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ORIGINAL CONTRIBUTION

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The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers

Summary *Background* Consumption of fruit and vegetables is associated with a decreased risk of heart disease and cancer. This has been ascribed in part to antioxidants in these foods inactivating reactive oxygen species involved in initiation or progression of these diseases. Non-nutritive anthocyanins are present in significant amounts in the human diet. However, it is unclear whether they have health benefits in humans. *Aim* To determine whether daily consumption of anthocyanin-rich cranberry juice could alter plasma antioxidant activity and biomarkers of oxidative stress. *Methods* 20 healthy female volunteers aged 18–40 y were recruited. Subjects consumed 750 ml/day of either cranberry juice or a placebo drink for 2 weeks. Fasted blood and urine samples were obtained over 4 weeks. The total phenol, anthocyanin and catechin content of the supplements and plasma were measured. Anthocyanin glycosides were identified by tandem mass spectrometry (MS-MS). Vitamin C, homocysteine (tHcy) and reduced glutathione (GSH) were measured by HPLC. Total antioxidant ability was determined using electron spin resonance (ESR) spectrometry and by the FRAP assay. Plasma total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL) cholesterol and triglycerides (TG) were measured. Glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) activities were measured in erythrocytes. Urine was collected for analysis of malondialdehyde (MDA) by HPLC and 8-oxo-deoxyguanosine (8-oxo-dG) by ELISA. Endogenous and induced DNA damage were measured by single cell gel electrophoresis (SCGE) in lymphocytes. *Results* Vitamin C, total phenol, anthocyanin and catechin concentrations and FRAP and ESR values were significantly higher in the cranberry juice compared with the placebo. Cyanidin and peonidin glycosides comprised the major anthocyanin metabolites [peonidin galactoside (29.2 %) > cyanidin arabinoside (26.1 %) > cyanidin galactoside (21.7 %) > peonidin arabinoside (17.5 %) > peonidin glucoside (4.1 %) > cyanidin glucoside (1.4 %)]. Plasma vitamin C increased significantly ($P < 0.01$) in volunteers consuming cranberry juice. No anthocyanins (plasma) or catechins (plasma or urine) were detectable and plasma total phenols, tHcy, TC, TG, HDL and LDL were unchanged. The antioxidant potential of the plasma, GSH-Px, CAT and SOD activities, and MDA were similar for both groups. Supplementation with cranberry juice did not affect 8-oxo-deoxyguanosine in urine or endogenous or H_2O_2-induced DNA damage in lymphocytes. *Conclusions* Cranberry juice consumption did not alter blood or cellular antioxidant status or several biomarkers of lipid status pertinent to heart disease. Similarly, cranberry juice had no effect on basal or induced oxidative DNA damage. These results show the importance of distinguishing between the *in vitro* and *in vivo* antioxidant activities of dietary anthocyanins in relation to human health.

Key words cranberry – human study – vitamin C – antioxidant capacity – oxidative DNA damage – anthocyanin

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Introduction

High fruit and vegetable consumption is consistently associated with a decreased risk of certain human pathologies such as cardiovascular disease and cancer. This has been ascribed in part to the recognised antioxidant micronutrients such as vitamins E, C and carotenoids that inactivate reactive oxygen species involved in the initiation or progression of these chronic diseases [1, 2]. Non-nutritive polyphenols, such as flavonoids, are also present in significant amounts in fruits and vegetables and have been shown to possess strong antioxidant properties in chemical systems [3]. Intake of one group of flavonoids, the anthocyanins, has been estimated to exceed 200 mg/day [4]. Anthocyanins are particularly prevalent in soft fruits such as red grapes, raspberries, blueberries and cranberries, reaching concentrations in excess of 10 g/kg in some berry cultivars [4]. Anthocyanins effectively modify biomarkers of both heart disease and cancer in vitro. They inhibit release of reactive oxygen species from activated human granulocytes [5] and suppress free-radical mediated lipid peroxidation and cell death in cultured aortic endothelial cells [6, 7]. Moreover, anthocyanin aglycones and glycosides are effective inhibitors of oxidant-induced DNA damage in immortalised normal human colon cells [8] and are potent inhibitors of tumour cell growth in vitro [9, 10]. Cranberries contain significant quantities of anthocyanins as well as flavonols and proanthocyanins [11, 12]. While the antibacterial capability of cranberries in protecting against urinary tract infections have long been recognised [13] it remains to be established whether cranberry anthocyanins possess anticarcinogenic and/or antiatherogenic properties. Cranberry extract strongly inhibits liver cancer cell growth in vitro [14], while cranberry anthocyanins decrease oxidation of human LDL in vitro and lower total and LDL cholesterol in animals [13, 15, 16].

