Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes1–3

Mostafa Noroozi, Wilson J Angerson, and Michael EJ Lean

ABSTRACT This study assessed the antioxidant potencies of several widespread dietary flavonoids across a range of concentrations and compared with vitamin C as a positive control. The antioxidant effects of pretreatment with flavonoids and vitamin C, at standardized concentrations (7.6, 23.2, 93, and 279.4 μmol/L), on oxygen radical–generated DNA damage from hydrogen peroxide (100 μmol/L) in human lymphocytes were examined by using the single-cell gel electrophoresis assay (comet assay). Pretreatment with all flavonoids and vitamin C produced dose-dependent reductions in oxidative DNA damage. At a concentration of 279 μmol/L, they were ranked in decreasing order of potency as follows: luteolin (9% of damage from unopposed hydrogen peroxide), myricetin (10%), quercetin (22%), kaempferol (32%), quercitrin (quercetin-3-L-rhamnoside) (45%), apigenin (59%), quercetin-3-glucoside (62%), rutin (quercetin-3-β-D-rutinoside) (82%), and vitamin C (78%). The protective effect of vitamin C against DNA damage at this concentration was significantly less than that of all the flavonoids except apigenin, quercetin-3-glucoside, and rutin. The ranking was similar with estimated ED50 (concentration to produce 50% protection) values. The protective effect of quercetin and vitamin C at a concentration of 23.2 μmol/L was found to be additive (quercetin: 71% of maximal DNA damage from unopposed hydrogen peroxide; vitamin C: 83%; both in combination: 62%). These data suggest that the free flavonoids are more protective than the conjugated flavonoids (eg, quercetin compared with its conjugate quercetin-3-glucoside, P < 0.001). Data are also consistent with the hypothesis that antioxidant activity of free flavonoids is related to the number and position of hydroxyl groups.

KEY WORDS Antioxidant, comet assay, DNA damage, flavonoids, quercetin, kaempferol, apigenin, quercitrin quercetin-3-L-rhamnoside, luteolin, myricetin, rutin, quercetin-3-β-D-rutinoside, quercetin-3-glucoside, vitamin C

INTRODUCTION The aim of this study was to determine the antioxidant capacities of various flavonoids and vitamin C against the oxidative DNA damage produced ex vivo in human lymphocytes by hydrogen peroxide. Flavonoids are polyphenolic compounds whose main dietary sources are fruit and vegetables and whose consumption has been linked to protection against heart disease and cancers (1). Flavonoids were initially considered to be substances without any nutritive value for humans. However, in 1936 Rusznyák and Szent-György (2) showed that two flavonoids derived from citrus fruits decreased capillary fragility and permeability in humans.

Antioxidant effect of flavonoids Quercetin, myricetin, kaempferol, rutin, and vitamin C are powerful antioxidants in the oxidation of LDL and provide a possible mechanism for the beneficial epidemiologic effect of dietary fruit and vegetables on heart disease (3). Evidence comes from several sources. Using a high-temperature incubation method, Mehta and Seshadri (4) first reported that quercetin has an antioxidant action. Hudson and Lewis (5) found that quercetin and luteolin have good primary antioxidant activity in the stability of lard, using the 100°C method. A study with ultraviolet-induced oxidation of LDL (6) showed that rutin, a polyphenolic flavonoid, vitamin C, and vitamin E were able to inhibit the peroxidation of LDL and its subsequent cytotoxicity. Quercetin (3,3',4',5,7-pentahydroxyflavone) prevents oxidation of LDL by macrophages in vitro by reducing the formation of free radicals (7, 8) and dietary intake of quercetin estimated from dietary records is inversely related to ischemic heart disease mortality (9). Several more recent studies using different assays have confirmed that quercetin is a strong antioxidant and that most flavonoids show antioxidant activity (10–14).

Single-cell gel electrophoresis (the comet assay) is a sensitive and rapid method for the detection of DNA damage at the individual cell level (15–17) and specifically for detecting oxidative DNA strand breaks (18, 19). It is considered a useful tool for investigating issues related to oxidative stress in human lymphocytes (20, 21) but has not been used previously with flavonoids. We therefore used the comet assay to evaluate the...
antioxidant capacity of some major dietary flavonoids, with vitamin C as a positive control.

