The Effect of Cruciferous and Leguminous Sprouts on Genotoxicity, *In vitro* and *In vivo*


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Abstract

Vegetable consumption is associated with a reduced risk of colorectal cancer, which is the second most common cancer after lung/breast cancer within Europe. Some putative protective phytochemicals are found in higher amounts in young sprouts than in mature plants. The effect of an extract of mixed cruciferous and legume sprouts on DNA damage induced by H₂O₂ was measured in HT29 cells using single cell microelectrophoresis (comet). Significant antigenotoxic effect (*P* ≤ 0.05) was observed when HT29 cells were pre-incubated with the extract (100 and 200 µL/mL) for 24 hours and then challenged with H₂O₂. A parallel design intervention study was carried out on 10 male and 10 female healthy adult volunteers (mean age = 25.5 years) fed 113 g of cruciferous and legume sprouts daily for 14 days. The effect of the supplementation was measured on a range of parameters, including DNA damage in lymphocytes (comet), the activity of various detoxifying enzymes (glutathione S-transferase, glutathione peroxidase, and superoxide dismutase), antioxidant status using the ferric reducing ability of plasma assay, plasma antioxidants (uric acid, ascorbic acid, and α-tocopherol), blood lipids, plasma levels of lutein, and lycopene. A significant antigenotoxic effect against H₂O₂-induced DNA damage was shown in peripheral blood lymphocytes of volunteers who consumed the supplemented diet when compared with the control diet (*P* = 0.04). No significant induction of detoxifying enzymes was observed during the study, neither were plasma antioxidant levels or activity altered. The results support the theory that consumption of cruciferous vegetables is linked to a reduced risk of cancer via decreased damage to DNA. (Cancer Epidemiol Biomarkers Prev 2004;13(7):1199–205)

Introduction

Colorectal cancer is the second most common cancer after lung (men)/breast (women) cancer within Europe, North America, Australia, and New Zealand (1). 190,000 new cases of this cancer were reported in Europe in 2000 and it affects 6% of men and women by age 75 in almost equal proportion. The incidence and mortality of the disease are generally increasing (1-3). Globally, the incident rates show an approximately 20-fold variation commonly attributed to both genetic and environmental factors, especially diet.

Diet seems to play a significant role in the etiology of colorectal cancer. The evidence for diets rich in vegetables protecting against several cancers, including colorectal, is convincing, in particular the evidence is strongest for raw vegetables, allium vegetables, and green vegetables (4). Within these broad categories of vegetables, specific groups seem to offer enhanced sources of protective phytochemicals, notably *Cruciferae* (5, 6) and especially *Brassicae* which contain many putative protective agents that regulate mammalian enzymes of xenobiotic metabolism. For example, isothiocyanates, such as sulforaphane (4-methylsulfinylbutyl isothiocyanate), present in the form of glucosinolate precursors, are potent inducers of phase II enzymes. Thus, the induction of these enzymes, some of which have been shown to detoxify risk factors associated with dietary carcinogenesis, by cruciferous vegetables (CFV; refs. 5, 7), provides a potential strategy for protecting against carcinogenesis and mutagenesis.

There is some experimental support for the protective role for brassica vegetables in colorectal cancer. *In vitro* brassica vegetables and their constituents exert antigenotoxic effects in human cell lines (8, 9) and consumption of brassica vegetables is consistently linked with reduction of chemically induced aberrant crypt foci (ACF) in animal studies (10-13). The evidence for link between legumes and reduced colorectal cancer risk is weaker than the case for CFVs, although Bostick et al. (14) did observe a decreased risk for colorectal cancer with the consumption of legumes. A recent study by Leuratti et al. (15) reported an inverse association with the quantity of DNA adducts (malondialdehyde-deoxyguanosine) in human colorectal mucosa and legume consumption. Additionally, the polyphenols found in legumes have been reported to possess antimutagenic and apoptosis-inducing properties (16-18).
While CFVs, and in particular brassica vegetables, seem to be a good source of protective phytochemicals, it is becoming evident that young sprouts of crucifers contain 10 to 100 times higher levels of certain glucosinolates than corresponding mature plants (19).

