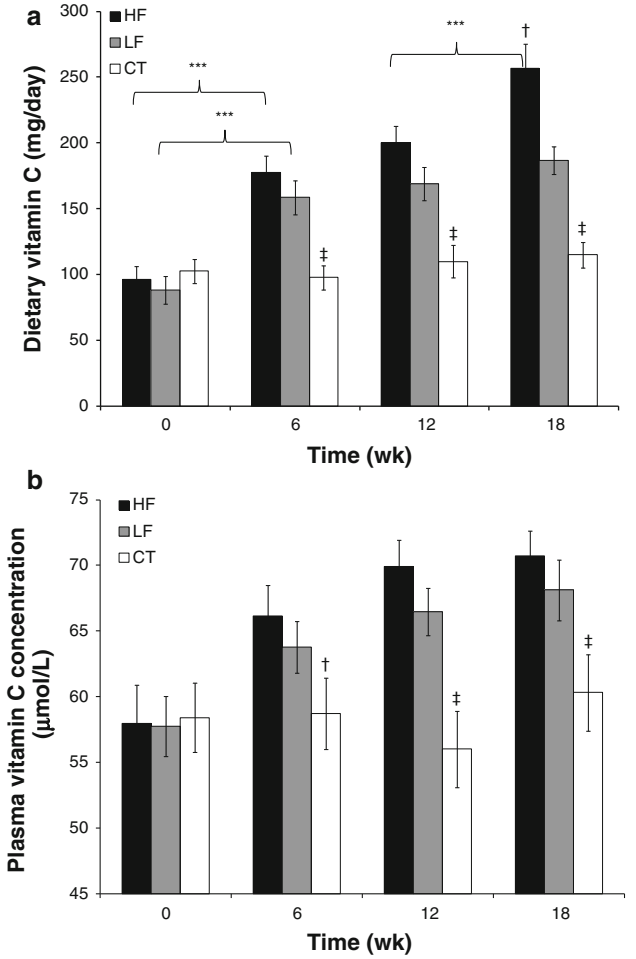

Fig. 2 Dietary vitamin C intakes (**A**) and plasma vitamin C concentrations (**B**) for the HF, LF and CT groups during the 18-week study period. Mean (±SEM). **a** A time × treatment interaction was observed ($P = 0.0001$). Intakes in LF and HF groups increased from baseline to 6 weeks (*** $P = 0.0001$), and in HF group from 12 to 18 weeks (***$P = 0.0001$). The CT group had lower intakes than the HF and LF groups at 6, 12 and 18 weeks ($^{\ddagger}P = 0.0001$). At 18 weeks, the HF group had higher vitamin C intakes compared to the LF and CT groups (†, $P = 0.002$). **b** A dose-dependent increase in the HF and LF groups was observed ($P = 0.0001$). The CT group had lower plasma vitamin C concentrations at 6, 12 and 18 weeks compared with the HF and LF groups($^{\dagger}P = 0.0019$; $^{\ddagger}P = 0.001$; ‡, $P = 0.001$, respectively)

