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Lymphocyte Lycopene Concentration and DNA Protection from Oxidative Damage Is Increased in Women after a Short Period of Tomato Consumption

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Marisa Porrini¹ and Patrizia Riso

Department of Food Science and Technology, Division of Human Nutrition, University of Milan, Milan, Italy

ABSTRACT Several epidemiologic studies have suggested a role of tomato products in protecting against cancer and chronic diseases. In nine adult women, we evaluated whether the consumption of 25 g tomato puree (containing 7 mg lycopene and 0.3 mg β -carotene) for 14 consecutive days increased plasma and lymphocyte carotenoid concentration and whether this was related to an improvement in lymphocyte resistance to an oxidative stress (500 μ mol/L hydrogen peroxide for 5 min). Before and after the period of tomato intake, carotenoid concentrations were analyzed by HPLC and lymphocyte resistance to oxidative stress by the Comet assay, which detects DNA strand breaks. Intake of tomato puree increased plasma ($P < 0.001$) and lymphocyte ($P < 0.005$) lycopene concentration and reduced lymphocyte DNA damage by $\sim 50\%$ ($P < 0.0001$). β -Carotene concentration increased in plasma ($P < 0.05$) but not in lymphocytes after tomato puree consumption. An inverse relationship was found between plasma lycopene concentration ($r = -0.82$, $P < 0.0001$) and lymphocyte lycopene concentration ($r = -0.62$, $P < 0.01$) and the oxidative DNA damage. In conclusion, small amounts of tomato puree added to the diet over a short period can increase carotenoid concentrations and the resistance of lymphocytes to oxidative stress. *J. Nutr.* 130: 189–192, 2000.

KEY WORDS: • lycopene • tomato • lymphocytes
• DNA damage • humans

There is increasing evidence that diet can play an important role in human health, providing important substances that increase the defense system against several diseases. Research has focused mainly on the action of single compounds. However, a need exists to identify real foods with healthy

properties, considering that little is yet known about the interaction of potentially relevant substances in foods.

Epidemiologic studies suggest that antioxidant capacity is improved by the consumption of tomato products, thereby decreasing the risk of the development of diseases related to oxidative stress (Burney et al. 1989, Franceschi et al. 1994, Giovannucci et al. 1995, Parfitt et al. 1994). Tomatoes contain different compounds (e.g., carotenoids, vitamin C, flavonoids) that may be responsible for the antioxidant properties suggested. In particular, lycopene, the main carotenoid in tomato products possesses the greatest quenching ability of singlet oxygen among the various carotenoids (Di Mascio et al. 1989) and is effective in protecting blood lymphocytes from NO_2 radical damage (Bohm et al. 1995).

The few data available show that lycopene, like the other carotenoids, is not equally distributed in tissues; this suggests a mechanism whereby certain carotenoids may exert unique biologic effects. However, further data about lycopene concentrations and antioxidant properties in specific cells are required.

In a previous study, we found that the daily consumption of 60 g tomato puree for 3 wk increased lycopene and β -carotene plasma concentrations and enhanced the resistance of lymphocyte DNA to an oxidative stress (Riso et al. 1999). In this study, to obtain data more consistent with the actual dietary intake, we evaluated whether the consumption of 25 g tomato puree for 2 wk was sufficient to produce the same effect. Furthermore, we were interested in studying the resistance of lymphocyte DNA to an oxidative stress in relation to lymphocyte lycopene concentration.

SUBJECTS AND METHODS

Subjects. Healthy female subjects ($n = 11$) were selected on the basis of their eating habits determined by means of a food-frequency questionnaire and a food preference list (Porrini et al. 1995). We wanted to exclude subjects who did not eat fruits and vegetables and those who followed a specific diet or regimen (e.g., vegetarians, vegans, macrobiotics).

Subjects selected were not taking any supplement, drug or medication. They were all lean (body mass index = $20.3 \pm 1.5 \text{ kg/m}^2$) nonsmokers, with a mean age of 25.4 ± 2.2 y. Informed written consent was obtained from each participant and the protocol was approved by the Local Ethics committee.

Experimental design. A week before the beginning of the study (-7 d) and during the entire experimental period (14 d), subjects were asked to follow precise instructions regarding their diet to limit carotenoid intake ($<600 \mu\text{g/d}$) without interfering with their own eating habits. They were provided with a list of foods that were and were not permitted as previously reported in detail (Riso et al. 1999) and were asked to avoid any source of lycopene other than the tomato puree they were given during the supplementation period.

