

Effect of dietary quercetin on oxidative DNA damage in healthy human subjects

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The effect of dietary intake of flavonols (predominantly quercetin) on oxidative DNA damage was studied in thirty-six healthy human subjects (sixteen men, twenty women). The study was a randomised crossover study, comprising two 14 d treatments of either a low-flavonol (LF) or high-flavonol (HF) diet with a 14 d wash-out period between treatments. Subjects were asked to avoid foods containing flavonols, flavones and flavanols during the LF dietary treatment period and to consume one 150 g onion (*Allium cepa*) cake (containing 89.7 mg quercetin) and one 300 ml cup of black tea (containing 1.4 mg quercetin) daily during the HF dietary treatment. A 7 d food diary was kept during each dietary period and blood samples were taken after each dietary treatment. Products of oxidative damage to DNA bases were measured in DNA from leucocytes. The study had more than 95 % power to detect a change of 20 % in DNA damage products ($P < 0.05$). Plasma vitamin C and plasma quercetin concentrations were also measured. No significant differences in intake of macronutrients or assessed micronutrients, measured DNA base damage products, or plasma vitamin C were found between the HF and LF dietary treatments. The plasma quercetin concentration was significantly higher after the HF dietary treatment period (228.5 (SEM 34.7) nmol/l) than after the LF dietary treatment period (less than the limit of detection, i.e. <66.2 nmol/l). These findings do not support the hypothesis that dietary quercetin intake substantially affects oxidative DNA damage in leucocytes.

Flavonols: Quercetin: Onions: Black tea: Oxidative DNA damage

Flavonoids are a group of more than 4000 polyphenolic compounds found naturally in many foods of plant origin (Cook & Saman, 1996; Hertog & Hollman, 1996; Wiseman, 1998). This group includes: flavonols such as quercetin found in onions (*Allium cepa*) and to a lesser extent in French beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), apples, apricots, cherries, grapes, wine, fruit juices, black tea and green tea (Hertog *et al.* 1992, 1993*b,c*; Crozier *et al.* 1997; McDonald *et al.* 1998); flavanols (or catechins including catechin, epicatechin, epigallocatechin and epigallocatechin gallate) found in tea; flavones such as apigenin; flavanones; anthocyanidins (Cook & Saman,

1996; Hollman, 1997; Wiseman, 1998), and the isoflavones genistein and daidzein found in soyabean products (Reinli & Block, 1996; Bingham *et al.* 1998; Wiseman, 1998, 1999).

Many of these compounds have been shown to display antioxidant action *in vitro* (de Whalley *et al.* 1990; Rice-Evans *et al.* 1996; O'Reilly *et al.* 1997; Wiseman *et al.* 1998), with quercetin being one of the most potent, depending on the assay used. In a study of the Dutch diet, Hertog *et al.* (1993*a*) found that tea, onions and apples appeared to provide some protection from CHD and this protective effect was attributed to the antioxidant activity

Abbreviations: LF, low-flavonol diet; HF, high-flavonol diet.

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of the constituent flavonols and flavones. However, this protective effect has not been confirmed by subsequent epidemiological studies, such as the male health professional non-fatal myocardial infarction study conducted in the USA (Rimm *et al.* 1996). The ability of flavonols to protect rodents against chemically induced carcinogenesis (Verma *et al.* 1988), and to induce chemo-protective phase II enzymes (Uda *et al.* 1997) is part of the evidence that has led to the proposal that flavonols have a role in the prevention of cancer. To date, there has been little epidemiological evidence to support this proposal (Hertog *et al.* 1994, 1995, 1996; Hertog & Hollman, 1996), although in a number of case-control studies flavonol-rich foods such as onions have been associated with reduced risk of stomach cancer (Boeing *et al.* 1991), lung cancer (Sankaranarayanan *et al.* 1994) and colon cancer (Steinmetz & Potter, 1993).

