Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage?[1–3]

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ABSTRACT

Background: Lycopene, the main carotenoid in tomato, has been shown to be a potent antioxidant in vitro. However, there is no significant evidence of its antioxidant action in vivo.

Objective: We evaluated the effect of tomato intake on plasma carotenoid concentrations and lymphocyte resistance to oxidative stress.

Design: Ten healthy women (divided into 2 groups of 5 subjects each) ate a diet containing tomato purée (providing 16.5 mg lycopene) and a tomato-free diet for 21 d each in a crossover design. Before and after each diet period, plasma carotenoid concentrations and primary lymphocyte resistance to oxidative stress (evaluated by means of single-cell gel electrophoresis) were analyzed.

Results: After the first 21-d experimental period, total plasma lycopene concentrations increased by 0.5 μmol/L (95% CI: 0.14, 0.87) in the group that consumed the tomato diet and decreased by 0.2 μmol/L (95% CI: −0.11, −0.30) in the group that consumed the tomato-free diet ($P < 0.001$). Tomato consumption also had an effect on cellular antioxidant capacity: lymphocyte DNA damage after ex vivo treatment with hydrogen peroxide decreased by 33% (95% CI: 0.8%, 61%; $P < 0.05$) and by 42% (95% CI: 5.1%, 78%; $P < 0.05$) in the 2 groups of subjects after consumption of the tomato diet.

Conclusion: The consumption of tomato products may reduce the susceptibility of lymphocyte DNA to oxidative damage. Am J Clin Nutr 1999;69:712–8.

KEY WORDS Tomato, lycopene, carotenoids, oxidative stress, antioxidants, single-cell gel electrophoresis, SCGE, lymphocytes, women

INTRODUCTION

There is evidence that the high incidence of several chronic diseases is linked to oxidative stress; accordingly, researchers have been interested in identifying factors in the human diet that could help to contain the damage caused by oxidative species. Epidemiologic studies have stressed the importance of consuming fruit and vegetables. These foods contain substances whose biological activity is not clear. Carotenoids, for example, are present in considerable amounts in plasma and human tissues and may have specific functions related to their high antioxidant capacity. It has been suggested that carotenoids decrease the potential stress caused by the reactive species of oxygen produced by aerobic metabolism (1, 2). In vitro studies showed that lycopene has the highest antioxidant capacity of the carotenoids, having the ability to quench singlet oxygen and trap peroxyl radicals (3, 4).

Tinkler et al (5) studied the quenching of singlet oxygen by β-carotene, astaxanthin, lycopene, and canthaxanthin bound to the surface of lymphoid cells and found that all 4 carotenoids protected the cells against the photodynamic reaction sensitized by rose bengal or meso-tetra(4-sulphonatophenyl)porphine, the highest protection being given by lycopene. It has also been shown that β-carotene and lycopene are effective protectors of lymphocyte cells from nitrogen dioxide radical damage, but lycopene is at least twice as effective as β-carotene (6). Ribaya-Mercado et al (7) showed that when skin is subjected to ultraviolet light stress, more lycopene is destroyed than β-carotene, suggesting a role of lycopene in mitigating oxidative damage in tissues.

Lycopene is the main carotenoid present in tomato and tomato products. Epidemiologic studies revealed an inverse relation between tomato intake and the development of some types of cancer (8–10). Furthermore, it has been reported that a high consumption of tomato improves plasma antioxidant status, lowers plasma lipid peroxidation (11), and improves the antioxidant defense of LDL against attack by singlet oxygen (12). Despite the evidence from epidemiologic studies and in vitro studies in cell culture, however, little information is available on the action of lycopene and other antioxidants in vivo.

The single-cell gel electrophoresis (SCGE) technique (also called the comet assay) was developed by Singh et al (13) and can be used to evaluate low degrees of DNA damage in individual

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[2] Some of the carotenoid standards were provided by the Istituto delle Vitamine (Milan, Italy) and Hoffmann-La Roche (Basel, Switzerland).

