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Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults¹⁻³

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ABSTRACT

Background: Cruciferous vegetable (CV) consumption is associated with a reduced risk of several cancers in epidemiologic studies.

Objective: The aim of this study was to determine the effects of watercress (a CV) supplementation on biomarkers related to cancer risk in healthy adults.

Design: A single-blind, randomized, crossover study was conducted in 30 men and 30 women (30 smokers and 30 nonsmokers) with a mean age of 33 y (range: 19–55 y). The subjects were fed 85 g raw watercress daily for 8 wk in addition to their habitual diet. The effect of supplementation was measured on a range of endpoints, including DNA damage in lymphocytes (with the comet assay), activity of detoxifying enzymes (glutathione peroxidase and superoxide dismutase) in erythrocytes, plasma antioxidants (retinol, ascorbic acid, α -tocopherol, lutein, and β -carotene), plasma total antioxidant status with the use of the ferric reducing ability of plasma assay, and plasma lipid profile.

Results: Watercress supplementation (active compared with control phase) was associated with reductions in basal DNA damage (by 17%; $P = 0.03$), in basal plus oxidative purine DNA damage (by 23.9%; $P = 0.002$), and in basal DNA damage in response to *ex vivo* hydrogen peroxide challenge (by 9.4%; $P = 0.07$). Beneficial changes seen after watercress intervention were greater and more significant in smokers than in nonsmokers. Plasma lutein and β -carotene increased significantly by 100% and 33% ($P < 0.001$), respectively, after watercress supplementation.

Conclusion: The results support the theory that consumption of watercress can be linked to a reduced risk of cancer via decreased damage to DNA and possible modulation of antioxidant status by increasing carotenoid concentrations. *Am J Clin Nutr* 2007;85:504–10.

KEY WORDS Watercress, cruciferous vegetables, DNA damage, antioxidants, humans, cancer biomarkers

INTRODUCTION

Increased vegetable intake, particularly of cruciferous vegetables (CVs) such as cabbage, cauliflower, broccoli, Brussels sprouts, watercress, and mustard greens, is associated with a decreased risk of several cancers in human population studies (1–9). However, not all associations, which have mostly been obtained from epidemiologic studies, are necessarily causal, and thus, intervention studies with specific dietary factors of interest are crucial.

CVs, especially those of the *Brassica* variety, have been shown to display several anticarcinogenic properties *in vivo*, as reviewed by Steinkellner et al (10) with the various underlying mechanisms having been summarized by others (11). Some of these mechanisms include alterations in the activities of metabolic enzymes (12), resulting in reduced carcinogenicity of dietary or environmental carcinogens *in vivo* (13), reduction of oxidative DNA damage levels in humans after supplementation with Brussels sprouts (14), and reduced DNA damage in human lymphocytes *ex vivo* in conditions of increased oxidative stress after supplementation with cruciferous and leguminous sprouts (15). Moreover, in *in vitro* studies, extracts of Brussels sprouts have been shown to reduce the genotoxic effects of hydrogen peroxide in human lymphocytes (16), and those of cruciferous and leguminous sprouts reduced genotoxic effects of hydrogen peroxide in human colon cancer (HT-29) cell lines (15). In terms of the active chemical species, CVs are rich sources of glucosinolates, a class of sulfur- and nitrogen-containing glycosides that are hydrolyzed (by plant myrosinase or intestinal microflora) to form isothiocyanates. These isothiocyanates have been shown, in several *in vitro* and *in vivo* studies, to display anticarcinogenic properties as reviewed previously (9, 17). Watercress (*Rorippa nasturtium-aquaticum*) in particular contains one of the highest concentrations of glucosinolates per gram weight of any vegetable (18, 19) as well as containing high concentrations of carotenoids such as lutein and β -carotene (20). These phytochemicals have also been associated with various anticarcinogenic properties, including antioxidant activities. Members of the *Cruciferae* family have also been shown to contain high amounts of phenolic compounds (21).