The aim of this study was to determine whether consumption of 750 ml of cranberry juice per day for 2 weeks could alter plasma antioxidant activity and several biomarkers of oxidative stress pertinent to heart disease and cancer in fasted blood from healthy human subjects. Total blood cholesterol and triglycerides, elevated low density lipoprotein (LDL) and high ratios of LDL to high density lipoprotein (HDL) cholesterol are associated with development of atherosclerosis [17]. Elevated total plasma homocysteine is an independent risk factor for heart disease, although the mechanism remains unknown [18]. The antioxidant status of fasted blood was assessed using several sensitive biomarkers including reduced glutathione levels (GSH), total phenol concentrations and plasma free radical trapping capacity (FRAP and ESR). In addition, the ability of cranberry juice to alter the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in washed red blood cells was measured. Urinary excretion of malondialdehyde (MDA) provides a marker for lipid oxidation. Oxidative damage to DNA has been implicated in the development of cancer and oxidised DNA base damage, such as that seen when 8-oxo-deoxyguanosine (O^8oxo-dG) is formed in DNA, is strongly mutagenic [2].

Materials and methods

Study subjects

A total of 20 healthy female volunteers aged between 18–40 y were recruited. None were taking medication or vitamin and mineral supplements. 2 subjects were light-smokers (3 cigarettes per day). Blood pressure, body weight and height were measured. All subjects were normotensive (systolic 100–150/diastolic 60–90 mmHg). The study was approved by the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen and subjects gave their informed consent in writing.

Subjects were randomly allocated to either the treatment or placebo group and in addition to their normal diet consumed 750 ml/day (3 x 250 ml) of either cranberry juice [Ocean Spray Cranberry Select)] or a placebo drink [Volvic Touch of Fruit (natural mineral water with strawberry flavour) + sucrose (9 g/100 ml, BDH Lab Supplies, Poole, UK)] for 2 weeks. Age, height and weight of the subjects in the cranberry juice (CJ; n = 11) and placebo (P; n = 9) groups were not significantly different and there was one light smoker in each group (age: 27.3 ± 6.5 vs. 28.3 ± 7.5 years; height: 1.66 ± 0.08 vs. 1.69 ± 0.06 m; weight: 62.72 ± 9.91 vs. 63.58 ± 7.98 kg, for CJ and P respectively). Volunteers were also asked to complete a simple diet questionnaire throughout the study to determine whether their diet and fluid intake changed over the course of the study. No significant changes were recorded (data not shown).

Blood and urine collection and storage

Fasted blood (30 ml) was removed by venepuncture from the arm into evacuated tubes with EDTA as an anticoagulant (Evacuette, Greiner Labortechnik, Austria). Blood and urine samples were obtained after an overnight fast at weeks –1 (baseline1), 0 (baseline 2), 1, 2 with weeks 0–2 representing the period of supplementation. Blood samples were stored on ice for a maximum of 1 h prior to centrifugation (4 °C, 2400 × g, 15 min). Plasma was divided into aliquots, snap frozen in liquid N_2, and stored at –80 °C. Erythrocytes were washed twice in phosphate buffered saline (PBS; pH 7.4), resuspended in PBS (to the original blood volume), snap frozen in liq-

uid N_2, and stored at –80°C. Plasma for vitamin C analysis was acidified using 10 % (w/v) filtered metaphosphoric acid (MPA; 500 µl MPA to 500 µl plasma) before freezing. Urine samples were stored with 2 % (w/v) butylated hydroxytoluene (BHT) (25 µl per 700 µl urine) at –80 °C for TBARS analysis. Samples of urine for 8-oxo-deoxyguanosine (O^8oxo-dG) analysis were acidified (9 µl of 2 M HCl added to 0.5 ml) before storage at –80 °C. Lymphocytes were isolated using Lymphoprep Lymphocyte Separation Medium, washed twice in RPMI, resuspended in RPMI + 10 % (v/v) heat inactivated fetal calf serum (FCS) and frozen at a cell density of 3×10^6 in FCS + 10 % dimethylsulfoxide (DMSO) at approximately –1 °C/min in polystyrene at –80 °C [19].

