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ORIGINAL CONTRIBUTION

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Comparison of lycopene and tomato effects on biomarkers of oxidative stress in vitamin E deficient rats

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Abstract *Background* Cohort studies suggested that individuals with higher intake of tomatoes and tomato products have a lower risk of degenerative diseases. Lycopene, an antioxidant and antiproliferative carotenoid, has been hypothesized to be responsible for the health benefits of tomatoes. However, studies demonstrated a higher potential of tomatoes compared to lycopene to reduce oxidative stress or carcinogenesis. *Aim of the study* Our study aimed at distinguishing lycopene effect from that of tomato on oxidative stress, by using yellow tomato, a tomato variety devoid of lycopene. *Methods* Effects of feeding with none (control), 16% freeze-dried yellow tomato (YT), 16% freeze-dried red tomato (RT) or 0.05% lycopene beadlets (LB) were compared in a rat model with mild oxidative stress induced by low vitamin E diet (LVED). Four groups of 10 rats were fed ad libitum for 6 weeks. Physiological parameters such as ingesta, body, spleen and liver weights, cholesterol and triglycerides (TG) levels were assessed. Lycopene and vitamin E concentrations and oxidative stress biomarkers were measured in the plasma and/or liver and/or heart tissue of the rats. *Results* RT, YT, and LB had no effect on rats' ingesta, body and spleen weights. RT, YT and LB had no effect on plasma cholesterol concentration. RT decreased TG level compared to control, YT and LB ($P < 0.05$). Rats fed RT or LB accumulated lycopene in plasma in contrast with rats fed YT. Heart level of thiobarbituric reactive species (TBARS) in rats fed RT or YT was lower than that in the control and the LB fed rats ($P < 0.05$). Despite similar concentrations of lycopene in plasma and liver, rats fed LB showed a significantly higher heart level of TBARS than rats fed tomatoes. RT increased erythrocyte superoxide dismutase (eSOD) activity compared with LB and nitric oxide (NO) level compared with control and LB. LB decreased ferric reducing ability of plasma (FRAP) level compared with control, RT and LB ($P < 0.05$). *Conclusion* Our study showed for the first time that tomatoes, containing or not containing lycopene, have a higher potential than lycopene to attenuate and or to reverse oxidative stress-related parameters in a mild oxidative stress context.

Key words tomato – lycopene – oxidative stress – vitamin E

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Introduction

Oxidative stress is characterized by an imbalance between antioxidant capacity and reactive oxygen species (ROS) generation. Overproduction of ROS increases during aging and contributes to many pathological events such as cancer and cardiovascular diseases. A lack of nutritional antioxidants, such as vitamin E (VitE), vitamin C (VitC), polyphenols and carotenoids can contribute to oxidative stress generation. In most Western countries, lower incidence of age-related chronic diseases is associated with a higher consumption of fruits and vegetables, which are the main dietary sources of protective micronutrients and phytochemicals [34, 39].

Recent epidemiologic studies focusing on tomato and tomato products associated their intake with a reduced risk of degenerative diseases [25–27]. High intake of tomato juice prevents low density lipoproteins (LDL) oxidation and thiobarbituric reactive species (TBARS) formation in healthy men [12]. In the Health Professionals Follow Up Study, Giovannucci et al. [27] first showed that high intake of tomato-based products was linked to lower prostate cancer risk. Later, the protective role of tomato was confirmed, but the mechanisms remain unclear [22]. Further investigations consolidated the epidemiologic evidence that lycopene could be a causal factor for the preventive effects of tomatoes/tomato products. Blood lycopene levels were inversely correlated with prostate cancer and cardiovascular [17, 28]. Note that, in these studies, lycopene may rather represent a biomarker of exposure of the subjects to tomato and/or tomato product consumption than a biomarker of effects. Lycopene is the most potent singlet oxygen quencher, in vitro, among the natural carotenoids and other micronutrients, such as VitE and VitC [20] and therefore delay or prevent oxidative damage [47]. Transported by low density lipoproteins (LDL), lycopene prevents their oxidation in vivo [4] and increases their degradation in vitro [23]. Lycopene also exerts direct anticarcinogenic effects including upregulation of gap-junction communication [9] and modulation of growth factors such as insulin-like growth factor 1 (IGF-1) or cytokines like interleukine 6 (Il-6) [43].