After the first week of consuming a diet low in carotenoids, subjects received 25 g of double-concentrate tomato puree (Sainsbury's, London, UK) providing ~ 7 mg lycopene and 0.3 mg β -carotene. The tomato puree was consumed uncooked at lunch with pasta

¹ To whom correspondence should be addressed.

and 5 g of olive oil for 14 consecutive days. Subjects consumed their meals at home. Compliance with the diet was assessed by a dietician.

Blood samples. Blood samples were collected at the beginning (d 0) and the end (d 14) of the experiment, early in the morning after overnight fasting. The blood was divided into two aliquots; 10 mL were used to separate lymphocytes, and the rest was centrifuged at $1000 \times g$ at 4°C to obtain the plasma.

Extraction of carotenoids from tomato, cells and plasma

Tomato. Tomato samples were extracted exhaustively with tetrahydrofuran, minimizing isomerization and degradation by performing the operation in the dark and using BHT as antioxidant. The extract was then recovered in petroleum ether, and aliquots of the organic phase were evaporated under nitrogen in the dark and redissolved in the HPLC mobile phase (Riso and Porrini 1997).

Lymphocytes. Lymphocytes were recovered from 10 mL of whole blood by means of a density gradient separation with Hystopaque 1077 (Sigma Chemical, St. Louis, MO). After being washed, lymphocytes were recovered in 1 mL of PBS and counted by hemocytometer. Cell membrane lysis was performed by adding 1 mL TRITON 1% (Sigma Chemical) and quickly freezing (in liquid nitrogen) and defrosting the samples. Carotenoid extraction was performed by adding 1 mL ethanol (containing echinenone as internal standard) and 2 mL hexane. After 1 min of vortexing, the organic layer was separated. A subsequent extraction with 2 mL hexane was performed, and the organic layer was separated and added to the previous one. The sample was then dried under N_2 and solubilized in $100 \mu\text{L}$ of the HPLC mobile phase for the carotenoid analysis.

Plasma. The extraction was performed in duplicate on $100 \mu\text{L}$ of plasma as previously reported (Porrini et al. 1998).

HPLC analysis of carotenoids. Carotenoid HPLC analysis was performed as previously described (Riso and Porrini 1997). Carotenoid concentrations were calculated by means of a mixture of standards containing lutein, zeaxanthin, β -cryptoxanthin (Hoffman-La Roche, Basel, Switzerland), α -carotene and β -carotene (Sigma Chemical); lycopene (Sigma Chemical) was prepared daily to avoid problems of degradation and injected separately. Lycopene concentration is the sum of the all-*trans* and *cis* isomers (Porrini et al. 1998). Carotenoid concentrations were corrected by the recovery of an internal standard. Detection limits, determined at a signal-to-noise ratio of 3, were between 20 and $70 \mu\text{g/L}$.

DNA damage evaluated by Comet assay

The procedure has been reported previously in detail (Riso et al. 1999).

Lymphocyte separation. Lymphocyte separation was performed on $70 \mu\text{L}$ of whole blood by density gradient with Hystopaque 1077 (Sigma) and then resuspended in PBS. Two samples for each subject were prepared.

Comet assay or single cell gel electrophoresis and H_2O_2 treatment. The Comet assay was conducted according to Singh et al. (1988) with little modification. It was used to evaluate DNA damage to primary lymphocytes after exposure to an oxidant. Cells embedded in agarose on microscope slides were exposed to a solution of H_2O_2 in PBS ($500 \mu\text{mol/L}$) for 5 min; other unexposed slides served as controls.

Quantification of DNA damage. Cells for each slide ($n = 50$) were captured electronically with an image analysis system and analyzed for fluorescence intensity. Undamaged DNA is recognized as a fluorescent core. The presence of strand breaks in the chain (damaged DNA) allows DNA to migrate during the electrophoresis to form a tail; the larger and more fluorescent the tail, the greater is the DNA damage.

DNA damage was calculated as Relative Tail Moment (RTM) as follows: Tail Moment/(Head Moment + Tail Moment). Tail/Head Moment is the sum of the intensity of each pixel in the Tail/Head multiplied by its distance from the center. $\text{CV} < 8\%$ were calculated on both control and treated slides. For each subject, the mean RTM of treated cells was subtracted from the mean RTM of control cells.

