JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 7421−7431 **7421**

Correlation Analyses of Phytochemical Composition, Chemical, and Cellular Measures of Antioxidant Activity of Broccoli (*Brassica oleracea* L. Var. italica)

MARIAN V. EBERHARDT,[†] KANTA KOBIRA,[§] ANNA-SIGRID KECK,[†] JOHN A. JUVIK,[§] AND ELIZABETH H. JEFFERY*,[†]

Department of Food Science and Human Nutrition and Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801

Chemical measures of antioxidant activity within the plant, such as the oxygen radical absorbance capacity (ORAC) assay, have been reported for many plant-based foods. However, the extent to which chemical measures relate to cellular measures of oxidative stress is unclear. The natural variation in the phytochemical content of 22 broccoli genotypes was used to determine correlations among chemical composition (carotenoids, tocopherols and polyphenolics), chemical antioxidant activity (ORAC), and measures of cellular antioxidation [prevention of DNA oxidative damage and of oxidation of the biomarker dichlorofluorescein (DCFH) in HepG2 cells] using hydrophilic and lipophilic extracts of broccoli. For lipophilic extracts, ORAC (ORAC-L) correlated with inhibition of cellular oxidation of DCFH (DCFH-L, $r = 0.596$, $p = 0.006$). Also, DNA damage in the presence of the lipophilic extract was negatively correlated with both chemical and cellular measures of antioxidant activity as measured by ORAC-L ($r = -0.705$, $p = 0.015$) and DCFH-L ($r = -0.671$, $p = 0.048$), respectively. However, no correlations were observed for hydrophilic (-H) extracts, except between polyphenol content and ORAC (ORAC-H; $r = 0.778$, $p < 0.001$). Inhibition of cellular oxidation by hydrophilic extracts (DCFH-H) and ORAC-H were ~ 8- and 4-fold greater than DCFH-L and ORAC-L, respectively. Whether ORAC-H has more biological relevance than ORAC-L because of its magnitude or whether ORAC-L bears more biological relevance because it relates to cellular estimates of antioxidant activity remains to be determined. Chemical estimates of antioxidant capacity within the plant may not accurately reflect the complex nature of the full antioxidant activity of broccoli extracts within cells.

KEYWORDS: ORAC; antioxidant; dichlorofluorescein; Comet assay; quinone reductase; thioredoxin reductase; broccoli; *Brassica oleracea* L. var. italica

INTRODUCTION

Oxidative stress, an imbalance between pro- and antioxidants, is associated with increased risk for chronic disease, including cancer and cardiovascular disease (*1*). An excess of reactive oxygen species (ROS) causes oxidative damage to cellular lipids, proteins, and DNA, an early stage for many chronic diseases. Therefore, risk for chronic disease may be lessened by enhancement of cellular systems that prevent such oxidative damage, including addition of exogenous antioxidants such as carotenoids and tocopherols, up-regulation of antioxidant enzymes, or improved production of cellular antioxidants such as the thiols glutathione and thioredoxin. Plant foods such as broccoli are rich sources of both antioxidant vitamins and nonessential nutrients, including polyphenols, that have been proposed to supplement the action of endogenous antioxidants (*2*). Following a meal of fruits and vegetables, plasma antioxidant status is improved and ex vivo measures show decreased low-density lipoprotein (LDL) oxidation (*3*−*5*). However, the various measures of antioxidant status are rarely evaluated for correlations with content of antioxidants.

A number of studies have shown a relationship between polyphenol content of fruits and vegetables and chemical measures of antioxidant capacity, although this relationship is not always seen (*6*, *7*). Few of these studies report the genotype under study. Broccoli contains numerous potential antioxidant phytochemicals, and their content is known to vary with genotype (*8*). The present study utilizes broccoli cultivars with genetically distinct antioxidant phytochemical profiles to investigate potential roles for these compounds in the overall antioxidant activity of plant foods. Genotypic differences in the antioxidant content in broccoli extracts provide the variation needed to investigate associations between phytochemical

* Address correspondence to this author at 499 Bevier Hall, University of Illinois, 905 S. Goodwin Ave., Urbana, IL 61801 [telephone (217) 333-3820; fax (217) 265-0925; e-mail ejeffery@uiuc.edu].
[†] Department of Food Science and Human Nutrition.
[§] Department of Natural Resources and Environmental Sciences.

10.1021/jf051495k CCC: $30.25
Published on Web 08/30/2005

content, chemical assays of antioxidation, and protection of cultured human cells from oxidative damage.

The potential for fruits and vegetables to prevent or reverse clinical oxidative stress has been proposed from data generated using a variety of different assays, many of which measure free radical scavenging activity of plant foods in vitro (*9*). A key chemical assay that has been widely used for the measurement of the antioxidant activity of pure compounds, food extracts, or plasma is the oxygen radical absorbance capacity (ORAC) assay. The ORAC assay was originally limited to measurement of hydrophilic antioxidant capacity because it utilized a hydrophilic radical generator and a hydrophilic detector (*10*). Modifications have improved both the sensitivity of the assay by utilizing a new indicator (*11*) and the breadth of components assayed by use of a solubility enhancer for lipophilic antioxidants (*12*), providing a more complete measurement of total antioxidant potential (*7*). However, validation that this chemical assay reflects cellular effects of antioxidant compounds remains to be determined.

Estimates of cellular oxidative stress and of oxidative damage to cellular macromolecules such as DNA have also been used to assess antioxidant activity (*13*). Although these assays are often more time-consuming and require more equipment than a chemical assay, cellular assays are frequently utilized to study the mechanism of action and may more closely reflect antioxidant activity within a biological environment (*14*). Intracellular ROS-induced oxidation can be monitored by loading cells with dichlorofluorescein (DCFH). Upon oxidation, fluorescent dichlorofluorescin (DCF^-) is formed. Thus, the change in fluorescence following exposure of cells to ROS and DCFH in the presence, compared to the absence, of the antioxidant extract can be used as an estimate of antioxidation (*15*). In addition to this direct, or acute, effect that antioxidants may have on the cell, plant extracts can increase cellular defenses by up-regulating antioxidant enzymes. Two enzymes with antioxidant activity that are up-regulated in response to plant secondary metabolites are quinone reductase (QR) and thioredoxin reductase (TR). QR, best known as a detoxification enzyme, has also been shown to regenerate reduced forms of α-tocopherol and coenzyme Q, thereby increasing the antioxidant capacity of the cell (*16*, *17*). TR is a protein disulfide oxidoreductase that reduces the disulfide at the active center of thioredoxin, a major cellular reductant (*18*). Therefore, the induction of QR and TR activities may enhance cellular antioxidant capacity.

Using the original ORAC assay and eight broccoli genotypes, we previously reported that the antioxidant capacity of aqueous broccoli extracts correlated with the ability of these broccoli extracts to decrease oxidative stress in ROS-treated human hepatoma HepG2 cells (*19*). The lipophilic fraction did not show good correlation between these two assays, which we considered could have been because the ORAC assay was formulated for hydrophilic products. Prevention of oxidative damage to cellular macromolecules, such as proteins, lipids, and DNA, was not evaluated. The present investigation extends that study, to determine if the improved ORAC assay reflects the content of water-soluble polyphenolics and/or lipid-soluble carotenoids and tocopherols and whether the improved ORAC assay reflects antioxidant actions of broccoli extracts added to cultured cells. Our aim was to evaluate the relationship between the chemical analysis of plant material and the effects of plant extracts on measures of cellular oxidative stress, including direct inhibition of oxidative damage and induction of preventative systems.

MATERIALS AND METHODS

Chemicals. Trappsol (randomly methylated β-cyclodextrin) was purchased from Cyclodextrin Technologies Development Inc. (High Springs, FL). 2,2′-Azobis(2-amidino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Broccoli Cultivation. Broccoli seeds were obtained from the Asgrow Seed Co. (Gem, Baccus, and Majestic), Peto Seeds (Peto 6, Peto 7, and Peto 13), the USDA Plant Genetics Resource Unit at Cornell University (Green Comet, Zeus, Shogun, Packman, Atlantic, Brigadier, Pirate, High Sierra, and Cavolo), and Dr. Mark Farnham of the USDA Vegetable Research Center (Charlestown, SC; EU8-1, EV6-1, SU003, VI 158, BNC, and MA191). In July 2001, seeds from all 22 broccoli cultivars were germinated in Fafard growing mix no. 2 (Conrad Fafard, New Brunswick, Canada) in a greenhouse under natural daylight. After 4 weeks, seedlings were transplanted into the University of Illinois vegetable research farm (Champaign, IL). Water, fertilizer, and pesticides were applied according to standard cultural practices developed for cruciferous vegetables in Illinois (*20*).

