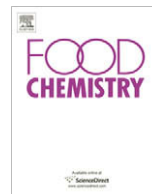




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## Berry juices, teas, antioxidants and the prevention of atherosclerosis in hamsters

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### ABSTRACT

The effects of raspberry, strawberry and bilberry juices and green and black tea on early atherosclerosis in hamsters were investigated. They received an atherogenic diet and at the same time either a juice or a tea at a daily dose corresponding to the consumption of 275 ml by a 70 kg human. After 12 weeks berry juices and teas inhibited aortic lipid deposition by 79–96% and triggered reduced activity of hepatic antioxidant enzymes, not accompanied by lowered plasma cholesterol. These findings suggest that moderate consumption of berry juices and teas can help prevent the development of early atherosclerosis. There were substantial differences between the five beverages in terms of composition and concentration of individual phenolic compounds that were present. This indicates that anti-atherosclerotic effects can be induced by a diversity of phenolic compounds rather than a few specific components. The possible mechanisms by which this is brought about are discussed.

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### 1. Introduction

The postulated involvement of lipid peroxidation in atherogenesis invoked intensive research on antioxidants. Consumption of fruits and vegetables has been linked with lower prevalence of coronary heart disease (Bazzano, 2006; Dauchet, Amouyel, Hercberg, & Dallongeville, 2006; Feldman, 2001; Liu et al., 2000). Drinking tea has also been linked with reduced mortality arising from cardiovascular disease (Kuriyama et al., 2006), although some epidemiological data are inconclusive (Yang & Landau, 2000). Fruits, vegetables and teas contain a wide range of antioxidant compounds, including phenolic compounds and vitamins. Phenolic compounds, such as anthocyanins, flavan-3-ols, flavonols, hydroxycinnamates and tannins, are widespread in fruits and vegetables, with especially high quantities being found in berries and teas. Berries are rich in anthocyanins and can also contain substantial quantities of ellagitannins, while flavan-3-ols and their related derivatives predominate in teas (Crozier, Jaganath, Marks, Saltmarsh, & Clifford, 2006).

Golden Syrian hamsters represent a useful test system because when fed a fat-rich diet they develop dyslipidemia and atherosclerotic plaques, similar in many respects to human atheroma (Auger et al., 2002).

A relatively straight-forward way to evaluate influences on atherosclerosis progression in animal models is to measure the extent of fatty streak development, the continuous accumulation of lipids (due mainly to large accumulations of macrophages) in the sub-endothelial space. Using this approach, we have evaluated the effects of raspberry, strawberry and bilberry juices and green and black tea, sources of potentially anti-atherogenic phenolic compounds, by feeding the beverages to golden Syrian hamsters on a high fat diet for a 12-week period.

### 2. Materials and methods

#### 2.1. Chemicals

5-O-Caffeoylquinic acid, procyanidin B2, (–)-epicatechin (+)-catechin, (–)-gallocatechin, (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin, (–)-epigallocatechin gallate, gallic acid, caffeine, theobromine, theaflavins and ellagic acid were purchased from Sigma–Aldrich (Poole, UK). Quercetin, myricetin, quercetin-3-O-rutinoside, quercetin-3-O-glucoside, quercetin-3-O-arabinoside, quercetin-3-O-galactoside, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, caffeic acid and *p*-coumaric acid were obtained from AASC Ltd. (Southampton, UK). Cyanidin-3-O-glucoside was purchased from Extrasynthese (Genay, France). Methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, Peebleshire, UK). Formic acid was obtained from Fisher Scientific (Loughborough, UK).

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## 2.2. Berries juices and teas

Bouvrage raspberry (*Rubus idaeus* L.; 1 ml = 0.6 g berries), bilberry (*Vaccinium myrtillus*; 1 ml = 0.32 g berries) and strawberry juices (*Fragaria ananassa*; 1 ml = 0.22 g berries) drinks were obtained from Ella Drinks Ltd. (Alloa, Clackmannanshire, UK). The green and black teas (The Tetley Group, Greenford, Middlesex, UK) were prepared by adding 300 ml of boiling water to 3 g of leaves. After brewing for 3 min with continuous stirring, tea solids were removed by filtration through a sieve and the resulting tea was allowed to cool before preparation of aliquots which were stored at  $-20^{\circ}\text{C}$  prior to use.

