Northumbria Research Link

Effects of acute consumption of a fruit and vegetable purée-based drink on vasodilation and oxidative status

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Abstract

Epidemiological studies indicate that diets rich in fruits and vegetables (F&V) are protective against CVD. Puréed F&V products retain many beneficial components, including flavonoids, carotenoids, vitamin C and dietary fibres. The present study aimed to establish the physiological effects of acute ingestion of a F&V purée-based drink (FVPD) on vasodilation, antioxidant status, phytochemical bioavailability and other CVD risk factors. A total of twenty-four subjects, aged 30–70 years, completed the randomised, single-blind, controlled, crossover test meal study. Subjects consumed 400 ml of the FVPD, or a fruit-flavoured sugar-matched control, after following a low-flavonoid diet for 5 d. Blood and urine samples were collected throughout the study day, and vascular reactivity was assessed at 90 min intervals using laser Doppler iontophoresis. The FVPD significantly increased plasma vitamin C ($P=0.002$) and total nitrate/nitrite ($P=0.001$) concentrations. There was a near significant time by treatment effect on *ex vivo* LDL oxidation ($P=0.068$), with a longer lag phase after consuming the FVPD. During the 6 h after juice consumption, the antioxidant capacity of plasma increased significantly ($P=0.003$) and there was a simultaneous increase in plasma and urinary phenolic metabolites ($P<0.05$). There were significantly lower glucose and insulin peaks after ingestion of the FVPD compared with control ($P=0.019$ and 0.003) and a trend towards increased endothelium-dependent vasodilation following FVPD consumption ($P=0.061$). Overall, FVPD consumption significantly increased plasma vitamin C and total nitrate/nitrite concentrations, with a trend towards increased endothelium-dependent vasodilation. Puréed F&V products are useful vehicles for increasing micronutrient status, plasma antioxidant capacity and *in vivo* NO generation, which may contribute to CVD risk reduction.

Key words: Juice: Flavonoids: Vascular reactivity: CVD

Many epidemiological studies have shown that a high consumption of fruits and vegetables is associated with a reduced risk of CVD and other chronic diseases[1–3]. CVD is responsible for about half (48%) of all deaths annually in Europe and is the major cause of death in men and women in the UK[4]. Endothelial dysfunction is a central feature in the early development of CVD, and impairment of endothelium-dependent vasodilation has been shown to precede structural atherosclerotic lesions[5]. The vascular endothelium displays systemic characteristics, which enables non-invasive measurements of peripheral circulation to be used as an indicator of the condition of the coronary arteries[6]. Laser Doppler imaging coupled with iontophoresis (LDI) is one such method of investigating the vasodilation of the peripheral microvasculature by delivering vasodilator agents across the skin under the influence of an applied electrical field[7]. Acetylcholine is administered at the anode and tests endothelial function via its vasodilator action of binding to muscarinic receptors on endothelial cells, subsequently generating NO via endothelial NO synthase (eNOS), thereby acting as an 'endothelium-dependent' vasodilator. A NO donor, sodium nitroprusside, is administered at the cathode and acts as an 'endothelium-independent' vasodilator, which acts as a control to test the integrity of vascular smooth muscle. Many fruits, vegetables and their juices contain polyphenolic flavonoid compounds[8,9] that have been shown to increase eNOS activity in cell and animal studies at physiological concentrations[10–15]. In addition, there is a growing body of evidence that supports the beneficial impact of chronic fruit and vegetable consumption[16–18] and isolated flavonoids[19,20] on

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Abbreviations: eNOS, endothelial NO synthase; FRAP, ferric-reducing antioxidant power; FVPD, fruit and vegetable purée-based drink; LDI, laser Doppler iontophoresis.

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improvements in measures of vascular function. However, the acute *in vivo* vascular response to fruit and vegetable consumption in human subjects is very limited[21] and has not been adequately assessed.

Oxidation of LDL has been recognised as an early stage in the development of atherosclerosis leading to CVD[22−25]. Many studies have reported the antioxidant effects of phytochemicals in fruits and vegetables, which include the retardation of the susceptibility of LDL to oxidation *in vitro* and *in vivo*[22−24,26]. Plant foods contain many components that could contribute to significant health benefits, including vitamins, fibre, carotenoids, sulphur compounds, nitrate and organic acids, as well as a wide variety of phenolic phytochemicals[26−29]. Compounds in the latter class may contribute to benefits by reducing oxidative stress[27], by regulating enzymes involved in phase 2 metabolism, as well as by other mechanisms. The beneficial components listed earlier are also retained in many fruit and vegetable juices and purée products[30−32]. Research has shown that fruit and vegetable juices also have a protective effect against CVD risk[17,33]. In the UK, the average adult consumes less than the recommended minimum of five 80 g portions of fruits and vegetables per d[34], but there has been a continued increase in fruit juice and liquidised/puréed product consumption in the UK[35], and these products represent an important source of micronutrients. The aim of the present study was to investigate the acute effects of the consumption of a concentrated fruit and vegetable purée-based drink on vascular function, antioxidant status and plasma concentrations of beneficial phytochemicals and vitamin C, which could contribute to a reduction in the risk of chronic diseases, such as CVD.