However, other investigations showed a higher in vivo effect of whole tomato compared to lycopene alone. Lycopene, through tomato sauce consumption, reduces leukocyte and prostate tissue oxidative DNA damage and decreases prostate specific antigen (PSA) level in prostate cancer patients [15]. In animal prostate cancer models, tomato supplementation showed higher effects than lycopene supplementation on carcinogenesis prevention and mortality [10, 30]. Few studies have investigated tomato or lycopene effect on cardiovascular diseases in contrast to prostate cancer. Recent investigations on ischemia-reperfusion model in the rat showed that tomato juice has a higher protective effect than lycopene, even if both reduced the extent of lipid peroxidation [19].

Most of the experiments comparing lycopene, tomato and tomato extracts have been made with the assumption that phytochemicals other than lycopene might also be involved in preventive effects of tomato. Obviously, there is a lack of experimental model that could really distinguish lycopene effect from that of tomato [24]. However, some tomato varieties, such as yellow tomato, do not contain lycopene. Yellow tomato contains lycopene cyclase, an enzyme that converts lycopene into beta-carotene and other xanthophyll, thus producing a fruit without lycopene [6]. The aim of this study was to compare the antioxidant potential of red tomato, yellow tomato and lycopene beadlets in a rat model with mild oxidative stress induced by low dietary vitamin E intake.

Materials and methods

Animals protocols

A total of 40 male Wistar rats from the husbandry of the Institut National de la Recherche Agronomique (Clermont-Ferrand/Theix, France) were maintained and handled according to the recommendations of the local and national ethic committees.

The rats (body weights: 180–200 g) were housed two per cage in a room maintained at 22°C with a 12 h light–dark cycle (light from 8:00 a.m. to 8:00 p.m.) and free access to food from 4:00 p.m. to 8:00 a.m.

Experimental design

Rats were randomized into four groups (10 rats per group) and fed ad libitum for 6 weeks with one of the four diets (Table 1): (1) low VitE diet (LVED) (AIN 93

Table 1 Diet composition (%)

	LVED supplemented with			
	Control	RT	YT	LB
Casein	15	15	15	15
Wheat starch	70.5	54.5	54.5	70.45
Groundnut oil	5	5	5	5
Mineral mix	3.5	3.5	3.5	3.5
Full vitamin mix	0.3	0.3	0.3	0.3
Vitamin mix without vitamin E	0.6	0.6	0.6	0.6
Cellulose	5	5	5	5
Lyophilized tomato	0	16	16	0
Lycopene beadlets	0	0	0	0.05

Freeze-dried red tomato (RT) and lycopene beadlets (LB) enriched diets contain 50 mg of lycopene per kg of diet

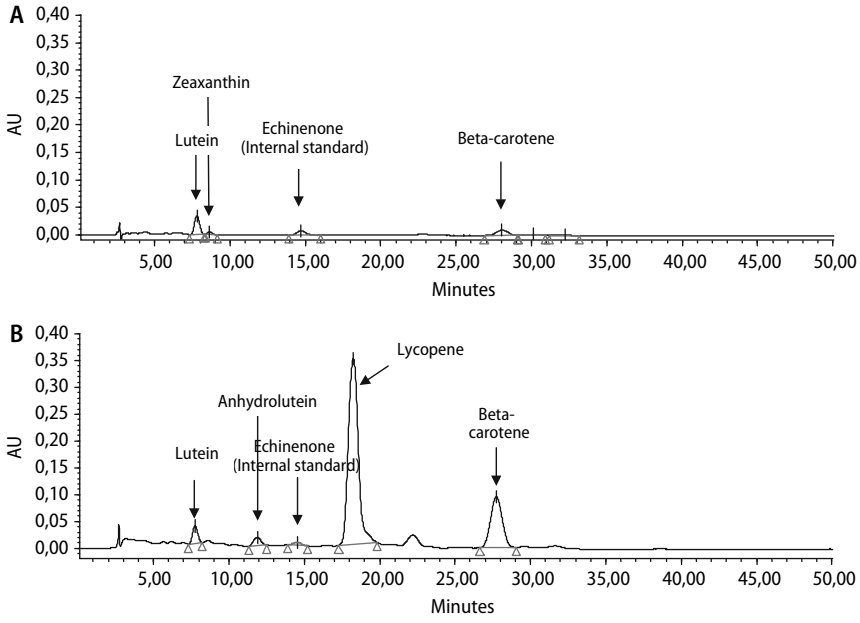

Fig. 1 HPLC profile at 470 nm obtained after carotenoids extraction of YT (**A**) and RT (**B**)

diet with 1/3 of vitamin E) control group, (2) LVED supplemented with freeze-dried red tomato (RT) to have a final concentration at 50 mg of lycopene per kg of diet, (3) LVED diet supplemented with freeze-dried yellow tomato (YT) (the same quantity as RT) and (4) LVED supplemented with lycopene beadlets (LB) to have a final concentration at 50 mg of lycopene per kg of diet. As shown in Fig. 1 and Table 2, YT used for the supplementation did not contain lycopene. Daily food consumption and body weight were recorded twice a week.