There is, however, *in vitro* evidence that flavonols and flavones can provide protection against oxidative damage to DNA (Cai *et al.* 1997; Duthie *et al.* 1997a; Noroozi *et al.* 1998). Quercetin and luteolin have been shown to reduce the formation of 8-hydroxydeoxyguanosine in isolated DNA exposed to u.v. irradiation or to FeCl₂ or H₂O₂ *in vitro* (Cai *et al.* 1997), although the biological relevance of such experiments is uncertain. Flavonols, when added to isolated human lymphocytes, have been demonstrated to protect against H₂O₂ (an oxidative challenge)-induced DNA strand breakage as measured by alkaline single-cell gel electrophoresis (Comet assay; Duthie *et al.* 1997a; Noroozi *et al.* 1998). Furthermore, consumption by diabetic patients of a high-flavonol (mostly quercetin from onions) or low-flavonol diet for 2 weeks in a crossover design, followed by isolation of lymphocytes, resulted in protection against H₂O₂-induced DNA strand breaks following the high-flavonol diet (Lean *et al.* 1999). In contrast, incubation of cultured human cells (colon, Caco-2; liver, HepG2; epithelial, HeLa) and isolated lymphocytes with high concentrations of flavonols (e.g. quercetin at concentrations up to 100 µM) in the absence of an oxidative challenge increased DNA strand breakage but did not induce oxidative damage to DNA bases (Duthie *et al.* 1997b).

A randomised crossover study in healthy human subjects was carried out to determine the effect of dietary intake of quercetin from onions and black tea on oxidative damage to DNA bases, measured by GC-MS, in leucocytes.

Materials and methods

Study design

The study was a randomised crossover study comprising two 14 d treatments of low-flavonol (LF) or high-flavonol (HF) diets with a 14 d wash-out period between treatments. During the HF dietary treatment period subjects were asked to consume one 150 g onion cake (89.7 mg quercetin) and one 300 ml cup of black tea (1.4 mg quercetin) daily. During the LF dietary treatment period subjects were asked to avoid the consumption of specific flavonol- and flavone-rich foods (as specified by Hertog *et al.* 1992) and tea, and to consume 6 g high-oleic acid sunflower oil (76 % 18 : 1, 14 % 18 : 2n 6; Anglia Oils Ltd, King's Lynn, Norfolk,

UK)/d, as contained in the onion cake. Subjects were asked to make no other changes to their diet or lifestyle except for those necessary for compliance with the study. During the last 7 d of each dietary treatment period, subjects were asked to maintain a 7 d food diary. At the end of each treatment period, fasting venous blood samples were collected into 10 ml lithium heparin- and 10 ml EDTA-coated vacutainers® (Becton Dickinson, Cowley, Oxon., UK), and the heights and weights of the subjects were recorded.

Subjects

Forty-two (twenty male, twenty-two female) healthy non-smoking non-supplement-taking (including antioxidant supplements) volunteers aged 20–60 (median 31.4, range 21–57 years) were recruited from staff and students at King's College London. All subjects had normal haematology, liver function and BMI (kg/m²; median 23.2, range 19.1–31.4).

Dietary intervention

Onion cakes were prepared from mild Spanish onions. The onions were peeled, chopped (approximately 10 × 30 mm) and fried for 8–10 min in batches of 1.2 kg in 45 g preheated high-oleic acid sunflower oil (for fatty acid composition, see p. 920) to which 160 g granulated quick-dried diced onions (McDougalls Ltd, High Wycombe, Bucks., UK) were added. The mixture was cooled, weighed into 150 g portions, compressed to form onion cakes and stored frozen at –20°C until used. Subjects were instructed to reheat the onion cakes from frozen in a microwave oven for 3 min at full power (800 W) for consumption at any time of the day, either alone or in combination with other foods. Tea bags were provided (mean weight 3.3 g; Marks & Spencer, London, UK) and subjects were instructed to prepare black tea by infusing one tea bag in 300 ml boiling water for 4 min without the subsequent addition of milk.

The combined onion cake and black tea dietary supplement provided a total of 131 mg flavonols, flavones and flavanols/d composing (mg/d): quercetin 91.1 (tea and onion cake); kaempferol 6.1 (tea and onion cake); apigenin 10.6 (onion cake); myricetin 0.5 (tea); epigallocatechin gallate 9.3 (tea); epicatechin gallate 5.1 (tea); epigallocatechin 4.8 (tea); epicatechin 2.5 (tea); catechin 0.6 (tea); for further details see p. 921.