Experimental methods

Study population

A total of twenty-four subjects (twenty males and four females), aged between 30 and 70 years, completed the study. The subjects were recruited from the University of Reading and general public around the Reading area, and were selected if they met the study criteria of no known liver disease, diabetes mellitus or a diagnosed mycocardial infarction; no gall bladder problems or abnormalities of fat metabolism; no subjects on weight-reducing diets or taking dietary supplements; no vigorous exercise or excess consumption of alcohol; BMI $<30 \text{ kg/m}^2$; blood pressure $<150/90 \text{ mmHg}$ and Hb $>125 \text{ g/l}$. All study subjects had basic anthropometric measurements recorded, and fasting blood was taken and analysed for liver function status, lipid levels, fasting glucose and a measure of potential alcohol abuse. A total of twenty-five subjects were required to be recruited to detect a 10 % difference in acetylcholine vasodilatory response measured by LDI based on the present data for intra-subject reproducibility response (within-day and between-day variation, both 12 %), with $P<0.05$, 80 % power and an allowance for a 10 % drop-out. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were assessed by the University of Reading Research Ethics Committee who gave a favourable ethical opinion for conduct. Each subject gave written informed consent before participating. The registration number for clinical trials is ISRCTN36287115.

Study design

The study was a randomised, single-blind, controlled, crossover, acute postprandial test meal study. The subjects were randomly assigned to either the fruit and vegetable purée-based drink (FVPD) or the control drink. The subjects consumed a low-flavonoid diet for a 5 d period preceding the study day. On the study day, a flexible cannula was inserted into the forearm and blood samples taken at baseline and at twelve additional time points after consumption of the relevant drink, eight samples 30 min apart followed by four samples 1 h apart. Urine was collected before the drink was consumed and then at 2 h intervals for 8 h of the study day. LDI was used to record a real-time measure of vascular reactivity for two baseline measurements and five measurements following drink consumption, all at 90 min intervals. The whole procedure was repeated with the other intervention drink after a 4-week washout.

Intervention drinks

Subjects were asked to consume 400 ml FVPD (Vie Shots; Unilever Bestfoods) or 400 ml fruit-flavoured cordial (Robinsons Lemon Barley Water; Robinsons Limited), which was matched for sugar composition and diluted with low-nitrate mineral water (The Buxton Mineral Water Company Limited). The FVPD was a fruit and vegetable drink made from 800 g fruit and vegetables in the form of concentrated juices and purées, which was composed of apple (56 %), carrot (29 %) and strawberry (8 %). The nutrient composition of the drinks is shown in Table 1.

Fruit and vegetable purée-based drink antioxidant components

FVPD extracts were prepared by extraction with acidified methanol[36]. Total phenolic compounds were determined by the Folin−Ciocalteu method[37], and flavonoid content and composition were determined by HPLC[24]. The polyphenol content of the FVPD was determined by the method of Garcia-Macias *et al.*[38]. Aqueous antioxidant activity was assessed by the oxygen radical absorbance capacity method[39]. A volume of FVPD extract containing 0.5 μM-gallic acid equivalents, determined by the Folin−Ciocalteu method, was incubated with isolated LDL containing 100 μg protein in the assay.

Anthropometric measurements

Measurements of height, weight, BMI and blood pressure were recorded at the start of each of the two study days.

Table 1. Nutrient composition of fruit and vegetable purée-based drink (FVPD) and control drink*

	FVPD (400 ml)	Control (400 ml)
Energy		
kcal	252	203
kJ	1054	849
Protein (g)	4·0	0·3
Carbohydrate (g)	54·4	50·4
Of which sugar (g)	50·4	50·4
Fat (g)	2·0	Trace
Of which saturates (g)	0·4	Trace
Fibre (g)	6·0	–
Na (g)	0·12	–
Ascorbic acid (mg)	120	–
Nitrate/nitrite (mg)	1·0	0·0
Total carotenoids (mg)	20·4	–
Total phenolics (mg GAE)	768	44
Caffeic acid (mg)	60	–
Ferulic acid (mg)	16	–
Epicatechin (mg)	457	–
Chlorogenic acid (mg)	31	–
Cyanidin (mg)	53	–
Pelargonidin-3-glucoside (mg)	37	–
Pelargonidin (mg)	11	–

GAE, gallic acid equivalents.
* Adapted from George et al.[65]

Plasma collection and analysis

Blood samples were collected via syringe from a cannula and transferred to citrate, EDTA and lithium heparin vacutainer tubes. The tubes were immediately wrapped in foil and kept on ice for transport to the laboratory. Following centrifugation at 4°C at 3000 rpm, plasma was aliquotted into separate cryogenic vials for storage at −80°C. Analysis did not commence until the intervention study was completed, and all samples from each subject were analysed within one batch to reduce inter-batch variation.

Analyses of plasma TAG, total and HDL-cholesterol, NEFA and glucose were performed using an Instrument Laboratory ILAB 600 autoanalyser and standard kits (Instrumental Laboratories Limited). Appropriate sero-normal, low and high quality control standards (Instrument Laboratories Limited) were included in all batches. Insulin was assessed by ELISA (Dako Cytomation; Ely) with in-house pooled plasma controls in each batch.

Plasma samples for ascorbic and uric acid analysis were treated with 5% metaphosphoric acid (1:1, v/v) and stored at −80°C prior to analysis by HPLC with UV detection[40,41]. Total nitrate/nitrite was measured using an ELISA kit (Active Motif; Rixensart) based on the Greiss reaction[42] in plasma, FVPD and the control drink.

