

## Daily intake of a formulated tomato drink affects carotenoid plasma and lymphocyte concentrations and improves cellular antioxidant protection

Marisa Porrini<sup>1</sup>, Patrizia Riso<sup>1\*</sup>, Antonella Brusamolino<sup>1</sup>, Cristiana Berti<sup>1</sup>, Serena Guarnieri<sup>1</sup> and Francesco Visioli<sup>2</sup>

<sup>1</sup>Department of Food Science and Technology, Division of Human Nutrition, University of Milan, Milan, Italy

<sup>2</sup>Department of Pharmacological Sciences, University of Milan, Milan, Italy

(Received 4 August 2004 – Revised 21 September 2004 – Accepted 27 September 2004)

The salutary characteristics of the tomato are normally related to its content of carotenoids, especially lycopene, and other antioxidants. Our purpose was to verify whether the daily intake of a beverage prototype called Lyc-o-Mato<sup>®</sup> containing a natural tomato extract (Lyc-o-Mato<sup>®</sup> oleoresin 6 %) was able to modify plasma and lymphocyte carotenoid concentrations, particularly those of lycopene, phytoene, phytofluene and  $\beta$ -carotene, and to evaluate whether this intake was sufficient to improve protection against DNA damage in lymphocytes. In a double-blind, cross-over study, twenty-six healthy subjects consumed 250 ml of the drink daily, providing about 6 mg lycopene, 4 mg phytoene, 3 mg phytofluene, 1 mg  $\beta$ -carotene and 1.8 mg  $\alpha$ -tocopherol, or a placebo drink. Treatments were separated by a wash-out period. Plasma and lymphocyte carotenoid and  $\alpha$ -tocopherol concentrations were determined by HPLC, and DNA damage by the comet assay. After 26 d of consumption of the drink, plasma carotenoid levels increased significantly: concentrations of lycopene were 1.7-fold higher ( $P < 0.0001$ ); of phytofluene were 1.6-fold higher ( $P < 0.0001$ ); of phytoene were doubled ( $P < 0.0005$ ); of  $\beta$ -carotene were 1.3-fold higher ( $P < 0.05$ ). Lymphocyte carotenoid concentrations also increased significantly: that of lycopene doubled ( $P < 0.001$ ); that of phytofluene was 1.8-fold higher ( $P < 0.005$ ); that of phytoene was 2.6-fold higher ( $P < 0.005$ ); that of  $\beta$ -carotene was 1.5-fold higher ( $P < 0.01$ ). In contrast, the  $\alpha$ -tocopherol concentration remained nearly constant. The intake of the tomato drink significantly reduced (by about 42%) DNA damage ( $P < 0.0001$ ) in lymphocytes subjected to oxidative stress. In conclusion, the present study supports the fact that a low intake of carotenoids from tomato products improves cell antioxidant protection.

### Lycopene: Phytoene: Phytofluene: Tomato drink: DNA damage: Lymphocytes

A number of recent studies have focused on tomatoes and tomato products because their high levels of intake by some populations have been associated with positive actions on human health. There is evidence that tomato consumption decreases the incidence of certain types of cancer (Gerber *et al.* 2001) and other degenerative diseases in part attributed to enhanced oxidative stress (Ness & Powles, 1997; Law & Morris, 1998). In fact, several compounds in tomatoes and their products may modulate radical-mediated oxidative damage that contributes to the initiation and progression of chronic disease processes (Lehucher-Michel *et al.* 2001).

Tomatoes (*Lycopersicon esculentum*) contain several antioxidant compounds, such as vitamin C, folate, polyphenols, etc. Their beneficial effects are, however, generally attributed to carotenoids, in particular, lycopene. Yet carotenoids other than lycopene, for example,  $\beta$ -carotene, phytoene and phytofluene, are present in considerable, albeit variable, amounts in both fresh tomatoes and their products (Müller, 1997; Beecher, 1998; Olmedilla *et al.* 1998). Notably, phytoene and phytofluene constitute about 10 and 4 %, respectively, of the total carotenoids of raw red tomato (Riso & Porrini, 2001; Khachik *et al.* 2002).