Vitamin Mix (AIN 93 VX) and Mineral Mix (AIN 93 MX) were purchased from MP Biomedicals. Vitamin Mix depleted in VitE were purchased from the Unité de Préparation des Aliments Expérimentaux (UPAE) (INRA, Jouy-en-Josas, France). Wheat starch and casein were from Louis François (France). Alphacel Non-Nutritiv Bulk was provided by GLS Strasbourg (Illkirch, France). Groundnut oil was purchased from Huileries de Lapalisse (France). One hundred and fifty kilogram of Red (Red Vine cultivar) and 150 kg of yellow (Yellow King cultivar) tomatoes were obtained from Rungis Market (Paris, France) and then freeze-dried by Greentech Society (Biopôle Clermont/Limagne), yielding 9.1 kg of RT and 10.7 kg of YT kept safe from air in a 4°C dark room. LB were a hydrosoluble powder (BASF, Germany) containing 11.3 mg lycopene per 100 g of powder.

Table 2 Carotenoids content of RT and YT used for the supplementation

Carotenoids content of freeze-dried tomatoes		
Carotenoids	RT	YT
Beta-carotene	31.8 ± 11.2	1.7 ± 0.1
Lycopene	312.6 ± 12.7	0
Lutein	6.82 ± 8.2	4.6 ± 0.1
Zaexanthin	0	1.9 ± 0.2

Sample preparation

At the end of the experiment, rats were anesthetized by i.p. injection of sodium pento-barbital (40 mg/kg of body weight). Blood was drawn from the abdominal aorta in dry- or heparin-containing tubes. Plasma was obtained by centrifugation (12,000 g, 4 min, 4°C) and was immediately stored at −80°C for future analyses.

After blood sampling, heart, liver, and spleen were removed and weighed. The heart and liver were rapidly washed in cold saline (9 g/l NaCl), placed into liquid nitrogen and stored at −80°C until use.

Analytical determination

Plasma and liver carotenoids and VitE were extracted twice with ethanol and hexane. The extract was dissolved in 200 µl methanol/dichloromethane (65/35, v/v). and injected into HPLC column [32].

Carotenoids from freeze-dried tomatoes and from diets were extracted first with methanol, trichloromethane and deionized water. After a 10 min-centrifugation at 400 g, the bottom layer (organic phase) was removed, put in a glass tube and then evaporated to dryness under nitrogen. A second extraction was made with tetrahydrofuran, dichloromethane and deionized water. After a 10 min-centrifugation at 400*g*, the second bottom layer was pooled to the first and evaporated to

dryness under nitrogen. The extracts were dissolved in 200 µl acetonitrile/dichloromethane (50/50, v/v) and injected into an HPLC apparatus.

Carotenoids and VitE were separated using two columns in a series (a Nucleosil C18, 150 × 4.6 mm, 3 µm followed by a Vydac C18, 250 × 4.6 mm) purchased from Interchim (Montluçon, France).

Carotenoids and VitE were quantified by reverse-phase HPLC on a Waters system composed of a pump, a cooled auto-sampler, a UV–Visible diode array detector system (Waters SA, Saint-Quentin en Yvelines, France). The mobile phase was an acetonitrile:dichloromethane:methanol (containing 50 mM ammonium acetate):water (70/10/15/5, v/v/v/v) mixture. The flow rate was isocratic (2 ml/min). Carotenoids and VitE were detected at 450 and 292 nm, respectively, and identified by comparison of their retention time and spectral analysis with those of pure mixture of lutein, zeaxanthin, lycopene, alpha- and beta-carotene, and alpha-tocopherol. An overall recovery yield of 75–100% was calculated with the internal standards (SI): echinenone (Hoffmann-La Roche, Basel, Switzerland) and tocopheryl acetate (Roche Vitamines France, Neuilly-sur Seine, France). Quantification was performed using Waters Millenium 32 Software (version 3.05.01). All solvents used were of HPLC grade from Carlo Erba (Chaussée à Vexin, France).