The combination of experimental and epidemiologic data provides suggestive evidence for a preventative effect of a high intake of brassica vegetables (reviewed in refs. 11, 20). Therefore, the aim of this work is to determine whether cruciferous and legume sprouts (CLS) possess antigenotoxic properties in vitro using the HT29 colorectal cell line and whether this can be shown in vivo in humans using peripheral blood lymphocytes. Furthermore, can the protective effect, if any, be related to modulation of antioxidative status (including the ferric reducing ability of plasma, uric acid, vitamin C) or induction of phase II enzymes, including glutathione S-transferase (GST), glutathione peroxidase, and superoxide dismutase?

Materials and Methods

Cruciferous and Legume Sprouts. While it would have been scientifically preferable to test only one variety of brassica, for example, broccoli sprouts, we were unable to obtain a sufficiently fresh or consistent source of such a product. As such, the CLSs used for the in vitro and in vivo studies represented the most suitable commercially available product (Super Sprouts, provided by Fresh Alternatives Ltd., Cookstown, Northern Ireland). Super Sprouts are an approximate equal mix of sprouts of CFVs, broccoli (Brassica oleracea), radish (Raphanus sativus), and leguminous vegetables alfalfa (Medicago sativa) and clover (Trifolium pratense), grown and harvested (day 3 after germination) under strictly controlled conditions. The CLSs were kept at 4°C and used within 3 days of purchase.

Preparation of CLS Juice for in vitro Studies. The method was modified from Kassie et al. (21). In brief, 250 g (contents of three randomly selected packets) of Super Sprouts were prepared with a Cookworks juice maker machine, the juices were kept on ice, centrifuged (9000 × g, 10 minutes, 4°C), the supernatants decanted, filter sterilized (0.22 μm), and immediately stored at –80°C.

Tissue Culture and Exposure to CLS. The HT29 cells were obtained from the European Collection of Animal Cell Cultures (ECACC), Salisbury, United Kingdom. DMEM was obtained from Life Technologies Ltd., Paisley, Scotland. HT29 cells were cultured in Roux flasks as monolayers in DMEM containing 10% fetal Paiseley, Scotland. HT29 cells were cultured in Roux flasks as monolayers in DMEM containing 10% fetal

In vitro Genotoxicity Studies (Comet Assay). The major cancer site thought to be protected by vegetables is the colon, so for our in vitro studies, we chose a cell line used widely as a model for colon cancer, that is, HT29. The HT29 cells were pre-incubated (1 or 24 hours) with CLS extract at various concentrations (0, 100, and 200 μL/mL). On completion of pre-incubations, cells were harvested, washed with PBS, and re-suspended by the addition of trypsin (0.25% trypsin-EDTA) at 37°C for 5 minutes. At this stage, a cell and viability count was done using a hemocytometer and trypan blue dye (Sigma Chemical Co., United Kingdom). Viability was approximately ≥97%. Cells were centrifuged at 1250 rpm for 3 minutes and cells re-suspended in appropriate medium. The comet assay was done essentially as described by Vetrini et al. (22). The incubations of HT29 cells, with and without H2O2 challenge (75 μM, 5 minutes, 4°C), were centrifuged for 5 minutes at 285 × g. The supernatant was discarded and the cell pellet re-suspended in 75 μL of 0.85% low melting point agarose in PBS and maintained in a water bath at 40°C. The suspension was added to previously prepared gels (normal agarose; 1%) on frosted slides and coverslips were added. The gels were chilled at 4°C and a further protective layer of low melting point agarose added. The slides were immersed in lysis buffer (2.5 mol/L NaCl, 100 mmol/L Na2 EDTA, 10 mmol/L Tris) for 1 hour at 4°C and then placed in electrophoresis buffer and allowed to unwind for 20 minutes before running at 26 V 300 mA for 20 minutes. After electrophoresis, gels were washed in neutralization buffer [0.4 mol/L Tris (pH 7.5) adjusted with HCl], 3 × 5-minute washes (4°C). Gels were stained with 20 μL of ethidium bromide (2 μg/mL) before scoring. Images were analyzed at 400× magnification using a fluorescence microscope. % Tail DNA was recorded using Komet 3.0 image analysis software (Kinetic Imaging Ltd., Liverpool). For each slide, 100 cells were scored. Positive (H2O2, 75 μmol/L) and negative (PBS) controls were included for all experiments. The mean % Tail DNA was calculated from 100 cells per gel (each sample in triplicate) and the mean of each independent experiment (n = 3) was used in the statistical analysis. Differences between means were evaluated by ANOVA, post hoc LSD (P < 0.05).

