Consumption of flavonoids in onions and black tea: lack of effect on F_2-isoprostanes and autoantibodies to oxidized LDL in healthy humans[1–3]

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ABSTRACT

Background: Oxidative damage to lipids in vivo may be involved in the development of atherosclerosis and cancer. Onions and black tea are foods rich in flavonoids, predominantly the flavonoid quercetin, which is a potent in vitro inhibitor of membrane lipid peroxidation and LDL oxidation.

Objective: Our objective was to investigate the effects of consuming a high-flavonoid (HF) diet enriched with onions and black tea on indexes of oxidative damage in vivo compared with a low-flavonoid (LF) diet.

Design: Thirty-two healthy humans were studied in a randomized crossover design. Indexes of oxidative damage used were plasma F_2-isoprostanes (a biomarker of lipid peroxidation in vivo) and the titer of antibodies to malondialdehyde (MDA)-modified LDL.

Results: There were no significant differences in the intake of macronutrients or assessed micronutrients, plasma F_2-isoprostane concentrations, and MDA-LDL autoantibody titer between the HF and LF dietary treatments. In the men, however, plasma concentrations of the F_2-isoprostane 8-*epi*-prostaglandin $F_{2\alpha}$ were slightly higher after the HF treatment phase than after the LF treatment [0.31 ± 0.029 nmol/L (111 ± 10.4 ng/L) compared with 0.26 ± 0.022 nmol/L (92 ± 7.8 ng/L); $P = 0.041$]. In all subjects, plasma quercetin concentrations were significantly higher after the HF treatment phase than after the LF treatment: 221.6 ± 37.4 nmol/L compared with less than the limit of detection of 66.2 nmol/L.

Conclusion: Flavonoid consumption in onions and tea had no significant effect on plasma F_2-isoprostane concentrations and MDA-LDL autoantibody titer in this study and thus does not seem to inhibit lipid peroxidation in humans. *Am J Clin Nutr* 2001;73:1040–4.

KEY WORDS Flavonoid, onions, black tea, antioxidant, lipid peroxidation, F_2-isoprostanes, oxidized-LDL-autoantibody titer

INTRODUCTION

Flavonoids, which include the flavonols quercetin, kaempferol, and myricetin (found in onions and tea), and catechins [eg, epicatechin, epigallocatechin, and epigallocatechin gallate (found in tea)] are polyphenolic compounds found in many foods of plant origin (1–9). In a study of the Dutch diet, tea, onions, and apples appeared to provide some protection from coronary heart disease and this protective effect was thought to be a result of the antioxidant action of the flavonoids (10). Although this effect was not confirmed by subsequent epidemiologic studies, eg, the US male health professionals nonfatal myocardial infarction study (11), a recent study showed tea intake to be associated with a low risk of myocardial infarction (12). There is currently no clear epidemiologic evidence to support the suggestion that flavonoids may have a role in the prevention of cancer (13–15).

Many flavonoids were shown to display antioxidant action in vitro (16–19). Quercetin, in particular, displays potent antioxidant properties in vitro, mostly against oxidative damage to membrane lipids and lipoprotein particles (16, 18) [oxidative damage to LDLs has been implicated in atherogenesis (20, 21)].

Plasma concentrations of F_2-isoprostanes (specific end products of the peroxidation of arachidonic acid residues) by mass spectrometry appear to currently be the biomarker of choice for lipid peroxidation in the human body (22–27). Although wide variations in F_2-isoprostane concentrations were found in the body fluids of healthy subjects, some healthy subjects appear to show higher rates of lipid peroxidation than do other subjects, even when consuming comparable diets (23, 25, 28), and could be at an increased risk of diseases involving lipid peroxidation, eg, atherosclerosis and cancer.

Autoantibodies that have been oxidized to LDL have been identified in serum and are evidence that LDL oxidation occurs in vivo (29). The titer of autoantibodies to malondialdehyde (MDA)-modified LDL was higher in Finnish males with carotid

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atherosclerosis than in age-matched control subjects (30). Furthermore, case control studies have suggested that titer of autoantibodies is a marker of atherosclerotic progression (31–33). In addition, autoantibodies against oxidized LDL were found in patients with type 2 diabetes (34). Measuring these autoantibodies may therefore provide a marker of the extent of LDL oxidation in vivo.

The objective of the present study was to determine the effect of consuming foods, such as onions and black tea, that are rich in flavonoids, predominantly the flavonoid quercetin. We used several different indexes of lipid peroxidation in vivo: plasma F_2-isoprostanes [eg, 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$)] and MDA-LDL autoantibody titre. This objective has not been investigated previously in a randomized crossover study of a high-flavonoid (HF) (using both onions and tea) compared with a low-flavonoid (LF) diet in healthy human subjects.