MATERIALS AND METHODS

The solutions and chemicals used in this study were purchased from the following companies. High-melting-point (HMP) agarose and low-melting-point (LMP) agarose, both electrophoresis grade, were from Gibco Ltd, Paisley, United Kingdom. Phosphate-buffered saline (PBS) tablets, ascorbic acid, kaempferol, quercetin, apigenin, myricetin, rutin (quercetin-3-β-D-rutinoside), Na₂EDTA, trypan blue solution (0.4%), RPMI (1640) medium with sodium bicarbonate without L-glutamine and phenol red, Histopaque 1077 (Ficoll-Hypaque, density: 1.077 g/L), ethidium bromide, and diamidine-2-phenylindol dihydrochloride (DAPI) were from Sigma Chemicals Co Ltd, Irvine, United Kingdom. Sodium hydroxide, hydrogen peroxide (10% v/v), sodium chloride were from BDH Ltd, Esher, United Kingdom. Fetal calf serum (FCS) was from Globepharm Ltd, Sussex, United Kingdom. Sodium bicarbonate, sodium hydroxide, hydrogen peroxide, Triton X-100, and sodium chloride were from BDH Ltd, Esher, United Kingdom. Na₂EDTA, trypan blue solution (0.4%), RPMI (1640) medium with sodium bicarbonate without L-glutamine and phenol red, Histopaque 1077 (Ficoll-Hypaque, density: 1.077 g/L), ethidium bromide, and diamidine-2-phenylindol dihydrochloride (DAPI) were from Sigma Chemicals Co Ltd, Irvine, United Kingdom. Fetal calf serum (FCS) was from Globepharm Ltd, Esher, United Kingdom. Sodium hydroxide, hydrogen peroxide, Triton X-100, and sodium chloride were from BDH Ltd, Esher, United Kingdom. Sodium hydroxide, hydrogen peroxide, Triton X-100, and sodium chloride were from BDH Ltd, Esher, United Kingdom.

Fatty acid-free human lymphocytes were isolated by centrifugation with Histopaque 1077 and incubated with different concentrations (0, 7.6, 23.2, 93, and 279.4 μM) of flavonoids or vitamin C for 30 min at 37°C. After pretreatment, cells were washed with PBS and were treated with hydrogen peroxide (100 μM/L for 5 min on ice). Then cells were suspended in LMP agarose, set on a microscope slide, and lysed with lysis solution containing 1% Triton X-100 for 1 h. Gel electrophoresis was then used to estimate tail DNA content of 600–1200 lysed nuclei at each concentration. Visual scoring was used routinely and validated by using image analysis (Imaging Research Inc, St Catherine’s, Canada) with the fluorescent dye ethidium bromide to quantify comet tail DNA.

Cell preparation

Human lymphocytes were isolated from fresh whole blood by adding 30 μL blood to 1 mL RPMI 1640 with 10% FCS on ice for 30 min, then underlaying it with Histopaque 1077 before centrifuging at 200 × g for 3 min at 4°C. Lymphocytes were separated as a pink layer at the top of the Histopaque 1077.

Antioxidant pretreatment

Cells were incubated with different concentrations of flavonoids or vitamin C for 30 min at 37°C in a dark incubator together with untreated control samples. Samples were then centrifuged at 200 × g for 3 min at 4°C. After pretreatment, cells were centrifuged and washed twice with PBS (0.01 mol) at 200 × g for 3 min at 4°C.

Oxygen-radical treatment

Samples were suspended in PBS with 100 μM H₂O₂/L for 5 min on ice. Samples were then centrifuged at 200 × g for 3 min at 4°C. Control samples were treated with PBS alone without hydrogen peroxide.