Despite the widely documented protective effects of CVs, only a few studies have investigated their effects on cancer risk

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with the use of human intervention trials. These trials have either been of parallel or crossover designs, with subject numbers ranging from 10–43, intervened with either broccoli, Brussels sprouts, or cruciferous and leguminous sprouts for between 3 d and 21 d in healthy subjects, measured improvements in a range of surrogate endpoints relating to cancer risks, including glutathione *S*-transferase (GST) activity, lymphocyte DNA damage, and urinary metabolite excretion profiles (14, 15, 22, 23). The aim of the present study was to investigate whether a diet supplemented with watercress had any effects on intermediate endpoints (biomarkers) of cancer risk by using blood as a surrogate tissue. We measured the concentrations of various antioxidants, activities of metabolizing enzymes, and DNA damage in lymphocytes.

SUBJECTS AND METHODS

Subjects

The study was conducted in sixty volunteers [mean (\pm SD) age: 32.98 \pm 10.97 y; range: 19–55 y]; 30 were men and 30 were women, 30 were nonsmokers and 30 were smokers (smoked 15–25 cigarettes/d). All subjects were healthy and nonusers of dietary supplements or medications, as determined using a pre-screening health and lifestyle questionnaire and standard clinical tests. The study was conducted with the prior approval of the ethics committee of the University of Ulster and with the informed consent of participants.

Study design

The study design was a single blind, randomized crossover trial. The volunteers were randomly assigned to either the treatment (watercress supplemented) or control group during the first phase of the study. It was ensured that each of these groups contained equal numbers of men and women and of smokers and nonsmokers. During the treatment phase, the subjects consumed one portion (85 g) of raw watercress daily for 8 wk in addition to their normal diet. During the control phase (8 wk), the subjects were asked to maintain their habitual diet. The control and the treatment phases were separated by a 7-wk washout phase. All volunteers completed a 7-d food diary during each phase of the trial. The watercress used for this intervention study was a commercially available product produced by Vitacress Ltd (Southampton, United Kingdom). The subjects were supplied with fresh watercress (85 g bag/d) during the supplementation phase, and it was purchased from a local supermarket every 2–3 days. Fasting blood samples were collected before and after each phase (week 0, week 8, week 15, and week 23) by venepuncture into EDTA- or lithium heparin-containing tubes, as required. All blood samples were processed on ice. Lymphocytes were isolated by using Histopaques-1077, according to the manufacturers instructions (Sigma Diagnostics, St Louis, MO), and plasma samples were prepared by centrifugation at 1000 \times *g* for 10 min, 4 °C. Red blood cell concentrate (washed twice with phosphate-buffered saline) samples were also collected. Plasma and red blood cells were immediately stored at –80 °C, whereas lymphocytes were stored frozen in liquid nitrogen. All biological measurements were carried out at the end of the intervention in batches containing equal number of active and control phase samples in each batch, and the researchers were blinded to these samples during analyses.

Methods

Watercress component analysis

A random bag of watercress was selected from each weekly supply during the 8-wk period that subjects consumed watercress. These samples were immediately stored at –80 °C (in an unopened form, stored in its original protective atmosphere) until analysis. Originally, sample bags of watercress were collected for both watercress intervention periods to allow comparison of levels throughout the 6-mo study. However, a freezer breakdown resulted in the loss of the material collected during the first watercress consumption period, and, consequently, all data related to samples of supplemented watercress are derived from the second intervention period. Watercress leaves were processed by using methods previously detailed by Mellon et al (24) with the use of liquid chromatography–mass spectroscopy (LC-MS). All glucosinolate standards had been previously purified, and flavonoid standards were either obtained from Extrasynthese (Genay, France) or had been previously purified from broccoli (quercetin-3-*O*-sophoroside and various hydroxycinnamic acid gentiobiose derivatives) and lettuce (quercetin-3-*O*-[6'-malonyl-glucoside]). The samples were freeze-dried and milled to a fine powder before extraction. All samples were analyzed in triplicate, by using ion-pair LC with UV-vis and also ion-pair LC/electrospray Ionisation (ESI) MS (to further confirm identities). The LC gradient for glucosinolate and phenolic analysis is a multipurpose chromatographic method that simultaneously separates glucosinolates and phenolics. The samples (40 mg) were extracted in 1 mL 70% acetonitrile (MeCN) at 70 °C for 20 min before being processed by the method previously detailed, with the use of sinigrin as the extraction standard. An injection volume of 20 μ L was used. Glucosinolate and phenolic analyses were performed by using the negative ion electrospray ramped cone voltage method. Hydrolysis product analyses were performed by using the same LC/MS system.