Plasma total cholesterol and triglyceride (TG) concentrations were enzymatically determined using a kit from BioMerieux (Charbonnières-les-bains, France) and Biotrol (Paris, France), respectively. The concentrations were determined spectrophotometrically (Hitachi U 2001, Japan) at 505 nm and 500 nm for triacylglycerols and cholesterol, respectively.

Ferric reducing ability of plasma (FRAP) was determined in 100 µl plasma sample diluted twice with distilled water. The tripyridyltriazine complex formed with the reduced ions was measured by a spectrophotometer at a wavelength of 593 nm (Uvikon 941 plus series, Kontron instruments, St. Quentin en Yvelines, France). Results were calculated from a standard scale of $FeSO_4$.

The susceptibility of heart to peroxidation was determined in tissue homogenates after lipid peroxidation induced with $FeSO_4$ (2 µmol/l)-ascorbate (50 µmol/l) for 30 min in a water bath at 37°C, using a standard of 1,1,3,3-tetrahethoxypropane [44].

Nitrite + nitrate (NO*x*), degradation products of nitrite oxide (NO), were measured in deproteinized plasma using the Griess reaction [36]. After protein precipitation of the sample and reduction of nitrate to nitrite with nitrate reductase, nitrite was quantified colorimetrically at 450 nm using a multiplate reader spectrophotometer (MR 700, Dynatech Laboratories, Guernesey, UK).

Erythrocyte superoxide dismutase (eSOD) activity was determined using a Ransod Kit (Randox Laboratories, Crumlin, U.K.). Briefly, the method uses xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a formazan dye. The eSOD activity was measured by the degree of inhibition of the reaction, using a spectrophotometer. The results were expressed as unit per mg of hemoglobin (Hb).

Total glutathione (tGSH) concentration was measured in liver. About 0.5 g of frozen tissue was homogenized in 0.2 M perchloric acid, 5 mM EDTA. After centrifugation (14,000*g*, 15 min), tGSH content was measured in the supernatant as described by Malmezat et al. [33].

Statistical analyses

Values are given as means ± SEM, and the differences between groups were determined by one way ANOVA followed by Student-Newman–Keuls (SNK) test. Values of $P < 0.05$ were considered significant.

Results

Pro-oxidant effects of LVED were assessed by comparison with a group fed with standard rat chow. LVED had no effect on food intake and rats' body, spleen and liver weights (data not shown). It reduced plasma and liver VitE concentrations by 3.8 and 1.8 times respectively ($P < 0.005$ for hepatic VitE concentration) while it increased 2.8 and 1.5 times heart tissue susceptibility to peroxidation and hepatic tGSH level respectively ($P < 0.005$ and $P < 0.05$ respectively) (data not illustrated).

Effects of tomatoes and lycopene

On lipemia, VitE status and lycopene status

As shown in Table 3, feeding LVED-rats with RT, YT and LB had no effect on rats' body, liver and spleen weights. RT feeding decreased plasma TG level compared to control ($P < 0.05$). Note that TG level in YT fed rats has a tendency to be lower than in control. There was no significant difference between groups for VitE concentration (Table 4). Lycopene was only present in the plasma and liver of RT and LB fed rats (Table 4).

On biomarkers of oxidative stress

As shown in Table 5, cardiac TBARS level was decreased in RT and YT fed groups as compared with

Table 3 Effects of tomato and lycopene supplementation on rat ingesta, body, spleen and liver weights, and on lipemia

	LVED supplemented with			
	Control	RT	YT	LB
Ingesta (g/day)	19.2 ± 0.5	22.4 ± 0.3	22.7 ± 0.3	21.7 ± 0.4
Rat body weight after 6 weeks of experimentation (g)	402.1 ± 12.2	423.4 ± 9.7	417.1 ± 6.7	426.3 ± 8.8
Liver weight (g)	14.1 ± 0.5	13.9 ± 0.6	14.7 ± 0.5	14.3 ± 0.7
Spleen weight (g)	0.95 ± 0.04	0.95 ± 0.04	0.88 ± 0.03	0.98 ± 0.03
Plasma Triglycerides (mM)	3.4 ± 0.4^{b}	2.2 ± 0.2^{a}	2.3 ± 0.3^{b}	2.7 ± 0.4^{b}
Plasma Cholesterol (mM)	0.74 ± 0.03	0.68 ± 0.04	0.72 ± 0.05	0.67 ± 0.03