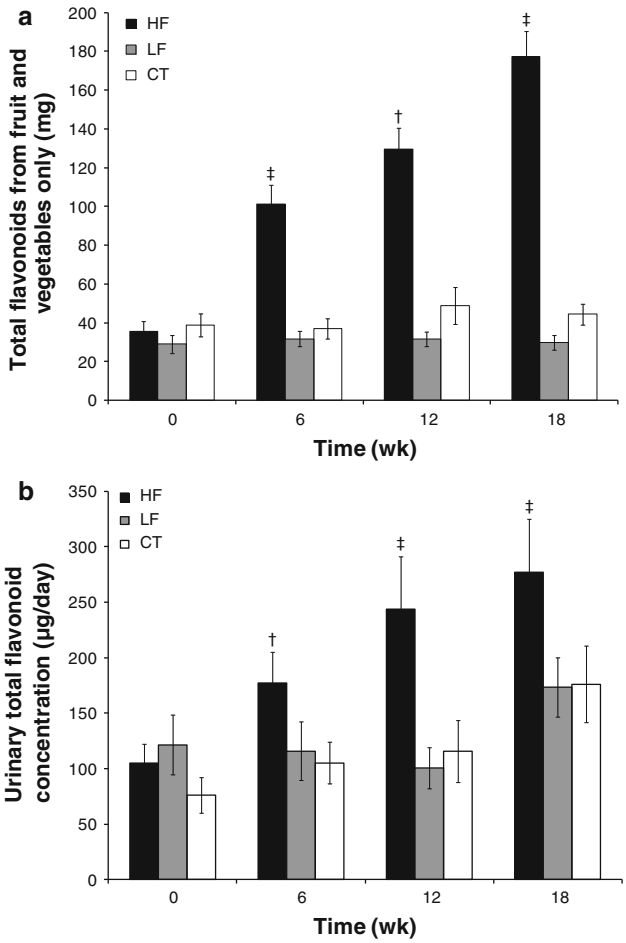

Fig. 3 Dietary flavonoid intakes from F&V (**A**) and total urinary flavonoid excretion (**B**) for the HF, LF and CT groups for the 18-week study period. Mean (±SEM). **a** A time × treatment interaction was observed ($P = 0.0001$). The HF group had a dose-dependent increase at 6 weeks (‡, $P = 0.0001$), 12 weeks (†, $P = 0.006$) and 18 weeks (‡, $P = 0.0002$). **b** A time × treatment interaction was observed ($P = 0.0001$) with a dose-dependent increase in total urinary flavonoid concentration in the HF group ($P = 0.0001$). The HF group also excreted significantly higher total flavonoids at 6, 12 and 18 weeks compared with the LF and CT groups (†, $P = 0.007$; ‡, $P = 0.001$; ‡, $P = 0.001$, respectively)

general increase in urinary excretion of kaempferol, luteolin, tamarexitin and naringenin in the HF group over time, which reached significance for naringenin ($P = 0.001$), where the HF group presented with between 13 and 75 % greater concentrations than the LF and CT groups (data not shown).

There was no significant effect of treatment on urinary potassium excretion (Table 5). There was, however, a significant time × treatment effect on urinary sodium excretion ($P = 0.03$), with a small but significant increase in the HF group from baseline (126 ± 6 mEq/day) to 6 weeks (130 ± 7 mEq/day ($P = 0.02$) and a significant reduction from baseline (122 ± 8 mEq/day) to 6 weeks (107 ± 7 mEq/day in the LF group ($P = 0.03$). The LF group had lower urinary sodium concentrations at 6, 12 and 18 weeks compared to baseline. There was no effect of treatment on the CT group (Table 6).

The baseline plasma vitamin C concentrations were similar in the HF (58.9 ± 2.9 μmol/L), LF (56.9 ± 2.2 μmol/L) and CT groups (58.4 ± 2.6 μmol/L). A significant time × treatment effect ($P = 0.0001$) was observed, with HF (66.3 ± 2.3; 70.3 ± 2.0; 70.6 ± 2.9 μmol/L) and LF groups (62.5 ± 2.0; 65.8 ± 1.7; 67.6 ± 2.3 μmol/L) exhibiting a sequential dose-related increase in plasma vitamin C at 6, 12 and 18 weeks, respectively. Whilst there was no significant difference between the HF and LF groups, their concentrations were significantly greater than the CT group ($P < 0.002$), and this difference remained stable throughout the intervention period (Fig. 2b; Table 6). The plasma uric acid concentrations were not significantly different between the HF, LF and CT group at baseline or throughout the study duration (Table 6). Baseline plasma folate concentrations were not significantly different between the HF (7.5 ± 0.8 μmol/L), LF (8.1 ± 0.8 μg/day) and CT (9.5 ± 0.8 μg/day) groups. There was a tendency for an increase from baseline to 12 weeks and a decrease thereafter in both the HF and LF groups, while values for the CT group remained relatively constant (Table 6).

There were no significant differences in the total (HF 2.36 ± 0.14 μmol/L; LF 2.43 ± 0.22 μmol/L; CT 2.07 ± 0.13 μmol/L) or individual plasma carotenoid concentrations between the intervention groups at baseline (Table 6). With the exception of plasma lutein, there was a significant time × treatment interaction in total plasma carotenoids ($P = 0.0001$) and the individual carotenoids: α-carotene ($P = 0.0001$), β-carotene ($P = 0.0001$), β-cryptoxanthin ($P = 0.016$) and lycopene ($P = 0.043$) (Table 6). Total plasma carotenoids in the HF and LF groups were significantly higher than CT after 12 weeks (HF 2.54 ± 0.15 μmol/L; LF 2.59 ± 0.16 μmol/L; CT 2.15 ± 0.15 μmol/L. $P = 0.0312$) and 18 weeks (HF 2.61 ± 0.17 μmol/L; LF 3.02 ± 0.21 μmol/L; CT 2.07 ± 0.13 μmol/L. $P = 0.0104$), with the LF group having significantly higher concentrations compared with the HF group on each occasion ($P < 0.002$). Plasma total carotenoids significantly increased in a dose-dependent manner in the LF group ($P = 0.001$) (Table 6). The HF and LF groups also had significantly higher plasma β-carotene concentrations than the CT group after 6 weeks (HF 0.80 ± 0.07 μmol/L; LF 0.86 ± 0.08 μmol/L; CT 0.63 ± 0.05 μmol/L. $P = 0.009$), 12 weeks (HF 0.93 ± 0.08 μmol/L; LF 0.96 ± 0.08 μmol/L; CT 0.71 ± 0.06 μmol/L. $P = 0.0100$) and 18 weeks (HF 0.98 ± 0.08 μmol/L; LF 1.22 ± 0.11 μmol/L; CT 0.73 ± 0.06 μmol/L) ($P = 0.0021$). Plasma β-carotene increased in a dose-dependent manner in the LF group ($P < 0.0001$). Plasma α-carotene was significantly higher in the LF group than HF and CT groups after 12 ($P < 0.0001$) and 18 weeks ($P < 0.0001$). Plasma lycopene was highest at 18 weeks, compared to 0, 6 and 12 weeks in the LF group ($P = 0.008$). The HF group had higher (between 9 and 87 %) β-cryptoxanthin concentrations compared with the LF and CT group, which reached significance after 12 ($P = 0.0151$) and 18 weeks ($P = 0.0011$). The HF group had higher β-cryptoxanthin concentrations than the LF group after 6 weeks ($P = 0.0128$) (Table 6).