DNA damage in lymphocytes

Peripheral blood lymphocytes, previously isolated and stored in liquid nitrogen, were thawed and screened for single strand breaks (SBs) in DNA by using the single cell gel electrophoresis (Comet) assay (25). Formamidopyrimidine DNA glycosylase (FPG) modification to the method was also used to allow additional oxidative purine damage to be assessed, according to the method of Collins et al (26). In brief, after the lysis stage, a separate slide to assess oxidative damage was washed in FPG reaction buffer (0.02 mmol/L Tris-HCL, 0.4 M NaCL, 1 mmol/L EDTA, and 0.5 mg/mL BSA, pH 7.5) for 3 \times 5 min. After this, 40 μ L of FPG (16 U/mL) was applied to the cells and incubated at 37 °C for 30 min. All slides were then transferred together to an electrophoresis chamber. In addition, basal DNA damage (SB) was measured in lymphocytes subjected to increased oxidative insult *ex vivo* by pretreating lymphocytes with 150 μ mol H₂O₂/L for 5 min, 4 °C, before the measurement of SBs. The mean (percentage DNA in tail) was calculated from 50 cells per gel (each sample in duplicate) and the mean of each set of data were used in the statistical analysis.

Biochemical assays with the use of plasma and erythrocytes

The ferric reducing/antioxidant and ascorbic acid concentration (FRASC) assay was used in the simultaneous measurement



of ascorbic acid and the total antioxidant capacity of plasma (ferric reducing ability of plasma, FRAP), as described in the methods of Benzie and Strain (27, 28). Working FRASC reagent was prepared by mixing 25 mL acetate buffer (300 mmol/L [3.1 g C₂H₃NaO₂ · 3H₂O (Riedel-de Haen, Seelze, Germany)], pH 3.6) and 16 mL C₂H₄O₂ [BDH Laboratory Supplies, Poole, UK] per liter of buffer solution, 2.5 mL TPTZ solution [10 mmol/L [2,4,6]-tripyrindyl-*s*-triazine (Fluka Chemicals, Buchs, Switzerland) in 40 mmol/L HCl (BDH)] and 2.5 mL FeCl₃ 6H₂O solution (20 mmol/L; BDH). The Hitachi 912 autoanalyser (Roche, Basel, Switzerland) was used to obtain both the FRAP value and the ascorbic acid concentration of the samples. This was done via monitoring the 0–4 min absorbance change of paired aliquots of water (40 μL added to 100 μL sample) and ascorbate oxidase (40 μL of a 4 μL/mL solution added to 100 μL sample)-treated samples run in parallel. The 4 min absorbance change of the aliquot diluted in water is referred to as the FRAP value. From this value, the absorbance change of the aliquot diluted with ascorbate oxidase was subtracted and the difference in the 2 readings was due specifically to the ascorbic acid (vitamin C) in the sample. By using the difference of the 2 readings (with and without ascorbate oxidase) and the stoichiometric factor of the reaction, ascorbic acid concentration of plasma was calculated. Glutathione peroxidase (GPX) in red blood cell concentrate was measured on the Hitachi 912 using a commercial kit (RANSEL kit; Randox Laboratories Ltd, Crumlin, CO Antrim, United Kingdom) according to kit manufacturers instructions. RCC aliquots were diluted in diluting buffer (40 μL RCC in 800 μL diluting buffer), followed by an addition of 800 μL Double Strength Drabkin's reagent (RANSEL kit). Superoxide dismutase (SOD) in red blood cell concentrate was measured by using the Hitachi 912 with a commercial kit (RANSOD kit; Randox Laboratories Ltd, Crumlin, CO Antrim, United Kingdom) according to manufacturer's instructions. RCC aliquots were diluted in phosphate buffer (20 μL RCC in 4 mL phosphate buffer). SOD and GPX controls (Randox Laboratories Ltd) were run every 20 tests. The % CVs of the interbatch controls for both the assays were <5%. The GPX and the SOD results were standardized to red blood cell concentrate (RCC) hemoglobin concentration, and the final results were expressed in U/g hemoglobin. Plasma lutein, retinol, α-tocopherol, and β-carotene were analyzed by simultaneous determination by using the HPLC method described by Thurnham et al (29). Plasma total cholesterol, HDL cholesterol, and triacylglycerols were measured on the Hitachi 912 autoanalyser by using commercial kits (Roche diagnostics, Lewis, United Kingdom) according to kit manufacturer's protocols, and plasma LDL cholesterol was calculated from the other 3 lipid profile parameters by using the Friedewald formula (30).