Results are expressed as means ± SEM for $n = 10$ animals per group. Means with different superscript letter (a,b) are significantly different ($P < 0.05$)

Table 4 Effects of tomato and lycopene supplementation on plasma and hepatic vitamin E and lycopene status

	LVED supplemented with			
	Control	RT	YT	LB
Plasma Vit E/TG[a] (μmol/mol TG)	3.2	5.6	5.8	4.3
Hepatic VitE (nmol/g wet tissue)	48.6 ± 0.9	54.9 ± 8.8	51.6 ± 5.4	50.1 ± 5.2
Plasma lycopene[a] (nM)	0	25.1	0	32.1
Plasma lycopene/TG[a] (nmol/mmol TG)	0	12.4	0	14.5
Hepatic lycopene (nmol/g wet tissue)	0	9.8 ± 1.8	0	7.4 ± 1.6

Results are expressed as means ± SEM for $n = 10$ animals per group
[a]Rats plasma ($n = 6$) samples were pooled for HPLC carotenoid and VitE analysis

control and LB fed groups ($P < 0.05$). FRAP value was lower in LB fed group as compared to the other groups (Table 5) ($P < 0.05$). FRAP values tended to increase in RT and YT fed rats compared to controls, but the difference was not significant. As also shown in Table 5, NO*x* status was higher in plasma of RT fed group than in plasma of LB fed and control group ($P < 0.05$), while YT fed rats exhibited an intermediary NO*x* plasma level. eSOD activity was significantly higher in the RT fed group than in the LB fed one (Table 5) ($P < 0.05$). YT feeding did not affect eSOD activity as compared with all the other conditions Finally, no significant differences were observed between the four groups regarding hepatic tGSH level.

Discussion

Demonstrating antioxidant effects of fruits and vegetables is difficult in a healthy context [18]. Studies using LVED as a mean of inducing oxidative stress have been repeatedly carried out in our laboratory [38] and in others [35, 48]. Cardiac TBARS and hepatic tGSH were the oxidative stress biomarkers mainly affected by LVED in the present work. In this context we demonstrated that the antioxidant potency of tomato was different from that of lycopene alone using yellow and red tomatoes comparatively.

Our main result is that feeding with RT or lycopene-free YT significantly decreased susceptibility of heart tissue to lipid peroxidation in rats compared with control and LB feedings. This result suggests a higher potential of tomato than lycopene alone to protect against oxidative stress. Even if lycopene is the main carotenoid of red tomatoes, the latter also contain other carotenoids and phytochemicals, such as vitamins, trace elements and polyphenols [13, 14].

The hypothesis that phytochemicals and micronutrients of tomato, other than lycopene, can act

Table 5 Effects of tomato and lycopene supplementation on oxidative stress parameters

	LVED supplemented with			
	Control	RT	YT	LB
Cardiac TBARS (nmol/g wet tissue)	168.4 ± 13.1^{b}	105.9 ± 6.7^{a}	105.3 ± 5.8^{a}	158 ± 15.8^{b}
Hepatic tGSH (μmol/g wet tissue)	3.7 ± 0.7	4.1 ± 0.7	3.6 ± 1	3.5 ± 0.9
eSOD activity (U/mg Hb)	2.9 ± 0.5^{ab}	3.3 ± 0.2^{a}	2.4 ± 0.2^{ab}	2.1 ± 0.2^{b}
FRAP (μM Fe^{2+})	263.3 ± 20.1^{a}	285.2 ± 25.6^{a}	280.1 ± 17.6^{a}	236.7 ± 22.3^{b}
Plasma NO*x* (μM)	15.3 ± 0.4^{b}	17.4 ± 0.6^{a}	16.3 ± 0.4^{ab}	15.8 ± 0.3^{b}

Results are expressed as means ± SEM for $n = 10$ animals per group
Means with different superscript letter (a,b) are significantly different ($P < 0.05$)

synergistically is strengthened by our results on the plasma antioxidant capacity in the different groups of rats. As assessed by FRAP analysis, plasma antioxidant capacity had a tendency to increase in rats fed RT and YT compared to the control group. It can be considered that hydrophilic antioxidant fraction was increased in the plasma of tomato fed rats [12, 45]. Other studies showed that polyphenols could counteract the lipid peroxidation induced by LVED [1, 40]. In particular flavonoids, such as naringenin, also present in tomatoes, could take over the role of VitE in biological membranes. FRAP level was significantly decreased in LB fed rats as compared with RT fed, YT fed or control rats. In this study, LB alone was not efficient to exhibit antioxidant effects.