Statistical analysis. Only complete sets of data were analyzed; thus nine of the eleven subjects were considered. One-way ANOVA for a repeated-measures design was used to investigate the effect of daily tomato consumption on lycopene and other carotenoid plasma concentrations. One-way ANOVA was also used to evaluate the effect of tomato consumption on lymphocyte DNA damage. Differences were considered significant if $P < 0.05$. The analysis of simple regression was used to evaluate the correlation between variables (plasma and lymphocyte carotenoid concentration vs. RTM). Statistical analyses were performed on a personal computer with Statistica Software (Statsoft, Tulsa, OK). Values are means \pm SD.

RESULTS

Plasma concentration of carotenoids. Plasma carotenoid concentrations at recruitment (-7 d) were $0.35 \pm 0.08 \mu\text{mol/L}$ for lycopene, $0.49 \pm 0.06 \mu\text{mol/L}$ for lutein, $0.07 \pm 0.02 \mu\text{mol/L}$ for zeaxanthin, $0.40 \pm 0.09 \mu\text{mol/L}$ for β -cryptoxanthin, $0.08 \pm 0.04 \mu\text{mol/L}$ for α -carotene, $0.40 \pm 0.11 \mu\text{mol/L}$ for all-*trans* β -carotene and $0.05 \pm 0.03 \mu\text{mol/L}$ for *cis* β -carotene. After 7 d of consuming a tomato-free diet (d 0) there were significant reductions in lycopene, lutein and β -cryptoxanthin ($P < 0.05$).

The plasma total lycopene, all-*trans* β -carotene and *cis* β -carotene concentrations increased significantly after 2 wk of consuming the diet with tomato puree (Table 1, $P < 0.05$). The plasma concentrations of lutein, zeaxanthin, β -cryptoxanthin and α -carotene were not significantly different before and after tomato consumption.

Lymphocyte concentration of carotenoids. There was no effect of tomato consumption on lutein, zeaxanthin, β -cryptoxanthin, α -carotene, all-*trans* β -carotene or *cis* β -carotene concentration of lymphocytes (Table 1). Tomato intake for 14 d significantly increased lycopene concentration ($P < 0.005$).

DNA damage. DNA damage of lymphocytes, quantified as RTM, significantly decreased with respect to baseline, after the 14 d of tomato intake ($P < 0.0001$). After tomato consumption, DNA damage to lymphocytes (RTM = 0.28 ± 0.08) was half ($P < 0.0001$) that of the basal level (RTM = 0.57 ± 0.05). The RTM of control cells at d 0 and 14 were 0.06 ± 0.02 and 0.07 ± 0.03 , respectively.

The regression analysis showed an inverse correlation between plasma lycopene concentration and oxidative DNA damage ($r = -0.82$, $P < 0.0001$) and between lymphocyte lycopene concentration and oxidative DNA damage ($r = -0.62$; $P < 0.01$).

DISCUSSION

It has been suggested that tomato consumption should be increased because of its antioxidant substances such as lycopene. However, the amount necessary to achieve the antioxidant activity and the bioavailability of lycopene and the other carotenoids to cells and tissues have not been clearly evaluated.

Some studies (Gartner et al. 1997, Porrini et al. 1998, Stahl and Sies 1992) have described the carotenoid plasma response after tomato product intake; however, little is known about the relationship between the intake of foods rich in carotenoids and their concentrations in specific cells or the amount necessary to ensure the antioxidant activity.

Some authors reported data obtained after supplementation of subjects with pure substances such as β -carotene. Murata et al. (1994) found that β -carotene concentrations in plasma, peripheral blood monocytes and platelets from subjects supplemented with 60 mg β -carotene for 44 wk were higher than

TABLE 1

Carotenoid concentrations in plasma and lymphocytes of women before (d 0) and after (d 14) tomato consumption¹

Carotenoid	Plasma		Lymphocytes	
	d 0	d 14	d 0	d 14
	$\mu\text{mol/L}$		$\text{nmol}/10^{12} \text{ cells}$	
Lutein	0.34 \pm 0.13	0.31 \pm 0.11	219.65 \pm 138.61	376.24 \pm 171.38
Zeaxanthin	0.05 \pm 0.01	0.05 \pm 0.01	32.86 \pm 20.98	59.91 \pm 33.33
β -Cryptoxanthin	0.16 \pm 0.06	0.14 \pm 0.05	123.85 \pm 89.35	161.65 \pm 76.18
α -Carotene	0.06 \pm 0.03	0.05 \pm 0.02	45.61 \pm 36.36	52.35 \pm 26.16
All-trans β -carotene	0.25 \pm 0.11	0.29 \pm 0.09 ^a	208.65 \pm 141.65	223.88 \pm 120.21
Cis β -carotene	0.02 \pm 0.01	0.05 \pm 0.03 ^b	28.35 \pm 28.45	47.72 \pm 29.78
Total lycopene	0.13 \pm 0.06	0.55 \pm 0.21 ^c	126.05 \pm 49.59	227.75 \pm 65.62 ^d