Dietary assessment

Subjects were asked to fill in a 7 d food diary (Bingham *et al.* 1995) which included food photographs for estimation of portion sizes. Subjects were asked to record everything they ate and drank at the time of eating, to record as much information about the foods as possible, and to provide weights of what they ate. If the latter was not possible, they were asked to use standard household measures or the food photographs provided to estimate portion sizes. Energy intake:BMR was used to assess adequacy of diet records, with cut-off points of 1.14 and 2.40 to detect under- and

overestimation of intake respectively (Goldberg *et al.* 1991).

Analytical methods

Chemicals. All chemicals were of the highest quality available from Sigma Chemical Co. (Poole, Dorset, UK), BDH Chemical Co. (Gillingham, Kent, UK) or Aldrich (Milwaukee, WI, USA), unless stated otherwise. Catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate were purchased from Apin Chemicals (Abingdon, Oxon., UK).

Flavonol, flavone and flavanol analysis. The HPLC system comprised a TSP spectra system P2000 pump, a TSP A1000 autoinjector and a TSP UV1000 detector (Thermo Separation Products, Rockford, IL, USA). The chromatography was performed using an Ultracarb ODS 20 C₁₈ column (4.6 mm i.d. × 250 mm, 5 µm film thickness; Phenomenex Ltd, Cheshire, UK) protected by an Ultracarb ODS 20 C₁₈ guard column (4.6 mm i.d. × 30 mm, 5 µm film thickness; Phenomenex Ltd). Catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (flavonols) were determined quantitatively in the aqueous tea extract, as described in McAnlis *et al.* (1998). Calibration curves ranging from 10 to 50 µg/ml were used. The aqueous tea extract was filtered through a 2 µm supor acrodisc syringe filter, 50 % methanol was added and the solution analysed by HPLC. The mobile phase was acetic acid–methanol–dimethylformamide–water (1:10:35:154, by vol.) with a flow rate of 1 ml/min and peaks were detected at 280 nm. Quercetin, kaempferol, myricetin (flavonols) and apigenin (a flavone) were determined quantitatively in the onion cake and tea according to a modified version of the method of Hertog *et al.* (1992), as described by McAnlis *et al.* (1998, 1999). Freeze-dried onion-cake powder (0.5 g) or aqueous tea extract (15 ml) was hydrolysed with 1.2 M-HCl for 2 h at 90°C to hydrolyse the glycoside. The mobile phase was ethanol (70–30 %) plus 1 % acetic acid, and peaks were detected at 375 nm (flavonols) and 340 nm (flavones). Calibration curves ranging from 1 to 50 µg/ml were used. The inter-assay CV was <5 % and the intra-assay CV was <1 %.

Plasma quercetin levels were measured by HPLC using a modified version of the technique developed by Hollman *et al.* (1996). In a 4 ml vial, 0.6 ml plasma was mixed with 1 ml methanol containing 2 g tert-butyl hydroxyquinone/l and 0.4 ml 10 M-HCl. The tightly sealed vial was heated at 90°C for 2 h. When cool, 2 ml methanol containing 2 g tert-butyl hydroxyquinone/l was added. The vials were centrifuged at 1000 g for 15 min and 20 µl of the upper layer was injected onto the HPLC column. Chromatographic separations were performed using an Inertsil ODS-2 column (4.6 mm i.d. × 150 mm, 5 µm film thickness; GL Sciences Inc., Tokyo, Japan) protected with an Ultracarb ODS guard column (Phenomenex Ltd). The main column was placed in a column oven at 30°C. The HPLC system utilised a fluorescence detector (emission wavelength 485 nm, excitation wavelength 422 nm; Waters, Watford, Herts, UK) for these measurements, instead of the u.v. detector used to measure quercetin in foods. The mobile

phase contained acetonitrile–methanol–0.025 M-phosphate buffer, pH 2.4, (10:38:52 by vol.), set at a flow rate of 1 ml/min. The post-column effluent was mixed with 1.5 M-Al(NO₃)₃ in methanol containing 7.5 % (v/v) acetic acid (0.4 ml/min) in a post-column reaction coil (3.65 m × 0.5 mm) placed in the column oven at 30°C. The inter-assay CV was <8 % and the intra-assay CV was <1 %.