SUBJECTS AND METHODS

Study design

The study was a randomized crossover study composed of two 14-d treatments of LF or HF diets, with a 14-d washout period between treatments. During the HF dietary treatment period, subjects were asked to consume one 150-g onion cake (containing 89.7 mg quercetin) and one 300-mL cup of black tea (containing 1.4 mg quercetin) daily. During the LF dietary treatment period, subjects were asked to avoid the consumption of specific flavonoid-rich foods (5) and tea and to consume 6 g high-oleic sunflower oil/d (76% 18:1, 14% 18:2n−6), as contained in the 150-g onion cake. Subjects were asked to make no changes to their diets or lifestyle other than those necessary for compliance with the study. During the last 7 d of each dietary treatment phase, subjects were asked to maintain a 7-d food diary. At the end of each treatment phase, venous blood samples were collected from subjects after an overnight fast and height and weight were recorded.

Subjects

Forty-two (20 male, 22 female) healthy, nonsmoking, nonsupplement-taking (including antioxidant supplements) subjects aged 20–60 y were recruited from among the staff and students at King's College London. All subjects had normal hematologic values, liver function, and body mass indexes (BMIs). Written informed consent from subjects and their doctors was obtained. The subjects received a modest financial payment for their participation in the study. The study protocol was reviewed and approved by the King's College London Research Ethics Committee.

Dietary intervention

Onion cakes were prepared from mild Spanish onions, chopped (≈10 × 30 mm), and fried in 1200-g batches for 8–10 min in 45 g high-oleic sunflower oil to which 160 g granulated quick-dried diced onions as added. The mixture was cooled, divided into 150-g portions, compressed to form onion cakes, and frozen at −20°C until consumed. Subjects were instructed to reheat the onion cakes in a microwave oven for 3 min at full power (800 W) to be consumed at any time of the day, either alone or in combination with other foods. Black tea bags (mean weight, 3.3 g) were also provided to the subjects, who were instructed to prepare the tea by infusing one tea bag into 300 mL boiling water for 4 min without the subsequent addition of milk.

Dietary assessment

Subjects were asked to complete a 7-d food diary (35), which included food photographs for the estimation of portion sizes. Subjects were asked to record everything they ate and drank at the time of consumption, to record as much information about foods consumed as possible, and to provide the weights of what they ate when possible; otherwise, subjects were to use standard household measures or the food photographs to estimate portion sizes.

Collection and handling of blood samples

Venous blood samples were collected on day 14 of each treatment period after the subjects had fasted overnight. To measure plasma F_2-isoprostane concentrations, blood samples were collected into 4.5-mL evacuated tubes containing sodium citrate (38 g/L), which were iced and to which indomethacin was added to achieve a final concentration of 15 (mol/L. The blood samples were centrifuged at 1500 × *g* for 10 min at 4°C and plasma was separated. Butylated hydroxytoluene (in 960 mL/L ethanol) was added to the plasma until a final concentration of 20 (mol/L. The plasma samples were snap-frozen with liquid nitrogen and stored at −70°C until analyzed. For plasma quercetin and autoantibody measurements, blood samples were collected into 10-mL evacuated tubes containing EDTA. The blood samples were centrifuged at 1500 × *g* for 10 min at 4°C and plasma was separated and snap-frozen with the use of liquid nitrogen and stored at −70°C until analyzed.

Analytic methods

Quercetin, kaempferol, myricetin, and apigenin were determined quantitatively in the onion cake and tea by the method of Hertog et al (5) as modified according to McAnlis et al (36, 37). Catechin, epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate were determined quantitatively in the aqueous tea extract according to McAnlis et al (36). Plasma quercetin concentrations were measured by HPLC with the use of a modified version of the technique developed by Hollman et al (38) according to McAnlis et al (37). Plasma autoantibodies to MDA-LDL were measured by using an enzyme-linked immunosorbent assay technique according to Woodside et al (39), and the values of autoantibody titer were expressed as a ratio of IgG binding to MDA-LDL compared with native LDL. Total (free and esterified) F_2-isoprostanes were isolated by using a solid-phase extraction procedure and 8-*epi*-$PGF_{2\alpha}$ concentrations were determined by gas chromatography–mass spectrometry according to the method of Nourooz-Zadeh et al (40).

Statistical analysis

Treatment order effects were tested with the use of repeated-measures analysis of variance (version 6; SPSS Inc, Chicago). Paired comparisons were made with the use of a paired *t* test.