In human plasma, lycopene accounts for at least 10 % of all carotenoids (Johnson, 1998). Lycopene is also present in other human tissues, for example, skin, adipose tissue (Walfisch *et al.* 2003), liver, testes, adrenal gland and prostate (Rao & Agarwal,

1999; Khachik *et al.* 2002), as well as being abundant in buccal mucosal cells (Paetau *et al.* 1999; Allen *et al.* 2003) and lymphocytes (Porrini & Riso, 2000). Moreover, phytoene and phytofluene are present in human plasma in concentrations of about 0.1–0.2  $\mu\text{mol/l}$  (Paetau *et al.* 1998; Edwards *et al.* 2003; Hoppe *et al.* 2003), although only a few studies report their concentration in human cells (Paetau *et al.* 1999) and tissues. Of the latter, phytoene has been found in lung tissue and phytofluene in breast tissue (Khachik *et al.* 2002). With regard to animal studies, a recent work by Werman *et al.* (2002) indicated liver as the principal storage site of phytoene, whereas only small amounts were found in the adrenal gland, spleen and kidney.

To our knowledge, very limited data are available on the bioavailability of phytoene and phytofluene, and their potential contribution to the health-promoting properties of tomato products has yet to be established, despite evidence of the involvement of tomato components other than lycopene in the DNA- and lipid-protecting properties of tomato intake (Riso *et al.* 1999; Porrini & Riso 2000; Riso & Porrini, 2001; Rao & Shen, 2002).

The aim of the present study was two-fold: (1) to assess the effect on plasma and cell carotenoid concentrations of the regular intake of a soft drink to which a well-characterized tomato extract had been added (2) to evaluate – in healthy subjects – whether this supplementation was able to protect lymphocyte DNA from oxidative damage.

\* Corresponding author: Dr P. Riso, fax +39 02 50316600, email patrizia.riso@unimi.it

## Subjects and methods

### Subjects

The protocol was approved by the local ethics committee. Twenty-six healthy men and women were recruited from within the University of Milan and selected according to the following criteria: (1) no history of cardiovascular, renal, hepatic or gastrointestinal disease; (2) not pregnant; (3) not taking dietary supplements or drugs. As we wanted to maintain subjects on their own diet during the experimentation, we selected a group of volunteers who were homogeneous for eating habits (in particular for fruit and vegetable consumption), as evaluated by a validated questionnaire.

Subjects who did not eat fruit and vegetables and those on vegetarian, macrobiotic or other alternative diets were excluded from the study.

The mean age of the selected subjects was 26.1 (SD 3.2) years for women and 25.4 (SD 2.4) years for men; BMI was also within the normal range both for women (20.1 (SD 1.5) kg/m<sup>2</sup>) and men (22.4 (SD 1.9) kg/m<sup>2</sup>). All subjects gave their written informed consent to participate in the study.

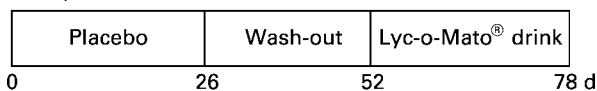
### Study

The experimental protocol followed a double-blind, cross-over design. Lyc-o-Mato<sup>®</sup> (LycRed Natural Products Industries Ltd, Beer-Sheva, Israel; prepared by the solubilization of Lyc-o-Mato<sup>®</sup> 6%, applying a micro-emulsification technique to yield a transparent, reddish drink) and a placebo drink were used for the treatments. Lyc-o-Mato<sup>®</sup> 250 ml contained 5.7 mg lycopene, 3.7 mg phytoene, 2.7 mg phytofluene, 1 mg  $\beta$ -carotene and 1.8 mg  $\alpha$ -tocopherol, as evaluated by HPLC analysis carried out by LycRed and confirmed in our laboratory. This drink was aromatized with grapefruit, pineapple and orange flavours. The placebo drink was identical except for the absence of the micro-emulsion containing lycopene and the other carotenoids. It also did not contain  $\alpha$ -tocopherol. Both beverages had an identical taste and flavour.

The subjects were divided into two groups, each with thirteen subjects: group 1 was assigned to the sequence placebo/wash-out/Lyc-o-Mato<sup>®</sup>, whereas group 2 followed the sequence Lyc-o-Mato<sup>®</sup>/wash-out/placebo. Each period lasted for 26 d; the wash-out period was required to avoid any carry-over effect in the Lyc-o-Mato<sup>®</sup>/placebo sequence (see Fig. 1). The bottles were given once per week to the subjects, who were instructed to store them in the fridge and drink one of them per d, in the morning after breakfast.