Analysis of oxidative DNA damage. DNA isolation from EDTA-anticoagulated whole blood was carried out as described in Rehman *et al.* (2000). Preparation of DNA and standards for GC–MS analysis of the products of oxidative damage to DNA bases, and the GC–MS measurements of the products of oxidative damage to DNA bases were carried out as described previously (England *et al.* 1998; Jenner *et al.* 1998; Rehman *et al.* 2000). Derivatisation of the DNA for GC–MS analysis was carried out at room temperature in the presence of ethanethiol, a protocol which appears to prevent artifactual oxidation of the DNA bases during sample processing (Jenner *et al.* 1998; Rehman *et al.* 2000).

Vitamin C analysis. Lithium heparin-treated plasma, prepared by centrifugation of the blood samples at 1500 g for 10 min at 4°C, was mixed with 10 % metaphosphoric acid (1:1, w/v) within 5 h of sample collection and stored at –80°C for vitamin C analyses. These conditions have been shown previously to maintain sample stability (Craft *et al.* 1988; Key *et al.* 1996). Vitamin C was determined using a COBAS BIO centrifugal analyzer (Roche Products, Welwyn Garden City, Herts., UK) fitted with a fluorescence detector (Vuilleumier & Keck, 1989). Samples and standards were kept on ice and out of direct light throughout the analysis.

Statistical analysis

Treatment-order effects were analysed using repeated-measures ANOVA, and paired comparisons were carried out using a paired *t* test. SPSS/PC version 6 was used (SPSS, Chicago, IL, USA).

Ethical considerations

Written informed consent of 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. There were no adverse reactions to the dietary treatments of the study, except mild flatulence and malodour associated with onion consumption reported by approximately half the subjects.

Results

Of the forty-two subjects recruited, thirty-six completed both dietary treatments. The physical characteristics of the latter are described in Table 1.

The dietary intakes of the subjects are shown in Table 2. No subjects reported energy intakes which resulted in an energy intake:BMR value of <1.14 or >2.40. There were no significant differences in dietary intakes between the HF and LF dietary treatment periods.

Table 1. Physical characteristics of the subjects (*n* 36)

	Mean	SD	Median	Range
Age (years)	31.4	7.7	30.5	21–57
BMI (kg/m ²)	23.7	2.3	23.2	19.1–29.6
Haemoglobin (g/l)	139	13	141	115–165
Erythrocyte count (10 ¹² /l)	4.63	0.42	4.64	3.95–5.61
Leucocyte count (10 ⁹ /l)	5.51	1.32	5.37	3.60–9.61
Lymphocytes (10 ⁹ /l)	1.82	0.49	1.83	0.77–3.18
Neutrophils (10 ⁹ /l)	2.95	1.01	2.78	1.56–5.41

The plasma quercetin and vitamin C concentrations following the HF and LF treatment periods are shown in Table 3. Plasma quercetin concentrations were greatly increased at the end of the HF treatment compared with the LF treatment (228.5 (SEM 34.7) nmol/l compared with less than the limit of detection, i.e. <66.2 nmol/l). Plasma vitamin C concentrations did not differ significantly between treatments.

The concentrations of the products of oxidative damage to DNA bases in leucocytes following the HF and LF treatment periods are shown in Table 4. The concentrations of the products of damage to the DNA bases did not differ significantly between the two dietary treatment periods for any of the products measured.