## 2.3. HPLC–PDA–MS<sup>2</sup> analysis of berry juices and teas

Berry juices and teas were analysed on a Surveyor HPLC system comprising of a HPLC pump, photodiode array detector (PDA) scanning from 250 to 700 nm, and an autosampler set at  $4^{\circ}\text{C}$  (Thermo Finnigan, San Jose, USA) with the separation carried out using a  $250 \times 4.6$  mm i.d.  $4 \mu\text{m}$  Synergi RP-Max column (Phenomenex, Macclesfield, UK) eluted at a flow rate of 1 ml/min. A mobile phase consisting of a 5–40% gradient over 60 min of acetonitrile in 0.1% formic acid was used for the analysis of all samples. 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 Deca XP ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, USA). Analysis was carried out using full scan mode from 100 to 2000 amu, with data dependent tandem MS (MS<sup>2</sup>) scanning, in both negative and positive ion mode.

A combination of co-chromatography with authentic standards, where available, absorbance spectra and mass spectra, using MS<sup>2</sup>, were used to confirm the identity of compounds previously reported in the literature (Stewart, Mullen, & Crozier, 2005). Quantitative estimates are based on calibrations generated by the PDA detector using the compound under study when a standard was available – see Chemicals. When this was not possible, a closely related derivative was used instead. For instance, all anthocyanins, were quantified by reference to cyanidin-3-O-glucoside, while chlorogenic acids, such as 3-O-p-coumaroylquinic acid were quantified by reference to the appropriate aglycone. In all instances the standard curve of reference compounds ranged from 2 to 500 ng.

The thearubigin content of the black tea was estimated as described by Stewart et al. (2005).

## 2.4. Animals, diets and experimental design

Sixty weanling male Syrian golden hamsters (Elevage Janvier, Le Genest-St-Isle, France) weighing ca. 100 g were maintained in plastic cages in a temperature controlled environment ( $23 \pm 1^{\circ}\text{C}$ ) subjected to a 12-h light/dark cycle and allowed free access to both food and water. Hamsters were handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines (National Research Council, 1985).

They were randomly assigned to six groups of 10 not statistically different for weight. For 12 weeks all the hamsters were fed a semi-purified atherogenic diet (Scientific Animal Food and Engineering, Augy, France) consisting of casein (200 g/kg), L-methionine (3 g/kg), corn starch (393 g/kg), sucrose (154 g/kg), cellulose (50 g/kg), lard (150 g/kg), and cholesterol (5 g/kg) (Auger et al., 2002). The diet also contained vitamin (10 g/kg) and mineral mixes (35 g/kg). It was formulated according to AIN-93 guidelines (Reeves, Nielsen, & Fahey, 1993) and was devoid of selenium, vitamin C and vitamin E. During the 12-week period, the hamsters received either water (control), raspberry juice, strawberry juice, bilberry juice, green tea or black tea daily by gavage. The volume of solutions fed was adjusted daily to the weight of hamsters. The calcu-

lation is based on a consumption of 275 ml/day for a 70 kg human as based on the US Food and Drug Administration Center for Drug Evaluation and Research dose calculator (<http://www.fda.gov/cder/cancer/animalframe.htm>).

## 2.5. Analytical procedures

At the end of the 12-week experimental period the hamsters were fasted overnight and blood was drawn by cardiac puncture under anesthesia. Plasma was prepared by centrifugation at 2000g for 10 min at  $4^{\circ}\text{C}$ , and then stored at  $-80^{\circ}\text{C}$  before analysis. The liver was perfused with saline to remove residual blood, rapidly excised, rinsed in ice cold saline, blotted dry, weighted, sectioned for analyses and stored in liquid nitrogen. The aortic tissues were then processed as described below.

Following blood collection and liver removal, the intact aorta was first perfused with phosphate buffered saline containing 1 mmol/l  $\text{CaCl}_2$  and 15 mmol/l glucose for 5 min, then with 0.1 mmol/l sodium cacodylate buffer pH 7.4 containing 2.5 mmol/l  $\text{CaCl}_2$ , 2.5% paraformaldehyde and 1.5% glutaraldehyde for the fixation of the vasculature. The aorta was carefully dissected and processed as previously described (Auger et al., 2002), lipids being stained in Oil red O. An image acquisition and analysis system (ImageJ, Scion Corporation, Frederick, MD) incorporated in an Olympus microscope was used to capture and analyse the total Oil Red O stained area of each aortic arch. The area covered by foam cells (aortic fatty streak area or AFSA) was expressed as a percentage of the total area.