Throughout the experimental period, subjects were instructed to maintain their own habitual diet, which was checked by weekly interviews.

#### Group 1



#### Group 2

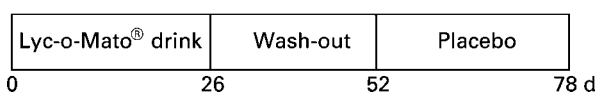


Fig. 1. Experimental design

### Blood and lymphocyte samples

Blood samples were collected at the beginning and the end of each treatment period, that is, before and after receiving placebo or Lyc-o-Mato<sup>®</sup>. Blood samples from fasting volunteers were drawn into evacuated tubes, using Li<sup>+</sup> heparin as the anticoagulant. Plasma was obtained by centrifugation at 800g for 15 min and was stored at  $-80^{\circ}\text{C}$ . Lymphocytes were separated by density gradient centrifugation of 10 ml whole blood (400 g for 30 min) with Histopaque 1077 (Sigma Chemicals Co, St Louis, MO, USA). The lymphocyte layer was removed from the gradient, washed with PBS, recovered in 1 ml PBS and stored at  $-80^{\circ}\text{C}$ .

### Carotenoids in beverages and biological samples

**Beverage samples.** Extraction of carotenoids from Lyc-o-Mato<sup>®</sup> was performed on 50 ml of the product by adding 25 ml 2-propanol, 25 ml dichloromethane and 100 ml of an extraction mixture (50 ml petroleum ether, 25 ml absolute ethanol and 25 ml acetone) previously prepared. The solution was mixed for 30 min and then transferred into a separation funnel, where 50 ml water were added. The solution was vigorously mixed for 5 min and then, after separation of the phases, an aliquot part of the upper phase was collected and dried under N. The dried extract was redissolved in mobile phase and was subjected to HPLC analysis.

**Plasma.** Carotenoid extraction was performed in duplicate on 100  $\mu\text{l}$  plasma as previously reported (Porrini & Riso, 2000).

**Lymphocytes.** Lymphocyte carotenoid extraction was performed following a published method (Riso *et al.* 2004). Carotenoid concentrations were expressed as  $\mu\text{mol}$  carotenoid/ $10^{12}$  cells.

**HPLC analysis.** An Alliance model 2695 (Waters, Milford, MI, USA) equipped with a model 2996 (Waters) photodiode array detector was used. Chromatographic data were acquired using a Millennium 4.0 workstation (Waters). Carotenoids and  $\alpha$ -tocopherol were separated on a 5  $\mu\text{m}$  Vydac 201TP54 C<sub>18</sub> (250  $\times$  4.6 mm, i.d.; Vidac, Esperia, CA, USA) at a flow-rate of 1.2 ml/min. The elution was carried out by linear gradient using methanol (A) and 2-propanol (B) as eluents.

Time	0	25	30	35
%B	0	20	20	0

The column and samples were thermostated at  $25 \pm 2^{\circ}\text{C}$  and  $6 \pm 1^{\circ}\text{C}$ , respectively, and 70  $\mu\text{l}$  was injected into the HPLC system. The chromatograms were acquired in the range 200–600 nm with 1.2 nm of spectral resolution. Analyses were integrated at 445 nm (lutein, zeaxanthin,  $\beta$ -cryptoxanthin, echinenone and  $\alpha$ - and  $\beta$ -carotene), 472 nm (lycopene), 286 nm (phytoene) and 366 nm (phytofluene).

Carotenoid concentrations were calculated by means of a mix of standards containing lutein, zeaxanthin,  $\beta$ -cryptoxanthin (Hoffman-La Roche, Basel, Switzerland),  $\alpha$ -carotene and  $\beta$ -carotene (Sigma Chemicals), whereas lycopene (Sigma Chemicals) was prepared daily (to avoid degradation) and injected separately;  $\alpha$ -tocopherol was calculated by using its specific standard (Sigma Chemicals) and integrated at 286 nm. Phytoene and phytofluene concentrations were estimated by considering lycopene as the reference standard and referring to  $E_{1\text{cm}} 1\%$  for each carotenoid (Davies, 1976). The data obtained were corrected by the recovery of echinenone used as the internal standard. Recovery was between 90 and 100%.