Discussion

There has been much interest in the beneficial effects on health of dietary flavonols, flavones and flavanols. This interest has been stimulated in part by their potent *in vitro* antioxidant activity (Rice-Evans *et al.* 1996) and their *in vivo* inhibition of chemically induced carcinogenesis in rats (Verma *et al.* 1988). Additionally, epidemiological evidence has shown a relationship between dietary intake of flavonols and flavones, and reduced risk of CHD (Hertog *et al.* 1993a);

Table 2. Energy and nutrient intakes of the subjects during the last 7 d of each of the low-flavonol and high-flavonol dietary treatment periods*

Diet ...	Low-flavonol		High-flavonol	
	Mean	SD	Mean	SD
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
SFA (g/d)	33.7	10.8	33.1	7.4
PUFA (g/d)	17.6	5.8	15.2	4.4
MUFA (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
Cholesterol (mg/d)	242.2	97.7	229	96.9
Fe (mg/d)	14.0	2.7	13.5	3.5
Vitamin E (mg/d)	10.1	4.3	9.5	3.0
β-Carotene (mg/d)	1.8	0.95	2.0	1.2
Vitamin C (mg/d)	146	70	138	68

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

* For details of diets and procedures, see pp. 920–921.

Table 3. Plasma quercetin and vitamin C concentrations of the subjects following the low-flavonol and high-flavonol dietary treatment periods*

Diet ...	Low-flavonol		High-flavonol	
	Mean	SEM	Mean	SEM
Quercetin (nmol/l)†	<LOD	–	228.5	34.7
Vitamin C (mmol/l)	69.8	4.5	70.5	4.2

LOD, limit of detection.

* For details of diets and procedures, see pp. 920–921.

† Limit of detection 66.2 nmol/l; 1 ng/ml = 3.3 nmol/l.

however, this relationship has not been confirmed by a subsequent epidemiological study conducted in the USA (Rimm *et al.* 1996). No relationship has been found between dietary intake of flavonols and flavones and reduced risk of cancer (Hertog *et al.* 1994, 1995, 1996; Hertog & Hollman, 1996). As yet, however, there are few controlled human intervention studies investigating the potential beneficial effects of dietary flavonols in relation to biomarkers of cancer risk, with most of the studies concentrating on the influence of green tea (Ishikawa *et al.* 1997) or red-wine polyphenols (Fenech *et al.* 1997; Carbonneau *et al.* 1998; Nigdikar *et al.* 1998) on LDL oxidation which, in the plasma, may be more accessible to flavonol-mediated effects than intracellular DNA.

In the present study, which provided 131 mg flavonols, flavones and flavanols from tea and onions/d, (91 mg quercetin/d), no significant differences were found in products of oxidative damage to DNA bases between the LF and HF dietary treatment periods. These results do not support the protection against H₂O₂-induced DNA strand breakage found in lymphocytes from diabetic patients following a similar dietary intervention with a high-flavonol diet (mostly quercetin from onions) or a low-flavonol diet in a crossover study design (Lean *et al.* 1999).

Table 4. Concentrations of products of oxidative damage to DNA bases (pmol/mg DNA) in leucocytes of subjects following the low-flavonol and high-flavonol dietary treatment periods*

Diet ...	Low-flavonol		High-flavonol	
	Mean	SEM	Mean	SEM
Product of DNA base damage				
8-hydroxyguanine	31	3.3	35	4
FAPy guanine	416	81	483	88
2-hydroxyadenine	129	17	147	21
8-hydroxyadenine	38	5	45	8
FAPy adenine	95	9	99	11
5-hydroxycytosine	72	7	77	7
5-hydroxyhydantoin	22	2	24	3
5-hydroxymethylhydantoin	43	5	45	6
5-hydroxyuracil	7	1	9	1
5-hydroxymethyluracil	12	1	15	2
Thymine glycol (<i>cis</i> + <i>trans</i>)	201	18	224	20
Total measured DNA base products	1037	88	1222	123

No significant differences between treatments were observed.

FAPy guanine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; FAPy adenine, 4,6-diamino-5-formamidopyrimidine.

* For details of diets and procedures, see pp. 920–921.

However, it is known that levels of oxidative damage to DNA bases are abnormally high in leucocytes from diabetic patients (Rehman *et al.* 1999).

In the present study there were no clear changes to oxidative damage to DNA bases following the HF diet, even though the intake of dietary flavonols, flavones and flavanols was approximately five times the estimated habitual intake in The Netherlands (Hertog *et al.* 1993b) and Denmark (Justesen *et al.* 1997), and approximately three times the mean intake allegedly associated with reduced risk of CHD (Hertog *et al.* 1993a). Although tea flavanol supplementation (750 ml/d) has been found to reduce the susceptibility of LDL to oxidative modification (more accessible to flavanol-mediated effects than intracellular DNA) as measured by Cu²⁺-induced LDL lag time (Ishikawa *et al.* 1997), these results have not been reproduced in all studies (McAnlis *et al.* 1998).