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cells. The technique has been used mainly to quantitate single-strand breaks in DNA and the repair capacity of DNA subsequent to ultraviolet light, ionizing radiation, or oxidative damage in in vitro models (14). This technique could be a valid tool for analyzing the effect of natural antioxidants on a reliable biomarker of oxidative stress such as the status of cellular DNA. In this study we used the SCGE technique to evaluate how the addition of a tomato product high in lycopene affected plasma carotenoid concentrations and the resistance of peripheral blood lymphocyte DNA to oxidative damage inflicted ex vivo.

SUBJECTS AND METHODS

Subjects

Ten women were recruited from the student population of the University of Milan according to the following inclusion criteria: no history of cardiovascular, hepatic, renal, or gastrointestinal disease; body mass index (BMI; kg/m^2) between 18.5 and 25 (15); nonsmoking; and not having taken any supplement, drug, or medication for ≥1 mo before the start of the study. Subjects were also selected on the basis of their eating habits, determined by means of a food-frequency questionnaire and a food preference list (16). This was necessary to exclude persons who did not eat fruit and vegetables or who followed a specific diet (eg, vegetarian, vegan, or macrobiotic). Subjects selected had a mean (±SD) age of 23.1 ± 1.1 y and a mean BMI of 20.5 ± 1.5. All 10 subjects selected completed the experiment. Informal, written consent was obtained from each participant and the protocol was approved by the local ethical committee.

Diet

A tomato purée (double-concentrate tomato purée; Sainsbury's, London) was used as the source of lycopene. The tomato purée (60 g) provided ≈16.5 mg lycopene and 0.6 mg β-carotene. This amount was chosen on the basis of results obtained in a previous study in which we investigated the effect of supplementation with tomato products on plasma lycopene concentrations (17).

To ensure that all subjects consumed the same diet throughout the experimental period, they were asked to follow a tomato-free diet low in carotenoids (<600 μg/d). A 1-wk menu was given with lists of foods that were allowed and not allowed. The daily menu consisted of a standard breakfast; a lunch with pasta as the first course, a second course chosen freely from the list of foods allowed, a fixed portion of a specific vegetable (lettuce, potato, eggplant, cauliflower, or fennel), and a specific fruit (apple, banana, pear, pineapple, or strawberry); and a dinner with a first course and second course chosen freely from the list of allowed foods, but without vegetables and fruit. Each day during the supplementation period, subjects ate uncooked tomato purée with 10 g olive oil and 70 g (raw weight) pasta with the main meal at home. The pasta was consumed with just olive oil during the tomato-free diet period. Compliance with the diet was monitored by a dietitian.

Experimental design

A crossover design was used. Subjects were randomly divided into 2 groups of 5 women each. All subjects started the tomato-free diet 1 wk before the start of the experiment to normalize plasma carotenoid concentrations at baseline. After this period, one group of subjects consumed the tomato diet daily for 21 d, while the other group continued to consume the tomato-free diet for 21 d. At the end of this period, the 2 groups switched treatments. There was no washout period between the 2 experimental sessions.

Blood samples

Plasma carotenoid concentrations were measured weekly throughout the experimental period; lymphocyte DNA damage was determined at the beginning and end of each experimental period and after 1 wk of tomato purée intake. About 500 μL blood, drawn by means of a finger prick, was collected in heparin-treated, 600-μL tubes early in the morning from fasting subjects. The blood was divided into 2 aliquots: 70 μL was used to separate lymphocytes while the rest was centrifuged for 5 min at 1000 × g at 4°C to obtain plasma for carotenoid analysis.

Extraction and HPLC analysis of carotenoids from foods and plasma

Carotenoids were extracted from vegetable foods and plasma and analyzed by HPLC according to a method described previously, with a partial modification of the extraction phase (18). Briefly, tomato samples were extracted exhaustively with tetrahydrofuran; isomerization and degradation were minimized by performing the operation in the dark and by using butylated hydroxytoluene as an antioxidant. The extracts were then recovered in petroleum ether and aliquots of the organic phase, evaporated under nitrogen in the dark, and redissolved in the HPLC mobile phase.

Plasma (100 μL) was extracted with 100 μL ethanol (with echinenone as an internal standard) and 200 μL hexane. After the sample was vortexed for ≈1 min and centrifuged for 5 min at 1000 × g at 4°C, 150 μL of the supernate was evaporated under nitrogen in the dark and redissolved in 100 μL of the mobile phase for the HPLC injection (20 μL).