Statistics

All values are expressed as mean ± SD, unless otherwise specified. The mean values are shown for all subjects (*n* = 60) during their supplementation (watercress) phase and during their control (no watercress) phase. The differences in the mean were calculated using paired samples *t* test for normally distributed data, whereas Mann-Whitney *U* test or Wilcoxon's signed-rank test were used for data that were not normally distributed. For the blood biomarker measurements, the results are presented as treatment effects, this was achieved by calculating individual differences between the values before and after treatment for both the

TABLE 1

Average concentration of phenolics, glucosinolates (GLS), and hydroxylated cinamic acid (HCA) derivatives in watercress leaves consumed by the subjects¹

Phenolic or glucosinolate	Fresh weight	Dry weight
	μmol/g	
Q-3- <i>O</i> -Sophoroside, 7- <i>O</i> -Glucoside	0.09 ± 0.02	1.0 ± 0.2
Q-3- <i>O</i> -Glc-(6"-Malonyl-Glc)	0.13 ± 0.029	1.43 ± 0.295
Q-3- <i>O</i> -Sophoroside	0.05 ± 0.005	0.59 ± 0.05
Q-3- <i>O</i> -Rutinoside (Rutin)	0.05 ± 0.004	0.6 ± 0.017
7-Methylsulfinylheptyl-GLS	0.1 ± 0.002	1.07 ± 0.025
8-Methylsulfinylheptyl-GLS	0.06 ± 0.07	0.68 ± 0.148
3-Indolylmethyl-GLS	0.04 ± 0.07	0.43 ± 0.26
2-Phenylethyl-GLS	1.53 ± 1.41	17.98 ± 4.31
4-Methoxy-3-indolylmethyl-GLS	0.065 ± 0.017	0.791 ± 0.05
Total HCA derivatives	9.40 ± 0.832	109.03 ± 4.22

¹ All values are $\bar{x} \pm SD$. Mean values of 8 bags of watercress, sampled once per week from one watercress intervention period (8 wk).

control and the supplementation phases for each subject. The statistical tests were then carried out on the difference (after – before) in values between treatment (watercress) and control phases. All blood biomarkers were measured in duplicates, and the average of the 2 values were taken as the final result. The results were also tested for dietary treatment and smoking interactions by using a univariate general linear model, and where significant interactions with smoking were observed, the data were further analyzed separately for subgroups of smokers and nonsmokers. All statistical analyses were performed by using the SPSS software version 11.0 (SPSS Inc, Chicago, IL).

RESULTS

Analysis of watercress leaves was carried out to determine average contents of key phenolic compounds and glucosinolates present in the leaves, as shown in Table 1. In terms of the phenolic components in watercress leaves, several glycosides of quercetin, including rutin, were present, although the major phenolics were derivatives of hydroxycinnamic acid. A number of glucosinolates were also present, with 2-phenylethylglucosinolate predominating.

Mean daily energy, macronutrients, and the relevant micronutrients intake of the subjects (*n* = 58) during the control phase (no watercress supplementation) and active phase (watercress supplementation) of the crossover trial are shown in Table 2. Two of the sixty subjects failed to return a completed food diary in each phase; body mass index data were available for 54 of the 60 subjects. However, all (*n* = 60) who started the intervention study finished it to completion, so all blood measurements were carried out with a full set of samples. No statistical differences in body mass index (*n* = 54), energy intake, and macronutrient intakes were observed between the control phase and the watercress phase of the study. However, the mean intakes of dietary fiber (*P* < 0.05), vitamin C (*P* < 0.001), vitamin E (*P* < 0.01), folate (*P* < 0.05) and carotene (*P* < 0.001), were higher during the watercress phase of the study than during the control phase.