While the 2 weeks used in the present study is a short time-course over which to observe significant changes in DNA base damage in leucocytes, one study has observed significant increases in some products of oxidative damage to DNA bases in a group of thirty subjects following 3 weeks of supplementation with vitamin C (500 mg/d; Podmore *et al.* 1998). As the present study was concerned with a potential decrease in oxidative damage, it has to be considered whether sufficient new leucocytes would be formed. While lymphocytes with a half-life of several months would probably not be affected, they represent only 30 % of the leucocytes; the remainder are granulocytes which have a sufficiently short half-life (7 d). However, as oxidative DNA damage occurs continuously, both in older cells and new ones, leucocyte turnover is probably not important and the lack of change suggests that neither the rate of damage nor the rate of DNA repair were being affected.

Although we failed to find a substantial effect of dietary quercetin on oxidative damage to DNA bases in the present study, this absence of an effect does not appear to reflect a lack of absorption and subsequent bioavailability (Hollman, 1997; Wiseman, 1999). Flavonols from onions and tea are absorbed both as aglycones and more readily as glycosides, the predominant form in food (Hollman, 1997; Hollman & Katan, 1997). For example, following the acute ingestion of 50 mg quercetin in fried onions, plasma quercetin increased from 94 nmol/l at baseline to 822 nmol/l after 2 h, decreasing to baseline by 24 h (McAnlis *et al.* 1999). In the present study fasting plasma concentrations of quercetin increased from less than the limit of detection (i.e. <66.2 nmol/l) following the LF diet to 228.5 (SEM 34.7) nmol/l following the HF diet. Furthermore, consumption of 13 mg quercetin/d from onions has been reported to result in plasma quercetin concentrations of 73 nmol/l (de Vries *et al.* 1998). The major flavonols present in lightly fried onions have been reported to be the quercetin conjugates, quercetin-3,4'-diglucoside and quercetin-4'-glucoside, together with much lower levels of isorhamnetin-4'-glucoside and quercetin aglycone (Aziz *et al.* 1998). However, following consumption of 300 g lightly fried onions, a proportionally higher accumulation of isorhamnetin-4'-glucoside than quercetin-4'-glucoside was found in plasma (10 % compared with 0.1 %) and excretion in urine (17 %

compared with 0.2 %), which could indicate either a preferential absorption of isorhamnetin-4'-glucoside or a post-absorption conversion of quercetin 4'-glucoside to isorhamnetin-4'-glucoside (Aziz *et al.* 1998).

The freedom of choice to eat the onion cake and black tea dietary supplements at any time of the day is probably not an important factor contributing to our failure to find an effect on DNA damage, because of the relatively long half-life of quercetin (25 h; Hollman *et al.* 1997). However, it can be argued that the highest quercetin concentration would be required at the moment of highest oxidative stress, and this factor would give some additional variation between subjects. From the within-subject variation in the products of DNA base damage observed in a previous study (Beatty *et al.* 1999), we calculated that with thirty-six subjects the present study should detect a change of 1 SD (20 %) at $P < 0.05$ with more than 95 % power. Thus, we could only have seen differences of ≥ 20 % between the two treatments had there been one. It is of considerable interest that all the products DNA base damage were higher after the HF diet than after the LF diet, even though this difference did not reach statistical significance. This phenomenon cannot be explained by an order of treatment effects because the order of the two treatments was truly randomly assigned, and when we statistically tested for an effect of the order of supplement treatment using repeated measures ANOVA, we did not find one. Furthermore, the analysis procedure cannot explain this phenomenon, because at the end of each treatment period all the samples (from subjects who had consumed either the HF or LF diet) were analysed for products of DNA base damage in random order and blind.

In conclusion, we failed to find a substantial effect of dietary quercetin from onions and black tea on oxidative damage to DNA bases (measured by GC-MS) in healthy human subjects.

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