RT feeding, unlike LB feeding, showed beneficial effects as concern the other oxidative stress biomarkers assessed, e.g. eSOD [41]. Superoxide dismutase activity has been shown to decrease when oxidative stress occurred [21]. Lipid peroxidation demonstrated in the erythrocytes can also alter antioxidant enzymatic system such as eSOD activity. As RT feeding reduced cardiac lipid peroxidation because of its antioxidant capacity, this effect could be extended to erythrocyte, thus preventing loss of eSOD activity. However, compared with the YT group, into which lipid peroxidation is similarly decreased but without significant effect on eSOD, it can be suggested a specific effect of RT on eSOD activity, different from that of YT or LB. Furthermore, RT has previously been shown to increase detoxifying enzyme activity in animals submitted to an oxidative stress [34]. Free radicals quenching is the mechanism by which red tomatoes protect eSOD [11], such an effect seems more efficient when lycopene acts synergistically with the other antioxidants of red tomatoes.

RT decreased triglyceride level unlike LB. In YT fed rats TG level was very close to that of RT fed rats. Other studies assessing tomato supplementation effects on lipemia and lipid peroxidation have shown reduced TG level after the supplementation [2, 42]. This effect has been attributed to the phenolics brought by tomatoes. The RT and YT effects could also be due to the fruit matrix and the presence of specific fibres in tomatoes, even in weak quantities, such as pectin [3]. The absence of such phenolics and specific fibres in LB fed rats may explain that lycopene as a single molecule cannot be efficient on this parameter.

NOx level in RT and YT, even if it was not significant for YT, could be linked with the prevention of lipidic peroxidation [7], as evidenced by cardiac TBARS level. Involvement of NO in peroxynitrite formation could have been lower in RT and YT compared with control and LB group.

The synergy of all the phytochemicals leads to the protective effect of tomato. Both lipophilic and hydrophilic compounds could be important, each type of these molecules being able to act at each stage of the complex oxidative stress mechanism. Further analysis aiming at comparing total antioxidant effects of yellow and red tomato extracts in vitro, as assessed by oxygen radical absorbance capacity assay (ORAC) previously shown to be closely related to phenolic compounds, could be of interest [29]. Metabolism of tomato compounds can lead to many bioactive molecules. Concerning lycopene, a range of metabolites and oxidative products called lycopenoids have been identified in vivo and vitro [31]. The antioxidant and antiproliferative action of these molecules have been demonstrated in vitro [5, 8, 37]. Metabolites and oxidative products of lycopene could act in synergy with other phytochemicals of tomato. This could provide an explanation why YT feeding had an intermediary effect on eSOD and NO compared with RT or LB feeding. Attempts to identify some of these compounds constitute an important challenge.

Two major concerns of our experimental design have to be considered. The first one is the choice of a murine model for carotenoid absorption. Rats are usually considered as poor absorbers of carotenoids even if recent studies used this model to investigate the effect of tomato and/or lycopene [30, 34, 43]. In the present study, lycopene was found to be readily available from a diet supplemented with RT or LB since it was detected both in plasma and liver. Moreover, lycopene concentrations we measured in these compartments have been shown to exhibit antioxidant effects in other studies [34]. In addition, as tomatoes were entirely freeze-dried, VitE originating from the seeds could have also been present in the freeze-dried tomato at a level higher than that in control and LB supplemented diets and sufficient to contribute to increased antioxidant status. But liver and plasma VitE concentrations in all groups were statistically similar.

It would be interesting to confirm these results in a human study. Feeding with 16% of freeze-dried tomato would correspond to a supplementation with 380 g of tomato puree or 330 of tomato paste per day, which is near from quantities found in clinical tries using tomato products [16, 46].

In conclusion, tomato feeding alleviates an experimentally induced oxidative stress more than lycopene alone. Effects of yellow and red tomatoes also strengthen the hypothesis that compounds other than lycopene could play a causative role in the health effects of tomato evidenced by epidemiologic studies. Yellow tomato may be a useful tool for future investigations on other parameters involved in oxidative stress related diseases.