The HPLC analysis was performed by using a 5-μm C_{18} column (201 TP 54; 250 × 4.6 mm internal diameter; Vydac, Hesperia, CA) fitted with a C_{18} guard column and biocompatible frits. The eluent consisted of methanol:tetrahydrofuran (95:5) at a flow rate of 1 mL/min. Tetrahydrofuran was stabilized with 0.1% butylated hydroxytoluene. The ultraviolet-visible detector (Varian 2010; Varian, Walnut Creek, CA) was set at 445 nm.

Carotenoid concentrations were calculated by means of a mix of standards containing lutein, zeaxanthin, β-cryptoxanthin (Hoffmann-La Roche, Basel, Switzerland), α-carotene, and β-carotene (Sigma Chemical Co, St Louis); lycopene (Sigma) was prepared daily to avoid degradation and was injected separately. A photodiode array detector supported by the MILLENNIUM 2010 CHROMATOGRAPHY MANAGER computing system (Waters, Milford, MA) was used to assess or confirm the spectrum identity of carotenoids; spectra were registered between 200 and 600 nm.

The separation of many different geometric isomers of lycopene has been reported in the literature (19–21). With our chromatographic conditions, however, only 2 peaks were separated: one had the same retention time as the lycopene standard and was considered to be all-trans-lycopene; the second had a similar absorbance spectrum and presumably consisted of cis isomers but we could not resolve or identify them. Data were corrected by the recovery of the internal standard. The recovery was between 90% and 100%.

DNA damage evaluated by SCGE

Lymphocyte separation

The 70-μL aliquot of whole blood (kept on ice after being drawn and processed within 15 min) was gently mixed in microfuge tubes with 900 μL cold RPMI 1640 medium (Sigma) supplemented with 10% fetal calf serum (Sigma). Then 100 μL Histopaque 1077 (Sigma) was carefully underlayered (added to the bottom of the microfuge tube). The samples were then centrifuged at 200 × g for 4 min at room temperature and ≈100 μL of the middle-top Histopaque layer was added to 1 mL phosphate-buffered saline (PBS; Ca^{2+} and Mg^{2+} free) to wash the cells. The samples were then centrifuged for a few seconds at 5000 × g at room temperature to pellet the cells and the supernate was poured off. The pellets were resuspended in ≈80 μL PBS. Cells recovered were confirmed to be 95% mononuclear cells. Two samples were prepared for each subject.

SCGE

The SCGE assay is a relatively simple, visual, and sensitive technique for measuring and analyzing DNA breakage in any eukaryotic cell population that can be obtained as a single-cell suspension. This assay was applied according to Singh et al (13) with little modification and used to evaluate DNA damage in primary lymphocytes after exposure to an oxidant as follows.

Ultrapure low-melting-point agarose (1.5%) and standard-melting-point agarose (1.0%) in tris, acetate, EDTA buffer (Sigma) were dissolved by microwave heating. Then 70 μL standard agarose was added to fully frosted microscope slides (Richardson Supply Co, London); the slides were immediately covered with a coverslip and the agarose was allowed to solidify at 4°C for 5 min. After the coverslips were removed, 100 μL low-melting-point agarose was quickly added to the cells and this mixture was layered on the slides; the slides were then covered with another coverslip and the agarose was allowed to solidify at 4°C for 5 min in the dark. After this solidification step, the coverslips were removed and the slides treated further.

Hydrogen peroxide treatment

DNA damage was induced ex vivo by exposing the lymphocytes to an oxidant. Two slides were prepared for each sample: one was immersed for 5 min in a solution of hydrogen peroxide in PBS (500 μmol/L) and the other was immersed in PBS alone. The slides were then placed in cold lysis buffer (2.5 mol NaCl/L, 0.1 mol Na_2EDTA/L, 10 mmol tris/L, 1% triton X-100, 1% dimethyl sulfoxide, pH 10) and kept at 4°C for 1 h in the dark. After this step the slides were placed side by side in a horizontal electrophoresis tank (Scotlab, Coatbridge, United Kingdom), avoiding spaces between the slides and placing the slides close to the anode, and then covered with fresh electrophoresis buffer (0.3 mol NaOH/L, 1 mmol Na_2EDTA/L). The slides were left at 4°C for 40 min before electrophoresis in the same solution at 25 V and 300 mA for 20 min at 4°C in the dark. After electrophoresis, the slides were washed in a neutralizing buffer (0.4 mol tris-HCl/L, pH 7.5) for 15 min to remove alkalis and detergents and were stained with ethidium bromide in neutralizing buffer (2 mg/L) for 20 min. After they were washed in PBS, the slides were drained, covered with coverslips, and observed under a microscope.