Dietary Intervention Study. The study was conducted with the approval of the ethics committee of the University of Ulster and with the informed consent of participants. Twenty healthy volunteers (10 male, 10 female, median age = 25.5 years, range = 21 to 45) were included in the parallel study. All volunteers completed a 7-day food diary before starting the study. The volunteers were randomly assigned to the gender-matched treatment and control groups. The treatment group consumed one portion (113 g) of CLS (raw) daily, for 14 days in addition to their normal diet. Fasting blood samples were collected on days 1 and 14 (week 0, week 2) by venipuncture into EDTA- or lithium heparin-contained tubes as required. Two subjects (one from each group) did not complete the study, reducing the total number to 18. All blood samples were processed on ice. Lymphocytes were isolated using Histopaque-1077, according to the manufacturer’s instructions (Sigma Diagnostics, St Louis, MO) and plasma samples were prepared by centrifugation at 1000 × g for 10 minutes. Washed red cells were also collected. All materials were immediately stored at –80°C or frozen in liquid nitrogen (lymphocytes).

COMET Assays (in vivo Study). Ideally in the in vivo study, colon biopsies would be taken and DNA damage assessed. Because this was not ethically possible in

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healthy volunteers, we chose to use lymphocytes as a surrogate, a minimally invasive end point used to evaluate in vivo antigenotoxicity. Peripheral blood lymphocytes were thawed from liquid nitrogen storage and screened for DNA damage with and without H2O2 insult (75 μm) according to the method previously described. In addition to single-stranded breaks (SSB) formamidopyrimidine DNA glycosylase modification to the method was used to allow oxidative purine damage to be assessed, according to the method of Rieger et al. (23). In brief, after the lysis stage, a slide to assess oxidative damage was washed in formamidopyrimidine DNA glycosylase reaction buffer [0.02 mmol/L Tris-HCl, 0.4 mol/L NaCl, 1 mmol/L EDTA, 0.5 mg/mL bovine serum albumin (pH 7.5)] for 3 × 5 minutes, 50 μL of formamidopyrimidine DNA glycosylase (10 μg/mL) were applied to the cells, and incubated at 37°C for 45 minutes. All slides were transferred to an electrophoresis chamber. The mean was calculated from 100 cells per gel (each sample in triplicate) and the mean of each set of data was used in the statistical analysis (22). Differences between means were evaluated by independent t test (P < 0.05).

Biochemical Assays Using Red Cell Concentrate. Total GST enzyme activity was determined in red cell concentrate according to the method of Lampe et al. (5) based on a modification of the method of Habig et al. (24). Glutathione was determined in red cell concentrate using a glutathione assay kit (Calbiochem, United Kingdom) in accordance with the manufacturer’s instructions. Both assays were run on a COBAS Fara centrifugal analyzer (Roche, United Kingdom).

Biochemical Assays Using Plasma. The ferric reducing ability of plasma assay was run according to the method of Benzie and Strain (25); glutathione peroxidase and superoxide dismutase were determined in plasma using the Ransel and Ransod kits, respectively (RANDOX, Northern Ireland), in accordance with the manufacturer’s instructions. The plasma assays were run on a Hitachi 912 analyzer. Plasma lutein, retinol, lycopene, α- and γ-tocopherol, and α- and β-carotene were analyzed by the HPLC method described by Thurnham et al. (26).

Uric acid and blood lipid profiles were carried out at Altnagelvin Hospital according to standard methods.