The ferric-reducing antioxidant power (FRAP) of plasma was determined using the method of Benzie & Strain[43], adapted for use with ninety-six-well microtitre plates[44]. A Genios spectrophotometer (Tecan Limited) was used to measure the absorbance at 593 nm. This method was used to estimate aqueous antioxidants. The method for LDL isolation and assessment of oxidative stability was based on that of Leigh-Firbank et al.[45]. Concentrations of 50 μg of LDL protein/ml and 5 μM-$CuSO_4$ were used for oxidation at pH

7·4. The formation of conjugated dienes (the breakdown products of lipid peroxidation) was monitored at 234 nm, 37°C every 2 min for 3 h using a Perkin-Elmer Lambda bio 20 UV/VIS Spectrometer[46].

Plasma (300 μl) was extracted four times with 0·5 ml of ethyl acetate by vortexing for 1 min after addition of 100 μl of 5 M-HCl. The mixture was then centrifuged at 13 200 rpm for 10 min, and the top layer removed. The ethyl acetate extracts were dried and derivatised by the addition of 200 μl of N,O-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane and heated at 70°C for 4 h[47]. An Agilent Technologies Model GC 6890N (G1530N) gas chromatograph coupled with an Agilent 5975 series Inert XL mass selective detector and a CTC analysis system autosampler were used for analyses. Samples (2·0 μl) were analysed on a 30 m, 0·25 mm inner diameter, 0·50 μm, DB-5-fused silica capillary column (Agilent Technologies), with temperature programming from 80°C (1 min) increasing up to 220°C at a rate of 10°C/min and from 220 to 310°C at a rate of 10°C/min and held for 6 min. Electron-impact mass spectra were recorded with ionisation energy of 70 eV. o-Phthalic acid was used as an internal standard.

Urinary creatinine, hippuric and phenolic acids

Analysis of urinary creatinine was performed using an Instrument Laboratory ILAB 600 autoanalyser. Urinary hippuric acid was measured as an indicator of polyphenol metabolism[48] and determined by the extraction method of Mohsen et al.[49] and analysed by HPLC by adapting the methods of Felgines et al.[50] and Kay et al.[51].

Laser Doppler imaging with iontophoresis

Subjects were in a supine position, in a quiet room at an ambient temperature of 22 ± 1°C for all measures. Two ION6 Perspex chambers (Moor Instruments Limited) with an internal platinum wire electrode were placed on the volar aspect of the forearm and attached to the skin using adhesive discs (MIC-1AD; Moor Instruments Limited) and connected to a MIC2 iontophoresis controller (Moor Instruments Limited). Skin temperature was recorded at the time of measurement. Acetylcholine chloride (2·5 ml, 1%; Sigma-Aldrich) in 0·5% NaCl solution was placed in the anodal chamber and 2·5 ml of 1% sodium nitroprusside (Sigma-Aldrich) in 0·5% NaCl solution was placed in the cathodal chamber. Circular glass coverslips were placed over each chamber to prevent loss of solutions. Current delivery was controlled by laser Doppler imager Windows software 5.1 (Moor Instruments Limited). Measurement of skin perfusion was carried out using a moorLDI2-IR laser Doppler imager (Moor Instruments Limited). The scanner head was positioned 30 cm above the chambers. The laser light was directed by a moving mirror in a raster fashion over both chambers. A total of ten repeat scans were taken, the first with no current to act as a control, then two scans at 10 μA, two at 20 μA, two at 30 μA and one at 40 μA to give a total charge of 8 mC, the final two scans were measured without any current. The area under the flux

Table 2. Baseline characteristics of subjects in acute fruit and vegetable purée-based drink consumption study

(Mean values and standard deviations)

	Males (n 20)		Females (n 4)	
	Mean	SD	Mean	SD
Age (years)	46	11	49	4
Weight (kg)	79·9	10·4	66·7	7·9
BMI (kg/m^2)	25·6	3·2	24·0	2·6
Systolic BP (mmHg)	125	12	106	12
Diastolic BP (mmHg)	79	7	71	7
Glucose (mmol/l)	5·0	0·3	4·9	0·1
Total cholesterol (mmol/l)	5·3	0·9	4·5	0·8
TAG (mmol/l)	1·4	0·9	0·8	0·2

BP, blood pressure.

v. time curve over the ten scans was deemed to indicate the microvascular response.

Statistical analyses

All statistical analyses were performed using SPSS 13.0 for Microsoft Windows (SPSS Inc.). The data were checked for normality using the Shapiro–Wilk test, as the number of subjects was less than fifty. Those data that were not normally-distributed were log transformed and reassessed. A repeated-measures ANOVA was used to detect significant differences between treatment groups with Bonferroni correction to reduce the likelihood of chance findings from multiple comparisons. A value of $P \leq 0.050$ was used to define significance and a 95% CI. The data presented in tables and graphs are displayed as means with their standard errors, unless otherwise stated.

Results

Antioxidant components of fruit and vegetable purée-based drink

The antioxidant components of the FVPD are listed in Table 1. The FVPD contained a high concentration of flavonoids, with 400 ml of the FVPD supplying 768 mg of total phenolics (gallic acid equivalents) including 100 mg of anthocyanins. The control drink contained only 44 mg of total phenolics (gallic acid equivalents) with no anthocyanins. The FVPD had a high antioxidant capacity when assessed by the oxygen radical absorbance capacity assay (6702 μM Trolox equivalents).

Anthropometric data

The baseline anthropometric data for the subjects are shown in Table 2. There was no change in weight, BMI or blood pressure between study days (data not shown).