Quantification of DNA damage

Individual cells or "comets" were viewed at a magnification of 400X under an epifluorescence microscope (BX 60; Olympus Italia, Milan, Italy) equipped with an excitation filter (BP520-550; Olympus Italia), dichroic beam splitter (DM565; Olympus Italia), and barrier filter (B580-IF; Olympus Italia). The light source was a mercury lamp (100 W; Olympus Italia). The microscope was attached to a high-sensitivity CCD videocamera (Variocam; PCO Computer Optics, Kelheim, Germany) and to a computer with an image analysis system.

With this assay, undamaged DNA is recognized as a fluorescent core whereas the presence of strand breaks in the chain (damaged DNA) allows the DNA to migrate during the electrophoresis to form a tail. The bigger and more fluorescent the tail, the greater the DNA damage that was induced. An example of comet images is shown in **Figure 1**.

Fifty cells on each slide were electronically captured and analyzed for fluorescence intensity with the COMET ANALYSIS program (Institute of Food Research, Norwich, United Kingdom) supported by the image processing environment VISILOG 4 (Noesis, Orsay, France). To quantify DNA damage, tail and head moment were evaluated and used to calculate relative tail moment. Tail and head moment is the sum of the intensity of each pixel in the tail and head multiplied by its distance from the center. Relative tail moment was calculated as follows:

$$\text{Relative tail moment} = \text{tail moment}/(\text{head moment} + \text{tail moment}) \quad (1)$$

For each subject, the mean relative tail moment of control cells was subtracted from the mean relative tail moment of treated cells.

Statistical analysis

Student's t test was used to evaluate the effect of the first week of the tomato-free diet on plasma carotenoid concentrations (days −7 to 0). A repeated-measures analysis of variance (ANOVA) with the sequence of diets (tomato diet then tomato-free diet or tomato-free diet then tomato diet) as the independent factor was used to investigate the effect of tomato consumption on plasma lycopene concentrations and relative tail moment. When a significant carryover effect was present, only the first sequence (group 1 fed tomato diet and group 2 fed tomato-free diet) was analyzed. The trend of plasma carotenoid concentration by time (days 0, 7, 14, 21, 28, 35, and 42) was evaluated by ANOVA with sequence of diets as the independent factor. Statistical analyses were performed on a personal computer with STATISTICA software (Statsoft Inc, Tulsa, OK).

RESULTS

Plasma concentration of carotenoids

Plasma concentrations of total lycopene throughout the experiment are reported in **Figure 2**. Total lycopene represents the sum of the 2 peaks on the chromatogram assumed to be all-trans-lycopene and cis isomers on the basis of spectra analysis, retention time, and addition of all-trans-lycopene standard to the samples. Total lycopene concentrations decreased significantly during the first week of the tomato-free diet in both groups (days −7 to 0; $P < 0.05$); as a result, total lycopene concentrations were not significantly different between the 2 groups at baseline. During the experimental period (days 0–42) there was a significant effect of the type of diet consumed ($P < 0.001$); however,