Slide preparation

Two layers of agarose were prepared. For the first layer, 85 μL 1% HMP agarose or standard agarose prepared at 40°C in PBS was dispensed onto fully frosted slides and covered with a 22 × 22 mm (no. 1) coverslip. To solidify the agarose, the slides were kept at 4°C for 10 min. Lymphocytes were suspended in 1% LMP agarose in PBS (prepared at 37°C) and 85 μL containing 20,000 lymphocytes were plated onto the first layer of agarose, covered with a coverslip, and kept for 10 min at 4°C to solidify. After the coverslips were removed, the slides were immersed in freshly prepared cold lysing solution.

Cell lysis

Slides were treated at 4°C for 60 min (vertically without coverslip) with lysis solution [2.5 mol NaCl/L, 100 mol Na₂EDTA/L, and 10 mol tris/L, adjusted to pH 10 with sodium hydroxide plus 1% Triton X-100 (added immediately before use)]. Different lysis tanks were used for control slides. The lysis solution was stored at 4°C before use.

Alkaline treatment and electrophoresis

After the slides were removed from the lysis solution they were placed in an electrophoresis tank horizontally, side by side. Up to
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Any gaps were filled with blank slides to avoid spaces between slides. Slides were covered with fresh electrophoresis buffer (300 mol NaOH/L and 1 mol Na₂EDTA/L, pH 13) at 4°C for 40 min to a depth of 2–3 mm above the slides. Buffer was made freshly each day and stored at 4°C before use. To prevent additional DNA damage, slides were processed in dark conditions. The electrophoresis was run at 25 V for 30 min at 4°C, covered with black paper against light.

Neutralization and staining

Before staining, slides were placed vertically without a cover slip in a neutralizing tank and gently washed three times for 5 min with neutralizing buffer (0.4 mol tris/L adjusted to pH 7.5 with hydrochloric acid) at 4°C in the dark. Sixty microliters of 20 mg DAPI/L or 60 μL of 20 mg ethidium bromide/L was dispensed directly onto slides and covered with a cover slip. Slides were kept for ≤12 h in a dark and air-tight moist chamber to prevent drying of the gel before viewing.

Quantification of DNA damage

Slides were examined at 400× magnification on an Olympus fluorescence microscope (Olympus Optical Co, Ltd, Tokyo) with excitation at 520 nm and a 620-nm emission barrier filter. Because the study involved the individual assessment of DNA damage in >30 000 cells, it was necessary to develop a rapid visual scoring system. Cells were assigned a score on a 5-point scale (range: 0–4) according to the amount of DNA in the tail of the comet as estimated by the observer (Figure 1). To validate this system, objective measurements of the distribution of DNA were performed for a sample of cells by using a BRS2 Image Analyser (Imaging Research, Inc). These measurements were made by quantifying the fluorescent intensity distribution of the comet as a function of distance from the leading edge of the head (Figure 2).

There was a close relation between the subjective visual score and the measurements of the percentage of DNA in the tail by image analysis, as shown in Figure 3. In 90% of cells, the percentage of DNA in the tail for different visual grades of damage fell in the following nonoverlapping ranges: grade 0 (no damage), < 5%; grade 1 (low damage), 5–25%; grade 2 (medium damage), 25–45%; grade 3 (high damage), 45–70%; grade 4 (very high damage), > 70%.

Slide scoring

Randomly selected lymphocytes were graded visually for each slide. A total damage score for the slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells of grade 4).

In a given experiment, duplicate slides were prepared and scored for each concentration of the antioxidant. Experiments were repeated three to six times. Therefore, for each concentration of each antioxidant, 6–12 samples of 100 randomly selected cells were analyzed in total. An example of the results for one of the flavonoids (kaempferol) is shown in Table 1.

Statistical analysis

Linear regression analysis of damage score versus log concentration of antioxidant was used to assess the dose dependency of the protective effect. The concentration that would reduce the percentage of cells showing DNA damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells of grade 4).

Figure 2. Fluorescent intensity profiles of comets (electrophoresed cell nuclei) with different grades of damage as measured by image analysis. The percentage of DNA in the tail of the comet was calculated as 100(A1 ~ 2A2)/A1, where A1 is the area under the curve for the whole comet and A2 is the area under the curve from the leading edge to the center of the head of the comet.