### DNA damage

The resistance of lymphocyte DNA to oxidative stress (500  $\mu\text{mol/l}$   $\text{H}_2\text{O}_2$ , 5 min) was evaluated by means of the comet assay, as previously reported in detail (Riso *et al.* 1999).

DNA damage was observed under an epifluorescence microscope (BX60; Olympus Italia, Milan, Italy) attached to a high-sensitivity CCD video-camera (Variocam; PCO Computer Optics, Kelheim, Germany) and via a computer provided with an image analysis system. For each slide, 100 cells were electronically captured and analysed for fluorescence intensity. Damaged DNA is recognized as a fluorescent core followed by a tail, caused by the electrophoretic migration of fragments away from the core. DNA damage was calculated as the percentage of DNA in the tail. For each subject, the percentage of DNA in the tail of control cells (i.e. cells that were not treated with  $\text{H}_2\text{O}_2$ ) was subtracted from the percentage of DNA in the tail of treated cells.

### Statistical analysis

Statistical analysis was performed using STATISTICA as the software (Statsoft Inc, Tulsa, OK, USA). A repeated-measures ANOVA with the sequence of treatments (placebo drink followed by Lyc-o-Mato<sup>®</sup> or vice versa) as the independent factor was used to investigate the effect of Lyc-o-Mato<sup>®</sup> intake on the variables under study (carotenoid variation in plasma and lymphocytes, and DNA resistance to oxidative stress). In the absence of a carry-over effect, all the data from the two groups of subjects were pooled and analysed together using ANOVA for repeated measure design with the treatment (Lyc-o-Mato<sup>®</sup> or placebo) as the factor. Differences between means were further evaluated by the least significant difference test. Differences were considered significant at  $P < 0.05$ . The analysis of simple regression was used to evaluate the correlation between variables.

### Results

Two subjects were omitted from the analysis as they did not exhibit good compliance with the protocol. In particular, one subject took the drink for 20 d instead of 26 d, whereas the other made considerable changes to his diet for about 1 week because of travelling. Hence, the results shown refer to twenty-four subjects. Supplementation with Lyc-o-Mato<sup>®</sup> was well tolerated, and no subject reported untoward side-effects. As no carry-over effect was observed, all the variables from groups 1 and 2 were matched and reported together.

### Plasma carotenoid and $\alpha$ -tocopherol concentrations

Plasma carotenoid and  $\alpha$ -tocopherol concentrations before and after each intervention period are reported in Table 1. After Lyc-o-Mato<sup>®</sup> consumption, lycopene, phytoene, phytofluene and  $\beta$ -carotene concentrations increased significantly. In particular, that of lycopene increased by about 0.21  $\mu\text{mol/l}$  (95% CI 0.16, 0.26;  $P < 0.0001$ ); phytoene concentration increased by about 0.12  $\mu\text{mol/l}$  (95% CI 0.08, 0.15;  $P < 0.0005$ ), phytofluene concentration by about 0.14  $\mu\text{mol/l}$  (95% CI 0.09, 0.19;  $P < 0.0001$ ) and  $\beta$ -carotene concentration by about 0.15  $\mu\text{mol/l}$  (95% CI 0.04, 0.25;  $P < 0.05$ ). Conversely, the consumption of Lyc-o-Mato<sup>®</sup> did not affect the plasma concentrations of lutein, zeaxanthin,  $\beta$ -cryptoxanthin and  $\alpha$ -carotene (which were not present in the drink).

All together, lycopene, phytoene, phytofluene and  $\beta$ -carotene concentrations increased by about 68, 92, 61 and 28%, respectively, after 26 d of Lyc-o-Mato<sup>®</sup> drinking but not after placebo treatment. Plasma concentrations of  $\alpha$ -tocopherol did not change throughout the intervention, possibly due to the relatively low amount of this vitamin present in the Lyc-o-Mato<sup>®</sup> drink.