Biochemical data

There was a significant time by treatment effect ($P<0.001$) and a significant increase in plasma ascorbic acid concentration following FVPD consumption at all time points from 60 min

Table 3. Plasma concentration of uric acid, glucose and insulin following acute consumption of fruit and vegetable purée-based drink (FVPD) or control

(Mean values with their standard errors, n 24)

Plasma analyte	Treatment	0		30		60		90		120		150		180		210		240		300		360		420		480		P (time × treatment)
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Vitamin C (μmol/l)	FVPD	58·7	3·4	65·1*	3·4	71·0*	3·4	75·0*	3·4	75·5*	3·6	77·1*	3·8	77·7*	3·5	74·2*	3·5	72·2*	3·9	71·4*	3·5	71·9*	3·5	68·8*	2·9	68·9*	4·0	0·001
	Control	57·5	4·1	58·1	4·5	60·1	3·9	59·0	3·9	56·4	4·0	52·0	3·5	55·9	3·5	57·3	4·3	57·3	4·7	55·0	4·1	56·8	3·5	54·5	4·6	53·8	4·8	
Uric acid (μmol/l)	FVPD	272	15	273	15	268	13	264	14	256	14	256	14	253	13	252	13	248	16	250	16	245	14	239	14	253	15	NS
	Control	257	14	258	14	258	13	258	14	258	13	248	12	244	11	248	11	254	14	253	14	250	12	232	11	251	10	
Glucose (mmol/l)	FVPD	5·5	0·1	7·7*	0·2	7·2	0·4	6·0	0·3	5·4	0·2	5·2	0·2	5·1	0·1	5·1	0·1	5·3	0·1	5·3	0·1	5·3	0·1	5·4	0·2	5·4	0·1	0·019
	Control	5·6	0·1	8·7	0·3	6·7	0·4	5·5	0·2	5·0	0·1	4·9	0·1	5·0	0·1	5·1	0·1	5·2	0·1	5·2	0·1	5·4	0·1	5·4	0·1	5·3	0·1	
Insulin (pmol/l)	FVPD	26·4	5·0	163·2*	28·7	156·5	21·3	99·2	13·3	52·5	6·9	33·1	4·9	22·7	4·9	17·1	2·4	22·2	6·7	15·4	2·7	15·1	2·7	17·6	2·9	18·5	2·9	0·003
	Control	22·4	4·3	229·8	30·3	150·0	19·7	63·0	9·4	34·6	4·6	17·9	2·6	16·8	2·2	21·9	2·2	16·0	1·9	14·7	2·2	15·7	2·2	17·6	2·0	17·3	3·2	

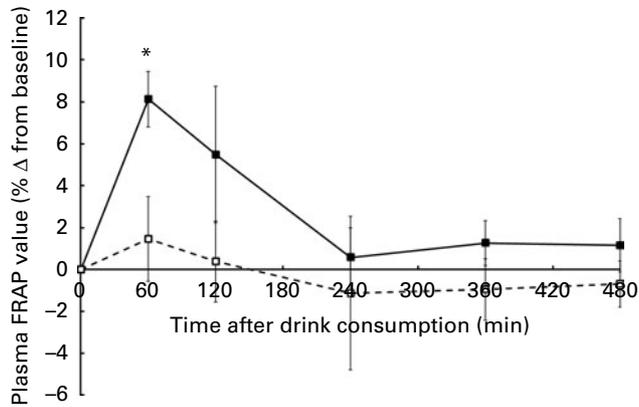* Mean values were significantly different from control (P<0·01).

Fig. 1. Ferric-reducing antioxidant potential (FRAP) of plasma (percentage change from baseline) following acute consumption of fruit and vegetable purée-based drink (FVPD) or control (*n* 24). The solid line represents the effect from FVPD consumption and the dashed line represents the effect from the control. There was a significant time by treatment effect (*P*=0·03). * Mean values were significantly different for effect between treatments after *post hoc* tests.

after ingestion, reaching a maximum value approximately 120 min after consumption. The plasma uric acid concentration did not change significantly (Table 3). There was a significant increase in oxidative stability determined by the FRAP assay after FVPD consumption, as shown in Fig. 1 (time by treatment, *P*=0·030). The peak FRAP response occurred 60 min after FVPD consumption (*P*=0·003). It was observed that there was a near significant attenuation in the reduction in the postprandial LDL oxidation lag phase response (time by treatment, *P*=0·068) after the FVPD compared with the control drink, with a significant difference in LDL oxidation lag phase between treatments at 60 min after FVPD consumption (*P*=0·016; Fig. 2). There was no correlation between the concentration of either plasma vitamin C or uric acid with plasma antioxidant capacity assessed by the FRAP and LDL oxidation assays (data not shown).