¹ Values are means \pm sd, $n = 9$.^a Significantly different from d 0, $P < 0.05$.^b Significantly different from d 0, $P < 0.01$.^c Significantly different from d 0, $P < 0.001$.^d Significantly different from d 0, $P < 0.005$.

in subjects given a placebo; however, no differences were present in red blood cells.

We found that the daily consumption of 25 g tomato puree for 14 consecutive days significantly increased plasma and lymphocyte lycopene concentration, whereas β -carotene concentration increased only in plasma, and the other carotenoids remained constant. The amount of lycopene delivered as tomato was quite low and the period of intake short, but the bioavailability of lycopene from tomato puree is probably very high (Porrini et al. 1998), resulting in a significant increase not only in plasma but also in cells. Previously, we reported an increase in plasma lycopene concentration of $\sim 0.5 \mu\text{mol/L}$ after the consumption of 60 g of the same tomato puree daily for 3 wk (Riso et al. 1999). In this study, the increase was $\sim 0.4 \mu\text{mol/L}$ after the consumption of less than half that quantity of tomato puree for 2 wk. Consequently, it seems that low amounts are sufficient to improve and maintain plasma levels, and that plasma lycopene concentrations do not respond in a dose-dependent manner. The 25 g tomato puree were sufficient to improve lycopene concentration even in lymphocytes, where we found almost a doubling effect.

To our knowledge, this is the first time that the cellular concentrations of specific antioxidants were studied relative to DNA oxidative damage. The interest in lymphocytes is due not only to the fact that they are considered a good marker of the actual body state, but also because lymphocytes could be a reliable model for studying the effect of the addition of specific antioxidants to the diet (Anderson et al. 1994, Duthie et al. 1996, Riso et al. 1999). We studied DNA damage because it is a useful biomarker of the oxidative status and the antioxidant defense system of the animal (Duthie et al. 1996). DNA damage in primary lymphocytes was induced ex vivo by means of H_2O_2 and measured by Comet assay. We chose a high concentration of H_2O_2 (500 $\mu\text{mol/L}$) to exacerbate DNA damage and highlight cells able to protect themselves from the oxidative stress.

After subjects consumed tomato puree for 14 d, DNA damage of lymphocytes challenged with H_2O_2 was reduced by $\sim 50\%$, demonstrating an improvement in cell antioxidant capacity. The regression analysis showed a strong inverse relation between plasma lycopene concentration and lymphocyte DNA damage. This indicates that when dietary antioxidant intake is consistent, plasma antioxidant concentrations reflect well the cellular antioxidant capacity, even if plasma is

more directly subjected to the fluctuation of dietary antioxidant intake than are cells.

The inverse relation between lycopene concentration and DNA damage was confirmed also in lymphocytes. From these data, we conclude that lycopene contributes to the protection of DNA from oxidative stress or, at least, that it is a good marker of tomato antioxidant properties. In fact, other substances present in tomato (β -carotene, lutein, phytoene and vitamin C) contribute to the antioxidant properties observed.

Few other studies have investigated the effect of the consumption of a real food on DNA damage. Pool-Zobel et al. (1997) supplemented 23 subjects for 2 wk with 300 mL tomato juice (40 mg lycopene), 330 mL carrot juice (22.3 mg β -carotene, 15.7 mg α -carotene) or 10 g dried spinach powder (11.3 mg lutein). Using the Comet assay, they found a significant decrease in endogenous levels of strand breaks in lymphocyte DNA after the intake of all the three foods, and a reduction of the levels of oxidized pyrimidine bases (detected by introducing an intermediate incubation step with endonuclease III) during the carrot juice supplementation.

Rao and Argawal (1998) evaluated the effect of dietary supplementation of lycopene from different tomato products (two different spaghetti sauces and a tomato juice) on lymphocyte DNA oxidation, measured by 8-oxo-2-deoxyguanosine. DNA damage tended to be lower when the products were consumed. Presumably, the supplementation period was too short for differences to be significant although the amount of lycopene provided was quite high. The authors found a significant increase in plasma lycopene concentrations but did not evaluate lymphocyte concentrations.