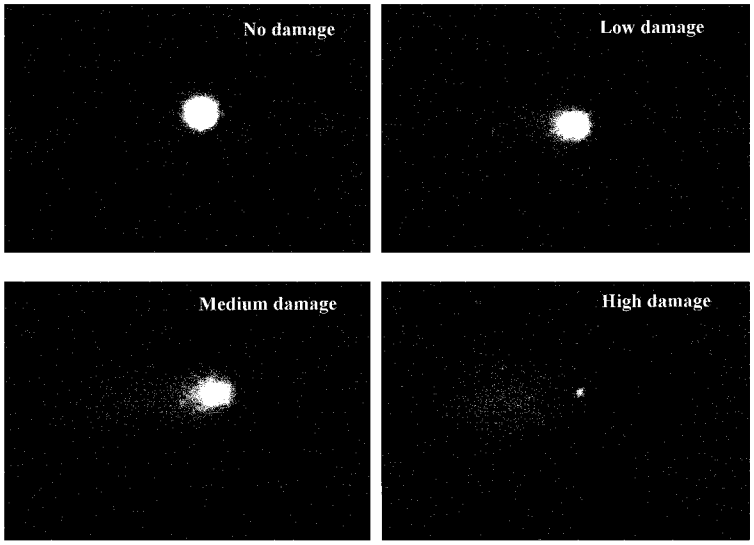

FIGURE 1. Images of comets obtained by single-cell gel electrophoresis representing different degrees of DNA damage.

there was also a significant carryover effect (P = 0.005). In the first 21-d experimental period (in which group 1 consumed the tomato diet and group 2 the tomato-free diet), plasma lycopene concentrations increased by 0.5 μmol/L (95% CI: 0.14, 0.87) in group 1 whereas they decreased by 0.2 μmol/L (95% CI: −0.11, −0.30) in group 2. ANOVA confirmed a significant increase in plasma lycopene concentrations after the tomato diet compared with the tomato-free diet (P < 0.0001). Steady state lycopene concentrations were reached after 7 d of tomato intake, whereas lycopene concentrations decreased progressively during consumption of the tomato-free diet and reached values lower than those at baseline. Under our chromatographic conditions, the all-trans form of lycopene predominated over the cis isomers.

Plasma concentrations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene are reported in **Figure 3**. Lutein (t = 2.506, P = 0.033) and β-cryptoxanthin (t = 2.465, P = 0.036) decreased significantly during the initial tomato-free diet period (days −7 to 0) whereas zeaxanthin, α-carotene, and β-carotene did not. During the experimental period, concentrations of lutein and zeaxanthin decreased significantly during the tomato-free diet (P < 0.05, ANOVA) but remained nearly constant during the period of tomato intake. β-Cryptoxanthin decreased continuously whereas α-carotene showed a slight modification with time. β-Carotene increased significantly during tomato consumption and decreased during the tomato-free diet in both groups (P < 0.01). No carryover effect was shown.

DNA damage

After 21 d of tomato intake, lymphocyte DNA damage, quantified as relative tail moment, decreased significantly with respect to baseline as shown in **Figure 4**. The differences in relative tail moments before and after each treatment period were significantly greater (P = 0.001 for both groups) after the tomato diet (mean for group 1: −0.09, 95% CI: −0.21, 0.02; mean for group 2: −0.14, 95% CI: −0.28, −0.01) than after the tomato-free diet (mean for group 1: 0.12, 95% CI: 0.02, 0.22; mean for

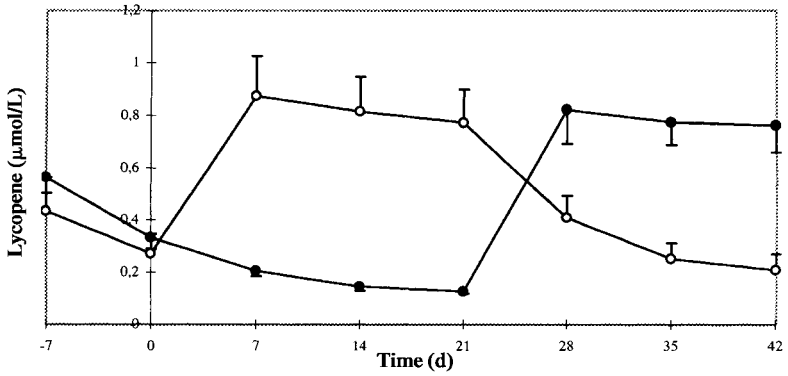

FIGURE 2. Mean (±SEM) plasma concentrations of total lycopene in group 1 (n = 5; ○) and group 2 (n = 5; ●) during the experiment. Both groups received a tomato-free diet from day −7 to day 0; group 1 then received the tomato diet for 21 d followed by the tomato-free diet and group 2 received the tomato-free diet first followed by the tomato diet. There was a significant carryover effect of diet (P = 0.005, ANOVA).