Plasma total cholesterol (TC) and HDL cholesterol (HDL-C) were determined by commercially available enzymatic methods (respectively Nos. CH 200 and CH 203, Randox Laboratories Ltd., Crumlin, UK) on a Pentra 400 automated analyser (HORIBA ABX, Montpellier, France). Plasma very low- + low-density lipoprotein cholesterol (referred to as « non-HDL-C in the data tables) was precipitated with phosphotungstate reagent and HDL-C was measured in the supernatant.

The liver was homogenised in 5 vol of 0.15 M KCl buffer (pH 7.4) and the homogenate was spun at 13,000g for 15 min at  $4^{\circ}\text{C}$ . The supernatant was stored at  $-80^{\circ}\text{C}$  prior to the assay of glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activity on a Pentra 400 analyser. GSHPx activity was measured by the method of Randox (Randox Laboratories Ltd., Crumlin, UK) using a commercial kit (Ransel, No. RS505). Superoxide dismutase (SOD) activity was determined using a Randox kit (Ransod, No. SD 125).

To extract and analyse livers by HPLC–PDA–MS<sup>2</sup>, the livers from two hamsters from each feeding group were combined and homogenised in 2 ml of methanol/water (v/v) containing 5% formic acid and 20 mM sodium diethyldithiocarbamate using an Ultraturax homogeniser. The resultant homogenate was shaken continuously for 30 min before being centrifuged at 2000g for 20 min. The supernatant was decanted and the pellet re-extracted twice. The three supernatants were combined and reduced to dryness *in vacuo*. The extract was dissolved in 25  $\mu\text{l}$  methanol in 475  $\mu\text{l}$  aqueous 1% formic acid and loaded onto an activated 2 g Sep–Pak C<sub>18</sub> cartridge (Waters, Milford, MA, USA) which was washed with 4 ml acidified water (pH 3.0) before elution with 4 ml methanol containing 1% formic acid. The methanolic eluates were reduced to dryness and resuspended in 50  $\mu\text{l}$  methanol in 950  $\mu\text{l}$  aqueous 1% formic acid before analysis for anthocyanin and flavan-3-ol metabolites by HPLC–PDA–MS<sup>2</sup> using single and selected ion monitoring.

## 2.6. Statistical analyses

Data are shown as the means  $\pm$  SEM,  $n = 10$  measurements/group. Tea and berry juices samples were analysed in triplicate.

Statistical analysis of the data was carried out using the Stat View IV software (Abacus Concepts, Berkeley, CA, USA) by one-way ANOVA followed by Fisher's Protected Least Significant Difference test. Differences were considered significant at  $p < 0.05$ .

### 3. Results

#### 3.1. Phenolic compounds in berry juices and teas

Twenty seven phenolic compounds were detected in the bilberry juice (Table 1) with the 13 anthocyanins comprising 599 nmoles/ml of a total flavonoid and phenolic content of 744 nmoles/ml. The juice also contained 76 nmoles/ml of gallic acid and smaller quantities of flavan-3-ols and a number of flavonols in low concentrations. The major components in the raspberry juice were anthocyanins (164 nmoles/ml), principally cyanidin-3-sophoroside and cyanidin-3- $2^G$ -glucosylrutinoside, and the ellagitannins, lambertianin C and sanguin H-6 (Table 2). The strawberry juice contained much lower overall levels of flavonoids and phenolics, 181 nmoles/ml (Table 3), than the bilberry and raspberry juices. The main constituents were pelargonidin-3-glucoside (91 nmoles/l) and a *p*-coumaric acid hexose conjugate (46 nmoles/l) (Table 3).

The compositions of the two teas are summarised in Table 4. Both green and black tea contained higher amounts of phenolic compounds than the juices, with 2894 and 2285 nmoles/ml, respectively for green and black tea. The main green tea constituents were catechins which comprised a group of eight flavan-3-ols, accounting for 2414 nmoles/ml with the major component being (–)-epigallocatechin (921 nmoles/ml). Black tea contained much lower concentrations of catechins (52 nmoles/ml) than green tea, but a large amount of theaflavins and thearubigins (1839 nmoles/ml) that were not present in green tea. Both teas also contained

**Table 1**

Concentration of phenolic compounds in bilberry juice. Data expressed as nmoles/ml  $\pm$  SEM ( $n = 3$ ).