Sample Preparation. Uniform broccoli heads, three to five per cultivar, were harvested at standard fresh market maturity, placed on ice, and transported to the laboratory. Equal amounts of tissue from each head of each cultivar were combined to obtain 100 g of sample, frozen in liquid nitrogen, and stored at −80 °C. Broccoli tissue was freeze-dried, ground to a fine powder, and stored at −20 °C until analysis. Hydrophilic extracts were prepared from 1 g of freeze-dried broccoli tissue added to 20 mL of dH_2O, vortexed for 20 s, and placed on an orbital shaker for 30 min at 2000 rpm. The samples were vortexed every 10 min for 20 s. The broccoli slurry was filtered through cheesecloth directly into a Büchner funnel fitted with Whatman no. 1 filter paper. The filtrate was collected and brought to 20 mL (final concentration equivalent to 50 mg of freeze-dried broccoli/mL). Lipophilic extracts for antioxidant assays were prepared by adding 5 mL of hexane to 0.5 g of freeze-dried broccoli tissue. Each sample was vortexed for 1 min. Samples were centrifuged for 10 min at 1300*g*, and the supernatant was collected. This procedure was repeated twice, and the combined supernatant was filtered through a Whatman no. 1 filter. Samples were dried on a Speed Vac (ARS2010; Savant, NY) and reconstituted with appropriate solvents before analysis. Separate tissue samples were extracted from each genotype for chemical (ORAC) and cellular (DCFH, DNA damage, QR and TR) assays.

Phytochemical Quantification. Samples for chemical analysis of carotenoids and tocopherols were extracted according to the method of Weber (*21*) with modifications as previously described (*22*). Briefly, 9 mL of ethanol with butylated hydroxytoluene (0.1 g/100 mL) was added to 0.3 g of freeze-dried broccoli powder and incubated at 35 °C for 10 min. Samples were saponified in 180 μL of 80% potassium hydroxide at 35 °C, with vortexing at the start and once during saponification. After 15 min, 3 mL of dH_2O was added to each sample, and tubes were placed on ice. Hexane (3 mL) was added to each sample followed by vortexing and centrifuging for 10 min at 1300*g*. The hexane fraction was collected, the procedure was repeated, and the two extracts were combined. Extracts were washed with 3 mL of dH_2O to remove water-soluble components, dried under a vacuum, and reconstituted in 300 μL of a mixture of acetonitrile/methanol/dichloromethane (45:20:35). Samples were filtered using a Costar Spin-x filter (Corning Life Sciences, Acton, MA) and stored at −80 °C.

Carotenoid and tocopherol concentrations were quantified simultaneously using an isocratic HPLC method with a reverse phase C30 (4.6 × 150 mm), 5 μm YMC carotenoid column (Waters, Milford, MA; *23*). Standard curves for a mixture of lutein, β-carotene, α-tocopherol, and γ-tocopherol at six concentrations were used for quantification. Two tissue samples were extracted and analyzed for each genotype.

Total polyphenols were measured using the Folin−Ciocalteu method (*24*) modified for use in 96-well microtiter plate. Hydrophilic broccoli extracts (10 μL of 50 mg/mL) or water (blank) were placed into each well, followed by 150 μL of Folin−Ciocalteu reagent (14 mL of water to 1 mL of Folin−Ciocalteu reagent). A standard curve was created using catechin concentrations ranging from 1.25 to 12.5 μM. After 3 min, 50 μL of sodium carbonate solution (2 mL of saturated sodium

bicarbonate/3 mL of dH_2O) was added, and the plate was left in the dark at room temperature for 2 h. Absorbance was measured at 725 nm, and results are expressed as micromoles of catechin equivalents per gram of broccoli dry weight.

Chemical Antioxidant Assay. For ORAC, the method of Davalos et al. (*25*) was used. Hydrophilic broccoli extracts were diluted with 75 mM phosphate buffer (pH 7.4) to provide a final concentration of 0.05 mg/mL. The assay was carried out in black-walled 96-well plates (Fisher Scientific, Hanover Park, IL), and each well contained a final volume of 200 μL. To each well were added 20 μL of broccoli extract and 120 μL of fluorescein (70 nM final concentration), and the plate was incubated at 37 °C for 15 min. Then AAPH (60 μL; 12 mM final concentration) was added to each well, and fluorescence intensity was estimated using an FLx800tbi plate reader (Bio-Tek Instruments, Winooski, VT) every 2 min for a total of 120 min using an excitation filter of 485/20 nm and an emission filter of 528/25 nm. A standard curve was constructed using Trolox (1−8 μM). Results were calculated on the basis of the differences in area under the curve between the control and the sample and expressed as micromoles of Trolox equivalents per gram of broccoli dry weight. The lipophilic ORAC assay (ORAC-L; *12*, *26*) followed the procedure as outlined above with the exception that the dried hexane extract was reconstituted in 20 mL of acetone and then diluted with 7% Trappsol solution, to provide a final concentration equivalent to 0.05 mg of broccoli/mL. All other reagents were prepared in 75 mM phosphate buffer (pH 7.4).

Cellular Assay for Oxidative Stress. Stock HepG2 cells were grown in 75 cm^2 polystyrene flasks (Corning Life Sciences) using Eagle's minimum essential medium with α-modification supplemented with 10% fetal bovine serum and incubated in 5% CO_2 at 37 °C. The ROS-induced oxidation assay measures the decrease in AAPH-induced oxidative stress in human hepatocarcinoma HepG2 cells acutely exposed to broccoli extracts. HepG2 cells (10^5) were plated into the wells of a 96-well plate. After 24 h, medium was removed and cells were washed with PBS. The cells were preincubated with 5 μM dichlorofluorescein diacetate (DCFH-DA) for 30 min at 37 °C to allow cellular uptake. Within the cell, DCFH-DA is hydrolyzed to release dichlorofluorescein (DCFH), which, by virtue of its polar nature, cannot exit the cell. Cells were then washed with PBS to remove any excess DCFH-DA from the medium. Broccoli extracts (equivalent to 0.5 mg/mL for hydrophilic extracts; 2 mg/mL for lipophilic extracts) were added, and oxidative stress was initiated by the addition of 1 mM AAPH. A control that had no added broccoli extract: a solvent blank (PBS or 0.1% DMSO), and a no-cell blank were included for each assay. Fluorescence (485/20 nm excitation, 530/25 nm emission) was monitored every 5 min for a total of 60 min at 37 °C. Data are expressed as percent decrease in fluorescence per milligram of cellular protein from control, at the 60 min time point. Data were calculated as follows: $100 - (f_B/f_C \times 100)$, where f_C = fluorescence intensity/mg of protein of control at 60 min and f_B = fluorescence intensity/mg of protein in cells to which broccoli extract has been added, read after 60 min.

Enzyme Activity. HepG2 cells (2×10^5 cells/well) were grown in 12-well plates (Corning Life Sciences) for 24 h prior to exposure to broccoli extracts (0.5 mg/mL) for a further 24 h. Cells were then detached using 0.04% trypsin/0.02% EDTA in 0.15 M phosphate buffer (pH 7.3), washed with 0.15 M phosphate buffer (pH 7.3), and lysed by sonication. Cell lysates were stored at −80 °C until analysis. The activity of QR was measured in microtiter plates using 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide as the substrate (*27*), and TR was measured following the method by Holmgren (*28*) as modified by Hill (*29*). Protein was measured according to the Bio-Rad assay (Hercules, CA) using bovine serum albumin as standard (*30*).