Biochemical analysis

The total phenol and catechin content of the cranberry juice and placebo were measured spectrophotometrically [20, 21]. Total anthocyanins in the drinks were assessed colorimetrically [22]. Individual anthocyanins in the cranberry juice were identified and quantified by gradient reversed phase HPLC with photodiode array and tandem mass spectrometric (MS-MS) detection. A Surveyor HPLC system (Thermo Finnigan, San Jose, USA) was used. This comprised a pump, diode array detector scanning from 250–700 nm, and an auto sampler set at 4 °C. Separation was carried out using a 250 × 4.6 mm i. d. 4 µm Synergi RP-Max column (Phenomenex, Macclesfield, UK) eluted at a flow rate of 1 mL/min with a 60 min, 5–30 % gradient of 0.1 % aqueous formic acid and acetonitrile. After passing through the flow cell of the diode array detector the column eluate was split and 0.3 mL was directed to a LCQ Duo ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan). Analysis of anthocyanins was carried out in full scan mode from 100–1000 amu using positive ionisation [23].

Vitamin C (plasma, cranberry juice and placebo) was measured by ion-paired reversed phase HPLC [24]. Plasma total phenols and catechins were measured as described [21, 25]. Two methods were employed to measure antioxidant activity. The ability of the plasma or juice to donate a hydrogen atom or electron to the synthetic free radical, potassium nitrosodisulphonate (Fremy's salt) was monitored by electron spin resonance spectroscopy (ESR) [26, 27] as previously described [28]. In brief, a 10-fold dilution of plasma or juice (3 ml) was mixed with an equal volume of Fremy's salt (50 µM and 1.0 mM solutions for plasma and juice, respectively) and kept for 5 minutes at room temperature before measurement. The spectrum of the low field resonance of the Fremy's radical was recorded after 5 min by ESR. Signal intensity was obtained by double integration and the concentration calculated by comparison with a control reaction with distilled water instead of fruit juice. Spectra were obtained at 21 °C on a Bruker ECS 106 spectrometer working at ca 9.5 GHz (X-band frequency) and equipped with a cylindrical (TM_{110} mode) cavity. The microwave power and modulation amplitude were set at 2 mW and 0.01 mT, respectively. Antioxidant capacity was expressed as the number of Fremy's radicals reduced by the plasma or juices. Antioxidant potential of the plasma and juice was also estimated from their ability to reduce Fe(III)–2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) complex to Fe(II)-TPTZ, the resulting intense blue colour being linearly related to the amount of reductant (antioxidant) present [29]. The ferric reducing antioxidant potential (FRAP value) was measured at 593 nm 7 min after 30 µl of plasma or a 10-fold dilution of the juice in distilled water was added to 900 µl of Fe(III)-TPTZ by which time the reaction was complete at 37 °C. Data are presented as change in FRAP value from baseline rather than absolute values as described previously [28] to accommodate effects of inter-individual variation in pre-intervention basal values.

Plasma homocysteine and reduced glutathione were assessed by reversed phase HPLC using a DS30 analyser [30]. Plasma total, HDL, and LDL cholesterol and triglycerides were estimated on a KONE dynamic discrete analyser using commercially available kits (Labmedics Ltd, Stockport, UK). Glutathione peroxidase (EC 1.11.1.9) [31]; catalase (EC 1.11.1.6) [32]; and superoxide dismutase (EC 1.15.1.1) [33] activities were measured in red blood cell extracts by previously published procedures. Haemoglobin was measured using Drabkins reagent (Sigma Diagnostics, Poole, UK). Lipid peroxidation was estimated by measurement of urinary MDA by HPLC [34] and urinary 8-oxo-deoxyguanosine was determined by ELISA (Genox Corp, Baltimore) [35]. Cellular DNA damage was measured using alkaline single-cell gel electrophoresis [19]. DNA strand breakage was assessed following treatment with and without 200 µM H_2O_2 [36]. Oxidised pyrimidines were detected as described previously [36] by incubating nucleoids for 45 min at 37 °C with endonuclease III (1 µg protein/ml). DNA damage was quantified by visual scoring and is expressed in arbitrary units [36].

Statistical analysis

All biomarker data approximated normality and were not log transformed. Repeated measures analysis of variance (ANOVA) using anti-dependence modelling [37] was performed using the statistical software Genstat for Windows version 3.2 to detect differences between the two groups across time. Treatment effect within group was tested with paired t-test using Microsoft Excel 2000. Differences were considered statistically significant where $P < 0.05$.