Percentage of cells showing DNA damage

Effect of kaempferol pretreatment against oxidative DNA damage in human lymphocytes in the comet assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No damage (≤5%)</th>
<th>Low damage (5–25%)</th>
<th>Medium damage (25–45%)</th>
<th>High damage (45–70%)</th>
<th>Very high damage (&gt;70%)</th>
<th>Total score (out of 400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μmol H₂O₂/L (no kaempferol)</td>
<td>14.0 ± 4.6</td>
<td>22.0 ± 2.5</td>
<td>10.4 ± 2.2</td>
<td>13.2 ± 2.2</td>
<td>40.3 ± 3.0</td>
<td>243.6 ± 12.7</td>
</tr>
<tr>
<td>Control</td>
<td>86.3 ± 2.1</td>
<td>12.3 ± 1.9</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>16.0 ± 2.5</td>
</tr>
<tr>
<td>7.6 μmol kaempferol/L + 100 μmol H₂O₂/L</td>
<td>27.4 ± 6.2</td>
<td>23.2 ± 3.7</td>
<td>7.5 ± 1.8</td>
<td>11.3 ± 1.4</td>
<td>30.6 ± 3.1</td>
<td>194.5 ± 15.2</td>
</tr>
<tr>
<td>Control</td>
<td>88.6 ± 3.1</td>
<td>9.8 ± 2.9</td>
<td>0.8 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.2</td>
<td>13.7 ± 3.4</td>
</tr>
<tr>
<td>23.3 μmol kaempferol/L + 100 μmol H₂O₂/L</td>
<td>33.0 ± 3.4</td>
<td>23.8 ± 3.9</td>
<td>6.2 ± 1.0</td>
<td>11.5 ± 1.0</td>
<td>25.6 ± 2.5</td>
<td>173.7 ± 8.2</td>
</tr>
<tr>
<td>Control</td>
<td>85.2 ± 2.7</td>
<td>12.2 ± 2.8</td>
<td>1.3 ± 0.5</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>19.3 ± 3.0</td>
</tr>
<tr>
<td>93 μmol kaempferol/L + 100 μmol H₂O₂/L</td>
<td>47.8 ± 4.3</td>
<td>17.8 ± 3.0</td>
<td>3.2 ± 1.0</td>
<td>8.5 ± 1.3</td>
<td>22.8 ± 2.4</td>
<td>140.9 ± 10.2</td>
</tr>
<tr>
<td>Control</td>
<td>94.3 ± 1.6</td>
<td>4.8 ± 1.6</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>279.4 μmol kaempferol/L + 100 μmol H₂O₂/L</td>
<td>68.8 ± 2.4</td>
<td>10.2 ± 2.1</td>
<td>4.5 ± 0.9</td>
<td>5.6 ± 1.0</td>
<td>11.0 ± 1.5</td>
<td>78.6 ± 8.2</td>
</tr>
<tr>
<td>Control</td>
<td>94.2 ± 1.3</td>
<td>4.8 ± 1.5</td>
<td>0.3 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>7.5 ± 1.2</td>
</tr>
</tbody>
</table>

* x ± SD; n = 6 repeat experiments. Control samples had no H₂O₂.
damage score obtained in the absence of the flavonoid by 50% \((ED_{50})\) was estimated from the regression line (in some cases by extrapolation) as a comparative measure of efficacy. Separate comparisons of the antioxidant activity of different agents were performed at each concentration by using one-way analysis of variance (ANOVA) together with Tukey’s method for all pairwise comparisons between flavonoids, and Dunnett’s method for comparing all flavonoids with vitamin C. The significance levels reported refer to error rates for each family of comparisons. The effect of quercetin and vitamin C in combination was analyzed by two-way ANOVA. For free flavonoids, the relation between the number of hydroxyl groups in the structure of the agents and the degree of protection against DNA damage was assessed by linear regression analysis. Statistical significance in all cases was assessed at the 5% level.

RESULTS

All the flavonoids and vitamin C produced dose-dependent reductions in oxidative DNA damage as assessed by linear regression analysis of the log-dose response curves. The CV was 19% for duplicate assessments of DNA damage and 20% for repeated identical experiments.