There was a significant time by treatment interaction (*P*=0·03) in the plasma concentration of salicylic acid, with a significant increase from 1 to 5 h after FVPD consumption (Table 4, *P*<0·0001). Plasma benzoic acid concentration tended to increase at 2 h after FVPD consumption and almost reached statistical significance at 6 h (Table 4, *P*=0·07). The benzoic acid concentration in the FVPD group remained higher than observed after consumption of the control drink throughout the intervention period. Plasma *p*-hydroxyphenylacetic acid and 3-(4-hydroxy phenyl)-2-hydroxypropanoic acid concentrations remained higher than the response to the control drink throughout the 8 h intervention period (Table 4). Both compounds peaked 90 min after FVPD consumption, but only *p*-hydroxyphenylacetic acid reached a maximum that was significantly higher than the control (Table 4, *P*=0·01). After consuming the FVPD, the urinary concentration of vanillic acid, *p*-hydroxybenzoic acid and ferulic acid remained higher than the control treatment throughout the intervention period (Fig. 3), the mean peak concentration occurred after 4 h for vanillic and *p*-hydroxybenzoic acid (*P*<0·001 and 0·04, respectively) as

shown in Fig. 3(a) and (b). Ferulic acid was only found in urine after subjects consumed the FVPD, and the concentration peaked after 2 h and remained constant until 6 h before reducing. There was a highly significant time by treatment effect (*P*<0·0001) in the plasma concentration of hippuric acid, with a significant increase from 3 to 8 h after FVPD consumption only (Table 4). There was also a significant time by treatment effect (*P*=0·008) in the urinary excretion of hippuric acid, with a significant increase at 360 and 480 min after FVPD consumption (*P*=0·0001 and 0·010, respectively) as shown in Fig. 3(d).

A significant time by treatment effect was observed for the glucose and insulin response (Table 3, *P*=0·019 and 0·003, respectively), with a significantly lower peak concentration after consumption of the FVPD compared to the sugar-matched control (*P*=0·004 and 0·029, respectively). There was no effect of FVPD consumption on plasma TAG, total or HDL-cholesterol or NEFA (data not shown). There was a significant time by treatment interaction (*P*<0·001) and a significant increase in total plasma nitrate/nitrite concentration following FVPD consumption at all time points after ingestion (Fig. 4).

Laser Doppler imaging with iontophoresis

The endothelium-dependent vasodilation response to acetylcholine is shown in Fig. 5. There was a trend towards increased vasodilation following FVPD consumption throughout the day, which almost reached statistical significance (*P*=0·061).

Discussion

The present study is the first to investigate the effects of an acute ingestion of fruits and vegetables, in the form of 400 ml of a purée-based drink, on vascular reactivity and plasma and urine phytochemical composition. The FVPD contained significant levels of vitamin C and polyphenols
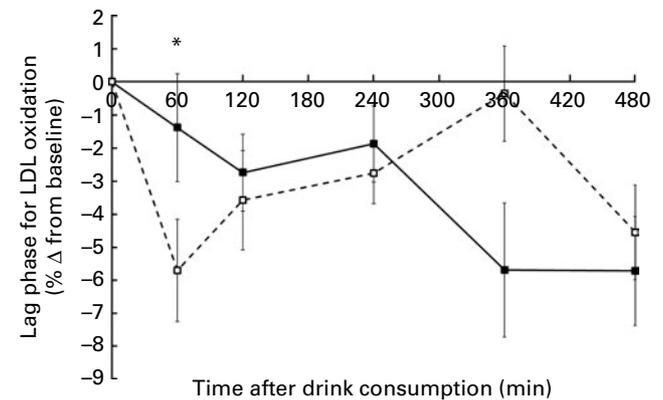

Fig. 2. LDL lag phase time (percentage change from baseline) following acute consumption of fruit and vegetable purée-based drink (FVPD) or control (*n* 24). The solid line represents the effect from FVPD consumption and the dashed line represents the effect from the control. There was a near significant time by treatment effect (*P*=0·068). * Mean values were significantly different for effect between treatments after *post hoc* tests.

Table 4. Plasma concentration of phenolics and hippuric acid following acute consumption of fruit and vegetable purée-based drink (FVPD) or control
(Mean values with their standard errors n 24)

| Plasma analyte (μmol) | Treatment | Time after drink consumption (min) | | | | | | | | | | | | | | | | P (time × treatment) |
| | | 0 | | 60 | | 90 | | 120 | | 180 | | 300 | | 360 | | 480 | | |
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Salicylic acid	FVPD	5.6	0.9	7.7*	1.2	6.7*	0.7	7.3*	1.0	5.9*	0.5	6.5*	0.8	6.2	0.8	4.8	0.6	0.03
	Control	4.1	1.0	2.9	0.7	3.4	1.1	3.1	0.5	3.2	0.9	3.6	0.8	3.0	0.4	3.0	0.6	
Benzoic acid	FVPD	31.2	2.9	32.1	8.5	26.0	3.4	41.4	11.1	48.0	12.7	65.5	21.6	65.6	13.4	41.3	7.4	0.07
	Control	25.3	3.4	19.5	2.9	15.3	3.2	30.2	8.9	31.2	6.9	35.6	5.0	26.9	8.5	26.6	5.3	
p-Hydroxyphenylacetic acid	FVPD	5.1	0.8	5.2	0.9	5.4	0.7	4.9	0.5	4.5	0.5	4.7	0.9	3.0	0.2	3.6	0.3	0.01
	Control	4.5	1.1	5.0	1.3	4.6	1.4	4.4	0.9	4.4	0.8	4.0	0.3	2.4	0.3	3.7	0.8	
3-(4OH)-4-phenyl-(2OH)-propanoic acid	FVPD	2.0	0.1	1.9	0.1	1.9	0.0*	1.89	0.1	1.8	0.0	1.8	0.0	1.7	0.0	1.8	0.0	NS
	Control	1.6	0.2	1.5	0.2	1.6	0.2	1.5	0.2	1.5	0.1	1.4	0.1	1.2	0.0	1.5	0.2	
Hippuric acid	FVPD	4.4	0.7	4.0	0.7	3.8	0.6	4.0	0.6	5.6*	0.7	11.1*	2.1	8.0*	1.1	7.1*	1.1	0.0001
	Control	4.4	0.7	3.1	0.7	2.8	0.6	2.7	0.6	2.7	0.5	2.5	0.5	2.1	0.5	2.0	0.4	