DNA Damage by Alkali Single Cell Gel Electrophoresis (Comet) Assay. HepG2 cells (4×10^4 cells/well) were grown in 24-well plates (Corning Life Sciences) for 24 h and then exposed to broccoli extracts equivalent to 0.5 mg/mL for 24 h. At the end of the treatment period, cells were incubated with 50 μM hydrogen peroxide (positive control) or medium only (negative control) for 30 min. Cells were detached with 0.04% trypsin/0.02% EDTA in 0.15 M phosphate buffer (pH 7.3), washed, and held on ice (<30 min) until analysis. The Comet assay was performed using reagents and CometSlides (Trevigen, Gaithersburg, MD), and the modified procedure was based on a previously published method (*31*). In brief, 50 μL of cell suspension was gently mixed with 500 μL of LM agarose (42 °C), and a 75 μL aliquot was transferred onto the CometSlide. After the gel had solidified at 4 °C for 10 min, the CometSlide was transferred to a prechilled lysis buffer for 30 min before being submerged in prechilled electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH >13) for 20 min. The slide was then electrophoresed on ice for 30 min at 300 A/25 v. After electrophoresis, the slide was placed in 80% ethanol for 5 min and dried for 24 h. The extent of DNA damage was evaluated using SYBR green staining solution and a fluorescence microscope with an excitation filter of 494 nm and emission filter of 521 nm. Samples were run in duplicate, and at least 200 nuclei were scored per slide.

Statistical Analysis. Statistical analysis was performed using ANOVA with Fisher's protected LSD and correlation analysis ($\alpha = 0.05$) using Statistical Analysis Systems software (SAS, Cary, NC).

RESULTS

Phytochemical Content. Variation in total polyphenol content (hydrophilic extract) and carotenoid and tocopherol concentrations (lipophilic extract) was observed among the 22 broccoli genotypes (**Table 1**). The content of total polyphenols ranged from 19.3 to 37.1 μmol of catechin equiv/g, with a mean value of 27.7 ± 5.6. For carotenoids and tocopherols, far larger ranges in content were observed across genotypes: 6-fold for lutein, 35-fold for β-carotene, 14-fold for γ-tocopherol, and 5-fold for α-tocopherol. α-Tocopherol (mean = 14.7 ± 7.2 μmol/100 g) and β-carotene (mean = 13.5 ± 8.9 μmol/100 g) were present in substantially higher amounts than γ-tocopherol (mean = 7.3 ± 3.4 μmol/100 g) or lutein (mean = 9.6 ± 4.7 μmol/100 g). Although there was no relationship between the contents of α- and γ-tocopherols ($r = 0.214$, $p = 0.339$), α-tocopherol content was positively correlated with total tocopherol content ($r = 0.92$, $p < 0.001$). β-Carotene and lutein were significantly correlated both between the individual carotenoids ($p = < 0.001$) and with total carotenoid content ($p = 0.005$ and 0.013, respectively).

Chemical Measures of Antioxidation. Chemical estimation of antioxidant capacity of 22 broccoli genotypes, as determined by ORAC analysis of hydrophilic and lipophilic extracts, is shown in **Figure 1A**. The antioxidant capacity of hydrophilic extracts (ORAC-H) was ~4-fold that of the lipophilic extracts (ORAC-L), accounting for 76−92% of the total, across broccoli genotypes. The mean ORAC-H and ORAC-L values across broccoli genotypes were 151.5 ± 43 and 28.2 ± 8 μmol of Trolox equiv/g of broccoli, respectively. There was no correlation between ORAC-H and ORAC-L ($r = 0.427$, $p = 0.0533$). For hydrophilic extracts, there was a significant correlation between ORAC-H and total polyphenol content ($r = 0.788$, $p < 0.001$). However, ORAC-L did not correlate with total or individual tocopherols or carotenoids.

Cellular Measures of Antioxidation. *ROS-Induced Oxidation*. The inhibition of cellular oxidative stress by broccoli extracts, as measured by a decrease of ROS-induced oxidation of DCFH to a fluorescent product, is shown in **Figure 1B**. Similar to the relative activities of ORAC-H and ORAC-L, hydrophilic extracts accounted for most quenching of DCFH oxidation (DCFH-H), with the exception of the genotype Green Comet. It was necessary to use 4-fold greater amounts of the lipophilic broccoli extracts (2 mg/mL) compared to the hydrophilic extracts (0.5 mg/mL) to provide reproducible readings, due to the low level of antioxidant activity of the lipophilic extract. Without correction for this difference, the mean percent losses in fluorescence for broccoli genotypes were 66.9 ± 13 and 35.1 ± 10 for hydrophilic and lipophilic extracts, respectively (**Figure 1B**). Thus, on a gram of broccoli basis, the mean

Table 1. HPLC Determination of Levels of Tocopherols, Carotenoids (Micromoles per 100 g of Dry Weight), and Polyphenols (Micromoles of Catechin Equivalents per Gram of Dry Weight) in Broccoli Extracts[a]

	lipophilic extract						hydrophilic extract
genotype	γ-tocopherol	α-tocopherol	total tocopherols	β-carotene	lutein	total carotenoids	total polyphenols
Atlantic	6.7 (6.6–6.9)	20.8 (20.2–21.5)	27.6 (27.0–28.1)	31.6 (30.3–32.9)	20.6 (18.3–22.8)	52.1 (51.2–53.1)	28.3 ± 0.68
Baccus	4.6 (4.5–4.7)	11.8 (11.2–12.5)	16.4 (15.7–17.2)	13.3 (12.6–14.0)	12.2 (12.0–12.3)	25.5 (24.9–26.1)	29.3 ± 0.37
BNC	14.1 (12.9–15.3)	15.4 (14.7–16.1)	29.4 (27.6–31.3)	30.2 (28.5–31.8)	14.2 (13.2–15.1)	44.3 (41.8–46.9)	34.0 ± 0.55
Brigadier	7.7 (7.4–8.0)	9.6 (6.3–12.8)	17.3 (13.7–20.8)	11.5 (10.7–12.3)	7.4 (7.3–7.5)	18.9 (18.2–19.5)	29.8 ± 0.33
Cavolo	7.7 (7.3–8.1)	6.6 (5.7–7.5)	14.3 (13.0–15.6)	9.6 (9.5–9.8)	4.8 (4.6–5.0)	14.4 (14.0–14.8)	37.1 ± 0.58
EU8-1	6.0 (5.8–6.2)	14.6 (14.5–14.6)	20.6 (20.3–20.8)	7.6 (6.6–8.6)	9.4 (8.5–10.2)	17.0 (15.1–18.8)	31.4 ± 0.69
Ev6-1	12.8 (10.9–14.7)	28.2 (26.4–30.0)	41.0 (37.3–44.7)	14.4 (13.8–15.1)	13.2 (10.7–15.8)	27.7 (24.5–30.9)	34.2 ± 0.69
Gem	8.5 (8.3–8.8)	11.5 (11.2–11.8)	20.0 (20.0–20.1)	9.3 (8.9–9.8)	8.5 (8.4–8.6)	17.8 (17.2–18.4)	20.2 ± 0.35
Green Comet	3.8 (3.4–4.1)	14.0 (12.5–15.4)	17.7 (16.6–18.8)	18.2 (16.2–20.1)	13.1 (12.3–13.9)	31.3 (28.6–34.0)	26.7 ± 0.51
High Sierra	12.1 (10.5–13.8)	17.1 (15.8–18.3)	29.2 (28.8–29.6)	27.7 (27.0–28.4)	17.0 (15.5–18.6)	44.7 (43.9–45.5)	26.3 ± 0.25
MA 191	1.0 (0.9–1.1)	12.4 (11.2–13.5)	13.3 (12.3–14.4)	13.8 (11.1–16.5)	9.7 (8.5–10.9)	23.5 (19.6–27.4)	26.0 ± 0.60
Majestic	4.2 (4.0–4.3)	29.0 (26.6–31.5)	33.2 (30.9–35.4)	14.9 (14.2–15.5)	7.3 (4.9–9.6)	22.1 (20.4–23.8)	22.0 ± 0.20
Marathon	8.4 (7.8–9.0)	13.5 (12.8–14.2)	21.9 (20.6–23.1)	8.5 (8.4–8.7)	4.1 (3.9–4.3)	12.7 (12.6–12.7)	24.8 ± 0.41
Packman	5.6 (5.2–6.1)	6.5 (5.3–7.7)	12.1 (11.4–12.9)	1.5 (1.3–1.6)	5.0 (4.5–5.4)	6.4 (5.8–7.1)	36.9 ± 0.14
Peto 6	5.3 (4.7–5.9)	5.4 (4.9–6.0)	10.7 (9.5–11.9)	8.5 (4.6–12.4)	7.8 (6.6–9.0)	16.3 (13.6–19.0)	19.9 ± 0.42
Peto 7	8.1 (5.9–10.2)	27.6 (25.4–29.8)	35.7 (35.6–35.7)	15.6 (13.4–17.7)	8.3 (8.2–8.5)	23.9 (21.6–26.2)	26.3 ± 0.50
Peto 13	7.5 (7.3–7.6)	8.4 (8.2–8.5)	15.8 (15.5–16.1)	1.4 (1.3–1.6)	3.4 (3.3–3.5)	4.8 (4.6–5.0)	37.1 ± 0.82
Pirate	7.3 (6.5–8.2)	19.8 (17.4–22.2)	27.1 (25.6–28.7)	21.0 (17.8–24.2)	9.7 (8.3–11.0)	30.7 (26.1–35.3)	24.8 ± 0.41
Shogun	9.5 (9.2–9.7)	22.9 (20.2–17.4)	32.3 (29.9–34.7)	21.8 (20.8–22.7)	10.6 (9.9–11.2)	32.3 (30.7–33.9)	24.3 ± 0.39
SU003	2.4 (2.0–2.9)	10.5 (9.9–11.0)	12.9 (11.9–13.9)	0.88 (0.87–0.88)	6.1 (6.0–6.3)	7.0 (6.8–7.1)	19.3 ± 0.43
VI 158	5.2 (4.1–6.2)	10.3 (7.8–12.8)	15.5 (14.0–16.9)	12.3 (12.0–12.7)	16.7 (12.9–20.6)	29.1 (24.9–33.2)	21.6 ± 0.20
Zeus	13.1 (12.4–13.8)	8.7 (7.8–9.6)	21.8 (20.3–23.4)	2.4 (2.1–2.8)	3.0 (2.5–3.4)	5.4 (5.4–5.5)	29.9 ± 0.30
mean	7.3 ± 3.4	14.7 ± 7.2	22.1 ± 8.6	13.5 ± 8.9	9.6 ± 4.7	23.1 ± 13.0	27.7 ± 5.6