The effects of watercress consumption on peripheral blood lymphocyte DNA damage in terms of basal DNA damage (SBs), basal plus oxidative purine DNA damage (basal plus oxidative), and finally the ability of the lymphocytes to resist exogenous (basal) DNA damage [150 μmol/L hydrogen peroxide challenge



TABLE 2
Nutrient intake and BMI during watercress-supplemented and control phases of the study

Variables	Watercress phase (<i>n</i> = 58) ¹	Control phase (<i>n</i> = 58) ¹	Change ² %	<i>P</i>
BMI (kg/m ²) ³	25.2 ± 4.1 ⁴	25.3 ± 4.0	0	NS
Energy intake (kcal)	1954.5 ± 848.1	1899 ± 792.1	3	NS
Total fat intake (g)	78.1 ± 36.6	72.2 ± 33.3	8	NS
Total protein intake (g)	77.8 ± 24.4	74.8 ± 29.1	4	NS
Total carbohydrate intake (g)	208.6 ± 96.5	212.9 ± 92.0	-2	NS
Fiber intake (g) ⁵	12.1 ± 5.0	11.2 ± 5.4	8	<0.05
Vitamin C intake (mg)	131.1 ± 52.4	97.6 ± 76.5	34	<0.001
Vitamin E intake (mg)	7.8 ± 4.0	6.2 ± 4.0	26	<0.01
Folate intake (μg)	266.2 ± 132.4	239.9 ± 129.4	11	<0.05
Carotene intake (μg)	4019.6 ± 1235.4	2253 ± 1856.0	78	<0.001

¹ Indicates the number of subjects who returned a completed food diary for that phase (from a total of 60 subjects). Mean treatment group values were significantly different between phases, *P* < 0.05 (Mann-Whitney *U* test).

² Calculated from watercress value - control value.

³ *n* = 54.

⁴ $\bar{x} \pm SD$ (all such values).

⁵ Calculated by using the Englyst method.

(H₂O₂ challenged) are shown in Figure 1. The percentage tail DNA values (\pm SEM) are presented as treatment effects; ie, the group means (active and control) of individual differences between the values after and before treatment, with all statistical analyses being carried out on mean of difference values between the watercress phase and control phase. Significant decreases in DNA damage were observed (basal, and basal + oxidative purine) for the subjects when consuming watercress. In the group as a whole, the mean differences between treatments were 17%, -2.65 ± 1.17 (*P* = 0.03) for basal DNA damage; 22.9%, -4.53 ± 1.14 (*P* = 0.002) for basal + oxidative-induced DNA damage; and 9.4%, -3.10 ± 1.66 (*P* = 0.07) for the H₂O₂ challenge. The data were further analyzed for smoking interactions by using a univariate general linear model (*P* < 0.01). We found that both basal DNA damage and H₂O₂ challenge had significant interactions, and hence we tested the effects of treatment in the 2 subgroups per se. In smokers, the mean difference between treatments for basal DNA damage was 25.6%, -3.98 ± 1.72 (*P* = 0.03); for H₂O₂ challenge was 15.5%, -5.05 ± 2.54 (*P* = 0.056). In the non-smoking group, the mean differences between treatments for basal DNA damage was 8.53%, -1.31 ± 1.58 (*P* = 0.4); for H₂O₂ challenge, was 3.4%, -1.14 ± 2.12 (*P* = 0.6).

The data shown in Table 3 show that watercress supplementation significantly increased the plasma concentration of the carotenoids measured. Plasma β -carotene concentration rose by \approx 33% and lutein by 100% after watercress supplementation, mean differences between treatments of 0.10 ± 0.024 and 0.17 ± 0.019 μmol/L (both *P* < 0.001, by using Wilcoxon's signed-rank test) were observed, respectively. Of the carotenoids, only β -carotene evidenced a significant smoking interaction, with a highly significant increase (*P* < 0.001) in β -carotene concentrations observed for nonsmokers and a lower significant increase (*P* < 0.05) observed for smokers during the watercress phase compared with the control phase. No significant changes due to watercress supplementation were observed for plasma concentrations of α -tocopherol, retinol, or vitamin C or in the total antioxidant potential of plasma (FRAP value). Generally, lipid

profiles (LDL, HDL, and total cholesterol) were unaffected by watercress consumption, with the exception of plasma triacylglycerol concentration, which showed a decrease of about 10% (-0.13 ± 0.1) during the active (supplemented) phase compared with the control phase (*P* = 0.07, Wilcoxon's signed-rank test). In response to watercress, red blood cell GPX and SOD activity did not change significantly in the total population.