Results

The antigenotoxic and genotoxic effects of two concentrations of CLS extract (100 and 200 μL/mL) on DNA damage in HT29 cells following various incubation periods (1 and 24 hours) are shown in Fig. 1A and B. The addition of 100 μL/mL of extract to HT29 cells for both 1 and 24 hours before harvest did not cause any significant increase in DNA damage compared with PBS (negative control). In contrast, the addition of 200 μL/mL of extract for 1 and 24 hours had a significant genotoxic effect compared with PBS, with mean differences (±SE) of 12.04 ± 3.33 (P = 0.011) and 26.31 ± 0.8416 (P < 0.05), respectively. Pre-incubation (24 hours) of HT29 cells with CLS extracts at 100 or 200 μL/mL produced a significant antigenotoxic effect subsequently when exposed to H2O2 challenge (75 μm). For cells pre-incubated with 100 or 200 μL/mL CLS for 24 hours, a decrease in genotoxicity of
approximately 50% and 30%, respectively, was seen. Significant antigenotoxic effects were only shown by the 200 μL/mL CLS extract concentration when pre-incubated for 1 hour with HT29 cells, which showed an 11% decrease in genotoxicity.

Total intakes of energy and other micronutrients in the control and treatment subjects are given in Table 1. There was no statistical difference between the two groups for total daily caloric intake and for the micronutrients protein, fat, carbohydrate, or vitamins (C and E) before the commencement of the study.

The effect of the 2-week supplementation of sprouts on peripheral lymphocyte DNA damage and their ability to resist exogenous damage are shown in Fig. 2A and B. The % Tail DNA values are presented as the difference (week 2 to week 0) for individuals and the group mean of these values was examined between post- and pretreatment for the two groups had different baseline values. All statistical analysis was carried out on the difference values when comparing effect of treatment. No significant change (week 2 to week 0) was observed in the level of DNA damage or oxidative damage between the treatment or control groups when the difference between pre- and post-supplementation values was compared.

The mean of the individual differences for DNA damage (% Tail DNA) in treatment and control groups when the difference between post- and pretreatment 2 to week 0) for individuals and the group mean of these % Tail DNA values are presented as the difference (week 0 baseline data—group mean for DNA damage and oxidative DNA damage: active treatment group, 15.96 ± 5.12 and 18.3 ± 7.64; control group, 15.41 ± 1.48 and 17.97 ± 4.11, respectively). However, the effect of sprout supplementation became evident when the response of the lymphocytes to H2O2 insult was examined (week 0 baseline data H2O2 challenged—group mean for DNA damage and oxidative DNA damage: active treatment group, 39.87 ± 4.85 and 44.10 ± 7.85; control group, 30.00 ± 9.11 and 35.51 ± 5.95, respectively). There was a highly significant mean difference between the DNA damage (% Tail DNA) in treatment and control groups when the difference between week 2 and baseline (week 0) values were compared (−14.43 ± 4.26; P = 0.04, independent t test). A highly significant difference was also noted for oxidative damage (% Tail DNA) over the same period (−13.82 ± 4.25; P = 0.05, independent t test). No gender-specific effects were observed.

The data in Table 2 show that a 2-week supplementation with CLSs had no significant effects on GST, glutathione peroxidase, superoxide dismutase, reduced glutathione, antioxidant potential (ferric reducing ability of plasma), vitamin C, plasma lutein, retinol, or lipid profiles when treatment and control groups were compared for difference in parameters between week 2 and baseline (week 0). No gender effect was observed.