Compound	Concentration
Gallic acid	76 $\pm$ 1
5-Caffeoylquinic acid	22 $\pm$ 1
Caffeic acid hexoside	12 $\pm$ 0
Total gallic and caffeic acid derivatives	110
Delphinidin-3-galactoside	38 $\pm$ 2
Delphinidin-3-glucoside	75 $\pm$ 2
Delphinidin-3-arabinoside	45 $\pm$ 0
Cyanidin-3-galactoside	32 $\pm$ 1
Cyanidin-3-glucoside	120 $\pm$ 1
Cyanidin-3-arabinoside	46 $\pm$ 1
Petunidin-3-glucoside	64 $\pm$ 1
Petunidin-3-arabinoside	12 $\pm$ 0
Peonidin-3-galactoside	4.8 $\pm$ 0.1
Peonidin-3-glucoside	45 $\pm$ 1
Malvidin-3-galactoside	31 $\pm$ 1
Malvidin-3-glucoside	74 $\pm$ 1
Malvidin-3-arabinoside	12 $\pm$ 1
Total anthocyanins	599
(–)-Epicatechin	8.1 $\pm$ 0.2
Procyanidin dimer	7.4 $\pm$ 0.5
Procyanidin trimer	3.2 $\pm$ 0.1
Total flavan-3-ols	19
Myricetin-3-galactoside	1.6 $\pm$ 0.0
Myricetin-3-glucoside	2.6 $\pm$ 0.1
Myricetin-3-glucuronide	1.1 $\pm$ 0.0
Quercetin-3-galactoside	2.9 $\pm$ 0.0
Quercetin-3-glucoside	1.5 $\pm$ 0.0
Quercetin-3-glucuronide	3.6 $\pm$ 0.1
Myricetin	1.2 $\pm$ 0.0
Quercetin	1.2 $\pm$ 0.0
Total flavonols	16
Total phenolics and flavonoids	744

broadly similar levels of chlorogenic acids and a diverse array of flavonols.

#### 3.2. Effects of berry juices and teas on fatty steak deposits

Fig. 1A shows the effects of bilberry, raspberry and strawberry juice consumption on aortic fatty streak deposits in hamsters fed a high-fat diet for 12 weeks. In the control animals, which ingested water rather than juice, fatty streaks covered 21.2  $\pm$  2.7% of the aortic wall. The extent of these deposits when juices or teas were administered to hamsters, were dramatically and significantly lower with respect to controls (all  $p < 0.001$  when compared to water control). The aortic fatty streak area was 4.5  $\pm$  0.5% for the group fed with bilberry juice, 2.4  $\pm$  0.5% with strawberry juice, 1.1  $\pm$  0.2% with raspberry juice and finally 0.75  $\pm$  0.13% and 1.40  $\pm$  0.31% for green and black tea, respectively. Representative pictures of fatty streak deposits are presented in Fig. 2.

**Table 2**

Concentration of phenolic compounds in raspberry juice. Data expressed as nmoles/ml  $\pm$  SEM ( $n = 3$ ).

Compound	Concentration
Cyanidin-3-sophoroside	108 $\pm$ 1
Cyanidin-3-( $2^G$ -glucosylrutinoside)	32 $\pm$ 1
Cyanidin-3-glucoside	12 $\pm$ 0
Pelargonidin-3-sophoroside	4.1 $\pm$ 0.1
Cyanidin-3-rutinoside	5.6 $\pm$ 0.1
Pelargonidin-3-( $2^G$ -glucosylrutinoside)	2.0 $\pm$ 0.1
Total anthocyanins	164
Procyanidin dimer B4	1.5 $\pm$ 0.1
Total flavan-3-ols	1.5
Sanguin H-10	46 $\pm$ 1
Lambertianin C	59 $\pm$ 2
Sanguin H-6	235 $\pm$ 6
Ellagic acid	16 $\pm$ 0.1
Ellagic acid-4-acetylptose	0.9 $\pm$ 0.0
Total hydrolysable tannins and ellagic acid derivatives	357
Quercetin-3-hexosyl-rhamnoside	0.2 $\pm$ 0.1
Quercetin-3-galactosylrhamnoside	0.6 $\pm$ 0.0
Quercetin-3-rutinoside	0.2 $\pm$ 0.0
Quercetin-3-galactoside	2.2 $\pm$ 0.1
Quercetin-3-glucoside	0.7 $\pm$ 0.0
Quercetin-3-glucuronide	2.4 $\pm$ 0.1
Quercetin	0.5 $\pm$ 0.0
Total flavonols	7.7
Total phenolics and flavonoids	530

**Table 3**

Concentration of phenolic compounds in strawberry juice. Data expressed as nmoles/ml  $\pm$  SEM ( $n = 3$ ).