* Mean values were significantly different from control ($P < 0.01$ after *post hoc* tests).

from the fruit sources, including anthocyanins, other flavonoids and phenolic acids. The present study is one of a few studies that have investigated the effect of an acute ingestion of polyphenol-rich drink on postprandial LDL oxidation. A major finding of the present study was that the susceptibility of LDL to oxidation increased throughout the 8 h of the intervention period after subjects fasted overnight and consumed either the FVPD or control. A fall in the lag phase time in subjects who had fasted for at least 12 h was also reported by Hodgson *et al.*[52], who found that LDL isolated at 60 min after consumption of water or water-containing caffeine had lag phases that were shorter than the baseline values. However, the acute consumption of the FVPD significantly increased the lag phase compared to control after 60 min for all subjects ($P = 0.016$) and the lag phase remained longer than control over a 4 h period. The increase in lag phase cannot be due to carotenoid components, because most of the increase in plasma carotenoids occurs after more than 2 h[53]. There was a significant increase in plasma ascorbic acid following FVPD consumption, with a maximum concentration reached at 150–180 min, indicating that the ascorbic acid in the FVPD was effectively absorbed. An increase in plasma oxidative stability, assessed by the FRAP assay from 60 to 180 min, suggested that the antioxidant components of the FVPD, including ascorbic acid and flavonoids, increased the antioxidant capacity. There was no effect of treatment on plasma uric acid, which was not surprising as the control drink was matched for sugar composition, which was necessary as previously it has been shown that fructose increases plasma uric acid[54]. Caccetta *et al.*[55] found a significant increase in plasma uric acid over 4 h, with a peak at 60 min after the subjects consumed 350 ml of red wine, but wine consumption had no effect on LDL oxidation.

In the present study hippuric acid was the main flavonoid metabolite found in plasma and urine. The result was comparable to previously reported studies, after consumption of phenolic compounds such as flavanols, quercetin or chlorogenic acid[26,48,56,57]. When these compounds reach the colon, they can be converted to valerolactone and then to phenolic acids by the action of gut microflora, which are then converted to benzoic acid by β oxidation in the liver and benzoic acid is conjugated with glycine to form N-benzoylglycine or hippuric acid, which can be absorbed or excreted in urine[48,56]. Valentova *et al.*[57] observed that after subjects consumed 1200 mg of dried cranberry juice/d for 8 weeks (estimated daily intake of 35 mg total phenolics), the major metabolite found in the urine was hippuric acid, which was present at a significantly higher concentration than the control. Additionally, the serum levels of advanced protein oxidation products were decreased at the end of the intervention. However, in contrast, no correlation between plasma/urinary hippuric acid and plasma antioxidant status was observed in the present study (data not shown). Hippuric acid has no antioxidant activity, due to the absence of a phenolic hydroxyl group in the molecule. In support of this, no correlation was observed between flavonoid metabolites in plasma and urine with antioxidant status after consumption of the FVPD. Plasma antioxidant status assessed by LDL oxidation and by
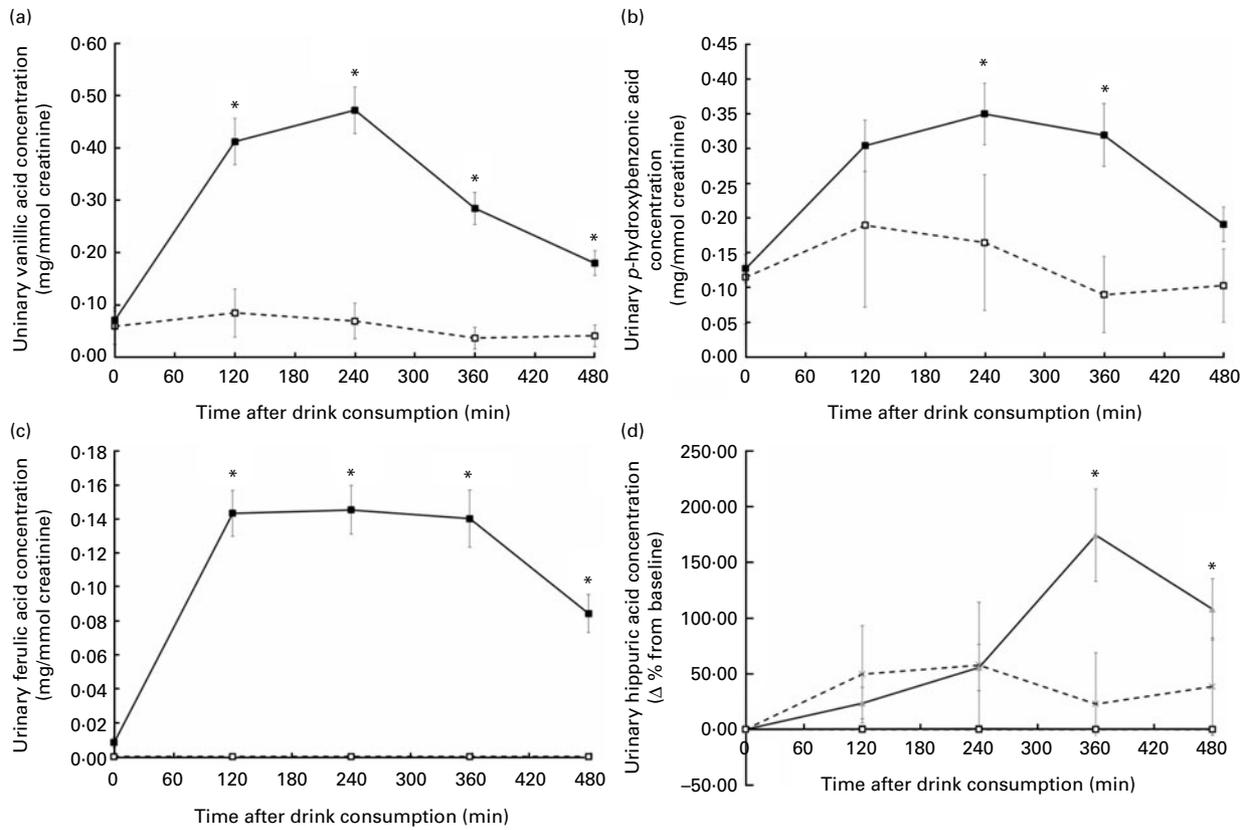
Fig. 3. Urinary phenolic acid ((a) vanillic acid; (b) p-hydroxybenzoic acid; and (c) ferulic acid) as mg/mmol creatinine, and (d) hippuric acid as % change from baseline following acute consumption of fruit and vegetable purée-based drink (FVPD) or control (n 24). The solid lines represent the effect from FVPD consumption and the dashed lines represent the effect from the control. The mean peak concentration occurred after 4 h for vanillic and p-hydroxybenzoic acid (P<0·001 and 0·04, respectively). Ferulic acid was only found in urine after subjects consumed the FVPD, and the concentration peaked after 2 h and remained constant until 6 h before reducing (time by treatment, P<0·001). There was a highly significant time by treatment effect in urinary hippuric acid (P<0·008). * Mean values were significantly different for effect between treatments after post hoc tests.