The baseline FRAP values were not significantly different between groups at baseline (HF 476 ± 12 μmol Trolox Eq; LF 496 ± 12 μmol Trolox Eq; CT 472 ± 9 μmol Trolox Eq). There was a significant time × treatment interaction on plasma FRAP values ($P = 0.009$). In the HF group, plasma FRAP was significantly higher after 6 weeks (492 ± 11 μmol Trolox Eq) and 12 weeks (507 ± 12 μmol Trolox Eq) compared to baseline ($P = 0.001$), with a further significant increase after 18 weeks (523 ± 15 μmol Trolox Eq) ($P = 0.001$). In the LF group, plasma FRAP was significantly higher after 12 (514 ± 13 μmol Trolox Eq) and 18 weeks (513 ± 12 μmol Trolox Eq) compared to baseline and 6 weeks (504 ± 12 μmol Trolox Eq) ($P = 0.002$). The FRAP values in the CT group remained constant across the study (Table 6).

Impact of intervention on anthropometric measures

There were no significant changes in measures of body weight, body mass index (BMI) or markers of body fat mass over the study period between or within the HF, LF and CT group (data not shown).

Discussion

The aim of this paper was to describe and evaluate the dietary strategies employed in the FLAVURS RCT giving detailed results of the dietary, plasma and urinary nutrient

Table 6 Mean ± SEM of urinary (24 h) and plasma biomarkers of fruit and vegetable intake for HF LF and CT groups during the 18-week study period

Biomarkers F&V intake	HF				LF			
	0 week	6 weeks	12 weeks	18 weeks	0 week	6 weeks	12 weeks	18 weeks
Urinary biomarkers								
Total flavonoids (μg/day)	92 ± 15	158 ± 25^{ABa‡}	211 ± 40^{ABb‡}	236 ± 41^{ABc‡}	91 ± 21	92 ± 20^{A‡}	75 ± 14^{A‡}	135 ± 20^{A‡}
Potassium (mEq/day)	59 ± 4	66 ± 4	54 ± 4	78 ± 4	53 ± 4	54 ± 4	52 ± 4	66 ± 4
Sodium (mEq/day)	126 ± 6	130 ± 7^{a+}	98 ± 6^{a}	114 ± 6	122 ± 8	107 ± 7^{a+}	102 ± 6^{b}	114 ± 7^{c}
Plasma biomarkers								
Total carotenoids (μmol/L)	2.4 ± 0.1	2.6 ± 0.2	2.5 ± 0.2$^{B+,C†}$	2.6 ± 0.2$^{B+,C‡}$	2.4 ± 0.2	2.4 ± 0.2^{a}	2.6 ± 0.2^{CDb†}	3.0 ± 0.2^{CDc‡}
α-Carotene (μmol/L)	0.43 ± 0.03	0.43 ± 0.03	0.45 ± 0.03^{C‡}	0.47 ± 0.04^{C‡}	0.55 ± 0.10	0.53 ± 0.08	0.53 ± 0.05^{CD‡}	0.68 ± 0.06^{CDc‡}
β-Carotene (μmol/L)	0.80 ± 0.07	0.96 ± 0.09^{B+}	0.93 ± 0.07^{B+}	0.98 ± 0.08^{B+}	0.85 ± 0.11	0.86 ± 0.08^{D+}	0.96 ± 0.08^{Db+}	1.22 ± 0.11^{Dc+}
β-Cryptoxanthin (μmol/L)	0.22 ± 0.02	0.24 ± 0.02^{A+}	0.24 ± 0.02^{AB‡}	0.23 ± 0.01^{AB‡}	0.21 ± 0.02	0.21 ± 0.02^{A+}	0.21 ± 0.02^{A‡}	0.21 ± 0.02^{A‡}
Lutein (μmol/L)	0.35 ± 0.02	0.44 ± 0.03	0.45 ± 0.03	0.46 ± 0.03	0.33 ± 0.02	0.36 ± 0.02	0.37 ± 0.02	0.37 ± 0.02
Lycopene (μmol/L)	0.60 ± 0.04	0.58 ± 0.03	0.54 ± 0.03	0.55 ± 0.03	0.50 ± 0.03	0.49 ± 0.03	0.53 ± 0.03	0.56 ± 0.03^{c}
Folate (μmol/L)	7.5 ± 0.8	9.5 ± 1.1	10.2 ± 0.9	8.8 ± 0.9	8.1 ± 0.8	10.0 ± 0.9	10.1 ± 0.9	8.6 ± 0.8
Vitamin C (μmol/L)	58.9 ± 2.9	66.3 ± 2.3^{Ba†}	70.3 ± 2.0^{Bb†}	70.6 ± 1.9^{Bc†}	56.9 ± 2.2	62.5 ± 2.0^{Da†}	65.8 ± 1.7^{Db†}	67.6 ± 2.3^{Dc†}
Uric acid (μmol/L)	277 ± 8	272 ± 9	275 ± 9	279 ± 10	298 ± 10	296 ± 10	293 ± 11	298 ± 11
FRAP (μmol Trolox Eq)	476 ± 12	492 ± 11^{a†}	507 ± 12^{b†}	523 ± 15^{c†}	496 ± 12	504 ± 12	514 ± 13^{b†}	513 ± 12