References

1. van Acker FA, Schouten O, Haenen GR, van der Vijgh WJ, Bast A (2000) Flavonoids can replace alpha-tocopherol as an antioxidant. FEBS Lett 473:145–148
2. Ahuja KD, Pittaway JK, Ball MJ (2006) Effects of olive oil and tomato lycopene combination on serum lycopene, lipid profile, and lipid oxidation. Nutrition 22:259–265
3. Aprikian O, Duclos V, Guyot S, Besson C, Manach C, Bernalier A, Morand C, Remesy C, Demigne C (2003) Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats. J Nutr 133:1860–1865
4. Arab L, Steck S (2000) Lycopene and cardiovascular disease. Am J Clin Nutr 71:1691S–1695S; discussion 1696S–1697S
5. Aust O, Ale-Agha N, Zhang L, Wollersen H, Sies H, Stahl W (2003) Lycopene oxidation product enhances gap junctional communication. Food Chem Toxicol 41:1399–1407
6. Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, visual attraction, and human health. Plant Cell 7:1027–1038
7. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87:1620–1624
8. Ben-Dor A, Steiner M, Gheber L, Danilenko M, Dubi N, Linnewiel K, Zick A, Sharoni Y, Levy J (2005) Carotenoids activate the antioxidant response element transcription system. Mol Cancer Ther 4:177–186
9. Bhuvaneswari V, Nagini S (2005) Lycopene: a review of its potential as an anticancer agent. Curr Med Chem Anticancer Agents 5:627–635
10. Boileau TW, Liao Z, Kim S, Lemeshow S, Erdman JW Jr, Clinton SK (2003) Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets. J Natl Cancer Inst 95:1578–1586
11. Bose KS, Agrawal BK (2007) Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease. Singapore Med J 48:415–420
12. Bub A, Watzl B, Abrahamse L, Delincee H, Adam S, Wever J, Muller H, Rechkemmer G (2000) Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in men. J Nutr 130:2200–2206
13. Bugianesi R, Salucci M, Leonardi C, Ferracane R, Catasta G, Azzini E, Maiani G (2004) Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and beta-carotene in cherry tomatoes. Eur J Nutr 43:360–366
14. Campbell JK, Canene-Adams K, Lindshield BL, Boileau TW, Clinton SK, Erdman JW Jr (2004) Tomato phytochemicals and prostate cancer risk. J Nutr 134:3486S–3492S
15. Chen L, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, van Breemen R, Ashton D, Bowen PE (2001) Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 93:1872–1879
16. Chopra M, O'Neill ME, Keogh N, Wortley G, Southon S, Thurnham DI (2000) Influence of increased fruit and vegetable intake on plasma and lipoprotein carotenoids and LDL oxidation in smokers and nonsmokers. Clin Chem 46:1818–1829
17. Clinton SK (1998) Lycopene: chemistry, biology, and implications for human health and disease. Nutr Rev 56:35–51
18. Collins JK, Arjmandi BH, Claypool PL, Perkins-Veazie P, Baker RA, Clevidence BA (2004) Lycopene from two food sources does not affect antioxidant or cholesterol status of middle-aged adults. Nutr J 3:15
19. Das S, Otani H, Maulik N, Das DK (2005) Lycopene, tomatoes, and coronary heart disease. Free Radic Res 39:449–455
20. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
21. Emre MH, Aktay G, Polat A, Vardt N (2007) Effects of benzo(a)pyrene and ethanol on oxidative stress of brain, lung tissues and lung morphology in rats. Chin J Physiol 50:143–148
22. Etminan M, Takkouche B, Caamano-Isorna F (2004) The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol Biomarkers Prev 13:340–345
23. Fuhrman B, Elis A, Aviram M (1997) Hypocholesterolemic effect of lycopene and beta-carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. Biochem Biophys Res Commun 233:658–662
24. Gann PH, Khachik F (2003) Tomatoes or lycopene versus prostate cancer: is evolution anti-reductionist? J Natl Cancer Inst 95:1563–1565
25. Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 91:317–331
26. Giovannucci E (2002) A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. Exp Biol Med (Maywood) 227:852–859
27. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87:1767–1776
28. Giovannucci E, Clinton SK (1998) Tomatoes, lycopene, and prostate cancer. Proc Soc Exp Biol Med 218:129–139
29. Huang Z, Wang B, Eaves DH, Shikany JM, Pace RD (2007) Total phenolics and antioxidant capacity of indigenous vegetables in the southeast United States: Alabama Collaboration for Cardiovascular Equality Project. Int J Food Sci Nutr 18:1–9
30. Imaida K, Tamano S, Kato K, Ikeda Y, Asamoto M, Takahashi S, Nir Z, Murakoshi M, Nishino H, Shirai T (2001) Lack of chemopreventive effects of lycopene and curcumin on experimental rat prostate carcinogenesis. Carcinogenesis 22:467–472
31. Lindshield BL, Canene-Adams K, Erdman JW Jr (2006) Lycopenoids: are lycopene metabolites bioactive? Arch Biochem Biophys 458(2):136–40
32. Lyan B, Azais-Braesco V, Cardinault N, Tyssandier V, Borel P, Alexandre-Gouabau MC, Grolier P (2001) Simple method for clinical determination of 13 carotenoids in human plasma using an isocratic high-performance liquid chromatographic method. J Chromatogr B Biomed Sci Appl 751:297–303
33. Malmezat T, Breuille D, Pouyet C, Mirand PP, Obled C (1998) Metabolism of cysteine is modified during the acute phase of sepsis in rats. J Nutr 128:97–105