the FRAP assay peaked at 60 min after consumption of the FVPD. The increase in oxidative stability at 60 min is expected to be mainly due to the protection of LDL by the increased plasma vitamin C concentration prior to isolation of the LDL. Hippuric acid reaching a peak at 5 and 6 h in plasma and urine, respectively. The delayed appearance of hippuric acid reflected the fact that this compound was formed in the colon due to bacterial metabolism and subsequently absorbed and excreted in the plasma and urine, respectively[26,48,56,57]. Epicatechin was the major flavonoid detected in the FVPD, followed by anthocyanins and caffeic acid (Table 1). However, apart from hippuric acid, which is the major metabolite of flavanols and hydroxycinnamates, other metabolites such as glucuronides, sulphates and o-methylates, in addition to anthocyanins, were not detected in the plasma or urine samples. The major metabolites detected were phenolic acids. These are secondary metabolites derived from the biotransformation of colonic microflora. The method used to analyse plasma was relatively insensitive in detecting intact flavonoid derivatives.

Several studies have investigated the effect of acute consumption of foods on Cu-catalysed LDL oxidation. Natella et al.[58] reported that acute consumption of a cup of coffee significantly reduced the susceptibility of LDL to oxidation

by increasing the lag phase of LDL isolated from blood removed at 30 and 60 min after consumption. Hodgson et al.[52] found no significant effect of the acute consumption of green tea, black tea and water containing 180 mg caffeine on LDL oxidation lag phase and total antioxidant capacity of
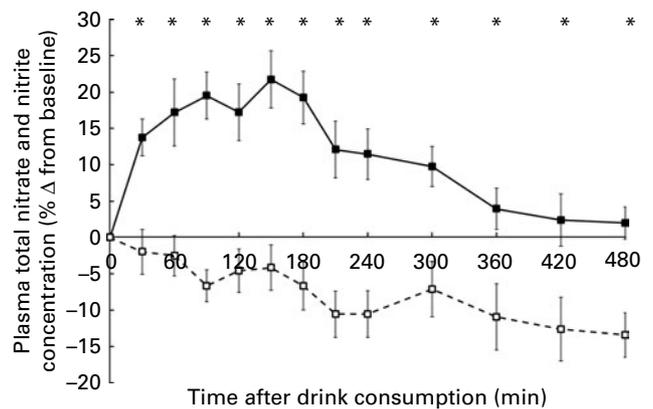

Fig. 4. Total plasma nitrate/nitrite concentration (% change from baseline) following acute consumption of fruit and vegetable purée-based drink (FVPD) or control (n 24). The solid line represents the values after FVPD consumption and the dashed line represents the values for the control. There was a significant time by treatment effect (P=0·001). * Mean values were significantly different for effect between treatments after post hoc tests.
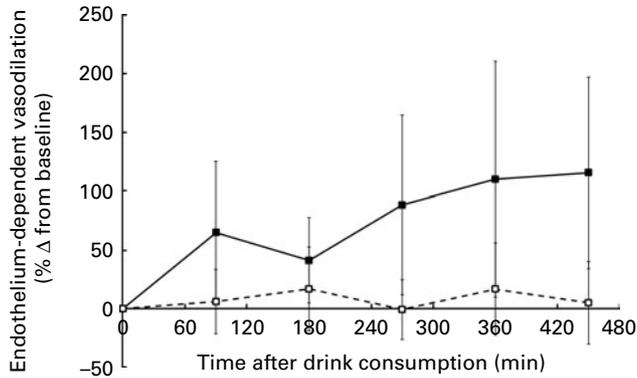
Fig. 5. Endothelium-dependent vasodilation in response to acetylcholine (percentage change from baseline) following acute consumption of fruit and vegetable purée-based drink (FVPD) or control (n 24). The solid line represents the values after FVPD consumption and the dashed lines represent the values for the control.