Compound	Concentration
<i>p</i> -Coumaric acid-hexose	46 $\pm$ 1
Total hydroxycinnamates	46
Pelargonidin-3-glucoside	91 $\pm$ 1
Pelargonidin-3-(6-malonylglucoside)	22 $\pm$ 0
Total anthocyanins	113
Procyanidin dimer B1	0.9 $\pm$ 0.0
Procyanidin dimer B3	4.9 $\pm$ 0.1
Procyanidin trimer	2.8 $\pm$ 0.0
Total flavan-3-ols	8.6
Sanguin	9.9 $\pm$ 0.2
Ellagic acid rhamnoside	2.0 $\pm$ 0.0
Total hydrolysable tannins and ellagic acid derivatives	12
Quercetin-3-glucuronide	0.9 $\pm$ 0.1
Kaempferol-3-glucoside	0.3 $\pm$ 0.0
Kaempferol-malonyl-hexoside	0.7 $\pm$ 0.0
Total flavonols	1.9
Total phenolics and flavonoids	181

**Table 4**  
Concentration of phenolic compounds, and purine alkaloids in green and black tea infusions. Data expressed as nmoles/ml  $\pm$  SEM ( $n = 3$ ).

Compound	Green tea	Black tea
Gallic acid	6.3 $\pm$ 0.2	132 $\pm$ 7
5-Galloylquinic acid	64 $\pm$ 1	77 $\pm$ 1
Total gallic acid derivatives	70	209
(-)-Gallocatechin	225 $\pm$ 2	n.d.
(-)-Epigallocatechin	921 $\pm$ 11	19 $\pm$ 1
(+)-Catechin	168 $\pm$ 6	7.4 $\pm$ 0.2
(-)-Epicatechin	459 $\pm$ 10	6.8 $\pm$ 0.1
(-)-Epigallocatechin-3-gallate	494 $\pm$ 25	7.4 $\pm$ 0
(-)-Epicatechin-3-gallate	147 $\pm$ 5	11 $\pm$ 0
Total flavan-3-ols	2,414	52
3-Caffeoylquinic acid	30 $\pm$ 1	5.0 $\pm$ 0.2
5-Caffeoylquinic acid	118 $\pm$ 1	32 $\pm$ 0.1
4- <i>p</i> -Coumaroylquinic acid	85 $\pm$ 2	76 $\pm$ 1
Total caffeic and coumaric acid derivatives	233	113
Quercetin-rhamnosylgalactoside	4.5 $\pm$ 0.2	3.6 $\pm$ 0.1
Quercetin-3-rutinoside	39 $\pm$ 1	29 $\pm$ 1
Quercetin-3-galactoside	46 $\pm$ 1	29 $\pm$ 1
Quercetin-rhamnose-hexose-rhamnose	7.2 $\pm$ 0.2	5.9 $\pm$ 0
Quercetin-3-glucoside	72 $\pm$ 1	46 $\pm$ 1
Kaempferol-rhamnose-hexose-rhamnose	7.7 $\pm$ 0	7.4 $\pm$ 0.2
Kaempferol-3-galactoside	17 $\pm$ 1	12 $\pm$ 1
Kaempferol-3-rutinoside	21 $\pm$ 1	18 $\pm$ 1
Kaempferol-3-glucoside	41 $\pm$ 0	28 $\pm$ 2
Kaempferol-3-arabinoside	2.0 $\pm$ 0.1	n.d.
Unknown quercetin conjugate	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1
Unknown quercetin conjugate	6.7 $\pm$ 0.2	4.9 $\pm$ 0.2
Unknown kaempferol conjugate	2.0 $\pm$ 0	n.d.
Unknown kaempferol conjugate	0.4 $\pm$ 0.0	10 $\pm$ 0.0
Total flavonols	267	185
Theaflavin	n.d.	20 $\pm$ 1
Theaflavin-3-gallate	n.d.	16 $\pm$ 1
Theaflavin-3'-gallate	n.d.	8.8 $\pm$ 0.1
Theaflavin-3,3'-digallate	n.d.	13 $\pm$ 0
Thearubigins	n.d.	1781
Total theaflavins and thearubigins	n.d.	1839
Total phenolics and flavonoids	2984	2285
Theobromine	57 $\pm$ 1	25 $\pm$ 1
Caffeine	804 $\pm$ 15	503 $\pm$ 3
Total purine alkaloids	861	528