[a] Mean and range, $n = 2$, for carotenoids and tocopherols; mean ± SD ($n = 3$) for total polyphenols. Moisture content of raw broccoli is 89.30% (*36*).

activity of the hydrophilic sample was almost 8-fold greater than that of the lipophilic extract. Whereas this minor activity from the lipophilic extract correlated well with ORAC-L, results from this cellular assay using the hydrophilic extracts, which constituted >80% of total activity of broccoli, showed no correlation with ORAC-H ($r = 0.317$, $p = 0.151$). For hydrophilic extracts, Zeus (80.9 ± 1.0%) caused the greatest decrease in fluorescence per milligram of protein, and the least effective was Green Comet (17.4 ± 1.6%). However, the lipophilic extract of Green Comet caused the greatest percent decrease in fluorescence per milligram of protein of all lipophilic extracts (49.2 ± 5%), with extracts of Packman causing the least inhibition of oxidative stress (11.1 ± 1.5%). Even given these data for the genotype Green Comet, there was no correlation between hydrophilic and lipophilic extracts in their abilities to quench cellular oxidative stress ($r = 0.064$, $p = 0.782$).

DNA Damage. The effect of hydrophilic and lipophilic extracts from a subset of 11 broccoli genotypes on hydrogen peroxide-induced DNA damage is shown in **Figure 2**. Exposure of HepG2 cells to 50 μM hydrogen peroxide increased DNA damage 10-fold over baseline. Neither hydrophilic nor lipophilic extracts from broccoli provided robust protection from hydrogen peroxide-induced DNA damage. The mean decreases in DNA damage due to the presence of broccoli extracts were 25.1 and 21.5% for hydrophilic and lipophilic extracts, respectively, although for the hydrophilic extracts, only Packman and Cavolo exhibited significant protection. For lipophilic extracts, Cavolo was most effective at preventing DNA damage by 45.6%, followed by BNC (30%), Peto 6 (27.7%), and SU003 (26.7%) ($p < 0.05$). A significant negative association ($r = -0.705$, $p = 0.015$) was observed between ORAC-L and DNA damage in the presence of the lipophilic extract, indicating that extracts with greater ORAC values provided enhanced DNA protection. No such relationship was found between ORAC-H and inhibition of DNA damage by the hydrophilic extract. Furthermore, protection from DNA damage correlated with the cellular

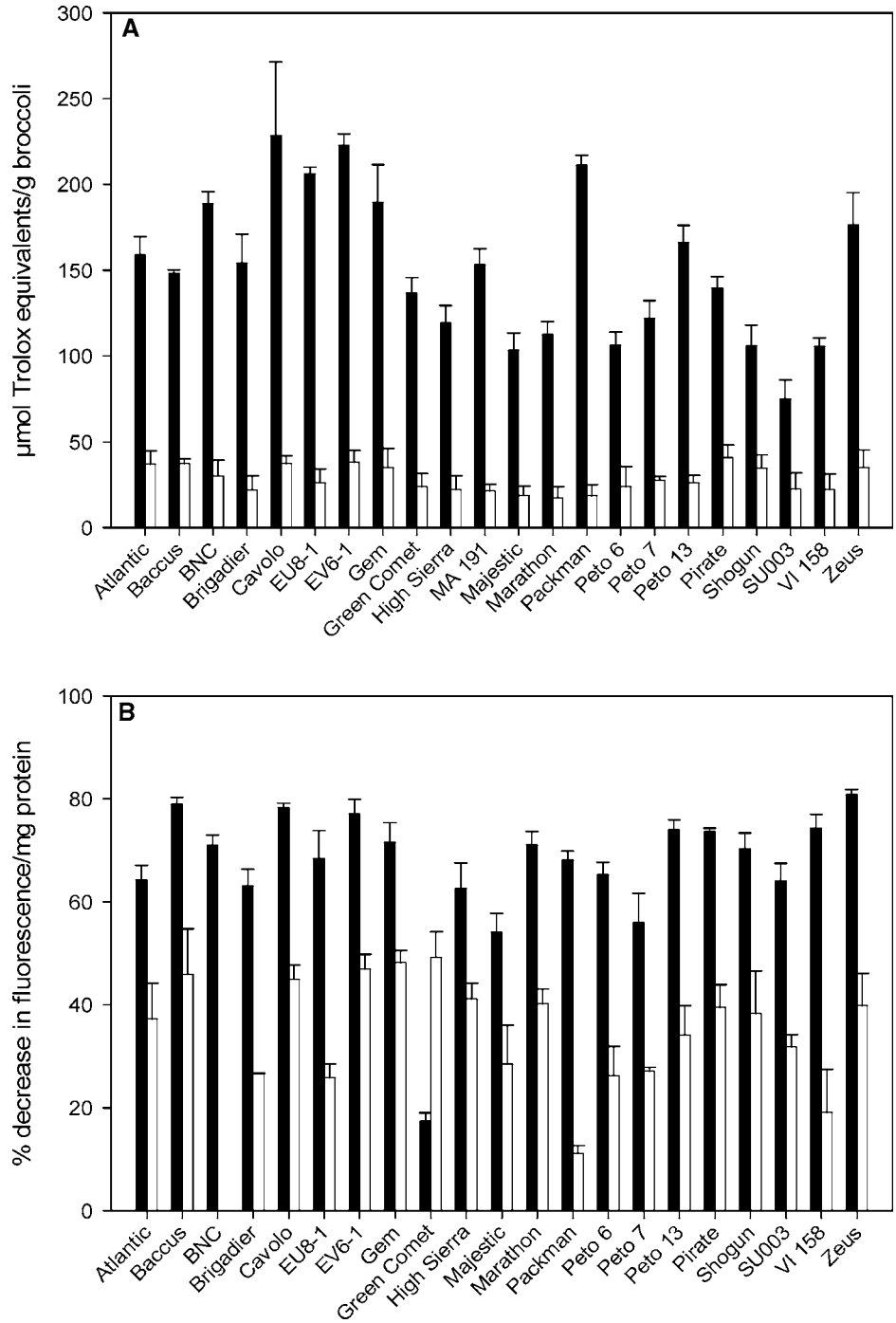

Figure 1. Antioxidant activity of broccoli extracts. Antioxidant activity of hydrophilic (solid bars) and lipophilic (open bars) extracts of 22 genotypes of broccoli was estimated by (**A**) the chemical ORAC assay, with a final concentration of extract equivalent to 0.05 mg of broccoli/mL, and (**B**) the cellular DCFH assay, with a final concentration of extract equivalent to 0.5 and 2.0 mg of broccoli/mL, for hydrophilic and lipophilic extracts, respectively. Data are mean ± SD ($n = 3$) from one representative experiment of three separate experiments.

estimate of antioxidant activity against ROS-induced oxidation only for the lipophilic extract ($r = -0.067$, $p < 0.048$).