RESULTS

Of the 42 subjects recruited, 36 completed both dietary treatments, yet only 32 provided the required samples for the analysis described here. The physical characteristics of these 32 subjects were as follows: a mean age of 30.4 ± 7.3 y, a mean BMI (in kg/m^2) of 23.6 ± 2.7, and a mean plasma cholesterol concentration of 5.2 ± 2.1 mmol/L. In addition, because of cost constraints, 8-*epi* $PGF_{2\alpha}$ concentrations were measured in plasma from only 20 of the subjects (with random sampling). There were no adverse

TABLE 1
Estimated dietary intake of subjects during the last 7 d of each of the low-flavonoid and high-flavonoid dietary treatment periods[1]

	Low-flavonoid diet	High-flavonoid diet
Energy (MJ/d)	10.7 ± 1.1	10.7 ± 1.1
Protein (g/d)	86 ± 18.6	84 ± 23.8
Total fat (g/d)	98.7 ± 25.2	90.9 ± 16.6
SFAs (g/d)	33.7 ± 10.8	33.1 ± 7.4
PUFAs (g/d)	17.6 ± 5.8	15.2 ± 4.4
MUFAs (g/d)	33.2 ± 10.4	28.3 ± 5.8
Cholesterol (mg/d)	242 ± 97.7	229 ± 96.9
Carbohydrate (g/d)	289 ± 52	294 ± 51.9
Alcohol (g/d)	17.6 ± 24.0	16.9 ± 22.7
NSP (g/d)	20.9 ± 6.9	21.6 ± 7.3
Vitamin C (mg/d)	146 ± 70	138 ± 68
Vitamin E (mg/d)	10.1 ± 4.3	9.5 ± 3.0
β-Carotene (mg/d)	1.8 ± 0.95	2.0 ± 1.2
Iron (mg/d)	14.0 ± 2.7	13.5 ± 3.5

[1] $\bar{x}$ ± SD. SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; NSP, nonstarch polysaccharides. There were no significant differences between treatments.

reactions to the dietary treatments of the study, except mild flatulence and malodor associated with onion consumption, reported by approximately one-half of the subjects.

The combined onion cake and tea dietary supplement provided 131 mg flavonoids/d, of which 91.1 mg/d was quercetin (tea and onion cake), 6.1 mg/d was kaempferol (tea and onion cake), 10.6 mg/d was apigenin (onion cake), 0.5 mg/d was myricetin (tea), 9.3 mg/d was epigallocatechin gallate (tea), 5.1 mg/d was epicatechin gallate (tea), 4.8 mg/d was epigallocatechin (tea), 2.5 mg/d was epicatechin (tea), and 0.6 mg /d was catechin (tea). The dietary intakes of the subjects are shown in **Table 1**. There were no significant differences in dietary intakes between the HF and LF dietary treatment periods.

The plasma 8-*epi*-$PGF_{2\alpha}$ concentrations, MDA-LDL autoantibody titer, and quercetin concentrations after the HF and LF treatment periods are shown in **Table 2**. Plasma quercetin concentrations were significantly higher at the end of the HF treatment than at the end of the LF treatment (221.6 ± 37.4 nmol/L compared with less than the limit of detection of 66.2 nmol/L). Plasma 8-*epi*-$PGF_{2\alpha}$ concentrations and MDA-LDL autoantibody titer did not differ significantly between treatments. In the men, however, plasma concentrations of 8-*epi*-$PGF_{2\alpha}$ were slightly higher after the HF treatment period than after the LF treatment period [0.31 ± 0.029 nmol/L (111 ± 10.4 ng/L) compared with 0.26 ± 0.022 nmol/L (92 ± 7.8 ng/L); $P = 0.041$].

DISCUSSION

There has been much interest in the beneficial effects of dietary flavonoids on well being. This is due in part to their potent antioxidant activity in vitro (17). Additionally, epidemiologic evidence has shown a relation between dietary intake of flavonoids and reduced risk of coronary heart disease (10, 12), but not cancer (13).

In the present study, which provided 131 mg flavonoids/d from tea and onions (91 mg quercetin/d), no significant differences were found in plasma F_2-isoprostane concentrations or MDA-LDL autoantibody titer between the LF and HF dietary treatment periods, even though the intake of dietary flavonoids during the HF period was ≈5 times the estimated habitual intake in the Netherlands (6) and Denmark (41) and nearly 3 times the mean intake associated with reduced risk of coronary heart disease (10).