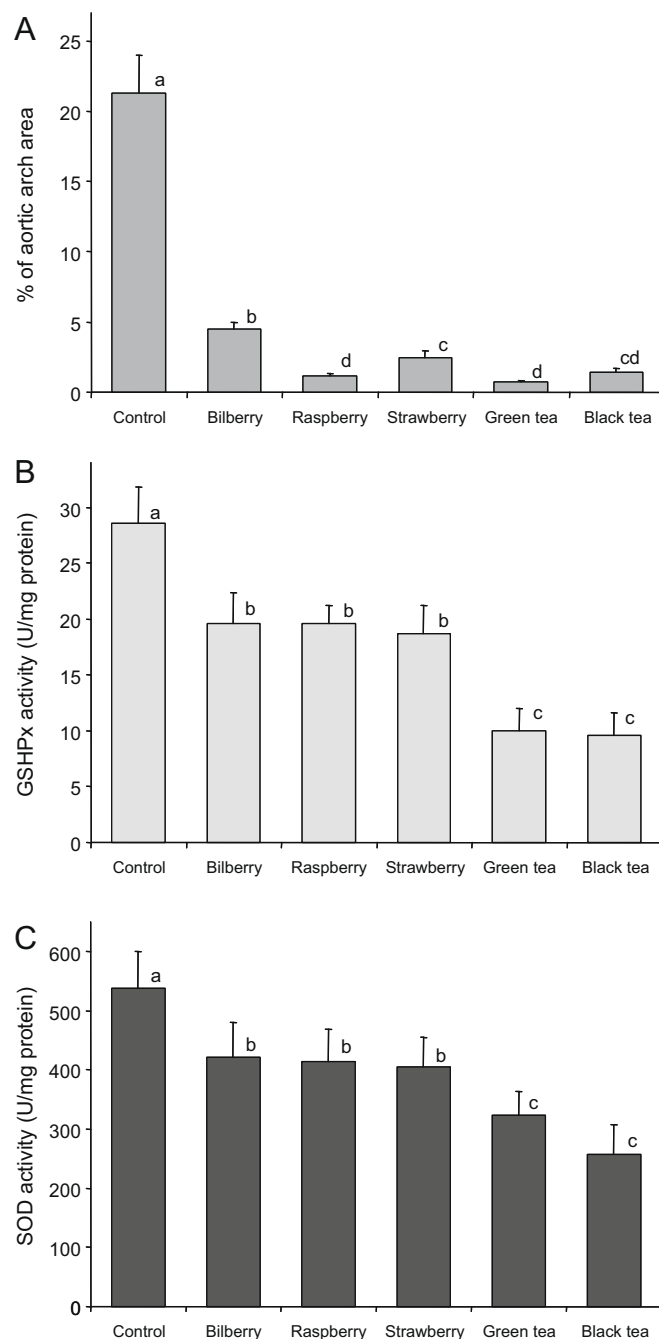
n.d. – not detected.

### 3.3. Effects of berry juices and teas on circulating cholesterol and hepatic antioxidant enzymes

The lower fatty streak deposition in juice and tea hamster groups was not accompanied by lower circulating cholesterol levels (total cholesterol, HDL-cholesterol and non-HDL-cholesterol were not significantly different between all groups; not shown here) but was associated to a reduced activity of liver antioxidant defense system in hamsters fed antioxidant rich beverages in comparison to controls (Fig. 1B and C). Teas induced a greater inhibition in the antioxidant enzymes compared to berry juices. Analysis of liver extracts by HPLC-mass spectrometry operating in selected ion and selected reaction monitoring mode did not detect the presence of any flavonoids or phenolic compounds derived from the teas or berry juices despite the hamsters being fed the supplements for a period of 12 weeks.