Enzyme Activity. Incubation of HepG2 cells with either hydrophilic or lipophilic extracts of a subset of 11 broccoli genotypes for 24 h increased QR activity (**Figure 3A**). With the exception of VI 158, all genotypes significantly induced QR activity over the control. The mean fold inductions of QR were 1.2 ± 0.1 and 1.3 ± 0.1 for hydrophilic and lipophilic extracts, respectively. For BNC, EU8-1, and Peto 13, the lipophilic extracts exerted a significantly greater effect than the hydrophilic extracts. However, for all other genotypes, inductions of QR were similar between hydrophilic and lipophilic extracts. The QR induction did not correlate with any of the acute chemical or cellular activities estimated.

The effect of broccoli extracts on TR activity in HepG2 cells is shown in **Figure 3B**. The mean fold inductions of TR were 1.7 ± 0.3 and 0.93 ± 0.1 for hydrophilic and lipophilic extracts, respectively. The activity of TR was significantly induced over control for all hydrophilic extracts with the exceptions of SU003, Peto 13, and Green Comet genotypes. In contrast to hydrophilic extracts, lipophilic extracts did not significantly induce TR activity. In fact, the activity of TR was slightly decreased from control by lipophilic extracts of VI 158, MA 191, and Peto 7 ($p < 0.05$). As with QR, induction of TR was not strongly

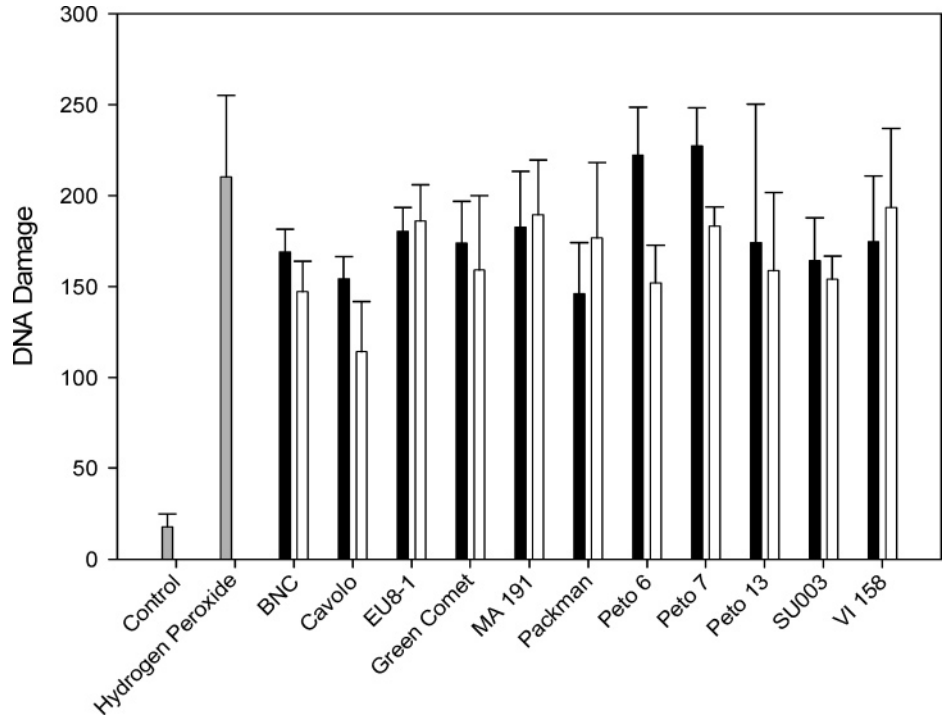

Figure 2. Effect of broccoli extracts on H_2O_2-induced DNA damage. Human hepatoma HepG2 cells were pretreated for 24 h with hydrophilic (solid bars) or lipophilic (open bars) extracts of 11 genotypes of broccoli equivalent to 0.5 mg of broccoli/mL and then subjected to 50 μM H_2O_2 treatment for 30 min. DNA damage was estimated by single cell gel electrophoresis (Comet assay), with a minimum of 200 nuclei evaluated per treatment. Control (–C), medium only; positive control (+C), H_2O_2 only. Data are mean ± SD ($n = 3$) from one representative experiment of three separate experiments.

correlated with any acute antioxidant measure, chemical or cellular. However, a weak negative correlation ($r = -0.57$, $p = 0.068$) was observed between total carotenoid content and the effect of lipophilic extracts on TR activity.

Correlations among Phytochemical Content and Chemical and Cellular Assays of Antioxidant Activity. Correlations were calculated between phytochemical content, antioxidant capacity, enzyme activity, inhibition of cellular oxidative stress, and inhibition of DNA damage for both lipophilic (**Table 2**) and hydrophilic (**Table 3**) extracts (**Figure 4**). For lipophilic broccoli extracts, antioxidant capacity (ORAC-L) significantly correlated with both inhibition of cellular oxidative stress (DCFH-L; $r = 0.596$, $p = 0.006$) and inhibition of DNA damage (DNA-L; $r = -0.705$, $p = 0.015$). However, hydrophilic extracts did not correlate with any cellular measures. The only significant correlation was between the chemical assay, ORAC-H, and total polyphenol content ($r = 0.788$, $p < 0.001$).

DISCUSSION

This study was conducted to determine the extent to which the content of tocopherols, carotenoids, and/or polyphenols in broccoli florets or a chemical estimate of antioxidant capacity within the food (ORAC assay) reflects cellular measures of oxidative damage within cultured human cells. The importance of this question is that if any of these broccoli tissue measures are reflected in improved protection against oxidative stress within cultured human cells, then these measures may be useful as markers in a breeding program to produce broccoli with improved antioxidant action.

Among vegetables, broccoli is reported to provide a relatively high antioxidant capacity, using the ORAC assay (400−700 μmol of Trolox equiv/serving; *13*, *32*). When a range of vegetables were evaluated by ORAC, a serving of broccoli was found to have an antioxidant capacity similar to that of yellow onions or spinach and substantially greater than that of a serving of many common vegetables such as corn, tomatoes, iceberg lettuce, or green peppers (*13*). We hypothesize that tocopherols and carotenoids, together with flavonoids and other polyphenolics, may be the components responsible for the antioxidation action of broccoli. The flavonoid content of broccoli is mostly kaempferol and quercetin (*33*, *34*), which are both reported to have antioxidant properties and to be absorbed from the diet (*35*). According to National Nutrient database values (*36*), broccoli is also a good source of several essential nutrients, including tocopherols (1 mg/100 g of broccoli; RDA = 10 mg) and carotenoids (2 mg/100 g of broccoli; RDA = 1 mg). The values reported here (**Table 1**), similar to values that we reported in an earlier study (*8*), support this information, showing mean values of 22 μmol (~1 mg, range = 0.4−1.5 mg) of tocopherols and 23 μmol (~0.6 mg, range = 0.1−1 mg) carotenoids per 100 g of fresh weight of broccoli.

The existence and magnitude of this broad variation in vitamin E and carotenoid contents across genotypes that we report here suggest that it is feasible to increase the content of these antioxidant vitamins in the broccoli floret, using available commercial germplasm in a breeding program to develop new broccoli germplasm with enhanced antioxidant content. Furthermore, this large range of observed variation in the phytochemical content across genotypes provides the power necessary for evaluating correlations among antioxidant compound concentrations and to determine associations between phytochemicals, antioxidant assays, and cellular assays for antioxidation. However, even broccoli genotypes available today can be recommended as a good source of these antioxidant vitamins. Only if increased levels can be shown to provide a substantial health advantage will it be worthwhile to develop advanced varieties for production.