### Discussion

Brussels sprouts and possibly other CFVs may have several cancer preventive mechanisms, including increased expression of defense enzymes as well as prevention of oxidant-induced DNA strand breaks by possible direct antioxidant effects (27). Sulforaphane, an isothiocyanate metabolite isolated from broccoli, is a potent inducer of quinone reductase GST (phase II enzymes) in mouse tissues (28-30). Several of the phase II enzymes, including GST and quinone reductase, induced by these compounds are vital in the defense against oxidative stress (31). Human studies support the experimentally based notion of oxidative DNA damage as an important mutagenic and apparently carcinogenic factor (32). The H2O2 challenge has been used in several studies to examine the effect of brassica extracts for this reason (27, 33). It was evident from our study that a crude extract of immature vegetable sprouts exerted significant antigenotoxic activity against a H2O2 challenge in HT29 human colon cells. Because the sprout extract was removed from the cells before exposure to the H2O2 challenge, the reduction (of about 50%) of induced DNA damage indicates an increased cellular capacity to protect against oxidative damage. These results are generally consistent with other authors as they show a protective effect. For example, Laky et al. (9) showed that an extract of mature brussels sprouts suppressed the genotoxic effect of benzo(a)pyrene in Hep G2 cells, and Zhu and Loft (33) reported that H2O2-induced DNA damage in human lymphocytes was reduced by exposure to a brussels sprout extract but only in cooked and autolysed samples that are not raw. In our study, although both concentrations of sprout extract exhibited protective activity, the lower concentration (100 μL/mL) was more effective than a higher level (200 μL/mL) but only after 24 hours of exposure. This is likely to be a consequence of the observed genotoxic effect of the 200 μL/mL dose, which presumably mitigated its antigenotoxic effect toward H2O2 to a certain extent. Kassie et al. (21) also showed that crude vegetable juices induced genotoxic effects in a differential DNA repair assay with the effects of radish and broccoli juices being pronounced at concentrations of 200 μL/mL and above. Furthermore, allyl isothiocyanate (AITC), a major breakdown product of the glucosinolate sinigrin in brussels sprouts, exhibits dose-related genotoxic effects in Hep G2 cells (9). The effects observed at the highest concentration after 1 hour may suggest a direct effect on the oxidative insult rather than enzymatic modulation given the exposure time, which would be supported by the lack of effect observed for the lower concentration. But the fact that after 24 hours of exposure

### Table 1. Subject parameters and estimated baseline of daily nutrient intakes (mean values ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Active treatment group (n = 9)</th>
<th>Control treatment group (n = 9)</th>
</tr>
</thead>
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<tr>
<td>Age (y)</td>
<td>27.7 (7.21)</td>
<td>28.44 (7.6)</td>
</tr>
<tr>
<td>Body mass (kg/m²)</td>
<td>28.86 (6.55)</td>
<td>24.61 (2.44)</td>
</tr>
<tr>
<td>Energy intake (MJ)</td>
<td>7.70 (2)</td>
<td>8.0 (2.38)</td>
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<tr>
<td>Vitamin C intake (mg)</td>
<td>79 (56.88)</td>
<td>84 (45.64)</td>
</tr>
<tr>
<td>Vitamin E intake (mg)</td>
<td>4.7 (2.03)</td>
<td>5.84 (2.86)</td>
</tr>
<tr>
<td>Selenium intake (g)</td>
<td>55.11 (21.05)</td>
<td>54.67 (17.92)</td>
</tr>
<tr>
<td>Total fat intake (g)</td>
<td>58.12 (21.06)</td>
<td>77.82 (30.15)</td>
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<tr>
<td>Total carbohydrate intake (g)</td>
<td>73.43 (9.44)</td>
<td>71.98 (17.52)</td>
</tr>
<tr>
<td>Total protein intake (g)</td>
<td>233.73 (71.11)</td>
<td>233.18 (71.38)</td>
</tr>
</tbody>
</table>
only the low dose effectively protected the cells from genetic damage may suggest that more than direct action was involved and that modulation of phase II may have been a potential reason for the protective effect, as it has also been reported that GST activity in HT29 cells can be modulated (34).