Biomarkers F&V intake	CT				*P* value
	0 week	6 weeks	12 weeks	18 weeks	Time × treatment
Urinary biomarkers					
Total flavonoids (μg/day)	57 ± 12	74 ± 14^{B‡}	76 ± 19^{B‡}	135 ± 27^{B‡}	0.0001
Potassium (mEq/day)	51 ± 4	58 ± 4	56 ± 4	67 ± 4	NS
Sodium (mEq/day)	112 ± 7	118 ± 7	108 ± 6	107 ± 7	0.0338
Plasma biomarkers					
Total carotenoids (μmol/L)	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.2$^{B+,D†}$	2.2 ± 0.2$^{B+,D‡}$	0.0001
α-Carotene (μmol/L)	0.36 ± 0.03	0.34 ± 0.02	0.40 ± 0.03^{D‡}	0.42 ± 0.04^{4‡}	0.0001
β-Carotene (μmol/L)	0.67 ± 0.05	0.63 ± 0.05^{BD+}	0.71 ± 0.06^{BD+}	0.73 ± 0.06^{244+}	0.0001
β-Cryptoxanthin (μmol/L)	0.25 ± 0.03	0.24 ± 0.03	0.22 ± 0.03^{B‡}	0.21 ± 0.03^{1‡}	0.016
Lutein (μmol/L)	0.32 ± 0.02	0.32 ± 0.03	0.34 ± 0.02	0.33 ± 0.02	NS
Lycopene (μmol/L)	0.55 ± 0.04	0.53 ± 0.05	0.57 ± 0.05	0.56 ± 0.04	0.0043
Folate (μmol/L)	9.5 ± 0.8	10.1 ± 0.9	9.8 ± 0.8	9.2 ± 0.9	NS

Table 6 continued

Biomarkers F&V intake	CT				*P* value
	0 week	6 weeks	12 weeks	18 weeks	Time × treatment
Vitamin C (μmol/L)	58.4 ± 2.6	58.7 ± 2.7^{BD†}	56.0 ± 2.9^{BD†}	60.3 ± 2.9^{24†}	0.0001
Uric acid (μmol/L)	270 ± 8	269 ± 9	272 ± 9	276 ± 9	NS
FRAP (μmol Trolox Eq)	472 ± 9	477 ± 11	470 ± 12	484 ± 10	0.0093

HF high-flavonoid F&V group, *LF* low-flavonoid F & V group, *CT* control group, *NS* non-significant, *Eq* equivalent

$^{+}$ $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$. Superscript uppercase letters ($^{A–D}$) refer to inter-treatment comparisons: ^{A} HF significantly higher than LF; ^{B} HF significantly higher than CT; ^{C} LF significantly higher than HF; ^{D} LF significantly higher than CT. Superscript lowercase letters ($^{a–c}$) refer to intra-treatment comparisons across time: ^{a} significant difference from 0 to 6 weeks; ^{b} significant difference from 6 to 12 weeks; ^{c} significant difference from 12 to 18 weeks

biomarker concentrations. The results indicated that participants who completed the study in general showed good compliance to the dietary prescription, particularly up to 12 weeks. This demonstrated the successful application and tolerance of the novel FLAVURS model of F&V intervention. Compliance was also facilitated by the supply and delivery of specified F&V to the two intervention groups, the clear instructions on what to consume, how to prepare the F&V and recipe ideas, in addition to the comprehensive dietetic support offered throughout the study duration.