Concentrations of β-carotene, γ-tocopherol, and α-tocopherol reported in this investigation were similar to those given in our earlier paper (*8*). Lutein concentrations were generally in

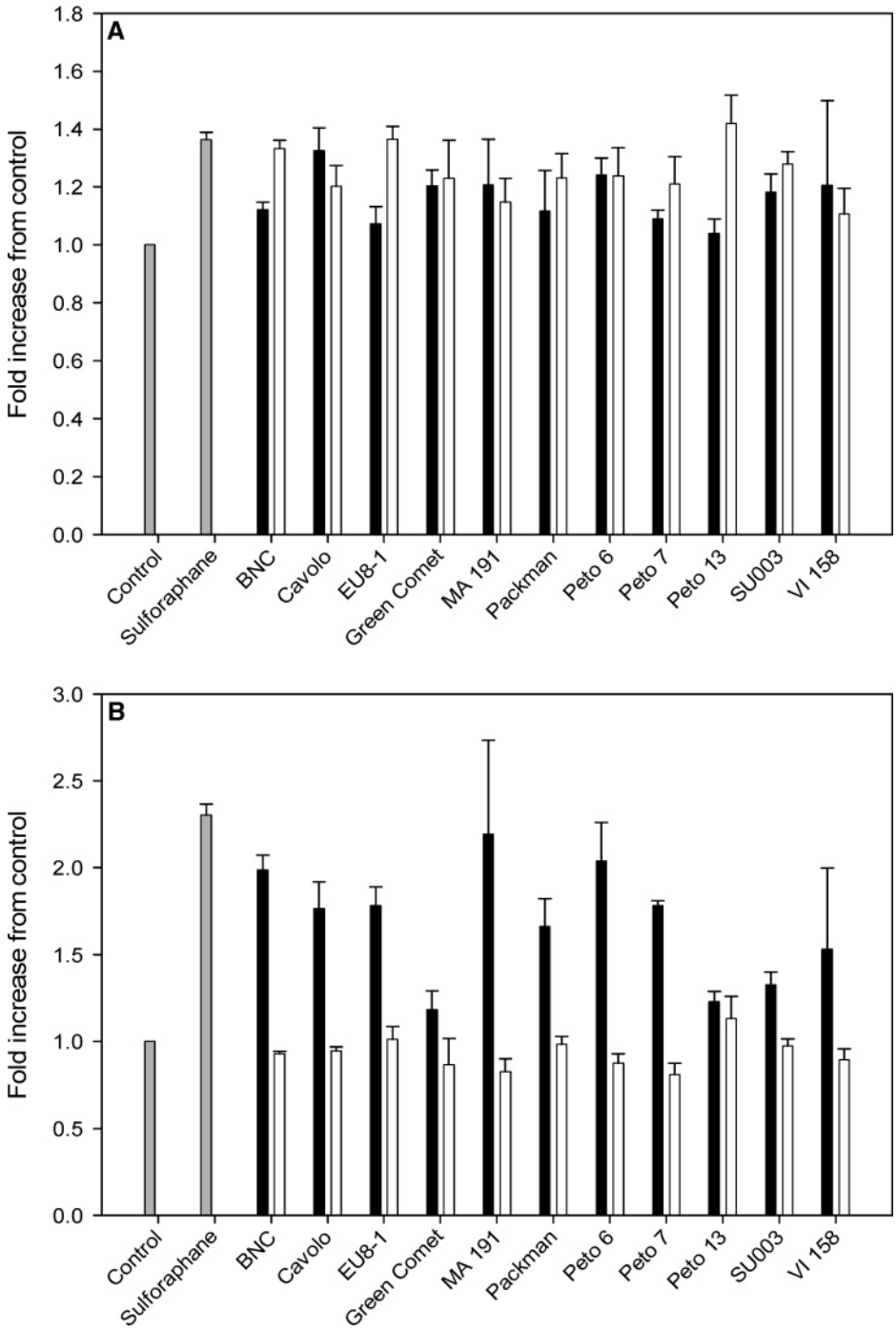

Figure 3. Effect of broccoli extracts on induction of enzyme activities. Human hepatoma HepG2 cells were pretreated for 24 h with hydrophilic (solid bars) or lipophilic (open bars) extracts of 11 genotypes of broccoli equivalent to 0.5 mg of broccoli/mL. Cells were analyzed for (**A**) quinone reductase (QR) and (**B**) thioredoxin reductase (TR) activity. Control (C), 0.1% DMSO; positive control (+C), 2 μM sulforaphane. Data are mean ± SD ($n = 3$) from one representative experiment of three separate experiments.

accordance with previously reported values also (*37*, *38*). As in our earlier study, a correlation was observed between total tocopherol content and β-carotene. This positive association may be due to increased geranylgeranyl diphosphates (GGPP) production, because both carotenoids and tocopherols are synthesized via the mevalonic-independent acid pathway (*39*). Condensation of two GGPP produced via the mevalonic-independent pathway gives rise to phytoene, which is the first committed step in the carotenoid pathway (*40*). Previous work suggests that rates of GGPP synthesis will influence both carotenoid and tocopherol biosynthesis, because GGPP is a direct precursor for the synthesis of the tocopherol side chain (*39*). No correlation was detected in this study between α- and γ-tocopherol, in agreement with previous reports for broccoli (*8*), canola (*Brassica napus*) (*41*), and sweet corn (*23*), suggesting that although α-tocopherol is the direct precursor of γ-tocopherol, accumulation of these compounds appears to be under independent regulation in these crop species. Our correlation analysis suggests that carotenoid and tocopherol concentrations in broccoli florets are either positively correlated or unassociated. These data suggest that it is feasible to design a breeding program to increase the content of carotenoids, tocopherols, and polyphenols, simultaneously.

Table 2. Lipophilic Broccoli Extracts; Correlations between Assays[a]

	γ-tocopherol	α-tocopherol	total tocopherols	lutein	β-carotene	total carotenoids	ORAC assay	ROS-induced oxidation	DNA damage	quinone reductase
α-tocopherol										
r value	0.214									
p value	0.339									
no. of data points	22									
total tocopherols										
r value	**0.579**	**0.92**								
p value	**0.005**	**<0.001**								
no. of data points	**22**	**22**								
lutein										
r value	0.096	0.372	0.349							
p value	0.673	0.088	0.112							
no. of data points	22	22	22							
β-carotene										
r value	0.305	**0.544**	**0.576**	**0.805**						
p value	0.167	**0.009**	**0.005**	**<0.001**						
no. of data points	22	**22**	**22**	**22**						
total carotenoids										
r value	0.243	**0.507**	**0.521**	**0.914**	**0.976**					
p value	0.275	**0.016**	**0.013**	**<0.001**	**<0.001**					
no. of data points	22	**22**	**22**	**22**	**22**					
ORAC assay										
r value	0.404	0.212	0.339	0.21	0.293	0.277				
p value	0.063	0.343	0.342	0.347	0.185	0.212				
no. of data points	22	22	22	22	22	22				
ROS-induced oxidation										
r value	0.358	0.173	0.272	0.157	0.325	0.276	**0.596**			
p value	0.122	0.466	0.246	0.509	0.162	0.238	**0.006**			
no. of data points	20	20	20	20	20	20	**20**			
DNA damage										
r value	–0.357	0.383	0.147	0.366	–0.052	0.091	**–0.705**	**–0.671**		
p value	0.281	0.245	0.666	0.269	0.879	0.79	**0.015**	**0.048**		
no. of data points	11	11	11	11	11	11	**11**	**12**		
quinone reductase										
r value	0.423	–0.018	0.172	–0.386	–0.158	–0.248	0.205	0.14	–0.239	
p value	0.195	0.959	0.613	0.241	0.644	0.462	0.546	0.719	0.479	
no. of data points	11	11	11	11	11	11	11	9	11	
thioredoxin reductase										
r value	0.193	–0.451	–0.274	–0.502	–0.548	–0.569	0.077	–0.049	–0.222	**0.761**
p value	0.57	0.163	0.414	0.115	0.081	0.068	0.821	0.9	0.511	**0.007**
no. of data points	11	11	11	11	11	11	11	9	11	**11**

[a] Significant differences are in bold.

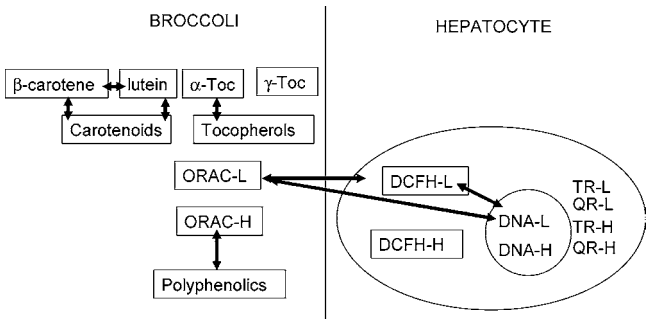

Figure 4. Relationship between biomarkers of antioxidation using lipophilic (-L) and hydrophilic (-H) extracts of broccoli. Significant correlations are shown by the joining arrows.