DISCUSSION

There is considerable scientific consensus from epidemiologic studies that CVs may reduce risk of cancers, including cancers of the lung (5, 6), prostate (7), colon (31), and of the lymphatic system (8, 32). However, debate on the issue concerns which types of Cruciferae are most important and whether the epidemiologic observations can be confirmed with experimental studies conducted in humans. The present study has focused on watercress, which has a particularly high content of glucosinolates and other potential anticancer phytochemicals including carotenoids. An in vitro study within our laboratory showed that watercress extract can protect cells (HT-29; colon cancer cell line) against DNA damage levels induced by genotoxic agents such as H₂O₂ and fecal water (33). In the present study, we reported a decrease in all our measures of DNA damage in lymphocytes in response to watercress consumption in humans in vivo. This is consistent with previous studies on vegetable consumption reported by Riso et al (34) and Pool-Zobel et al (35). Two of the 3 measures of DNA damage (basal DNA damage and H₂O₂ challenge, Figure 1) were found to have significant interactions with smoking, and, on further subgroup analyses, we found the reductions in these variables as a result of watercress supplementation were of greater magnitude and more significance in smokers than in nonsmokers. This may reflect a higher toxin burden present in the smoking group, as has been indicated by a significantly lower total antioxidant status at baseline in smokers than in nonsmokers (*P* < 0.01, results not shown), possibly suggesting a greater benefit of consumption of watercress for this compromised group. A recent study by Kang et al