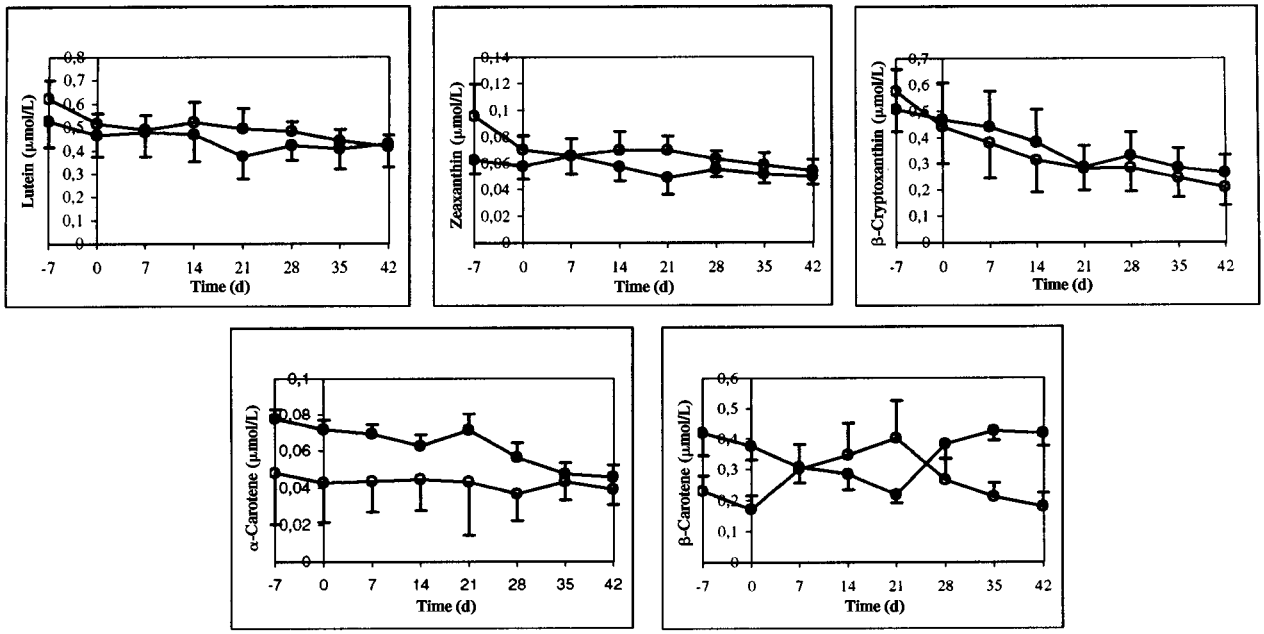

FIGURE 3. Mean (±SEM) plasma concentrations of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene in group 1 (n = 5; ○) and group 2 (n = 5; ●) during the experiment. There was no significant carryover effect of diet by ANOVA.

group 2: 0.01, 95% CI: −0.05, 0.07). During the tomato diet, relative tail moment decreased by 33% (95% CI: 0.8%, 61%) and 42% (95% CI: 5.1%, 78%) in groups 1 and 2, respectively. No carryover effect was noted. After the tomato-free diet, relative tail moments were not significantly different from baseline in either group. The trend in relative tail moment over the experimental period was similar to that for plasma lycopene concentrations, suggesting a relation between lycopene concentrations and oxidative stress.

To verify whether the resistance of lymphocytes to oxidative stress responded rapidly to lycopene intake, DNA damage was evaluated after just 7 d of supplementation with tomato. The variations in relative tail moment were significantly lower ($P = 0.009$ for both groups) after 7 d of tomato consumption (mean variation in group 1: −0.05, 95% CI: −0.22, 0.13; mean variation in group 2: −0.15, 95% CI: −0.25, −0.05) than after 21 d of the tomato-free diet.