Although in vitro data are valuable for evaluating potential protective effects of vegetables, it is crucial to establish whether such antigenotoxic activity could be observed in humans. Thus, the most important finding in this study was the in vivo antigenotoxic effect of CLS consumption in lymphocytes. Currently, few studies exist that have measured the effect of (edible) plant consumption on genotoxicity in humans. A study by Verhagen et al. (35) supported the idea that brassica consumption may diminish cancer risk, using excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) from human urine. A recent study by Leuratti et al. (15) reported an inverse association with the quantity of DNA adducts (malondialdehyde-deoxyxynogosine) in human colorectal mucosa and legume consumption. This short-term supplementation study with CLS significantly reduced DNA damage and oxidative (purine) DNA damage in human lymphocytes in response to a subsequent H₂O₂ challenge ex vivo, a result that was entirely consistent with our preliminary in vitro experiment. While no change was observed in background oxidative damage in the above in vitro study, our findings on the effects of vegetable consumption and antigenotoxicity agree with those of Riso et al. (36). The latter study showed that a 21-day supplementation with tomato puree in healthy women significantly decreased DNA damage in blood lymphocytes in response to ex vivo H₂O₂ insult as measured by the comet assay. The first study on lymphocyte DNA damage after consumption of vegetables was by Pool-Zobel et al. (37), which showed that vegetable consumption decreased basal DNA damage, but did not effect the response to challenge. A recent observational study, which examined nutritional and lifestyle determinants of lymphocyte DNA damage in a healthy population, failed to show a significant effect for fruit and vegetable consumption. However, the authors did report a significant inverse association between CFV consumption and basal oxidative DNA damage (38). Previous dietary intervention trials with vegetables have yielded variable results. The most recent study by Moller et al. (39) reported that consumption of fruits and vegetables (600 g) or vitamins and minerals (placebo) had no effect on oxidative DNA damage measured in mononuclear cell DNA or urine. H₂O₂ sensitivity, detected by the comet assay, did not differ between the groups. Use of juices or purees (40) seems to be more effective than whole vegetables (38), suggesting that bioavailability may be important. It is noteworthy, therefore, that our study using intact vegetables gave positive results. It is possible that this reflects a higher concentration of phytochemicals in the young plants compared with mature ones or the use of one specific vegetable type compared with another.

The reasons for the observed antigenotoxic effects are unknown; the effect may be related to antioxidant status. Torbergsen and Collins (41) concluded that carotenoid supplementation (7 days) in humans appeared to enhance lymphocyte recovery but it was believed that this was a result of an antioxidant protective effect rather than a stimulation of DNA repair. As such, the ferric reducing ability of plasma assay was undertaken to examine overall antioxidant effect of the major species of non-enzymatic antioxidants (including uric acid, ascorbic acid, and α-tocopherol). In our study, the reported antigenotoxic...
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment group</th>
<th>Control group</th>
<th>Difference in mean group response to treatment (week 2 to week 0) ± SD</th>
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<tr>
<td></td>
<td>(means ± SD)</td>
<td>(means ± SD)</td>
<td></td>
</tr>
<tr>
<td>GST (units/g Hb)</td>
<td>3.26 (1.92)</td>
<td>3.00 (1.64)</td>
<td>−0.26 (0.54)</td>
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<tr>
<td>GSH (μmol/g Hb)</td>
<td>5.76 (1.55)</td>
<td>5.99 (1.05)</td>
<td>0.37 (1.34)</td>
</tr>
<tr>
<td>SOD (units/mL)</td>
<td>266.53 (22.63)</td>
<td>292.34 (22.80)</td>
<td>−6.83 (43.22)</td>
</tr>
<tr>
<td>GPX (units/mL)</td>
<td>396.05 (129.86)</td>
<td>389.72 (98.59)</td>
<td>−23.44 (27.40)</td>
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<tr>
<td>FRAP</td>
<td>1,184.80 (182.41)</td>
<td>1,142.00 (105.87)</td>
<td>−16.67 (218.43)</td>
</tr>
<tr>
<td>Vitamin C (μg/L)</td>
<td>10.61 (2.26)</td>
<td>11.01 (3.36)</td>
<td>−0.40 (2.52)</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.94 (0.75)</td>
<td>4.69 (0.75)</td>
<td>−0.26 (0.43)</td>
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<tr>
<td>HDL (mmol/L)</td>
<td>1.39 (0.46)</td>
<td>1.37 (0.45)</td>
<td>−0.26 (0.09)</td>
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<tr>
<td>LDL (mmol/L)</td>
<td>1.40 (0.81)</td>
<td>1.13 (0.60)</td>
<td>−0.27 (0.71)</td>
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<tr>
<td>HDL/LDL ratio</td>
<td>3.27 (0.86)</td>
<td>3.09 (0.67)</td>
<td>−0.18 (0.42)</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.66 (1.20)</td>
<td>2.49 (0.96)</td>
<td>−0.17 (0.29)</td>
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<td>Retinol</td>
<td>2.71 (0.66)</td>
<td>2.82 (0.85)</td>
<td>−0.02 (0.29)</td>
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<td>Lutein</td>
<td>0.36 (0.22)</td>
<td>0.49 (0.30)</td>
<td>0.11 (0.51)</td>
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<td>γ-Tocopherol</td>
<td>2.73 (1.26)</td>
<td>2.81 (1.92)</td>
<td>0.08 (0.93)</td>
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<tr>
<td>α-Tocopherol</td>
<td>35.28 (4.23)</td>
<td>35.64 (6.01)</td>
<td>0.36 (3.75)</td>
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<tr>
<td>α-Cryptoxanthine</td>
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<td>0.10 (0.08)</td>
<td>0.01 (0.02)</td>
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<tr>
<td>β-Cryptoxanthine</td>
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<td>0.09 (0.07)</td>
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<td>Lycopene</td>
<td>0.98 (0.53)</td>
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<tr>
<td>α-Carotene</td>
<td>0.20 (0.14)</td>
<td>0.17 (0.12)</td>
<td>0.05 (0.23)</td>
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<tr>
<td>β-Carotene</td>
<td>0.66 (0.48)</td>
<td>0.57 (0.40)</td>
<td>0.31 (0.70)</td>
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</table>