### Lymphocyte carotenoid and $\alpha$ -tocopherol concentrations

Lymphocyte carotenoid and  $\alpha$ -tocopherol concentrations registered during the study are reported in Table 2.

Lycopene concentration increased by about 1.36  $\mu\text{mol}/10^{12}$  cells (95% CI 0.78, 1.93;  $P < 0.001$ ); phytoene increased by about 0.54  $\mu\text{mol}/10^{12}$  cells (95% CI 0.29, 0.79;  $P < 0.005$ ), phytofluene by about 1.12  $\mu\text{mol}/10^{12}$  cells (95% CI 0.54, 1.71;  $P < 0.005$ ) and  $\beta$ -carotene by about 0.73  $\mu\text{mol}/10^{12}$  cells (95% CI 0.44, 1.02;  $P < 0.01$ ). Concentrations of the other carotenoids were not significantly affected by the intake of Lyc-o-Mato<sup>®</sup>.

All together, lycopene, phytoene, phytofluene and  $\beta$ -carotene concentrations increased by about 105, 159, 84 and 51%, respectively, after the consumption of Lyc-o-Mato<sup>®</sup> for 26 d. In contrast, the placebo treatment did not modify lymphocyte carotenoid concentrations.  $\alpha$ -Tocopherol concentrations remained constant throughout the entire experiment.

### DNA damage

Figure 2 shows lymphocyte DNA damage (as percentage of DNA in the tail), registered before and after each experimental period and evaluated after the oxidative treatment of the cells.

After 26 d of Lyc-o-Mato<sup>®</sup> intake, lymphocyte DNA damage decreased significantly. The differences between the percentages

**Table 1.** Carotenoid and  $\alpha$ -tocopherol plasma concentrations ( $\mu\text{mol/l}$ ) before and after each intervention period

	Before placebo		After placebo		Before Lyc-o-Mato <sup>®</sup>		After Lyc-o-Mato <sup>®</sup>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lycopene	0.34	0.12	0.32	0.12	0.31	0.17	0.52 <sup>a</sup>	0.17
Phytofluene	0.23	0.10	0.22	0.10	0.23	0.12	0.37 <sup>a</sup>	0.17
Phytoene	0.13	0.08	0.14	0.08	0.12	0.09	0.23 <sup>a</sup>	0.16
$\beta$ -Carotene	0.60	0.42 <sup>b</sup>	0.56	0.39	0.54	0.35	0.69 <sup>b</sup>	0.43
$\alpha$ -Carotene	0.11	0.09	0.12	0.18	0.11	0.11	0.12	0.11
Lutein	0.53	0.26	0.50	0.23	0.50	0.28	0.49	0.23
Zeaxanthin	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04
$\beta$ -Cryptoxanthin	0.30	0.20	0.32	0.20	0.32	0.29	0.29	0.20
$\alpha$ -Tocopherol	48.6	23.7	47.8	24.2	47.3	20.7	48.3	22.5

<sup>a</sup> Significantly different from each other point of the same group,  $P < 0.0001$ .

<sup>b</sup> Significantly different from other points,  $P < 0.05$ .

**Table 2.** Carotenoid and  $\alpha$ -tocopherol lymphocyte concentrations ( $\mu\text{mol}/10^{12}$  cells) before and after each intervention period

	Before placebo		After placebo		Before Lyc-o-Mato <sup>®</sup>		After Lyc-o-Mato <sup>®</sup>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lycopene	1.20	0.60	1.33	0.56	1.30	0.47	2.66*	1.43
Phytofluene	1.20	0.60	1.22	0.63	1.34	0.80	2.47*	1.60
Phytoene	0.32	0.22	0.40	0.24	0.34	0.22	0.88*	0.71
$\beta$ -Carotene	1.28	1.05	1.31	0.71	1.43	0.87	2.16†	1.35
$\alpha$ -Carotene	0.23	0.31	0.27	0.29	0.21	0.26	0.29	0.26
Lutein	1.14	0.65	1.25	0.69	1.24	0.56	1.40	0.63
Zeaxanthin	0.19	0.12	0.24	0.15	0.22	0.13	0.23	0.13
$\beta$ -Cryptoxanthin	0.52	0.28	0.59	0.30	0.60	0.25	0.62	0.29
$\alpha$ -Tocopherol	48.8	23.3	48.9	24.0	52.2	15.4	51.1	20.1

\* Significantly different from each other point of the same group,  $P < 0.0001$ .