DISCUSSION

There were 2 aims of this work: to evaluate the use of the SCGE technique in studying the in vivo action of natural antioxidants and to verify the effect of the intake of a tomato product on plasma lycopene concentrations and lymphocyte DNA resistance to oxidative stress. Consumption of the tomato purée for 21 d significantly increased both plasma lycopene concentrations and the resistance of lymphocyte DNA to oxidative stress inflicted ex vivo (exogenous damage).

Because we used a study design without a washout period, the 2 groups started the second 21-d experimental period with different plasma lycopene concentrations. Group 1 (who consumed the tomato diet during the first 21 d) needed ≈7 d for plasma lycopene concentrations to return to basal concentrations, a significant carryover effect. Nevertheless, tomato consumption for 21 d had a clear effect on plasma lycopene concentrations. Lymphocyte resistance to oxidative stress was also affected by the type of diet consumed; however, no carryover effect was present. Thus, it seems that whereas plasma lycopene concentrations are strictly linked to the presence of lycopene in the diet, cell resistance to oxidative stress is the result of a complex antioxidant defense system that could be helped by the consumption of foods such as tomato, even if these foods are not the only determinant.

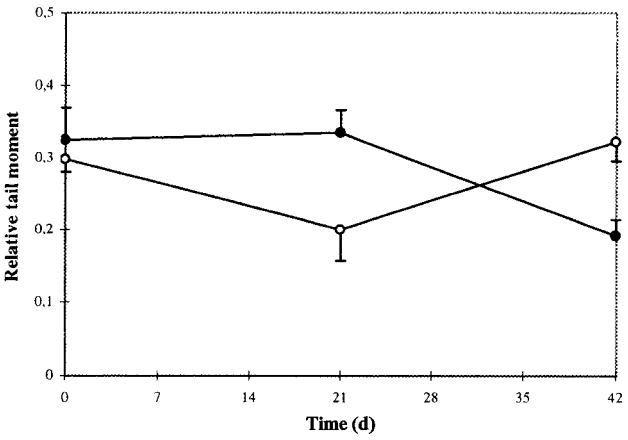

FIGURE 4. Mean (±SEM) DNA damage in lymphocytes challenged ex vivo with hydrogen peroxide in group 1 (n = 5; ○) and group 2 (n = 5; ●). Group 1 received the tomato diet for 21 d followed by the tomato-free diet and group 2 received the tomato-free diet first followed by the tomato diet. Damage was quantified as relative tail moment as defined in equation 1. There was no significant carryover effect of diet by ANOVA.

The SCGE technique is considered to be a useful tool for assessing DNA damage in individual cells. The advantages of this method are its sensitivity to DNA single-strand breaks and alkaline-labile sites in single cells and the small quantity of blood or tissue required. The assay is also easy to perform and gives results in a relatively short time. Furthermore, the use of primary cells representative of the actual body state make this procedure particularly useful for screening antioxidant action. As far as we know this is the first time this assay has been used to assess in vivo the effect of the intake of a food claimed to have antioxidant properties. Only a few other studies reported in the literature used the SCGE technique to analyze the effect of other antioxidant substances on oxygen-radical-generated DNA damage in human lymphocytes. Anderson et al (22) studied the effect in vitro of supplementation with several endogenous and exogenous antioxidants on lymphocytes from 2 donors and found that hydrosoluble vitamin C produced clear dose-related responses when cells were challenged with hydrogen peroxide. Under these conditions, vitamin C had a small protective effect at low doses (40 and 200 μmol/L) but exacerbating effects at high doses (5 mmol/L). In contrast, the authors found no effect of the fat-soluble vitamin E on DNA damage and hypothesized that this could have been the result of an insufficient treatment time.