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Results

Phytochemical content and antioxidant potential of cranberry juice and placebo

Vitamin C, total phenols, total anthocyanins and total catechins in the cranberry juice supplement were determined and compared with placebo drink. As expected, the levels of all the phytochemicals measured were significantly higher in the supplement compared with the placebo (Table 1; $P < 0.001$). The antioxidant capacity of the cranberry juice, assessed using the FRAP assay and by ESR markedly exceeded that of the placebo drink (Table 1). HPLC-MS-MS was used to identify the main cranberry juice anthocyanins. HPLC with detection at 520 nm revealed the presence of six peaks (Fig. 1), which were identified as known cranberry anthocyanins [12] by MS-MS analysis (Table 2). Both peaks 1 and 2 (retention times [t_R] 14.2 and 15.3 min) yielded a positively charged molecular ion ($[M+H]^+$) at m/z 449, which with a loss of 162 amu corresponding to cleavage of a hexose unit, produced a cyanidin-like MS^2 fragment at m/z 287. On the basis of the elution order, peak 1 is identified as cyanidin-3-galactoside, a known major anthocyanin in cranberries, and peak 2 is identified as cyanidin-3-glucoside, which is an established minor component [12]. Peak 3 (t_R – 16.4 min) was one of four major peaks and had a $([M+H]^+$ at m/z 419 which on MS^2 produced an ion at 287 m/z formed by a m/z 132 loss corresponding to the cleavage of a pentose. The MS-MS spectra and the HPLC elution order are in keeping with the presence of cyanidin-3-arabinoside, also identified previously [12]. Peaks 4–6 (t_R – 17.7, 18.8 and 21.4 min) had respective ($[M+H]^+$ at m/z 463, 463 and 433 all of which produced a MS^2 peonidin ion at m/z 301. On the basis of these MS-MS spectra, the HPLC elution order, previous identifications and the relative amounts of each component [12], peak 4 is identified as peonidin-3-galactoside, peak 5 is peonidin-3-glucoside and peak 6 is peonidin-3-arabinoside (Table 2).

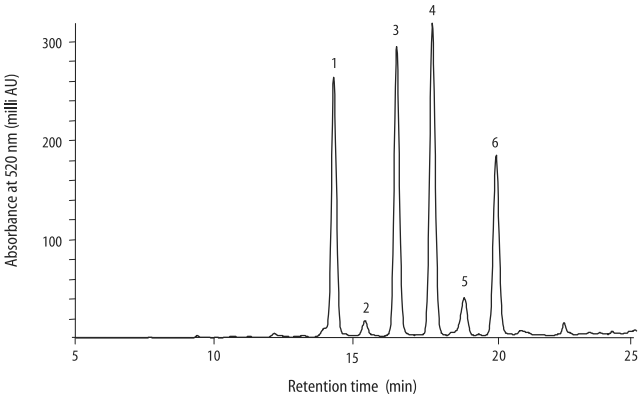

Fig. 1 Mass spectrometer trace of cranberry juice analysis for anthocyanins. Six anthocyanin glycosides were quantified by mass spectrometry. **A** cyanidin galactoside (21.7), **B** cyanidin glucoside (1.4 %), **C** cyanidin arabinoside (26.1 %), **D** peonidin galactoside (29.2 %), **E** peonidin glucoside (4.1 %), **F** peonidin arabinoside (17.5). Percentage area for each compound is shown in parentheses. Absorbance was 520 nm

Table 1 Phytochemical content and antioxidant potential of cranberry juice and placebo

	Cranberry Juice	Placebo
Vitamin C (mg/l)	897 ± 5.3	1.84 ± 0.02
Total phenols (mg/l GAE)	1136 ± 3.5	8.96 ± 0.14
Catechins (mg/l)	29.06 ± 0.36	ND
Anthocyanins (mg/l) (malvidin-3-glycoside equivalents)	2.80 ± 0.19	ND
FRAP (mM FeII)	14.00 ± 0.01	0.06 ± 0.01
ESR (radicals reduced/10^{18}/ml)	9.16 ± 0.07	0.02 ± 0.00

ND not detectable

Bioavailability biomarkers

Plasma total phenols were unaffected by supplementation with cranberry juice (Table 3). No anthocyanins (plasma) or catechins (plasma or urine) were detectable in samples isolated from either group (data not shown). Plasma vitamin C increased significantly (from 63.0 ± 6.9 μM at baseline to 89.6 ± 6.3 μM; $P < 0.01$) in

Table 2 HPLC-MS-MS identification of anthocyanins in cranberry juice. Peak numbers and HPLC retention times refer to HPLC trace in Fig. 1. Percent refers to the contribution of individual anthocyanins to total anthocyanins

