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.

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, 1993b,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 soybean 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. (1993a) 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 mm diameter) and lightly cooked 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 (flavanols) 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 polyacryllic supor (Millipore, Hertfordshire, UK) and 0-4 ml 10M-HCl. The tightly sealed vial was heated at 100°C for 15 min at 4°C, was mixed with 10 % metaphosphoric acid (1:1, v/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.
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 health of dietary flavonols, flavones and flavanols. This has shown a relationship between dietary intake of flavonols and flavones, and reduced risk of CHD (Hertog et al. 1993a); 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 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* (Mean values and standard deviations for thirty-two subjects)

<table>
<thead>
<tr>
<th>Diet …</th>
<th>Low-flavonol</th>
<th>High-flavonol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ/d)</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>10-7</td>
<td>1.1</td>
<td>10-7</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>86</td>
<td>18-6</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>98.7</td>
<td>25-2</td>
</tr>
<tr>
<td>SFA (g/d)</td>
<td>33.7</td>
<td>10-8</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>17.6</td>
<td>5.8</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>33.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>242</td>
<td>97.7</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>289</td>
<td>52</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>17.6</td>
<td>24.0</td>
</tr>
<tr>
<td>NNS (g/d)</td>
<td>20.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>242.2</td>
<td>97.7</td>
</tr>
<tr>
<td>Fe (mg/d)</td>
<td>14.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>10.1</td>
<td>4.3</td>
</tr>
<tr>
<td>β-Carotene (mg/d)</td>
<td>1.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>146</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 3. Plasma quercetin and vitamin C concentrations of the subjects following the low-flavonol and high-flavonol dietary treatment periods* (Mean values with their standard errors for thirty-six subjects)

<table>
<thead>
<tr>
<th>Diet …</th>
<th>Low-flavonol</th>
<th>High-flavonol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin (nmol/l)</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>LOD ± 228.5 34.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (mmol/l)</td>
<td>69.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

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* (Mean values with their standard errors for thirty-six subjects)

<table>
<thead>
<tr>
<th>Product of DNA base damage</th>
<th>Low-flavonol</th>
<th>High-flavonol</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-hydroxyguanine</td>
<td>31</td>
<td>3.3</td>
</tr>
<tr>
<td>FAPy guanine</td>
<td>416</td>
<td>81</td>
</tr>
<tr>
<td>2-hydroxyadenine</td>
<td>129</td>
<td>17</td>
</tr>
<tr>
<td>8-hydroxyadenine</td>
<td>38</td>
<td>5</td>
</tr>
<tr>
<td>FAPy adenine</td>
<td>95</td>
<td>9</td>
</tr>
<tr>
<td>5-hydroxyctosine</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>5-hydroxyhydantoin</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>5-hydroxymethylhydantoin</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>5-hydroxuracil</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>5-hydroxymethyluracil</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Thymine glycol (cis + trans)</td>
<td>201</td>
<td>18</td>
</tr>
</tbody>
</table>

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 Cu$^{2+}$-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 ofisorhamnetin-4'-glucoside and quercetin aglycone (Aziz et al. 1998). However, following consumption of 300 g lightly fried onions, a proportionally higher accumulation ofisorhamnetin-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 ofisorhamnetin-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 $\geq 20 \%$ 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.

Acknowledgements

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