More recently, Duthie et al (23) carried out a double-blind supplementation study in groups of smokers and nonsmokers. Half of each group received a daily antioxidant supplement containing vitamin C (100 mg/d), vitamin E (280 mg/d), and β-carotene (25 mg/d) and the other half received a placebo for ≤40 wk. The authors evaluated both endogenous DNA damage (strand breaks by means of SCGE and oxidized bases by means of a modified SCGE technique) and exogenous DNA damage in lymphocytes (by SCGE after an ex vivo treatment of cells with hydrogen peroxide). After 20 wk of supplementation there was a highly significant decrease in endogenous oxidative damage evaluated as oxidized bases but not as strand breaks in the lymphocyte DNA in both smokers and nonsmokers. However, the lymphocytes of subjects supplemented for 20 (or in some cases 40) wk showed an increased resistance to oxidative damage when challenged ex vivo with hydrogen peroxide. The decrease in DNA damage was ≈25% when 100 or 300 μmol H_2O_2/L was used as the oxidant. In our study the decrease in DNA damage after tomato intake was ≈30–40%. Despite the different methodologic approaches, in both studies supplementation with antioxidants resulted in a comparable effect. Duthie et al (23) suggested that their results may support the hypothesis that fruit and vegetables exert a protective effect against cancer via a decrease in oxidative damage to DNA.

More research should be carried out on the healthful properties of real foods. For this reason, we chose to evaluate the potential protective effect of lycopene by giving subjects a tomato product instead of a supplement. However, we cannot say that the protective effect was solely due to lycopene (which is the main antioxidant present in tomato); lycopene may have interacted with other carotenoids (β-carotene, lutein, phytoene, and phytofluen) or other antioxidants (nutrients and nonnutrients) such as vitamin C or flavonoids.

In the present study we did not evaluate endogenous DNA damage but the susceptibility of cells to an ex vivo insult that produces chain breaks in DNA resulting in comet tailing. Tomato consumption may prevent DNA damage resulting from peroxide insult. This can be explained by considering a direct physical reaction with oxidant species of lycopene or other antioxidants introduced with the tomato and present in high quantities in the cells. Tinkler et al (5) and Bohm et al (6) suggested that lycopene is an efficient protector against singlet oxygen and the nitrogen dioxide radical, a major toxic component of polluted air and cigarette smoke. Our results suggest that lycopene may also be an efficient protector against peroxide. This protective effect was evident after the first week of tomato intake and by the end of the 21 d it had improved further.

Recently, Lowe et al (GH Lowe, LA Booth, AJ Young, RF Bilton, unpublished observations, 1998) evaluated the antioxidant efficiency of lycopene delivered to adenocarcinoma HT29 cells at concentrations between 1 and 5 μmol/L. The xanthine–xanthine oxidase superoxide anion and hydrogen peroxide generating system was used to produce single-strand DNA breaks in lycopene-supplemented and -unsupplemented cells. Single-strand DNA breaks were evaluated by SCGE. The damage induced by xanthine–xanthine oxidase was reduced when the cells were supplemented with low concentrations of lycopene. Supplementation with 2 μmol lycopene/L resulted in a 20% reduction in relative tail moment; however, when lycopene doses >3 μmol/L were used, a progressive loss of activity was observed. Note that these concentrations are greater than those generally observed in human plasma. Our subjects reached their highest plasma lycopene concentrations after the first week of tomato consumption (0.9 μmol/L) and these concentrations were maintained over the subsequent 2 wk. These data confirm our previous study in which tomato purée was consumed for only 7 d and a steady state was reached after 4 d (17). Humans seem to have a homeostatic mechanism that keeps the concentration of this carotenoid within a constant range for a given dose (ie, at a steady state).

The other carotenoids analyzed, apart from β-carotene, decreased during the period of the tomato-free diet, suggesting that fruit and vegetables should be eaten regularly to maintain plasma carotenoid concentrations. β-Carotene concentrations, in contrast, increased during tomato consumption and decreased during the tomato-free diet. This finding was probably dependent on the absorption of the small amount of β-carotene present in the tomato and seems to suggest that β-carotene absorption was not affected by the high quantity of lycopene in the lumen, as reported recently by Johnson et al (24). It is likely that β-carotene played a part in improving the antioxidant capacity of the cell and in protecting DNA from oxidative damage. Further studies are needed to understand the mutual interactions of the numerous antioxidants present in foods, so that foods that have a protective effect on human health can be identified.

Our results support the hypothesis that the consumption of tomato products has a significant moderating effect on oxidative damage to lymphocyte DNA inflicted ex vivo by hydrogen peroxide. The SCGE technique can be used to discern the different effects of a tomato-free diet and a tomato diet on DNA damage and consequently can be proposed as a simple bioassay for studying the effect of different foods on oxidative stress.

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