In general, participants were compliant in the first 6 weeks (target of 2 additional portions) and between the 6- and 12-week period (target of 4 additional portions). However, in the last 6 weeks of the study (target of 6 additional portions), participants appeared to struggle to fully comply. This was reflected in data from both the record forms and dietary recalls, but more so in the dietary recalls. Although the participants in the intervention groups were advised to add extra portions of study F&V to their habitual diet, when comparing the recalls and record forms, it was evident that some participants had replaced 1–2 habitual portions of F&V with the study foods, particularly at the later stages of the study. This has been observed in previous studies published by our group [37]. It was of note that participants in the LF group were generally able to achieve higher intakes of F&V than participants in the HF group at the highest level of advised intervention. This could be because the types of F&V in the LF list were more commonly consumed products (for example carrots, peas and broccoli) and, therefore, more acceptable and more easily incorporated into the participants' habitual diet. Although the individuals in the control group were generally compliant, despite the instructions not to change their dietary habits, it appeared that they also increased their consumption of F&V by a mean intake of half a portion/day for the study duration. This is not unexpected given that the participants were aware that they represented the control group in a F&V intervention trial.

The dietary intake data for NSP, vitamin C, folate and carotenoids supported a dose–response increase in F&V in the HF and LF groups, but not the CT group. The two intervention groups consumed similar levels for the study duration with the exception of NSP and vitamin C during the last 6 weeks of the study, where higher intakes were observed in the LF and HF groups, respectively. While there was limited information on individual dietary carotenoid intake, due to limitations in the nutrient database, plasma analysis was able to provide more data, revealing a near dose-dependent increase in plasma total carotenoids, α-carotene and β-carotene concentrations in both intervention groups, irrespective of the types of F&V consumed. These data are consistent with dose–response

increases in lutein and β-cryptoxanthin [38], α-carotene and β-carotene [39] and α-carotene, β-carotene, lycopene, lutein and zeaxanthin [37] following higher F&V intake in other human dietary intervention studies. The strength of the association invariably depends on the types of foods consumed, but it is encouraging that in FLAVURS, similar increases were observed in the two intervention groups that contained very diverse F&V.

Both dietary and plasma data showed a dose-dependent increase in vitamin C intake in the HF and LF groups. However, while the dietary data showed higher intakes by the HF group at 18 weeks compared to both the LF and CT groups, no difference in the plasma vitamin C concentrations was detected at 18 weeks between the intervention groups. Plasma vitamin C concentrations are representative of dietary intakes until a plasma threshold concentration of ~80 μmol/L, above which plasma vitamin C is lost in the urine [40]. In FLAVURS, estimated dietary vitamin C intakes of 257 mg/day reported in the HF group at 18 weeks would not manifest as a higher plasma vitamin C concentration as plasma saturation was reached at 12 weeks (4 additional portions of F&V) with an estimated dietary intake of 200 ± 13 mg/day. This observation supports previous data on dose-dependent increases in plasma vitamin C with F&V consumption [38, 41]. Thus, it appears that plasma vitamin C may be useful as a biomarker of F&V up to an intake of approximately 7 portions/day.

Higher F&V intake has been associated with increased potassium intake [37, 42–44]. In FLAVURS, the increases in dietary potassium in the HF and LF groups were quantitatively higher than in the CT group, but this did not reach statistical significance. Potassium intake is dependent on the particular foods chosen. In FLAVURS, the changes in urinary potassium concentrations were broadly similar to the dietary potassium changes, although in contrast to other studies, neither of the potassium concentrations mimics the increase in the intake of F&V [42, 44]. This probably reflects the choice of intervention F&V in the FLAVURS study, which were not chosen specifically for their high potassium content, in contrast to other studies [44]. A significant reduction in dietary sodium intake was also apparent from the dietary data, and lower urinary sodium excretion was detected in the HF and LF groups at some time points. This probably reflects an exchange of habitually consumed high-sodium foods with study F&V and is an additional potential benefit of an increased F&V intake (irrespective of flavonoid content) in relation to the dietary treatment of hypertension [42]. The higher sugar intake in the HF and LF groups at 12 and 18 weeks was possibly a reflection of the increased amounts of sugar-containing fruit. It was also observed that, despite the addition of up to 6 portions of F&V to the diets of those in the intervention groups, there was no significant increase in reported dietary energy intake. These data were supported by a lack of change in body weight, BMI, or measures of adiposity and reflect the substitution of habitually consumed foods with the intervention F&V.