Peak	t_R (min)	λ_{max} (nm)	$[M+H]^+$ (m/z)	MS^2 fragment ions (m/z)	Compound	%
1	14.2	520	449	287 [C]([M + H]$^+$-Gal)	cyanidin-3-galactoside	27.1
2	15.3	520	449	287 [C]([M + H]$^+$-Glc)	cyanidin-3-glucoside	1.4
3	16.4	520	419	287 [C]([M + H]$^+$-Arab)	cyanidin-3-arabinoside	26.1
4	17.7	520	463	301 [Peo]([M + H]$^+$-Gal)	peonidin-3-galactoside	29.2
5	18.8	520	463	301 [Peo]([M + H]$^+$-Glc)	peonidin-3-glucoside	4.1
6	21.4	520	433	301 [Peo]([M + H]$^+$-Arab)	peonidin-3-arabinoside	17.5

t_R retention time; *[M + H]*$^+$ positively charged molecular ion; *C* cyanidin; *Peo* peonidin; *Arab* arabinoside; *Gal* galactoside; *Glc* glucoside unit

Table 3 The effect of cranberry juice on blood biomarkers in human volunteers. Results are mean ± SEM for volunteers given placebo (P, n = 9) or cranberry juice (CJ, n = 11) for 14 days

Biomarkers		Baseline 1	Baseline 2	Week 1	Week 2
Total phenols (μg/ml GAE)	CJ	6.84±0.36	7.99±0.32	8.22±0.52	7.06±0.24
	P	7.16±0.5	7.21±0.5	7.20±0.50	6.92±0.45
TC (mmol/l)	CJ	4.76±0.19	4.91±0.16	4.80±0.21	4.90±0.22
	P	4.39±0.28	4.49±0.25	4.20±0.19	4.46±0.25
HDL (mmol/l)	CJ	1.37±0.10	1.40±0.09	1.40±0.09	1.40±0.10
	P	1.32±0.08	1.32±0.08	1.26±0.05	1.30±0.06
LDL (mmol/l)	CJ	2.96±0.22	3.19±0.18	2.97±0.20	2.81±0.21
	P	2.56±0.21	2.71±0.25	2.35±0.19	2.51±0.20
TG (mmol/l)	CJ	0.99±0.09	0.86±0.10	1.03±0.11	1.10±0.12
	P	1.07±0.12	1.04±0.09	1.28±0.17	1.03±0.13
TC/HDL ratio	CJ	3.59±0.22	3.61±0.21	3.52±0.19	3.58±0.19
	P	3.35±0.18	3.43±0.18	3.34±0.15	3.47±0.21
LDL/HDL ratio	CJ	2.28±0.25	2.38±0.23	2.20±0.19	2.09±0.21
	P	1.98±0.17	2.09±0.22	1.87±0.16	1.97±0.19
Homocysteine (μM)	CJ	7.69±0.9	8.05±1.3	7.88±1.1	7.82±1.1
	P	7.08±0.5	7.24±0.3	6.58±0.3	7.02±0.4
GSH (μM)	CJ	6.1±1.8	6.1±1.7	5.9±1.9	5.7±1.8
	P	5.9±1.6	5.5±1.3	5.5±1.2	5.7±1.4
MDA (nmol/mg creatinine)	CJ	1.69±0.27	1.10±0.20	1.70±0.18*	1.34±0.23
	P	1.82±0.24	1.13±0.1	1.14±0.18	1.42±0.15
ΔFRAP (μM Fe II)	CJ	25.5±1.8	26.1±1.9	26.1±1.5	24.8±1.5
	P	25.4±1.5	25.4±0.7	25.5±1.2	25.4±1.2
ESR (radicals reduced/10^{18}/ml)	CJ	7.02±0.20	7.21±0.32	6.87±0.32	6.73±0.23
	P	6.99±0.14	6.91±0.88	6.84±0.15	7.10±0.17
GSHPx (U/gHb)	CJ	3.01±0.39	2.99±0.25	3.29±0.16	2.98±0.13
	P	3.17±0.14	3.47±0.14	3.50±0.22	3.55±0.16
Catalase (k/g Hb)	CJ	44.7±3.4	45.7±3.2	48.4±3.6	47.7±3.2
	P	45.8±2.8	44.9±2.4	45.6±3.4	44.9±3.4
SOD (μg/g Hb)	CJ	1366±98	1233±75	1200±69	1176±65
	P	1246±95	1227±80	1181±98	1251±73

TC total cholesterol; *HDL* high density lipoprotein, *LDL* low density lipoprotein; *TG* triglycerides; *MDA* malondialdehyde; *ESR* Electron spin resonance; *FRAP* ferric reducing antioxidant potential; *GSH* reduced glutathione; *GSHPx* glutathione peroxidase; *SOD* superoxide dismutase. * $P < 0.05$ where significance refers to differences compared with baseline values within treatment groups

volunteers fed cranberry juice for 1 week. This increase (approx. 50 % above baseline) was maintained at week 2 of the study. Vitamin C concentrations were similar in the placebo group throughout the supplementation trial (Fig. 2).