samples isolated 60 min after consumption. Miyagi *et al.*[59] reported that acute consumption of red wine containing 433 mg of total phenolics caused a significant increase in the lag phase time of samples isolated 1 and 2 h after consumption. However, consumption of red grape juice containing 511 mg of total phenolics had no effect. Caccetta *et al.*[55] reported findings that contradict the latter study, with no effect on the susceptibility of LDL to oxidation after acute consumption of red wine containing 700 mg of total polyphenols. Another postprandial study investigated the effect of consuming olive oil containing 2·7, 164 and 366 mg/kg of phenolic compounds on LDL oxidation. The results showed that only the consumption of 366 mg/kg olive oil had an effect, retarding LDL oxidation by 9·5 and 15·2 % in LDL isolated from blood removed 2 and 6 h, respectively, after consumption of olive oil[60]. Consequently, the effect of polyphenol-rich foods on the susceptibility of LDL to oxidation in acute interventions depends on the nature and amount of polyphenols in the food, as well as on other variables. The fall in oxidative stability of LDL isolated from both test and control groups after baseline may be due to the effects of fasting. However, absorption or formation of flavonoid metabolites in the plasma of subjects may be the reason for the increase in plasma antioxidant capacity due to the FVPD.

The present study also found that there was a significantly lower glucose and insulin peak concentration after consumption of the FVPD compared to the sugar-matched control. As the FVPD contained a mixture of purées and concentrated juices, this may have been due to the matrices of the fruit and vegetable particles slowing down the absorption of the sugars, whereas the sugars in the control drink were dissolved in mineral water. This highlights an additional benefit to puréed fruits and vegetables over juices that contain minimal food matrix components. There was a significant increase in total plasma nitrate and nitrite following FVPD consumption. The control drink was specifically diluted with Buxton mineral water that had the lowest nitrate content of all commercially available mineral waters. This was chosen to reduce any confounding effects of nitrate in the control, affecting

vasodilation measured by LDI. Although the FVPD contained a minimal concentration of nitrate/nitrite from the fruits and vegetables (1·0 mg in 400 ml), the plasma concentrations of nitrate/nitrite at each time point after consumption of the FVPD were significantly higher than the expected concentration provided by the drink. The increase in plasma nitrate/nitrite was maintained throughout the day, whereas research has shown that the plasma nitrate concentration from the ingestion of dietary sources reaches a peak after approximately 60 min and then declines[28,61]. Therefore, the increase in nitrate/nitrite following FVPD consumption could be due to flavonoid components within the drink increasing eNOS activity. *In vitro* studies have shown that flavonoids[10–12] at physiological concentrations[13] can increase eNOS gene expression. The FVPD was particularly rich in (−)-epicatechin (457 mg/400 ml) and this compound has been shown to increase eNOS activity[14,15] and endothelium-1 release[15]. In support of these findings, acute consumption of epicatechin-rich apples and grapes (consumed as champagne) have been associated with beneficial effects on postprandial NO status and on *in vivo* vasodilation measured by LDI[62] and flow-mediated dilation[21] in randomised control human studies. In addition, increased plasma NO and increased vasodilation have been reported after chronic consumption of fruits and vegetables, which may be due in part to flavonoids and/or other bioactive components[16–18]. The significant increase in urinary hippuric acid at 360 and 480 min indicated that the flavonoid components of the FVPD were absorbed with metabolism into hippuric acid, which is a metabolite of several polyphenol compounds[48,56].

The novel LDI results were encouraging. There was a trend towards increased endothelium-dependent vasodilation following ingestion of the FVPD compared to the control drink. The initial increase could be due to flavonoid components present in the FVPD increasing eNOS activity by scavenging superoxide[14], which may explain the increase in total plasma nitrate and nitrite found after FVPD consumption. The largest increase in vasodilation was observed at approximately 7·5 h after FVPD consumption, which could be attributed, at least in part, to methylated flavonoid metabolites from the small intestine[63]. These metabolites have been shown to have an even greater ability to increase eNOS activity compared with the parent molecules by inhibiting endothelial NADPH oxidase, thereby reducing superoxide production[64]. There was no effect of the FVPD on plasma TAG, total and HDL-cholesterol or NEFA, but this was not unexpected as no fat meal had been given with the drinks.

Overall, the present study provides evidence that consumption of fruits and vegetables in the form of purée-based drinks acutely increases plasma ascorbic acid, total plasma nitrate and nitrite concentrations, oxidative stability of plasma assessed by the FRAP and *ex vivo* Cu-catalysed LDL oxidation. Increased concentrations of phenolic metabolites were detected in plasma and urine after consumption of the FVPD, and it was concluded that the increase in the oxidative stability of LDL and antioxidant capacity of plasma could be due to components in the FVPD such as phenolic acids, flavonoids and their metabolites. There was a trend towards

increased endothelium-dependent vasodilation following FVPD consumption, which may be due to increased eNOS activity or increased NO sparing indicated by the increased plasma nitrate and nitrite. Purée-based fruit and vegetable drinks are suitable vehicles for micronutrient and phytochemical ingestion, which may contribute to CVD risk reduction.

Acknowledgements

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