34. Moreira EA, Fagundes RL, Filho DW, Neves D, Sell F, Bellisle F, Kupek E (2005) Effects of diet energy level and tomato powder consumption on antioxidant status in rats. Clin Nutr 24:1038–1046
35. Moroni F, Recchioni R, Marcheselli F, Fattoretti P, Bertoni-Freddari C (2004) Effect of dietary restriction on DNA synthesis in vitamin E-deficient rats. Ann N Y Acad Sci 1030:462–467
36. Moshage H, Kok B, Huizenga JR, Jansen PL (1995) Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 41:892–896
37. Nara E, Hayashi H, Kotake M, Miyashita K, Nagao A (2001) Acyclic carotenoids and their oxidation mixtures inhibit the growth of HL-60 human promyelocytic leukemia cells. Nutr Cancer 39:273–283
38. Nicolle C, Cardinault N, Gueux E, Jaffrelo L, Rock E, Mazur A, Amouroux P, Remesy C (2004) Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. Clin Nutr 23:605–614
39. Pajk T, Rezar V, Levart A, Salobir J (2006) Efficiency of apples, strawberries, and tomatoes for reduction of oxidative stress in pigs as a model for humans. Nutrition 22:376–384
40. Ramirez-Tortosa C, Andersen OM, Gardner PT, Morrice PC, Wood SG, Duthie SJ, Collins AR, Duthie GG (2001) Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic Biol Med 31:1033–1037
41. Sarkar S, Yadav P, Bhatnagar D (1997) Cadmium-induced lipid peroxidation and the antioxidant system in rat erythrocytes: the role of antioxidants. J Trace Elem Med Biol 11:8–13
42. Shen YC, Chen SL, Wang CK (2007) Contribution of tomato phenolics to antioxidation and down-regulation of blood lipids. J Agric Food Chem 55:6475–6481
43. Siler U, Herzog A, Spitzer V, Seifert N, Denelavas A, Hunziker PB, Barella L, Hunziker W, Lein M, Goralczyk R, Wertz K (2005) Lycopene effects on rat normal prostate and prostate tumor tissue. J Nutr 135:2050S–2052S
44. Sunderman FW Jr, Marzouk A, Hopfer SM, Zaharia O, Reid MC (1985) Increased lipid peroxidation in tissues of nickel chloride-treated rats. Ann Clin Lab Sci 15:229–236
45. Takeoka GR, Dao L, Flessa S, Gillespie DM, Jewell WT, Huebner B, Bertow D, Ebeler SE (2001) Processing effects on lycopene content and antioxidant activity of tomatoes. J Agric Food Chem 49:3713–3717
46. Watzl B, Bub A, Briviba K, Rechkemmer G (2003) Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. Ann Nutr Metab 47:255–261
47. Willis MS, Wians FH (2003) The role of nutrition in preventing prostate cancer: a review of the proposed mechanism of action of various dietary substances. Clin Chim Acta 330:57–83
48. Winklhofer-Roob BM, Meinitzer A, Maritschnegg M, Roob JM, Khoschsorur G, Ribalta J, Sundl I, Wuga S, Wonisch W, Tiran B, Rock E (2004) Effects of vitamin E depletion/repletion on biomarkers of oxidative stress in healthy aging. Ann N Y Acad Sci 1031:361–364