The effect of cranberry juice on plasma, red blood cell and urinary biomarkers

TC, TG, HDL and LDL were unaffected by the intervention (Table 3). Plasma total homocysteine remained unchanged. Similarly, the antioxidant potential of the plasma [measured as reduced glutathione (GSH), FRAP and by ESR] and the activity of the Phase II metabolising enzymes glutathione peroxidase, catalase and superoxide dismutase did not differ significantly from baseline values in both groups (Table 3). Urinary MDA was unchanged by cranberry juice consumption (Table 3).

The effect of cranberry juice on endogenous and induced DNA damage

Endogenous DNA damage in lymphocytes remained unchanged in both treatment groups over the study period (Table 4). Similarly, despite a significant decrease ($P < 0.05$) in both treatment groups with time there was no difference in the background excretion rate of the oxidised purine nucleoside, 8-oxo-deoxyguanosine (measured in urine) in volunteers fed cranberry juice or placebo (Table 4) at the conclusion of the intervention. Supplementation with cranberry juice did not affect either endogenous DNA strand breakage or oxidised

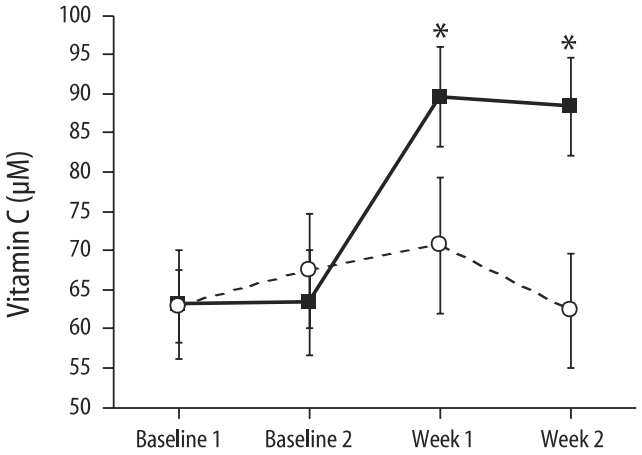

Fig. 2 Plasma vitamin C concentration. Results are mean ± SEM for subjects on placebo (circles; n = 9) or cranberry juice (squares; n = 11). * $P < 0.01$, where * refers to a significant difference compared with baseline

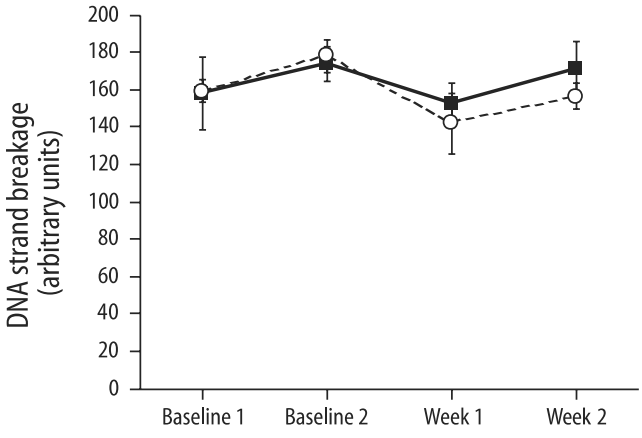

Fig. 3 Induced DNA strand breakage. Lymphocytes were treated ex vivo with hydrogen peroxide (200 µM) and DNA strand breakage measured using single cell gel electrophoresis. Results are mean ± SEM for subjects on placebo (circles; n = 9) or cranberry juice (squares; n = 11)

pyrimidines in isolated lymphocytes. Moreover, DNA strand breakage induced in response to oxidative stress (H_2O_2 treatment) was similar in lymphocytes isolated from both treatment groups indicating that the cellular antioxidant capacity of the volunteers was unchanged by supplementation (Fig. 3).