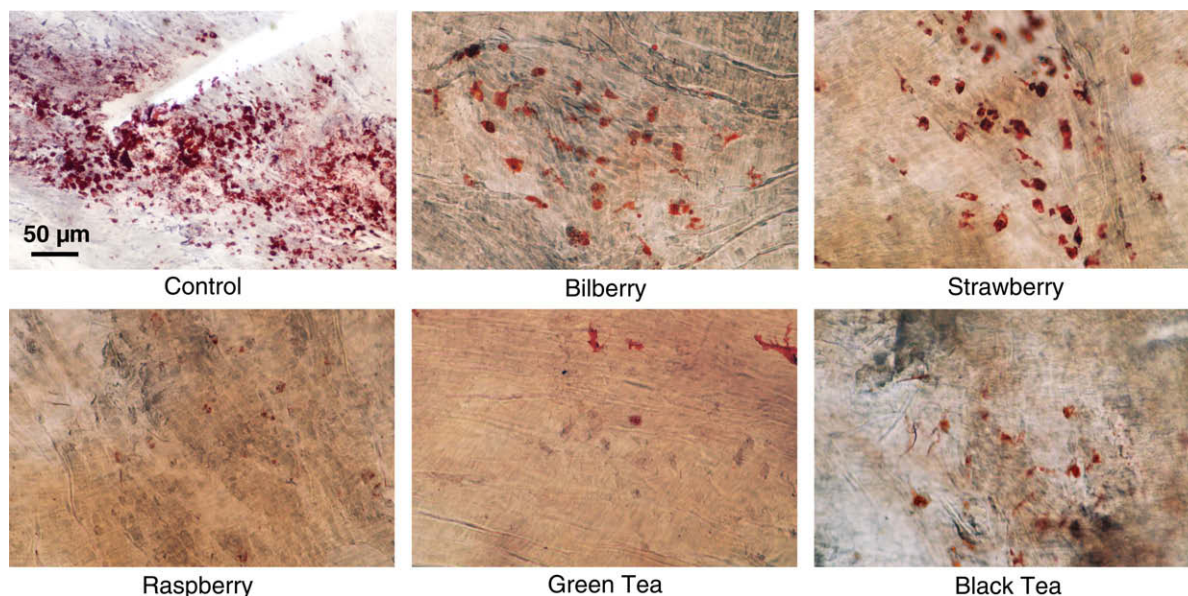
## 4. Discussion

Daily consumption of each of the test beverages for a 12-week period resulted in a substantially lower fatty streak deposition in the arteries of the hamsters compared to water-treated controls, with stronger effects for green tea and raspberry juice (Figs. 1A and 2). This marked limitation of the onset of atherosclerosis was not associated with any significant change in plasma cholesterol profile.



**Fig. 1.** Effects of dietary treatments on aortic fatty streak area and hepatic antioxidant enzyme activities. Mean values expressed as (A) a percent of aortic arch area  $\pm$  SEM ( $n = 10$ ) for aortic arch area, and as units per mg of hepatic proteins  $\pm$  SEM ( $n = 10$ ) for (B) SOD and (C) GSHPx activity. Bars with different letters are significantly different ( $p < 0.05$ ).

The observation that the plasma cholesterol profile did not change among groups of hamsters (Table 5) is in keeping with the work of Andrews et al. (1995) that demonstrates that absolute cholesterolaemia is not pivotal in determining the aortic fatty streak deposition. These results thus strengthen the hypothesis that oxidation of LDL, more than their plasma level, must be implicated in the pathogenesis of atherosclerosis (Breinholt, Lauridsen, & Dragsted, 1999). This can explain, at least in part, the effects observed on aortic atherosclerosis after antioxidant juices and tea consumption.



**Fig. 2.** Photomicrographs of hamster aortic arches after 12 weeks on an atherogenic diet (control) and 12 weeks on an atherogenic diet supplemented with either strawberry juice, bilberry juice, raspberry juice, green tea or black tea. The micrographs are examples of the aortic arch surface covered by lipid inclusion in the intima with lipids coloured red using Oil Red O stain. Quantifications of fatty streaks are summarised in and Fig. 1A. All micrographs have the same scale.