Examples of dose-response curves are shown in Figure 4. The concentrations that would produce a 50% reduction in DNA damage as estimated from the regression equations were, in decreasing order of efficacy, quercetin \((47 \, \mu mol/L)\), luteolin \((51 \, \mu mol/L)\), myricetin \((64 \, \mu mol/L)\), kaempferol \((104 \, \mu mol/L)\), quercitrin \((288 \, \mu mol/L)\), quercetin-3-glucoside \((984 \, \mu mol/L)\), apigenin \((1.5 \, mmol/L)\), rutin \((43 \, mmol/L)\), and vitamin C \((233 \, mmol/L)\) (Figure 5). The values for quercetin, quercetin-3-glucoside, apigenin, rutin, and vitamin C were derived by extrapolation of the dose-response curves and should be regarded as approximate measures of relative efficacy rather than true ED_{50} values.

The results of all pairwise comparisons of the antioxidant effect of the nine agents at a concentration of 279 \(\mu\)mol/L are shown in Table 2. Although there were minor differences in the ranking of the agents as compared with the ED_{50} values, none of these relate to any significant differences. The four most potent agents studied (luteolin, myricetin, quercetin, and kaempferol) are all members of the group of free flavonoids (which also includes apigenin), and in most pairwise comparisons were significantly more effective than the conjugated flavonoids (quercitrin, quercetin-3-glucoside, and rutin). Vitamin C was significantly less potent than luteolin, myricetin, quercetin, kaempferol, and quercitrin. It did not differ significantly in efficacy from quercetin-3-glucoside, apigenin, and rutin. At lower concentrations, there were fewer significant differences between the agents. Rutin was consistently the weakest antioxidant of all the flavonoids tested, and vitamin C was also less effective than most of the other agents. Quercetin was consistently one of the most potent.
In a single experiment, the antioxidant effects of vitamin C, quercetin, and both these agents combined were assessed at a relatively low fixed concentration of 23.2 μmol/L. The results are shown in Figure 6. Quercetin alone reduced oxidative DNA damage by 29% relative to untreated control cells, whereas vitamin C alone reduced damage by 17%. The two agents combined reduced damage by 38%. Two-way ANOVA showed that the treatment effects of both agents were significant (quercetin: F[1,16] = 18.49, P < 0.001; vitamin C: F[1,16] = 5.08, P = 0.04) and that the interaction between them was insignificant. The analysis therefore suggests that the protective effects of these agents at the doses investigated were additive.

The mean damage score for the five free flavonoids at a concentration of 279 μmol/L is plotted against the number of hydroxyl groups in the structure of the molecule in Figure 7. There was a negative correlation between these variables, although for the small number of agents studied it was not significant (r = -0.60, P = 0.17; n = 5).

**DISCUSSION**

Evidence is accumulating to indicate the biomedical importance of flavonoids as antioxidants. Daily intake in Netherlands has been estimated at 23 mg/d, of which 16 mg is quercetin (22). Tea (48%), onions (29%), and apples (7%) were the main food sources of flavonoids in Netherlands (22). In other cultures (eg, Mediterranean countries) red wine, which contains 10–20 mg/L combined flavonoids, could be an important source (23). Grape juice or grapes also provide flavonoids (3) as do berries (24). In the Dutch diet, total flavonoid consumption predicts ischemic heart disease (23) and stroke (25) but not cancers (22). Hertog and Hollman (22) quote Dorant (26) as having shown an inverse association between consumption of onions and cancer risk, particularly cancer of the stomach, colon, and rectum. These data are derived from crude analyses of food frequencies coupled with knowledge of flavonoid contents of certain foods. Because many hundreds of flavonoids exist in foods, it is obviously important to consider the relative potency of specific compounds.
The results of the present study indicate that the aglycons quercetin, luteolin, myricetin, and kaempferol (Figure 8) have a greater antioxidative capacity than do the conjugate flavonoids, such as quercetin-3-glucoside, quercitrin, and rutin (Figure 9). This agrees with the results of several other studies using a wide range of methods for assessing antioxidant activity, as shown in Table 3. In our study, apigenin was the least potent of the free flavonoids, and this agrees with previous studies (27, 3). Also, Chen et al (8) reported that apigenin showed no antioxidant activity in rape seed oil heated to 105°C.