Discussion

Epidemiological studies consistently report that a high intake of fruit and vegetables is associated with a significantly reduced risk of cancer and heart disease [38, 39]. The potential health benefits of increasing intake of fruit and vegetables to the population at large are therefore significant, and current nutritional guidelines recommend individual consumption of no less than 5 portions per day. However, it remains unclear which components of a plant-based diet are protective and what is their mechanism of action. The beneficial effects of these foods has been ascribed at least in part to certain phenolics that are potent antioxidants in vitro and prevent DNA damage and lipid oxidation in cultured cells [6, 40, 41]. Berry anthocyanins (cranberry and blackberry) have been shown to protect cultured human vascular endothelial cells against both oxidative membrane and DNA strand breakage [7, 42] and to inhibit colon cancer cell proliferation in vitro [43]. However, their ability to modify markers of oxidative stress or carcinogenesis in vivo, especially in human subjects, is highly contentious. Feeding grape juice to rats has been observed to significantly inhibit mammary adenocarcinoma multiplicity and tumour mass [44], while anthocyanins from camellia, hibiscus and glutinous red rice increase survival rates in mice inoculated with syngeneic tumor cells [45]. Basal oxidative DNA damage is decreased in the colon of rats fed polyphenol and tannin extracts from red wine [46], while lipid peroxidation and oxidative DNA damage in response to vitamin E depletion is decreased in the liver of rats fed cyanidin-3-glycoside [47, 48]. Hibiscus extract similarly protects against oxidative cell membrane damage and lipid oxidation in rats exposed to tertbutylhydroperoxide [49]. However, in those studies where complex anthocyanin extracts or single anthocyanin glycosides have been shown to be effective against oxidative damage, the polyphenols have gene-

Table 4 The effect of cranberry juice on indices of endogenous DNA stability in human volunteers. Results are mean ± SEM for volunteers given placebo (P, n = 9) or cranberry juice (CJ, n = 11) for 14 days

Endogenous DNA damage		Baseline 1	Baseline 2	Week 1	Week 2
Strand breakage (lymphocyte) (arbitrary units)	CJ	45.6±4.1	38.7±4.5	39.9±2.8	37.4±4.8
	P	42.1±5.0	37.8±4.8	36.4±4.1	33.8±4.0
Oxidised pyrimidines (lymphocyte) (arbitrary units)	CJ	57.4±8.8	62.0±8.8	60.8±7.5	54.2±5.8
	P	62.8±7.1	61.7±6.8	51.6±6.7	49.7±5.9
8-Oxo-dG (urine) (ng/mg creatinine)	CJ	ND	19.1±5.4	17.4±2.4	9.5±0.6*
	P	ND	17.2±1.0	13.8±1.0	10.2±1.5*

8-oxo-dG 8-oxo-deoxyguanosine; *ND* not determined. * $P < 0.05$ where significance refers to differences compared with baseline values within treatment groups

rally been fed at supra-physiological or pharmacological doses [47, 49].

The objective of the present study was to investigate the bioavailability and in vivo antioxidant potential of anthocyanins from cranberry juice, at nutritionally relevant concentrations, and to assess the cytoprotective effects of cranberry juice consumption on several biomarkers of oxidative stress in healthy volunteers.

Cranberry juice contained significant levels of vitamin C, total phenols, catechins and anthocyanins with associated antioxidant activity (measured as FRAP and by ESR). Despite an estimated intake of 850 mg/day of total phenols, 2.2 mg/day of anthocyanins and 22 mg/day of catechins, no anthocyanins or catechins were detected in the blood or urine of volunteers consuming cranberry juice daily for 2 weeks. Anthocyanins have been detected at very low concentrations in plasma and urine from subjects fed elderberry juice [50–52], but only when consumed at pharmacological doses (500 mg–1500 mg) and not in unsupplemented subjects. Moreover, it has been reported that less than 1 % of ingested anthocyanins are excreted, suggesting poor absorption of these compounds compared with other flavonoids [51–53]. However, in this study, blood samples were taken 10–12 h after the consumption of juice and anthocyanins may not have been detected if they had cleared from the circulation within this period. Moreover, anthocyanins do appear to undergo significant metabolism and transformation by human colon microflora in vitro, indicating that bioavailability may be greater than previously supposed [54]. In the present study, anthocyanins and catechins were fed daily in cranberry juice at nutritionally relevant levels and correspondingly there was no change in the antioxidant capacity of the plasma. Serum antioxidant status (measured ex vivo) has been reported to increase following consumption of 100 g wild blueberry powder containing 1.2 g anthocyanins [55]. However, it is unlikely that this level is relevant to the normal human diet. In addition to the lack of effect on antioxidant status in the present study, feeding cranberry juice did not alter lipid profile (total cholesterol, HDL and LDL), cellular antioxidant enzyme activity (SOD, catalase or glutathione peroxidase) or DNA stability measured in blood from the volunteers. While the lack of effect of the intervention on plasma lipid profiles may reflect the relatively short duration of the study, alternatively, these compounds, at levels relevant to the human diet, may not be effective cytoprotectants in vivo. However, it should be considered that while these dietary compounds proved ineffective in this small-scale study of relatively young and healthy volunteers, their effect in a larger study or on individuals with higher indices of endogenous oxidative stress (such as cigarette smokers) remains to be investigated. In contrast, plasma vitamin C levels rose significantly following supplementation with cranberry juice indicating efficient absorption of this recognised antioxidant. Surprisingly, given that the estimated intake in this study was in excess of 600 mg/day, there was no corresponding increase in the total antioxidant potential of the plasma and no changes in plasma lipid metabolites, homocysteine and reduced glutathione, malondialdehyde (MDA) or cellular antioxidant enzyme activities. The results of vitamin C intervention on plasma antioxidant capacity and indices of lipid peroxidation in humans are conflicting. Supplementation with vitamin C (1 g/day) for 4 weeks has been reported not to increase plasma total antioxidant capacity (measured as FRAP), despite significantly increasing plasma ascorbate levels [56]. In contrast, FRAP was increased after 4 hours in elderly women consuming 1.25 g of vitamin C [57]. Supplementation of young men with vitamin C (1 g/day) failed to alter urinary TBARS after 30 days [58], while plasma MDA levels declined significantly after the same dosing regime in an unconnected study [59]. In a placebo-controlled crossover intervention trial, supplementation for 2 weeks with physiological (60 mg/day) and pharmacological (6 g/day) doses of vitamin C did not alter plasma MDA/4-hydroxynonenal levels [60]. Moreover, plasma TBARS in smokers have actually been found to increase after vitamin C (500 mg/day for 2 months) supplementation [61].