Both hydrophilic and lipophilic broccoli extracts exhibited antioxidant activity, as measured by the ORAC assay. However, the majority of activity was found in the hydrophilic extract, 76−92% of the total antioxidant capacity. In an earlier study of broccoli (*42*) utilizing the original ORAC assay with water-soluble reagents (*10*), the lipophilic extract provided 10% of the antioxidant activity. In the present study, using the modified assay that employs Trappsol to encircle the hydrocarbon chain of tocopherols and thus enhance their water solubility (*12*), only a small increase was seen, raising the fraction of ORAC activity due to lipophilic components to 16%. The ORAC system utilizes the peroxyl radical generator AAPH to measure chain-breaking antioxidants, such as tocopherols. As carotenoid antioxidant activity is due to its function as a singlet oxygen quencher, a different assay may be needed to accurately quantify the contribution of carotenoids to antioxidant activity in broccoli. Recently, the carotenoid lycopene has been shown to cause a dose-dependent increase in ORAC values, when 0.8% of β-cyclodextrin was used (*43*). To our knowledge, the ORAC assay has not been evaluated for measurement of the antioxidant action of β-carotene and lutein, the major carotenoids in broccoli. However, it appears to be contradictory that these carotenoids, known for their antioxidant behavior in vivo, should provide only a minor portion of the overall antioxidant action. As recently suggested, there may not be a single chemical assay that can accurately quantify the contribution of lipophilic and hydrophilic components to the total antioxidant action of the plant food (*44*).

The effect of broccoli extracts on the prevention of oxidative damage to cellular macromolecules has not previously been reported. Cellular measures were chosen to reflect antioxidant activities measured in whole animals, shown to relate to health benefits (*45*). Our results indicate that an antioxidant effect was evident for both lipophilic and hydrophilic extracts for all except the effect of the lipophilic extract on TR induction, which

Table 3. Hydrophilic Broccoli Extracts; Correlations between Assays[a]

	total polyphenols	ORAC assay	ROS-induced oxidation	DNA damage	quinone reductase
ORAC assay					
r value	**0.788**				
p value	**<0.001**				
no. of data points	**22**				
ROS-induced oxidation					
r value	0.256	0.317			
p value	0.249	0.151			
no. of data points	22	22			
DNA damage					
r value	–0.515	–0.495	–0.183		
p value	0.105	0.122	0.590		
no. of data points	11	11	11		
quinone reductase					
r value	–0.299	–0.096	–0.066	–0.09	
p value	0.372	0.779	0.847	0.784	
no. of data points	11	11	11	11	
thioredoxin reductase					
r value	–0.040	0.210	0.276	0.390	0.189
p value	0.907	0.536	0.411	0.236	0.578
no. of data points	11	11	11	11	11

[a] Significant differences are in bold.

showed a negative relationship. However, there was a lack of correlation between chemical and cellular measures of antioxidation, using the hydrophilic extracts (**Table 3**; **Figure 4**). This indicates that the chemical ORAC assay may not be the best tool for directing the development of genotypes with greater antioxidant effects within the body and/or that conditions of the cellular assays such as cell type used may not reflect the full range of activity described by the chemical assay. In contrast, and as reported for other fruits and vegetables (*9*), the content of polyphenols correlated well with ORAC-H, so that one may be used to predict the other. Total phenols, as measured by the Folin−Ciocalteu assay, are based on an oxidation/reduction reaction and can therefore be considered an antioxidant assay. Therefore, a correlation between the Folin−Ciocalteu and ORAC assay, both chemical antioxidant assays, is expected. Direct quantification of the flavonoids quercetin and kaempferol in broccoli cultivars was previously found not to correlate with ORAC-H (*42*).

Broccoli extracts prevented ROS-induced oxidative stress in HepG2 cells. As with the chemical ORAC assay, hydrophilic broccoli extracts accounted for the majority of the inhibition of oxidative stress within these cultured cells. Interestingly, for lipophilic, but not for hydrophilic, fractions, prevention of ROS-induced oxidative stress correlated with the chemical ORAC measure across genotypes ($r = 0.596$, $p = 0.006$). This relationship was not seen in our earlier study, which did not use the β-cyclodextrin-modified ORAC assay, and thus this change may be responsible for the correlation seen here. However, as neither ORAC nor the cellular ROS-induced oxidation assay was able to measure >16% contribution to total antioxidant activity by lipophilic extracts, the question remains as to whether the lipophilic components truly contribute to antioxidant activity clinically or if current assays do not provide accurate measures of their antioxidant activity. This is of particular interest, because protection from cardiovascular disease may be expected to relate to lipid-soluble antioxidants such as the tocopherols and carotenoids, within plasma lipoproteins such as LDL.

Genes for the enzymes QR and TR both contain an antioxidant response element (ARE) in the promoter sequence, which has been shown to be required for up-regulation of QR and TR synthesis by a variety of electrophilic compounds, such as the amphipathic isothiocyanate sulforaphane from broccoli (*46*, *47*). Induction of QR activity was similar for hydrophilic and lipophilic extracts. Although induction was significant, it was not substantial, being 1.2- and 1.3-fold over control for lipophilic and hydrophilic extracts, respectively (**Figure 3**). In contrast, for TR, hydrophilic extracts caused a significant and substantial induction of activity, whereas lipophilic extracts were without effect, suggesting that any effect of the lipophilic extract on QR was not ARE-dependent. Further work is needed to explore this. Induction of QR and TR did not correlate with any other antioxidation assay. HepG2 cells were preincubated with broccoli extracts for 24 h to study the up-regulation of QR and TR, whereas the cellular assay for the inhibition of ROS-induced oxidation depends on acute exposure to broccoli extracts. In future studies it would be of interest to evaluate the prevention of ROS-induced oxidation within cells after 24 h of exposure, when QR, TR, and other antioxidant enzymes have been up-regulated, to determine if the enzymes protect against oxidative stress.

In designing this experiment, we expected that ORAC would correlate with one or more cellular measures of antioxidant activity. We particularly expected a correlation between the inhibition of cellular oxidative stress and the inhibition of DNA damage, as both are considered to measure the radical scavenging power of antioxidants within cells. However, only lipophilic broccoli extracts showed any correlation, accounting for a very small fraction of total ORAC (**Figure 4**). For hydrophilic extracts, not only did we see little relationship between the analysis of broccoli and the cell-based estimates of protection from oxidative damage, but even the cell-based assays that we chose did not correlate one with another. The ORAC assay does provide a measure of antioxidant capacity of plant extracts like those of broccoli evaluated here. Furthermore, the extracts did decrease oxidative damage within the cell. However, the ORAC values did not correlate with cellular measures of antioxidant activity, indicating a need to develop assay conditions and/or an assay that more accurately predicts the antioxidant activity of plant extracts within cells or biological matrices.

In conclusion, the present data suggest that estimates of antioxidant potential of plant-based foods do not accurately reflect the antioxidant activity of broccoli extracts within cultured human cells. There are a number of studies showing that increased dietary levels of antioxidants, including carotenoids and polyphenolics but not tocopherols, are reflected in increased plasma levels of these components. Furthermore, studies have shown that increased dietary levels of some antioxidants have direct effects on lowering LDL oxidation measured ex vivo. Yet in clinical trials, the health benefit of purified antioxidants, such as β-carotene and vitamin E, is ambiguous and paradoxical. Before extensive breeding programs are undertaken to increase the antioxidant capacity of plant foods, it is necessary to verify assays to show that they reflect clinical effects and to carry out feeding studies to determine the benefit of increased dietary levels of these products.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ARE, antioxidant response element; DCFH, dichlorofluorescein; DCFH-DA, dichlorofluorescein diacetate; DCF, dichlorofluorescin; GGPP, geranylgeranyl diphosphates; ORAC, oxygen radical absorbance capacity; QR, quinone reductase; ROS, reactive oxygen species; SAS, statistical analysis software; TR, thioredoxin reductase.

ACKNOWLEDGMENT

We thank Dr. Mark Farnham of the USDA Vegetable Research Center for providing broccoli seeds, Dr. Barbara Schmidt for assistance with the Folin−Ciocalteu method, and Dr. John Finley (USDA, Grand Forks, ND) for helpful discussions.

LITERATURE CITED

(1) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: Oxford, U.K., 1999.