† Significantly different from each other point of the same group,  $P < 0.0005$ .

of DNA in the tail before and after each treatment were significantly greater ( $P < 0.0001$ ) after Lyc-o-Mato<sup>®</sup> (mean  $-24.1$ , 95% CI  $-29.2$ ,  $-18.9$ ) than after placebo (mean  $-3.1$ , 95% CI  $-7.7$ ,  $1.6$ ). During the intervention period, the percentage of DNA in the tail decreased by about 42% ( $P < 0.0001$ ), as calculated by considering the variation between the percentage of DNA in the tail registered before and after Lyc-o-Mato<sup>®</sup> intake with regard to the value recorded before Lyc-o-Mato<sup>®</sup>.

#### Regression analysis

The regression analysis showed a significant ( $P < 0.05$ ) inverse correlation between plasma lycopene ( $r = 0.31$ ,  $P < 0.005$ ), phytoene ( $r = 0.29$ ,  $P < 0.005$ ) and phytofluene ( $r = 0.21$ ,  $P < 0.05$ ), and DNA damage. The values of  $R^2$  were, however, extremely low. The same results were obtained by analysing lymphocyte carotenoid concentrations and DNA damage (lycopene:  $r = 0.34$ ,  $P < 0.005$ ; phytoene:  $r = 0.30$ ,  $P < 0.005$ ; phytofluene  $r = 0.30$ ,  $P < 0.01$ ). A positive correlation was observed between lycopene, phytoene and phytofluene in the plasma (lycopene/phytoene:  $r = 0.67$ ,  $P < 0.0001$ ; lycopene/phytofluene:  $r = 0.67$ ,  $P < 0.0001$ ; phytoene/phytofluene:  $r = 0.60$ ,  $P < 0.0001$ ) and in the lymphocytes (lycopene/phytoene:  $r = 0.66$ ,  $P < 0.0001$ ; lycopene/phytofluene:  $r = 0.65$ ,  $P < 0.0001$ ; phytoene/phytofluene:  $r = 0.86$ ,  $P < 0.0001$ ). With regard to  $\beta$ -carotene, we observed a positive correlation with

lycopene ( $r = 0.40$ ,  $P < 0.0005$ ), phytoene ( $r = 0.46$ ,  $P < 0.0001$ ) and phytofluene ( $r = 0.39$ ,  $P < 0.0005$ ) in the lymphocytes.

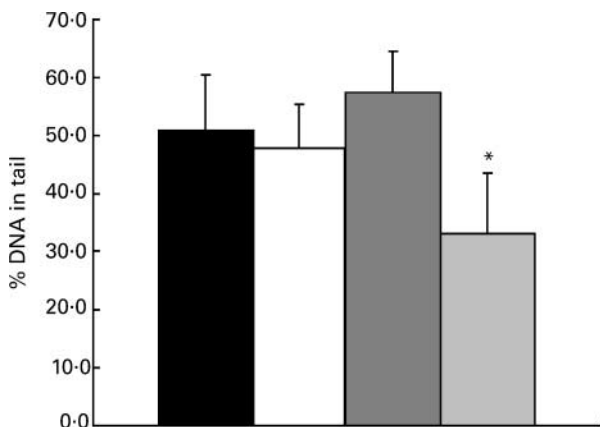
#### Discussion

Several studies suggest that tomatoes and their products might exert beneficial effects on human health due to their carotenoid content, which has the potential to increase the antioxidant defences of the human body. Although lycopene and  $\beta$ -carotene are the most widely studied components of tomato, phytoene and phytofluene could also play a relevant role. In the present study, we investigated the effect of the regular intake of a soft drink, Lyc-o-mato<sup>®</sup>, containing a tomato extract that provides lycopene, phytoene, phytofluene and  $\beta$ -carotene in amounts easily attainable from a diet rich in fruit and vegetables.

The consumption of Lyc-o-Mato<sup>®</sup> for 26 d significantly increased all the carotenoids in both plasma and lymphocytes. It is noteworthy that no oil – which facilitates absorption (Stahl & Sies, 1992) – was added to the soft drink, as the extraction technique adopted for the formulation of Lyc-o-Mato<sup>®</sup> provides carotenoids in a natural tomato oil medium.