There are reports that consumption of fruit juice and green tea both increase the activity of hepatic antioxidant enzymes (Lin et al., 1998; Young et al., 1999). This contrasts with the findings of the present study where berry juice and tea consumption lowered hepatic GSHPx and SOD activities (Fig. 1B and C), and agree with previous results in hamsters receiving either pure catechin, quercetin or resveratrol (Auger et al., 2005) or phenolics from purple grape, apple, purple grape juice and apple juice (Décordé, Teissèdre, Auger, Cristol, & Rouanet, 2008). One explanation for this down regulation is that it is a consequence of dietary antioxidants being able to scavenge oxygen radicals and thus reduce the need for enzymatic endogenous antioxidants.

The prevention of fatty streak deposition by berry juice and tea consumption does seemingly involve mechanisms allowing the possibility of phenolic compounds to induce local antioxidant effects which cannot be ruled out. Recent data suggests that dietary phenolics can modulate inflammatory pathways, hence reducing the severity of local inflammation (Rahman, Biswas, & Kirkham, 2006). The pathogenesis of atherosclerosis has been linked to the occurrence of inflammatory processes inside the arterial wall during the initiation of lesions (Call, Deliargyris, & Newby, 2004). Compounds derived from the ingested phenolics could, therefore, delay the progression of atherosclerosis by inhibition of arterial wall inflammation.

Endothelial dysfunction is also associated with the increased production of the vasoconstrictive peptide endothelin-1, which has been linked with chronic inflammation of the arterial wall (Feletou & Vanhoutte, 2006; Schiffrin, 2005). Endothelin is also related with the onset and development of atherosclerosis, with atherosclerotic plaques containing an increased endothelin concentration (Bacon, Cary, & Davenport, 1996). Moreover, endothelin-1 production is induced by oxidised LDL, which in turn can recruit macrophages and monocytes in the arterial wall (Schiffrin, 2001). Endothelin, thus, plays a pivotal role in development of diseases related to vascular function. As demonstrated by Corder et al. (2006), phenolic compounds, principally procyanidins, are able to reduce the production of endothelin-1 by endothelial cells. The green tea polyphenol (–)-epigallocatechin gallate is also reported to reduce endothelin expression (Spinella et al., 2006). Inhibition of endothelin-1 over-expression is, therefore, a further potential

mechanism for the observed protective effects of juice and tea consumption.

Tea antioxidants, in particular catechins, act either as activators or inhibitors of signal transduction kinases interfering with multiple pathways of signal transduction in cardiovascular relevant cells (Stangl, Dreger, Stangl, & Lorenz, 2007). Arguably, this could explain the strong effect of green tea with respect to the other beverages in this study.

The fact that the five beverages, which reduced the onset of atherosclerosis, contained a very different spectrum of phenolics implies that a wide variety of compounds may be bioactive and that the observed preventive effects may be due to the influence of several constituents working either independently or in tandem. What compounds enter the circulatory system, reduce the arterial fatty streaks deposition and the activity of hepatic SOD and GSHPx remains to be determined. Analysis of the livers of hamsters after 12 weeks plus an overnight treatment between last feed and sacrifice showed no trace of either the parent compounds from the beverages or their glucuronyl-, methyl- or sulfo-metabolites, indicating that they do not accumulate in these tissues, at least in detectable quantities.

In conclusion, we have demonstrated that berry juices and teas fed to hamsters under atherogenic diet are able to facilitate a very strong inhibition of aortic fatty streaks deposition. These effects are physiologically relevant as they were induced by a daily supplement equivalent to 275 ml of beverage consumed on a daily base by a 70 kg human. The features and progression of the lesions observed in the hamster model of atherosclerosis are morphologically similar to atheromatous lesions observed in humans. The hamster is therefore considered to be a good animal model to study the formation of atheromas in humans (Yamanouchi et al., 2000). It is also of interest to note while both bilberry and strawberry juices prevent aortic lipid deposition in hamsters, blueberry, a close relative of bilberry, and strawberry extracts both bring about improvements in neuronal function and behaviour in a rodent model of accelerated aging (Shukitt-Hale, Carey, Jenkins, Rabin, & Joseph, 2007).

Carotid atherosclerosis is associated with aortic atherosclerosis (Shimizu et al., 2003) and the intima-media thickness of the common carotid artery has been shown to predict coronary events and

is, therefore, a non-invasive predictor of future ischemic stroke incidence (Chambless, Folsom, Clegg, Sharrett, & Shahar, 2000). Thus, polyphenol-rich berry juices and green and black tea intake may be of significant relevance to clinical and public health.

### Conflict of interest statement

The authors declare that there are no conflicts of interest.

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