The position and number of hydroxyl groups has an important role in antioxidant activity (8, 28). In our study, the protective effect of myricetin, quercetin, kaempferol, and apigenin against DNA damage (each at 279.4 μmol/L) would be consistent with a relation to the number of hydroxyl groups although the position of hydroxyl groups is also critical. For apigenin, the three hydroxyl groups at positions 5, 7, and 4’ were associated with a small but definite antioxidant effect. Kaempferol, with an additional hydroxyl group at position 3, was more protective than apigenin, quercetin, and myricetin, with groups at the 3’ and 5’ positions, were still more effective (Figure 10). The antioxidant activity of quercetin involves hydrogen atom donation to peroxy radicals, thus terminating the radical chain reaction (29). Shahidi and Wanasundara (30) showed that flavonoids are excellent hydrogen donors and those with a 3,4’ dihydroxy configuration such as quercetin possess strong antioxidant activity. Luteolin, with several hydroxyl groups similar to those of kaempferol, was significantly more effective in the present study, as others also reported (27, 28). This may be because the hydroxyl group at the 3’ position in luteolin confers greater antioxidant activity than the group at the 3’ position in kaempferol.

### TABLE 3

Rankings from the literature of antioxidant activity of flavonoids tested in the present study and vitamins in order of decreasing potency as determined with different methods

<table>
<thead>
<tr>
<th>Rankings of antioxidant activity in order of decreasing potency</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin, myricetin, rutin, trolox, vitamin C, kaempferol, α-tocopherol, β-carotene, apigenin</td>
<td>Lipoprotein oxidation model</td>
<td>(3)</td>
</tr>
<tr>
<td>Myricetin, quercetin</td>
<td>Antioxidant activity against peroxide (induction period of lard 60 °C)</td>
<td>(4)</td>
</tr>
<tr>
<td>Quercetin, luteolin, quercitrin</td>
<td>Stability of lard at 100 °C</td>
<td>(5)</td>
</tr>
<tr>
<td>Quercetin, myricetin, kaempferol, apigenin</td>
<td>Lipid peroxidation in red blood cell membranes</td>
<td>(8)</td>
</tr>
<tr>
<td>Quercetin, myricetin, rutin, quercitrin</td>
<td>Xanthine-xantine oxidase system</td>
<td>(11)</td>
</tr>
<tr>
<td>Myricetin, quercetin, rutin, quercitrin</td>
<td>Superoxide generation by Fenton methosulfate model</td>
<td>(11)</td>
</tr>
<tr>
<td>Myricetin, quercetin, rhamnetin, apigenin, kaempferol</td>
<td>Hydroxyl radical-scavenging activity</td>
<td>(12)</td>
</tr>
<tr>
<td>Luteolin, quercetin, kaempferol, apigenin</td>
<td>Nonenzymatic lipid peroxidation in rat liver</td>
<td>(27)</td>
</tr>
<tr>
<td>Luteolin, kaempferol, rutin, quercetin, myricetin</td>
<td>Fenton reagent assay (Fe²⁺/H₂O₂)</td>
<td>(28)</td>
</tr>
<tr>
<td>Kaempferol, luteolin = rutin, quercitin</td>
<td>Antioxidation of linoleic acid</td>
<td>(29)</td>
</tr>
<tr>
<td>Quercetin, kaempferol, rutin</td>
<td>Free radical-scavenging mechanism in meat</td>
<td>(30)</td>
</tr>
<tr>
<td>Luteolin, apigenin</td>
<td>CCl₄-induced microsomal lipid peroxidation</td>
<td>(31)</td>
</tr>
<tr>
<td>Myricetin, α-tocopherol, β-carotene</td>
<td>Inhibition of strand breaks in plasmid by singlet molecular oxygen</td>
<td>(32)</td>
</tr>
<tr>
<td>Quercetin, α-tocopherol,</td>
<td>Inhibition of human low-density lipoprotein model</td>
<td>(33)</td>
</tr>
<tr>
<td>Myricetin, quercetin, quercitrin, rutin</td>
<td>Lipid peroxidation in corn oil</td>
<td>(34)</td>
</tr>
<tr>
<td>Quercetin, luteolin, rutin</td>
<td>Lipid peroxidation in lard</td>
<td>(34)</td>
</tr>
<tr>
<td>Quercetin, rutin</td>
<td>Autooxidation of rat cerebral membranes assay</td>
<td>(35)</td>
</tr>
<tr>
<td>Quercetin, luteolin, myricetin, kaempferol, quercetin, quercetin-3-glucoside, apigenin, rutin, vitamin C</td>
<td>ED₅₀ in the comet assay of human lymphocyte DNA</td>
<td>present study. (36)</td>
</tr>
</tbody>
</table>
The comet assay has been explored as a potential tool for detecting the antioxidant effect of foods or nutrients. Supplementation with vitamin C (100 mg/d), vitamin E (280 mg/d), and β-carotene (25 mg/d) for 20 wk significantly decreased endogenous oxidative DNA damage in human lymphocytes (37). Hartmann et al (38) used the comet assay to study DNA damage in peripheral white blood cells of humans after exhaustive exercise and reported that vitamin E supplementation prevented exercise-induced DNA damage. Green et al (39) found a reduction in radiation-induced DNA damage after vitamin C ingestion (35 mg/kg). The effect of various antioxidants on oxygen radical–generated DNA damage in human lymphocytes was investigated by using the comet assay. There were small protective effects of vitamin C at low doses (40 μmol/L) and no protection at high doses (5 mmol/L). Trolox (Aldrich Chemical Co, Ltd, Dorset, United Kingdom), a water-soluble analogue of vitamin E, had no effect on DNA damage (20).