The subjects in the present study, as opposed to those in our previous investigations, were not on a low-carotenoid diet before and during the entire experiment; hence basal carotenoid concentrations were relatively high. It has been shown, for example, that the lower the initial lycopene level, the higher the increase in plasma concentration after its intake. Despite this, the current results on lycopene concentration are comparable to those of our two previous studies (Porrini & Riso, 2000; Riso *et al.* 2004), in which we gave female volunteers 7 or 8 mg lycopene per d through food, and other observations reporting lycopene increases following 5 mg lycopene consumed as a food component or as capsules (Böhm & Bitsch, 1999; Rao & Shen, 2002). The intake of Lyc-o-Mato<sup>®</sup> was also associated with a significant increase in lymphocyte lycopene concentration.  $\beta$ -Carotene concentrations also increased, in both plasma and lymphocytes, after the intake of 1 mg/d provided by the drink, consistent with our previous observations (Porrini & Riso, 2000).

Concerning the other carotenoids, we now report that phytoene and phytofluene, administered through Lyc-o-Mato<sup>®</sup> (about 4 and 3 mg, respectively) increase in plasma and in lymphocytes after their intake. Phytoene and phytofluene are normally present in moderate amounts in raw tomatoes, tomato products and other fruits and vegetables (Müller, 1997; Khachik *et al.* 2002; Edwards



**Fig. 2.** Lymphocyte DNA damage after *ex vivo* treatment with  $\text{H}_2\text{O}_2$  (500  $\mu\text{mol/l}$ ) evaluated by the comet assay (% DNA in tail) ( $n = 24$ , means  $\pm$  s.d.) before and after each intervention period. (■) before placebo; (□) after placebo; (▒) before Lyc-o-mato; (◐) after Lyc-o-mato. \*Significantly different from each other points ( $P < 0.0001$ ).

**Table 3.** Recent intervention studies reporting phytoene and phytofluene plasma concentrations

Authors	Study characteristics	Plasma phytoene ( $\mu\text{mol/l}$ )	Plasma phytofluene ( $\mu\text{mol/l}$ )
Paetau <i>et al.</i> 1998, 1999	Tomato juice 4 weeks 5.8 mg phytoene 5.1 mg phytofluene	0.109–0.299*	0.269–0.854
	Oleoresin 4 weeks 4.4 mg phytoene 4.9 mg phytofluene	0.106–0.166*	0.269–0.384
	Lycopene beadlets 4 weeks 2.5 mg phytoene 3.7 mg phytofluene	0.097–0.19*	0.266–0.464
Müller <i>et al.</i> 1999	Tomato juice 6 weeks 7.3 mg phytoene 2.3 mg phytofluene	0.09–0.46	0.12–0.41
Richelle <i>et al.</i> 2002	Tomato paste 8 weeks 1.98 mg/d phytofluene		0.16–0.49
	Lactolycopene 8 weeks 1.98 mg/d phytofluene		0.18–0.41
Edwards <i>et al.</i> 2003	Tomato juice 3 weeks 2.1 mg phytoene 1.1 mg phytofluene	0.067–0.140	0.052–0.124

\* Data after treatment were extrapolated from articles.

*et al.* 2003), but data concerning their bioavailability or their *in vivo* antioxidant action are scarce.

In Table 3, we have outlined some of the studies that have evaluated plasma phytoene and phytofluene concentrations after the intake of tomato products or lycopene formulations. In agreement with these studies, our data suggest that the absorption of these compounds from the Lyc-o-Mato<sup>®</sup> drink used in the current study is comparable to that recorded after tomato juice intake. Furthermore, it appears that the increase in phytoene and phytofluene in the plasma is dependent on the dose and the duration of intake. In contrast, data on the bioavailability of phytoene and phytofluene to the cells following intervention with foods or supplements are very scarce. One such report is that of Paetau *et al.* (1999), who found an increase in phytofluene in human buccal mucosal cells after a daily intake of about 5 mg phytofluene for 3 weeks. It is noteworthy that we found an increase in the lymphocytes of approximately 159 % for phytoene and approximately 84 % for phytofluene after the intake of Lyc-o-Mato<sup>®</sup>, suggesting that these compounds may interact to increase cell antioxidant protection.