Abbreviations: GSH, reduced glutathione; SOD, superoxide dismutase; GPX, glutathione peroxidase; FRAP, ferric reducing ability of plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

effect of CLS consumption was not linked to an increase in antioxidant status reflected in ferric reducing ability of plasma activity, ascorbic acid, uric acid, or α-tocopherol plasma levels. Neither did the plasma levels of lycopene or α- and β-carotene change significantly.

It is known that CFVs, especially the brassicas, contain a high concentration of isothiocyanates, which are potent inducers of detoxification enzymes, such as GSTs. It has also been reported that GST activity in HT29 cells can be modulated (34). GSTs can reduce carcinogenicity by conjugation reaction with reduced glutathione, transforming a carcinogen to a compound with lower biological activity and increased excreetability (42). The consumption of CFV has been shown to increase human plasma and urine clearance of various compounds, including heterocyclic amines [refs. 42-44; reviewed recently by Steinkellner et al. (11)]. CFV do not seem to alter plasma GST activity, but have been shown to enhance specific isozymes GSTπ and GSTμ (5, 7). In both the studies of Lampe et al. (5) and Nijhoff et al. (7), the CFV feeding periods were only 6 and 5 days, respectively. Our study represented a feeding period of CFVs approximately 2 to 3 times longer than either of these previous studies, but as with these trials, our study also failed to show an effect on GST activity. Neither did it show any effects on reduced glutathione level, superoxide dismutase, or glutathione peroxidase activity. These data seem to suggest that the antigenotoxic effects observed in this study are not related to an induction effect on detoxifying enzymes.

There was, however, a small decrease (5%) in cholesterol levels although it was not statistically significant over the 2-week supplementation, while control values decreased by around 2%. Only observation over a longer supplementation period may determine if these effects are the result of the treatment or simply normal fluctuation.

In a dietary non-controlled intervention, such as the study reported here, there is always a risk that unrecorded dietary differences are actually behind the effects observed. However, due to the already high burden placed on the subjects by consumption of 115 g sprouts daily for 14 days and the issue of further increasing the participant burden, subject dissatisfaction and failure to complete became a very real concern. As such, the second food record was not taken, instead we relied on continual reinforcement every 2 or 3 days (with delivery of fresh sprouts) that the sprout consumption was in addition to the habitual diet. Although not ideal, given that there is a risk, unrecorded dietary differences may be behind the effects, we felt that the decision was warranted and the risk of effect being attributed to other factors small especially given the short duration and high potency feeding.

To summarize, our in vitro and in vivo data provide important evidence that supports the belief that consumption of CFV is linked to a reduced risk of cancer via a decrease in oxidative and other damage to DNA in humans. However, we were unable to link this effect to concomitant changes in detoxification enzymes or to antioxidants in plasma. It is possible that young sprouts with their high concentrations of phytochemicals may be a potent source of protective chemicals against cancer.
Acknowledgments

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