Cranberry juice consumption did not alter basal DNA damage in lymphocytes isolated from the volunteers in the present study. Levels of oxidised pyrimidines and DNA strand breakage in lymphocytes were similar for both groups at the end of the intervention study as was the urinary excretion rate of 8-oxodG, a lesion found in significant quantities in human DNA and a commonly used biomarker for measuring overall oxidative DNA damage [2]. Similarly, cranberry juice supplementation did not increase the resistance of lymphocytes to oxidative stress ex vivo. The reported effect of vitamin C supplementation on biomarkers of oxidative DNA damage in human studies is inconsistent. Vitamin C has been reported to increase resistance to ionising radiation-induced DNA strand breakage in lymphocytes ex vivo [62], while 8-oxodG levels are substantially increased in sperm from men made vitamin C deficient (5 mg/day for 32 days) and decreased following resupplementation (60–250 mg/day for 28 days) with ascorbate [63]. Conversely, excretion of 8-oxodG was unchanged in men taking vitamin C (500 mg/day) for 36 months, despite a 91 % increase in plasma total ascorbate [64]. We have shown previously that prolonged supplementation (20 weeks) with a combination of vitamin C (100 mg/day), vitamin E (280 mg/day) and B-carotene (25 mg/day) significantly decreased endogenous oxidised pyrimidines and oxidant-induced DNA strand breakage in lymphocytes from men aged 50–59 years [36]. Subjects in the present study would have consumed approx. 670 mg of vitamin C per day (from the cranberry

juice), considerably more than in the previous study (100 mg/day), suggesting that genoprotection in the early study resulted from increased intake of lipid- rather that the water-soluble antioxidants. However, in the present study, baseline plasma vitamin C levels were significantly higher compared with that recorded for male subjects previously [approx, 63 μM, vs. 29 μM in smokers and 37 μM in non-smokers] and it is conceivable that increasing vitamin C in volunteers with a relatively low endogenous antioxidant level, might offer significant protection.

Discrepancies described for the effects of vitamin C on plasma antioxidant capacity and biomarkers of oxidative damage may reflect different blood sampling regimes employed in the intervention studies. Vitamin C is highly water-soluble and fasted morning samples processed several hours following the last vitamin C dose may not show elevated antioxidant capacity above basal levels, as described here. An increase in plasma antioxidant capacity may be detected by blood sampling earlier post-supplementation, as previously reported [28]. In any case, increasing vitamin C intake through consumption of cranberry juice over a period of weeks did not alter markers of oxidative damage lipid or DNA damage in this study. We have also reported that endogenous and induced oxidative damage is decreased in human volunteers in response to short-term intervention with nutritionally-relevant concentrations of flavonoids [65, 66].

In conclusion, we report here that short-term supplementation with cranberry juice did not influence several biomarkers of blood lipid status and had no cytoprotective effect on either basal or induced oxidative DNA damage in a group of young, healthy volunteers. These results show the importance of taking bioavailability and metabolism into consideration when distinguishing between the *in vitro* and *in vivo* antioxidant activities of dietary anthocyanins in relation to human health.

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