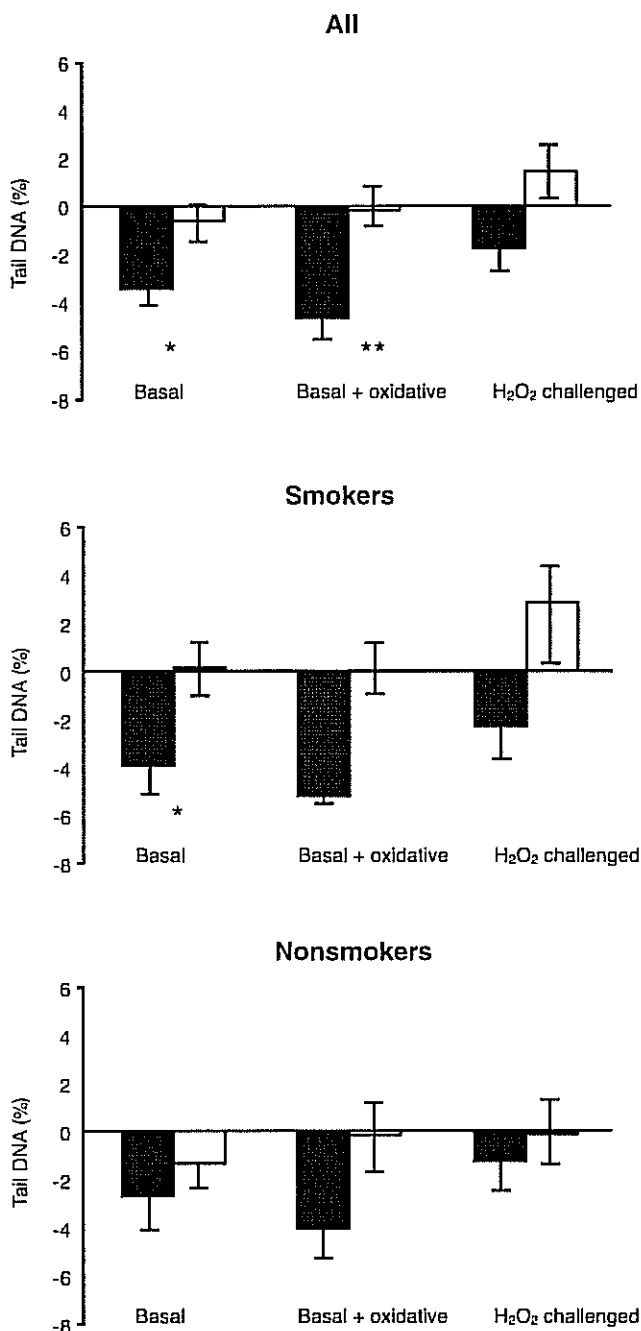


FIGURE 1. Comparison of the effects of watercress supplementation phase (■) with that of a control phase (□) on lymphocyte DNA damage in the total study population ($n = 60$), in smokers ($n = 30$), and in nonsmokers ($n = 30$) determined by using the Comet assay. Lymphocytes were collected from the subjects before and after watercress supplementation and were untreated (basal) or treated ex vivo with formamidopyrimidine DNA glycosylase (basal + oxidative) to assess oxidated purines or hydrogen peroxide (H_2O_2 challenged) to test for DNA damage resistance. DNA damage was measured as the mean of the individual difference (after - before supplementation values) in the active and control phases. * $P \leq 0.05$, ** $P \leq 0.01$ (paired-samples t test). Interactions between smoking and DNA damage measures were tested by a univariate general linear model, and, when such an interaction was evident, the smokers and nonsmokers were analyzed for treatment effect per se.

(36) which used a commercially available green vegetable drink (*Angelica keiskei*-based juice) given to smokers daily for 8 wk, also reported a significant decrease in lymphocyte DNA damage.

However, in contrast to our data and that of other authors, some recent articles have shown little or no association between vegetable consumption and biomarkers of cancer risk. In a 24 d, parallel design, intervention study performed in 43 healthy male and female subjects (nonsmokers), consumption of fruit and vegetables (600 g) or supplementation with vitamins and minerals compared with placebo had no effect on oxidative DNA damage measured in mononuclear cells or urine (37). A further article on the "6 a day study" reported that the fruit and vegetable diet enhanced resistance of plasma lipoproteins to oxidation and increased erythrocyte glutathione peroxidase activity (38). The reasons for the limited or different effects observed in this study in comparison with our study may be related to the choice of study design (parallel compared with crossover), population size (15 subjects compared with 60) or the difference in exposure period (24 d compared with 56 d). Moller and Dragsted et al (37, 38) did, however, exercise dietary control over their subjects, whereas our study simply supplemented the normal diet of the participants. However, dietary assessment with 7-d food diaries indicated that watercress supplementation did not alter the overall dietary habits of our subjects, as evidenced from similar energy and macronutrient intakes during the supplemented and control phases of the study. The increased intakes of some micro- or extra nutrients (vitamin C, vitamin E, folate, and carotene) during the active phase were most likely to be attributable to watercress supplementation.

The mechanisms that produced the antigenotoxic effects in this study due to watercress supplementation are unknown, although this may be related to antioxidant status (39). Watercress is known to be a rich source of the carotenoids lutein and β -carotene (20). Therefore, it was not surprising that plasma concentrations of these carotenoids were significantly elevated after watercress intervention. A recent placebo controlled trial with carotenoid supplements (lutein, β -carotene, and mixed carotenoids) conducted in postmenopausal women for 56 d showed a decrease in endogenous lymphocyte DNA damage as a result of carotenoid supplementation (40). Therefore, increases in the in vivo concentrations of lutein and β -carotene may contribute to the decrease in DNA damage levels in lymphocytes observed in the present study. The greater difference in the concentrations of β -carotene in nonsmokers than in smokers as a result of watercress supplementation may indicate that this carotenoid was used up to a higher extent in smokers, perhaps reflecting a greater requirement of this antioxidant in smokers. In support for this concept, a similar observation was also made in a previous study, in which plasma lutein and β -carotene status increased more markedly in nonsmokers than in smokers as a result of green vegetable supplementation (41). Furthermore, the elevated plasma lutein in particular may provide additional health benefits to watercress consumers in terms of cardiovascular risk (42) and macular degeneration (43). A recent prospective study also reported that high plasma concentrations of carotene (including β -carotene) were associated with a reduced risk of mortality from cancers and cardiovascular diseases (44). Finally, because plasma lutein can reflect intake of green leafy vegetables (45) as well as of CVs (46), the increased concentrations of the lutein as a result of watercress supplementation indicated good compliance in the study participants during the