F&V contain a number of components (including vitamin C, E, A, carotenoids, flavonoids) with moderate to high antioxidant capacity, and an increase in plasma antioxidant capacity and/or reduction in oxidative stress would be predicted following an increase in F&V consumption. This was confirmed in FLAVURS that reported a significant dose-dependent rise in plasma antioxidant potential (measured by FRAP) in both the HF and LF groups. This result was encouraging, as it supported the dietary and biomarker data for an increase in dietary antioxidant intake. Plasma uric acid is the major endogenous antioxidant and is present in excess quantities compared with dietary antioxidants [45]. Nevertheless, in FLAVURS, there was no significant impact of intervention on plasma uric acid, suggesting that the increase in FRAP was due, in part, to the increased F&V-derived nutrient and non-nutrient antioxidants. Thus, according to dietary and plasma/urinary biomarkers, the FLAVURS dietary intervention tool and specific dietary advice adequately addressed the large fundamental compositional differences in the nutrient content of the F&V consumed by the HF and LF groups, with close matching of these potentially bioactive components between the two intervention groups maintained throughout the study.

The successful relative matching of the LF and HF diets for other potential bioactive components was of paramount importance to the study outcome, as FLAVURS aimed to determine the impact of not only the dose of F&V, but also specific dose–response relationships between flavonoid intake and vascular function and numerous other established and putative CVD risk factors.

Results for the dietary intake of flavonoids from F&V and total flavonoids provide evidence of a marked and significant increase in flavonoid-rich F&V in the HF group reaching a mean (±SEM) intake of 177 ± 13 mg/day from approximately 7.6 ± 0.4 portions F&V at 18 weeks. In addition, there was a close matching of the study groups for background habitual flavonoid intake and no change in the consumption of flavonoid-rich foods or drinks (such as tea, coffee and red wine) in any of the dietary groups throughout the study period, except for that provided to the HF group. However, quantitatively (approximately 500 mg/day), the flavonoids from flavonoid-rich foods and drinks not included in the FLAVURS intervention model made a greater contribution to the total daily flavonoid intake than those from the additional F&V prescribed for the intervention. The background dietary flavonoids were derived predominantly from black tea and red wine

consumption, as was evident from the significantly higher total urinary flavonoid concentrations in the high and moderate black tea and red wine drinkers, irrespective of the designated dietary intervention group, and also the high levels of dietary flavan-3-ols consumed (Table 5). However, a large proportion of this 500 mg/day intake consisted of large polymeric polyphenols (e.g. thearubigins, theaflavins) generated from the oxidation of gallated flavanols during black tea production or flavanol–anthocyanin complexes formed during red wine maturation. Whilst these are polyphenols due to their size and structure, it is predicted that they would not be detected in blood and urine, primarily as they are not absorbed in the small intestine (due to their high molecular weight) [46].

The total urinary flavonoid concentrations in the 3 dietary groups showed a similar pattern to the dietary intake. Urinary concentrations of individual flavonoids have been shown to reflect acute ingestion of specific flavonoid-rich F&V [12, 47]. In FLAVURS, a combination of flavonoid-rich F&V was consumed, resulting in a large range of ingested flavonoids, but all in relatively small amounts in the 24 h urine collection sample, compared with samples from postprandial studies. This may account for the significant number of urine samples from all 3 dietary groups in which urinary flavonoids could not be detected. Those flavonoids that were identified in the 24 h urine samples (hesperetin, kaempferol, luteolin, myricetin, naringenin and tamarexitin) represented only a proportion of those consumed. Another contributing factor could be the rapid metabolism or low absorption of certain flavonoids. For example, no anthocyanidins or metabolites were detected in any of the urine samples, yet they contributed to an estimated 30–40 % of the total flavonoid intake from the HF F&V. This probably reflects the poor absorption of these compounds reported to be 0.036 ± 0.043 % of the ingested dose [48, 49]. In FLAVURS, total urinary flavonoid concentrations were a valid biomarker of flavonoid-rich F&V intake, yet more sensitive methods of detection would be required to reflect specific dietary flavonoid intakes.

In conclusion, application of the FLAVURS dietary intervention model successfully achieved increases in F&V in the HF and LF groups with a quantitative increase in flavonoid intake in the HF group only. This dietary tool could have wide applications for other intervention studies or in the context of increasing specific F&V intakes in population groups. The successful matching of other potentially bioactive components present in F&V will allow the specific impact of flavonoids on the CVD risk profile to be determined in subsequent analytical work.