We also analysed  $\alpha$ -tocopherol concentrations in plasma and lymphocytes; however, due to its relatively low amount in Lyc-o-Mato<sup>®</sup>, its baseline concentrations were not affected by the supplementation.

The second aim of the present study was to verify whether the increase in lymphocyte carotenoid concentration was able to improve cellular defences against oxidative stress. We chose lymphocytes as the target cells to evaluate the effects of the carotenoids against DNA oxidative damage. We found that a moderate consumption of Lyc-o-Mato<sup>®</sup> was sufficient to afford an approximately 42 % reduction in DNA damage with regard to baseline levels, suggesting a beneficial effect of the drink on a surrogate marker of mutagenesis.

These results are consistent with our previous observations, in which up to 50 % lymphocyte DNA protection was registered after the daily consumption of tomato puree providing about 7 or 16 mg lycopene (Riso *et al.* 1999; Porrini & Riso, 2000). Recently, Bowen *et al.* (2002) observed a significant decrease of leucocyte 8-hydroxydeoxyguanosine–deoxyguanosine (–21 %) in patients with localized prostate adenocarcinoma who consumed tomato sauce-based pasta dishes for 3 weeks (30 mg lycopene daily) before undergoing radical prostatectomy. In addition, a decrease in 8-hydroxydeoxyguanosine–deoxyguanosine was demonstrated in the prostate tissue. Furthermore, Rao & Agarwal (1998) found that 1 week of supplementation with either lycopene or a tomato product (tomato oleoresin capsules) led to a fall in leucocyte 8-oxo-2-deoxyguanosine level. Conversely, Kucuk *et al.* (2002) found no significant changes in lymphocyte DNA damage (evaluated as 5-Hydroxymethyluridine concentration) in patients with diagnosed prostate cancer who were supplemented with a tomato oleoresin extract, providing 30 mg lycopene and other carotenoids (namely, phytoene and  $\beta$ -carotene), for 3 weeks. A reduction in cancer extension was, however, reported. In addition, Steck *et al.* (1999, 2000) did not find any effect on lymphocyte DNA damage of a supplementation with a vegetable juice that provided 23 mg lycopene and other carotenoids per d. The authors did, however, observe a decrease in DNA damage, as measured by the comet assay, in lung epithelial cells exposed to air or ozone in subjects supplemented with the vegetable juice.

We can formulate some hypotheses concerning the negative results reported in the literature on lymphocyte DNA damage. First, the choice of different markers of DNA damage can affect the interpretation of the results; this can explain the differences between the studies by Bowen *et al.* (2002) and Kucuk *et al.* (2002). Also, the use of patients instead of healthy subjects might represent an important variable. In our study, we used the comet

assay to analyse DNA damage in healthy subjects or, better still, lymphocyte resistance to DNA damage after the *ex vivo* treatment of cells with H<sub>2</sub>O<sub>2</sub>. Steck *et al.* used the same technique but after an *in vivo* treatment with ozone; hence, we hypothesize that their intervention may be ineffective in protecting against this oxidant or that the stress inflicted was too small.

We did not find strong correlations between individual plasma and lymphocyte carotenoid concentrations and DNA damage. This may suggest that the protection from oxidative stress cannot be attributed to one single compound. In addition, in our previous studies with tomato products, we stressed that it is difficult to extrapolate the impact of each antioxidant compound present in foods or formulations in terms of protection from oxidative stress. Further studies with pure compounds are necessary to answer this question. The positive correlation we found between lycopene and phytoene and phytofluene in both plasma and lymphocytes suggests that they do not compete for absorption.

In summary, this study confirms that low intakes of carotenoids from tomatoes increase the antioxidant defence system of the lymphocytes. Moreover, a fruit-aromatized soft drink containing some of the bioavailable and active compounds present in tomato products might constitute an additional or alternative source of bioactive carotenoids for people who do not consume tomato products.

### Acknowledgement

We gratefully acknowledge LycoRed Natural Products Industries Ltd (Beer-Sheva, Israel) for supplying the Lyc-o-Mato<sup>®</sup> drink.

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