Overall, no significant differences were found in plasma F_2-isoprostane concentrations between the LF and HF dietary treatment periods. The sample size was large enough to identify a difference of biological importance; the study had >95% power to detect a concentration change of 20% ($P < 0.05$). Furthermore, the 14-d treatment period was probably long enough in duration to note changes had there been any because we recently showed a significant decrease in F_2-isoprostane concentrations after the consumption of soy isoflavone phytoestrogens for 14 d (42).

In the men in the present study, however, plasma 8-*epi* $PGF_{2\alpha}$ concentrations were slightly higher after the HF treatment phase compared with the LF treatment phase [0.31 ± 0.029 nmol/L (111 ± 10.4 ng/L) compared with 0.26 ± 0.022 nmol/L (92 ± 7.8 ng/L); $P = 0.041$]. This finding is opposite to what was expected, and although it may be explained by the prooxidant effects that flavonoids can display (43) or possibly because males could be more susceptible to such an increase because differences in hormonal status (eg, they mostly lack the antioxidant female hormone estrogen (44)), it is currently unclear whether this is an actual phenomenon. This finding suggests that further studies in males are warranted, although it is uncertain whether this elevation in plasma 8-*epi* $PGF_{2\alpha}$ concentrations would be observed again. Measurements of plasma F_2-isoprostane concentrations do not appear to have been made in other published studies of flavonoid consumption in onions and tea; however, in heavy smokers, plasma F_2-isoprostane concentrations were ≈2-fold higher than those in age and sex-matched control subjects (32). Furthermore, consumption of quercetin-rich black currant and apple juice (1500 mL) was shown to have a prooxidant effect, increasing oxidative protein damage (measured as an increase in plasma 2-amino-adipic semialdehyde residues) (43). This may be explained by the relatively high iron content of black currants because some flavonoids are known to display prooxidant effects in the presence of iron or copper (45).

Our finding of no significant difference in plasma MDA-LDL autoantibody titer between the LF and HF dietary treatment periods suggests that the concentration of oxidized LDL in vivo was not altered by the different dietary treatments. Furthermore, the titer of autoantibodies from healthy subjects may reflect minimal concentrations that cannot be lowered by dietary intervention. Indeed, there are currently no studies that show a decrease in autoantibody titer in healthy persons because of drug or dietary intervention. In addition, many case-control studies showed no difference in autoantibody titer between healthy individuals and patients with extensive atherosclerosis (46, 47).

TABLE 2
Plasma 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*-$PGF_{2\alpha}$), malondialdehyde-modified (MDA)-LDL autoantibody titer, and quercetin concentrations after low-flavonoid and high-flavonoid dietary treatment periods[1]

	Low-flavonoid diet	High-flavonoid diet
8-*epi*-$PGF_{2\alpha}$		
(ng/L)	107 ± 8.4	117 ± 9.7
(nmol/L)	0.30 ± 0.024	0.33 ± 0.027
MDA-LDL autoantibody titer	1.34 ± 0.02	1.33 ± 0.02
Quercetin (nmol/L)	<LOD ± —	221.6[2] ± 37.4

[1] $\bar{x}$ ± SEM. $n = 32$ except for 8-*epi*-$PGF_{2\alpha}$, $n = 20$. LOD, limit of detection.
[2] Significantly different from low-flavonoid diet, $P < 0.01$ (paired t test).

Therefore, it is uncertain whether different results would have been found had we studied patients with cardiovascular disease in addition to healthy subjects.

Although we failed to find an effect of dietary flavonoid consumption on plasma F_2-isoprostane concentrations and MDA-LDL autoantibody titer in the present study, this does not appear to reflect a lack of absorption and subsequent bioavailability (3, 4). Flavonoids from onions and tea are absorbed by the body, both as aglycones and more readily as glycosides, the predominant form in food (3). For example, in one study the consumption of 13 mg quercetin/d from onions resulted in plasma quercetin concentrations of 73 nmol/L (48). Furthermore, after the acute ingestion of 50 mg quercetin aglycone in fried onions, plasma quercetin increased from 94 nmol/L at baseline to 822 nmol/L after 2 h, decreasing to baseline after 24 h (37). In the current study, fasting plasma concentrations of quercetin after the LF diet were lower than the limit of detection of 66.2 nmol/L but were 221.6 ± 37.4 nmol/L after the HF diet. However, a measure of plasma antioxidant capacity would have been useful in this study because it might have shown whether enough antioxidants were absorbed to expect changes in antioxidant status. In conclusion, we failed to find an effect of flavonoid intake (predominantly quercetin) from onions and tea on plasma F_2-isoprostane concentrations and MDA-LDL autoantibody titer in healthy humans.

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