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References

1. Bazzano LA, He J, Ogden LG, Loria CM, Whelton PK (2003) Dietary fiber intake and reduced risk of coronary heart disease in US men and women—The national health and nutrition examination survey I epidemiologic follow-up study. Arch Internal Med 163(16):1897–1904
2. Joshipura KJ, Hu FB, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Colditz G, Ascherio A, Rosner B, Spiegelman D, Willett WC (2001) The effect of fruit and vegetable intake on risk for coronary heart disease. Ann Internal Med 134(12):1106–1114
3. Law MR, Morris JK (1998) By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? Eur J Clin Nutr 52(8):549–556
4. WHO (2003) Food based dietary guidelines in the WHO European region, Eur/03/5045414. WHO, Copenhagen
5. Hughes DA (1999) Effects of carotenoids on human immune function. Proc Nutr Soc 58(3):713–718
6. Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH, Boeing H, Hofman A, Grobbee DE, Witteman JCM (1999) Dietary antioxidants and risk of myocardial infarction in the elderly: the Rotterdam Study. Am J Clin Nutr 69(2):261–266
7. Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R (2003) Olive oil and red wine antioxidant polyphenols inhibit endothelial activation - Antiatherogenic properties of Mediterranean diet phytochemicals. Arteriosclerosis Thrombosis Vasc Biol 23(4):622–629. doi:10.1161/01.atv.0000062884.69432.a0
8. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M (2006) (−)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci USA 103(4):1024–1029. doi:10.1073/pnas.0510168103

9. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D (1993) Dietary antioxidant flavonoids and risk of coronary heart-disease—the Zutphen elderly study. Lancet 342(8878): 1007–1011
10. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C (2008) Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. J Nutr 138(9):1671–1676
11. Egert S, Bosy-Westphal A, Seiberl J, Kurbitz C, Settler U, Plachta-Danielzik S, Wagner AE, Frank J, Schrezenmeir J, Rimbach G, Wolffram S, Muller MJ (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr 102(7):1065–1074. doi: 10.1017/s0007114509359127
12. Hubbard GP, Wolffram S, de Vos R, Bovy A, Gibbins JM, Lovegrove JA (2006) Ingestion of onion soup high in quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in man: a pilot study. Br J Nutr 96(3):482–488. doi:10.1079/bjn20061831
13. Bates B, Lennox A, Swan G (2010) National Diet and Nutrition Survey: headline results from year 1 of the Rolling Programme (2008/09). [Online]: Available from: http://www.food.gov.uk/science/dietarysurveys/ndnsdocuments/ndns0809year0801
14. Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB (1998) Prediction of coronary heart disease using risk factor categories. Circulation 97(18):1837–1847
15. Grundy SM, Balady GJ, Criqui MH, Fletcher G, Greenland P, Hiratzka LF, Houston-Miller N, Kris-Etherton P, Krumholz HM, LaRosa J, Ockene IS, Pearson TA, Reed J, Washington R, Smith SC Jr (1998) Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA task force on risk reduction. American heart association. Circulation 97(18):1876–1887
16. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB (1998) Prediction of coronary heart disease using risk factor categories. Circulation 97(18):1837–1847
17. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R (2002) Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 360(9349):1903–1913
18. Doll R, Peto R (1976) Mortality in relation to smoking: 20 years' observations on male British doctors. Br Med J 2(6051):1525–1536
19. Lean ME (2000) Pathophysiology of obesity. Proc Nutr Soc 59(3):331–336
20. Treasure T, MacRae KD (1998) Minimisation: the platinum standard for trials? Randomisation doesn't guarantee similarity of groups; minimisation does. Br Med J 317(7155):362–363
21. Pocock SJ, Simon R (1975) Sequential treatment assignment with balancing for prognostic factors in controlled clinical trial. Biometrics 31(1):103–115
22. USDA (2007) USDA Database for the Flavonoid Content of Selected Foods (Release 2.1). http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02-1.pdf
23. Nelson M, Atkinson M, Meyer J (1997) Food portion sizes: a photographic atlas. MAFF Publications
24. White BL, Howard LR, Prior RL (2011) Impact of different stages of juice processing on the anthocyanin, flavonol, and procyanidin contents of cranberries. J Agric Food Chem 59(9): 4692–4698. doi:10.1021/jf200149a
25. Lee SU, Lee JH, Choi SH, Lee JS, Ohnisi-Kameyama M, Kozukue N, Levin CE, Friedman M (2008) Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial onion products. J Agric Food Chem 56(18):8541–8548. doi:10.1021/jf801009p
26. Thurnham DI, Smith E, Flora PS (1988) Concurrent liquid-chromatographic assay of retinol, alpha-tocopherol, beta-carotene, alpha-carotene, lycopene and beta-cryptoxanthin in plasma, with tocopherol acetate as internal standard. Clin Chem 34(2): 377–381
27. Liau LS, Lee BL, New AL, Ong CN (1993) Determination of plasma ascorbic acid by high-performance liquid chromatography with ultraviolet and electrochemical detection. J Chromatogr Biomed Appl 612(1):63–70
28. O'Broin S, Kelleher B (1992) Microbiological assay on microtitre plates of folate in serum and red cells. J Clin Pathol 45(4): 344–347
29. Hald PM (1946) The flame photometer for the measurement of sodium and potassium in biological materials. J Clin Chem 163: 429
30. Nielsen SE, Sandstrom B (2003) Simultaneous determination of hydroxycinnamates and catechins in human urine samples by column switching liquid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry. J Chromatogr B 787(2):369–379
31. El Mohsen MA, Marks J, Kuhnle G, Moore K, Debnam E, Kaila Srai S, Rice-Evans C, Spencer JP (2006) Absorption, tissue distribution and excretion of pelargonidin and its metabolites following oral administration to rats. Br J Nutr 95(1):51–58
32. Benzie IFF, Strain JJ (1999) Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Oxid Antioxid Pt A 299:15–27
33. Bub A, Watzl B, Abrahamse L, Delincee H, Adam S, Wever J, Muller H, Rechkemmer G (2000) Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in men. J Nutr 130(9):2200–2206
34. Brown H, Prescott R (2006) Applied mixed models in medicine, 2nd edn. John Wiley & Sons, New York
35. Kenward M, Roger J (1997) Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53(3): 983
36. Smirnov N (1948) Table for estimating the goodness of fit of empirical distributions. Ann Math Stat 19(2):279–281
37. Paterson E, Gordon MH, Niwat C, George TW, Parr L, Waroonphan S, Lovegrove JA (2006) Supplementation with fruit and vegetable soups and beverages increases plasma carotenoid concentrations but does not alter markers of oxidative stress or cardiovascular risk factors. J Nutr 136(11):2849–2855
38. McCall DO, McGartland CP, McKinley MC, Sharpe P, McCance DR, Young IS, Woodside JV (2010) The effect of increased dietary fruit and vegetable consumption on endothelial activation, inflammation and oxidative stress in hypertensive volunteers. Nutrition, Metabolism and Cardiovascular Diseases [online] 1–7
39. Zino S, Skeaff M, Williams S, Mann J (1997) Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. Br Med J 314(7097):1787–1791
40. Levine M, ConryCantilena C, Wang YH, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR (1996) Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 93(8):3704–3709
41. Vioque J, Weinbrenner T, Asensio L, Castello A, Young IS, Fletcher A (2007) Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. Br J Nutr 97(5):977–986. doi:10.1017/s0007114507659017

42. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N (1997) A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 336(16):1117–1124
43. Troyer JL, Racine EF, Ngugi GW, McAuley WJ (2010) The effect of home-delivered Dietary Approach to Stop Hypertension (DASH) meals on the diets of older adults with cardiovascular disease. Am J Clin Nutr 91(5):1204–1212. doi:10.3945/ajcn.2009.28780
44. Berry SE, Mulla UZ, Chowienczyk PJ, Sanders TAB (2010) Increased potassium intake from fruit and vegetables or supplements does not lower blood pressure or improve vascular function in UK men and women with early hypertension: a randomised controlled trial. Br J Nutr 104:1839–1847
45. Wayner D, Burton G, Ingold K, Barclay L, Locke S (1987) The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical trapping antioxidant activity of human blood plasma. Biochim Biophys Acta 924:408–419
46. Mulder TP, Rietveld AG, van Amelsvoort JM (2005) Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. Am J Clin Nutr 81(1 Suppl):256S–260S
47. Vauzour D, Houseman EJ, George TW, Corona G, Garnotel R, Jackson KG, Sellier C, Gillery P, Kennedy OB, Lovegrove JA, Spencer JPE (2010) Moderate Champagne consumption promotes an acute improvement in acute endothelial-independent vascular function in healthy human volunteers. Br J Nutr 103(8):1168–1178. doi:10.1017/s0007114509992959
48. Jin Y, Alimbetov D, George T, Gordon MH, Lovegrove JA (2011) A randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects. Eur J Clin Nutr 65(7):849–856. doi:10.1038/ejcn.2011.55
49. Hollands W, Brett GM, Radreau P, Saha S, Teucher B, Bennett RN, Kroon PA (2008) Processing blackcurrants dramatically reduces the content and does not enhance the urinary yield of anthocyanins in human subjects. Food Chem 108(3):869–878. doi:10.1016/j.foodchem.2007.11.052