To our knowledge, this is the first report describing the effect of the consumption of a small quantity of tomato, and consequently lycopene [7 mg vs. 20.5–50.4 mg lycopene used by Rao and Argawal (1998) and 40 mg lycopene used by Pool-Zobel et al. (1997)], for a short period of time on oxidative damage. Previously, large amounts of foods and/or antioxidants likely have been used to clearly establish their effects on specific targets. Now it is important to begin considering the effect of reasonable amounts of dietary antioxidants to determine healthy dietary habits to recommend to the population.

This is particularly important in light of the results of a cell culture study (Lowe et al. 1999) in which high lycopene concentrations ($>3 \mu\text{mol/L}$) did not afford protection against

DNA damage in HT29 cells but seemed to act as a prooxidant. However, these high lycopene concentrations cannot be achieved even by consuming a diet very rich in lycopene, probably due to a mechanism of control that maintains plasma lycopene concentrations at values less than $\sim 1 \mu\text{mol/L}$ (Porrini et al. 1998).

In conclusion, our results emphasize the importance of the consumption of foods such as tomato in improving antioxidant capacity and decreasing the risk of the development of diseases related to oxidative stress.

LITERATURE CITED

- Anderson, D., Yu, T. W., Phillips, B. J. & Schmezer, P. (1994) The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the Comet assay. *Mutat. Res.* 307: 261–271.
- Bohm, F., Tinkler, J. H. & Truscott, T. G. (1995) Carotenoids protect against cell membrane damage by nitrogen dioxide radical. *Nat. Med.* 1: 98–99.
- Burney, P.G.J., Comstock, G. W. & Morris, J. S. (1989) Serologic precursors of cancer: serum micronutrients and the subsequent risk of pancreatic cancer. *Am. J. Clin. Nutr.* 49: 895–900.
- Di Mascio P., Kaiser S. & Sies H. (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 274: 532–538.
- Duthie, S. J., Ma, A., Ross, M. A. & Collins, A. R. (1996) Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res.* 56: 1291–1295.
- Franceschi, S., Bidoli, E., La Vecchia, C., Talamini, R., D'Avanzo, B. & Negri, E. (1994) Tomatoes and risk of digestive-tract cancers. *Int. J. Cancer* 59: 181–184.
- Gartner, C., Stahl, W. & Sies, H. (1997) Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am. J. Clin. Nutr.* 66: 116–122.
- Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A. & Willett, W. C. (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.* 87: 1767–1776.
- Lowe, G. M., Booth, L. A., Young, A. J. & Bilton, R. F. (1999) Lycopene and β -carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses. *Free Radic. Res.* 30: 141–151.
- Murata, T., Tamai, H., Morinobu, T., Manago, M., Takenaka, H., Hayashi, K & Mino, M. (1994) Effect of long-term administration of β -carotene on lymphocyte subsets in humans. *Am. J. Clin. Nutr.* 60: 597–602.
- Parfitt, V. J., Rubba, P., Bolton, C., Marotta, G., Hartog, M. & Mancini, M. (1994) A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *Eur. Heart J.* 15: 871–876.
- Pool-Zobel, B. L., Bub, A., Muller, H., Wollowski I. & Rechkemmer, G. (1997) Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trial with carotenoid-rich foods. *Carcinogenesis* 18: 1847–1850.
- Porrini, M., Crovetti, R., Silva, S. & Testolin, G. (1995) Evaluation of satiety sensations and food intake after different preloads. *Appetite* 25: 17–30.
- Porrini, M., Riso, P. & Testolin, G. (1998) Absorption of lycopene from single or daily portions of raw and processed tomato. *Br. J. Nutr.* 80: 353–361.
- Rao, A.V. & Argawal, S. (1998) Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutr. Cancer* 3: 199–203.
- Riso, P., Pinder, A., Santangelo, A. & Porrini M. (1999) Does tomato consumption effectively increase the resistance of lymphocyte DNA to oxidative damage? *Am. J. Clin. Nutr.* 69: 712–718.
- Riso, P. & Porrini, M. (1997) Determination of carotenoids in vegetable foods and plasma. *Int. J. Vitam. Nutr. Res.* 67: 47–54.
- Singh N. P., McCoy, M. T., Tice, R. R. & Schneider, E. L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175: 184–191.
- Stahl, W. & Sies, H. (1992) Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice. *J. Nutr.* 122: 2161–2166.