TABLE 3
Effects of watercress supplementation on biological variables in blood¹

Variables	Watercress phase (n = 60)		Control phase (n = 60)	
	Before treatment	After treatment	Before treatment	After treatment
β -Carotene ($\mu\text{mol/L}$)	0.33 \pm 0.19	0.43 \pm 0.26 ²	0.32 \pm 0.21	0.33 \pm 0.2
Lutein ($\mu\text{mol/L}$)	0.18 \pm 0.07	0.35 \pm 0.18 ²	0.18 \pm 0.07	0.17 \pm 0.07
α -Tocopherol ($\mu\text{mol/L}$)	27.29 \pm 6.23	26.61 \pm 6.13	27.28 \pm 6.71	26.31 \pm 5.37
Retinol ($\mu\text{mol/L}$)	1.89 \pm 0.39	1.84 \pm 0.36	1.89 \pm 0.43	1.79 \pm 0.37
Vitamin C ($\mu\text{mol/L}$)	57.6 \pm 30.6	59.3 \pm 29.13	57.25 \pm 28	51.5 \pm 32.64
FRAP (arbitrary units)	1227 \pm 239	1203 \pm 184.24	1230 \pm 240	1169 \pm 194
LDL (mmol/L)	3.07 \pm 0.97	2.94 \pm 1.08	3.13 \pm 0.9	3.03 \pm 0.91
HDL (mmol/L)	1.47 \pm 0.36	1.42 \pm 0.44	1.42 \pm 0.44	1.42 \pm 0.39
Cholesterol (mmol/L)	5.12 \pm 1.17	4.89 \pm 1.42	5.21 \pm 1.01	5.08 \pm 0.92
Triacylglycerol (mmol/L)	1.28 \pm 0.8	1.13 \pm 0.79	1.29 \pm 0.8	1.28 \pm 0.72
GPX (U/g hemoglobin)	43.95 \pm 11.38	44.66 \pm 12.01	43.74 \pm 11.54	44 \pm 11.95
SOD (U/g hemoglobin)	1283 \pm 243	1297 \pm 239	1291 \pm 240	1304 \pm 247

¹ All values are $\bar{x} \pm$ SD. SOD, superoxide dismutase; GPX, glutathione peroxidase; FRAP, ferric reducing ability of plasma. All measurements were carried out in plasma, except GPX and SOD, which were carried out by using red blood cell concentrate.

² Values (after – before) were significantly different from the control phase, $P < 0.001$ (Wilcoxon signed-rank test).

supplement phase of the present study. Watercress contains a high concentration of glucosinolates along with other bioactive phytochemicals (eg, lutein) that may have also contributed to the antigenotoxicity observed in lymphocytes as a result of supplementation. Note that consumption of watercress had no effect on the activity of antioxidant enzymes such as SOD or GPX. This observation further supports previous observations from our laboratory (15) that CVs do not exert their protective activity via modulation of SOD or GPX enzyme activity, although Dragsted et al (38) reported that a fruit and vegetable diet (a component of which was CV) did significantly increase GPX activity. Another potential mechanism of the antigenotoxicity observed as a result of watercress supplementation is a change in GST activity, as has been previously observed with brassica vegetables (12, 22). However, the effects of watercress on GST activity in the present study and the correlations with GST single nucleotide polymorphisms will be dealt with in a separate article.

In conclusion, data from our randomized, single blind human dietary intervention study provided important evidence that supports the hypothesis that consumption of watercress, a CV, can reduce cancer risk in humans via a decrease in DNA damage. This effect was observed with a concomitant elevation in plasma antioxidant concentrations of β -carotene and lutein. However, it should be noted that lymphocyte DNA damage is only a surrogate marker of whole-body cancer risk, and the effects of watercress on cancer risks at different sites could well be different to that observed in lymphocytes. This, among other things, could depend on tissue exposure, bioavailability of watercress phytochemicals in the various tissues, or both. 

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JRR and CIRG were the principal investigators of the study and were responsible for the study design and in charge of the overall operation. SH, LAB, CIRG, and JRP were the intervention study conducts. SH and LAB were responsible for the blood biomarker analyses. JW and MB were responsible for dietary intake assessment and coding of the food diaries. RB was responsible for the watercress component analyses. IB was responsible for the statistical analyses and data interpretation. CIRG, SH, and LAB was

responsible for the manuscript preparation, and IR was responsible for critically reviewing the manuscript. None of the authors had any financial or personal conflict of interest.

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