In the present study we showed that the comet assay can be used to give reproducible results in estimating the extent of oxidative DNA damage to human lymphocytes. It thus proved possible to rank the potency of the antioxidant agents tested with high confidence. It was clear that vitamin C, well recognized as a dietary antioxidant, provides definite protection against oxidative DNA damage at normal human physiologic concentrations. The concentrations of vitamin C tested were chosen to span the normal plasma concentrations from 34 to 114 μmol/L (40). Much higher concentrations (150 mg/L) are found in leukocytes, including lymphocytes, which concentrate vitamin C to concentrations 14-fold greater than those in plasma (40, 41). Intracellular concentrations were not measured in the present study. Fruit and vegetables provide >60% of total vitamin C intake and these foods are likely to contribute other antioxidants, including flavonoids. At equimolar concentrations, the results show clearly a greater antioxidant potency for most
of the flavonoids tested than for vitamin C. The results also showed that the effects of quercetin, one of the most potent antioxidant flavonoids, and vitamin C could be additive when cells were pretreated with both at concentrations of 23.2 μmol/L.

The conclusions from these in vitro experiments must remain tentative until more is known about the absorption, distribution, metabolism, and biological effects of flavonoids within the body. Epidemiologic studies have shown consistent associations between consumption of fruit and vegetables and protection against a range of cancers (42–44), ischemic heart disease (45), and type 2 diabetes (46). These observational associations have suggested a causal relation mediated by dietary antioxidants limiting lipid peroxidation and DNA damage. Most analyses have sought mechanisms based on antioxidant actions (Table 3), may be important factors for health that coexist with the more familiar antioxidant vitamins. Flavonoid analysis in human diets is now becoming possible (22, 48) and the factors that govern their concentrations in foods are becoming better understood.

The importance of quercetin, one of the most widespread and also most potent antioxidant flavonoids, is highlighted by the results of the present study.

We thank Andrew Collins (Rowett Research Institute, Aberdeen) for close support in study design and training, and Alan Crozier (Plant Molecular Science Group, Division of Biochemistry and Molecular Biology, University of Glasgow) for criticism and guidance.

REFERENCES