(2) Prior, R. L. Fruits and vegetables in the prevention of cellular oxidative damage. *Am. J. Clin. Nutr.* **2003**, *78* (3 Suppl.), 570S−578S.

(3) Cao, G.; Russell, R. M.; Lischner, N.; Prior, R. L. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J. Nutr.* **1998**, *128*, 2383−2390.

(4) Jimenez-Escrig, A.; Dragsted, L. O.; Daneshvar, B.; Pulido, R.; Saura-Calixto, F. In vitro antioxidant activities of edible artichoke (*Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. *J. Agric. Food Chem.* **2003**, *51*, 5540−5545.

(5) Roberts, W. G.; Gordon, M. H.; Walker, A. F. Effects of enhanced consumption of fruits and vegetables on plasma antioxidant status and oxidative resistance of LDL in smokers supplemented with fish oil. *Eur. J. Clin. Nutr.* **2003**, *57*, 1303−1310.

(6) Kaur, C.; Kapoor, H. C. Anti-oxidant activity and total polyphenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.* **2002**, *37*, 153−161.

(7) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **2004**, *52*, 4026−4037.

(8) Kurilich, A. C.; Tsau, G. J.; Brown, A.; Howard, L.; Klein, B. P.; Jeffery, E. H.; Kushad, M.; Wallig, M. A.; Juvik, J. A. Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *J. Agric. Food Chem.* **1999**, *47*, 1576−1581.

(9) Proteggente, A. R.; Pannala, A. S.; Paganga, G., Van Buren, L.; Wagner, E.; Wiseman, S.; Van De Put, F.; Dacombe, C.; Rice-Evans, C. A. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Res.* **2002**, *36*, 217−233.

(10) Cao, G.; Verdon, C. P.; Wu, A. H.; Wang, H.; Prior, R. L. Automated assay of oxygen radical absorbance capacity with the COBAS FARA II. *Clin. Chem.* **1995**, *41*, 1738−1744.

(11) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(12) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Deemer, E. K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**, *50*, 1815−1821.

(13) Zhu, C. Y.; Loft, S. Effects of Brussels sprouts extracts on hydrogen peroxide-induced DNA strand breaks in human lymphocytes. *Food Chem. Toxicol.* **2001**, *39*, 1191−1197.

(14) Liu, R. H.; Finley, J. Potential cell culture models for antioxidant research. *J. Agric. Food Chem.* **2005**, *53*, 4311−4314.

(15) Wang, H.; Joseph, J. A. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radical Biol. Med.* **1999**, *27*, 612−616.

(16) Beyer, R. E.; Segura-Aguilar, J.; Di Bernardo, S.; Cavazzoni, M.; Fato, R.; Fiorentini, D.; Galli, M.; Setti, M.; Landi, L.; Lenaz, G. The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2528−2532.

(17) Siegel, D.; Bolton, E. M.; Burr, J. A.; Liebler, D. C.; Ross, D. The reduction of α-tocopherolquinone by human NAD(P)H: quinone oxidoreductase: the role of α-tocopherolhydroquinone as a cellular antioxidant. *Mol. Pharmacol.* **1997**, *52*, 300−305.

(18) Nordberg, J.; Arner, E. S. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biol. Med.* **2001**, *31*, 1287−1312.

(19) Kurilich, A. C.; Jeffery, E. H.; Juvik, J. A.; Wallig, M. A.; Klein, B. P. Broccoli extracts protect against reactive oxygen species in HepG2 cells. *J. Nutraceuticals, Funct. Med. Foods* **2003**, *4*, 5−16.

(20) Foster, R.; Maynard, E. *Midwest Vegetable Production Guide for Commercial Growers*; University of Illinois Cooperative Extension Services C-1356; 1998; pp 40−44.

(21) Weber, E. J. Carotenoids and tocols of corn grain determined by HPLC. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1129−1134.

(22) Kurilich, A. C.; Juvik, J. A. Quantification of carotenoid and tocopherol antioxidants in *Zea mays*. *J. Agric. Food Chem.* **1999**, *47*, 1948−1955.

(23) Kurilich, A. C.; Juvik, J. A. Simultaneous quantification of carotenoids and tocopherols in corn kernel extracts by HPLC. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 2925−2934.

(24) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics and phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(25) Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J. Agric. Food Chem.* **2004**, *52*, 48−54.

(26) Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsh-Woodhill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273−3279.

(27) Prochaska, H. J.; Santamaria, A. B. Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **1988**, *169*, 328−336.

(28) Holmgren, A.; Bjornstedt, M. Thioredoxin and thioredoxin reductase. *Methods Enzymol.* **1995**, *252*, 199−208.

(29) Hill, K. E.; McCollum, G. W.; Boeglin, M. E.; Burk, R. F. Thioredoxin reductase activity is decreased by selenium deficiency. *Biochem. Biophys. Res. Commun.* **1997**, *234*, 293−295.

(30) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(31) Burdette, J. E.; Chen, S. N.; Lu, Z. Z.; Xu, H.; White, B. E.; Fabricant, D. S.; Liu, J.; Fong, H. H.; Farnsworth, N. R.; Constantinou, A. I.; Van Breemen, R. B.; Pezzuto, J. M.; Bolton, J. L. Black cohosh (*Cimicifuga racemosa* L.) protects against menadione-induced DNA damage through scavenging of reactive oxygen species: bioassay-directed isolation and characterization of active principles. *J. Agric. Food Chem.* **2002**, *50*, 7022−7028.

(32) Cao, G.; Sofic, E.; Prior, R. Antioxidant activity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(33) Plumb, G. W.; Price, K. R.; Rhodes, M. J.; Williamson, G. Antioxidant properties of the major polyphenolic compounds in broccoli. *Free Radical Res.* **1997**, *27*, 429−435.

(34) Vallejo, F.; Tomas-Barberan, F. A.; Ferreres, F. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. italica) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. *J. Chromatogr. A.* **2004**, *29*, 181−193.

(35) Hollman, P. C.; Katan, M. B. Health effects and bioavailability of dietary flavonoids. *Free Radical Res.* **1999**, *31*, s75−80.

(36) USDA National Nutrient Database for Standard Reference, release 17; 2004; http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl.

(37) Granado, F.; Olmedilla, B.; Blanco, I.; Rojas-Hidalgo, E. Carotenoid composition in raw and cooked Spanish vegetables. *J. Agric. Food Chem.* **1992**, *40*, 2135−2140.

(38) Hart, D. J.; Scott, K. J. Development and evaluation of an HPLC method for the analysis of carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem*. **1995**, *54*, 101−111.
(39) Fraser, P. D.; Bramley, P. M. The biosynthesis and nutritional use of carotenoids. *Prog. Lipid Res.* **2004**, *43*, 228−265.
(40) The Arabidopsis Genome Initiative. *Nature* **2000**, *408*, 796−815.
(41) Marwede, V.; Schierholt, A.; Mollers, C.; Becher, H. C. Genotype *x* environment interactions and heritability of tocopherol contents in canola. *Crop Sci.* **2004**, *44*, 728−731.
(42) Kurilich, A. C.; Jeffery, E. H.; Juvik, J. A.; Wallig, M. A.; Klein, B. P. Antioxidant capacity of different broccoli (*Brassica oleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay. *J. Agric. Food Chem.* **2002**, *50*, 5053−5057.
(43) Bangalore, D. V.; McGlynn, W.; Scott, D. D. Effect of β-cyclodextrin in improving the correlation between lycopene concentration and ORAC values. *J. Agric. Food Chem.* **2005**, *53*, 1878−1883.
(44) Prior, R. L.; Wu, X.; Schaich, K. Standardardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(45) Crespy, V.; Williamson, G. A review of the health effects of green tea catechins in in vivo animal models. *J. Nutr*. **2004**, *134*, 3431s−3440s.
(46) Hayes, J. D.; McMahon, M. Molecular basis for the contribution of the antioxidant responsive element to cancer chemoprevention. *Cancer Lett.* **2001**, *174*, 103−113.
(47) Hintze, K. J.; Wald, K. A.; Zeng, H.; Jeffery, E. H.; Finley, J. W. Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. *J. Nutr*. **2003**, *133*, 2721−2727.

Received for review June 23, 2005. Revised manuscript received July 18, 2005. Accepted July 25, 2005. This research was supported by a grant from USDA-IFAFS 00-52102-9695.

JF051495K