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ORIGINAL CONTRIBUTION

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Protective activity of tomato products on *in vivo* markers of lipid oxidation

Summary *Background* It has been suggested that regular consumption of tomato products improves antioxidant defenses due to their endogenous antioxidant compounds, notably lycopene. *Aim of the study* We evaluated the effects of tomato consumption on parameters of lipid oxidation in healthy human volunteers. *Methods* Twelve females (enrolled at T-7), after a one-week of carotenoid-poor diet (T0), were instructed to supplement the same diet with different tomato products (raw, sauce, and paste), thereby providing approximately eight mg lycopene/day for three weeks (T21). Blood samples were periodically collected in order to evaluate plasma carotenoid concentrations, plasma antioxidant capacity, and susceptibility of LDL to metal ion-induced oxidation. Furthermore, 8-iso-$PGF_{2\alpha}$, a marker of *in vivo* oxidative stress, was analyzed in the 24-hour urine. *Results* Carotenoid concentrations decreased significantly during the carotenoid-poor diet ($P < 0.05$), while lycopene concentrations increased significantly after tomato consumption ($P < 0.001$). The antioxidant capacity of plasma did not vary during the study. Conversely, LDL oxidizability decreased after tomato consumption, as demonstrated by a shortening of the lag phase ($P < 0.001$). This parameter was significantly correlated with lycopene concentration ($r = 0.36$, $P < 0.05$). The excretion of 8-iso-$PGF_{2\alpha}$ in urine was also significantly lower (–53 %, $P < 0.05$ compared with T0) after tomato supplementation. *Conclusions* These results further support a role for tomato products in the prevention of lipid peroxidation, a risk factor of atherosclerosis and cardiovascular disease.

Key words lipid peroxidation – LDL – isoprostane – tomato – lycopene – humans

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Introduction

Lipid peroxidation, namely low-density lipoprotein (LDL) oxidation, is thought to contribute to the onset and development of degenerative diseases, such as cardiovascular disease and atherosclerosis [1–3], and may be indirectly involved in the promotion of DNA damage and consequently cancer [4, 5]. The study of dietary factors that may limit lipid damage triggered by radicals is crucial for optimization of diets aimed at reducing the risk of degenerative disease through potentiation of the endogenous antioxidant defense system.

Although it is usually difficult to attribute outright health effects to individual dietary components, some, albeit not all [6], epidemiological studies have shown inverse correlations between high lycopene consumption, notably through tomatoes, and the incidence of diseases such as coronary heart disease (CHD) and cancer [7–11]. These results, together with an understanding of the contribution of excessive free radical generation in the onset of these diseases [2], led to the hypothesis that a high proportion of dietary antioxidants – including tomato-derived carotenoids and lycopene – might play a preventive role [11, 12].

Lycopene is an open-chain, unsaturated, red-colored

carotenoid found in tomatoes, guava, watermelon and pink grapefruit [8, 13]. Lycopene accounts for about 50 % of carotenoids in human serum, and it must be entirely derived from the diet. The antioxidant (quenching) properties of lycopene have been demonstrated primarily on singlet oxygen ($^1O_2$), a reactive oxygen species that is at least partially responsible for the initiation of degenerative diseases [14, 15]. Other studies also reported protection against damage induced by NO_2 [16], CCl_3O_2 [17], H_2O_2 [18], and UV radiation [19, 20].

We previously demonstrated that consumption of small amounts of tomato puree, providing approximately seven mg/day lycopene for two weeks, was sufficient to afford lymphocyte DNA protection from H_2O_2 oxidative damage induced *ex vivo* [21]. The increased antioxidant capacity was significantly correlated with the increase in lymphocyte lycopene concentrations; however, the potential contribution of other antioxidants present in tomato products was not excluded. Other investigators have shown reduced lipid peroxidation in human serum after consumption of carotenoid-rich vegetables [22] or supplemented lycopene [23].

To further investigate the protective potential of tomato products against oxidative stress *in vivo*, in the present study we evaluated several parameters of oxidative damage, especially related to lipid peroxidation, in human volunteers before and after a defined period of dietary supplementation.

Materials and methods

Subjects

This investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the local ethic's committee.

Twelve healthy, normolipidemic female volunteers (age 22–38 years, nonsmokers, BMI 18–24) were recruited from the University of Milan and gave informed consent to the study. Volunteers were selected after the administration of a questionnaire concerning their dietary habits and lifestyle. Women following a hypocaloric, vegetarian or vegan diets, and women habitually consuming dietary supplements or drugs were excluded from the study.

Dietary instructions

Subjects were asked to follow a controlled carotenoid-poor diet for one week prior to the beginning of the study (the "run-in period"). This diet was standardized for its carotenoid content (< 600 µg carotenoid/day) and was devoid of tomatoes and their derivatives. Each volunteer was given a one-week menu and a list of food items that were allowed and not allowed. The daily menu consisted of a standard breakfast; a lunch with a first course and a second course taken from the list, together with a fixed portion of a specific vegetable (lettuce, potato, eggplant, etc.) and a specific fruit (apple, banana, pear, etc.); and dinner, consisting of a first course and a second course and without fruit and vegetables.

Subsequently, the same diet was supplemented for three weeks with tomato products, according to typical Italian dietary habits (nearly daily consumption of tomato products). In particular, subjects had to consume – per week – two supplements of fresh tomato (100 g), three supplements of tomato sauce (60 g each), and two supplements of tomato paste (15 g each). All products were eaten together with 5 g olive oil, in order to optimize absorption [24–26]. Table 1 reports the frequency and amount of consumption, as well as the carotenoid content and profile of the tomato products, used in the study. Carotenoids in tomato products were analyzed and quantified by HPLC as previously described [25].

The amounts of tomato products to be consumed were selected based on the results of a previous study, where approximately seven mg/day lycopene provided by tomato paste were able to significantly increase plasma and lymphocyte lycopene concentrations [21].

At the beginning of the study (T-7), at the end of the run-in period (T0), and at the end of the three-week supplementation period (T21), blood samples were drawn from fasting volunteers into Vacutainers™ containing Li^+ heparin as the anticoagulant. Plasma was obtained by centrifugation at 800 x g for 15 min, and samples were stored at –80 °C.

To evaluate F2-isoprostane excretion, a marker of *in vivo* oxidative stress, 24-hour urine was collected at T-7, T0, and T21. Total collected volumes per individual were measured, and aliquots were stored at –80 °C.

Carotenoids in plasma were quantified by HPLC after hexane extraction, using equinenone as the internal standard [25].

LDL (d = 1.019–1.063) were isolated by ultracentrifu-

Table 1 Carotenoid composition of tomato products used in the study

Product	Frequency (day per week)	Amount (g/portion)	Lycopene (mg/portion)	β-carotene (mg/portion)
Raw tomatoes	2	100	3.3	0.6
Tomato sauce	3	60	11.8	0.6
Tomato paste	2	15	7.0	0.2

gation from freshly separated plasma [27]. After protein quantitation [28], LDL samples were diluted to 200 µg/mL with phosphate-buffered saline (PBS). LDL oxidation was induced by the addition of copper sulphate (final concentration 5 µmol/L) and assessed continually by UV-spectrometry at 234 nm [29]. The time required to form conjugated dienes (the "lag phase") was used as an index of LDL oxidizability [30]. The antioxidant capacity of plasma to reduce Cu^{++} was evaluated by the use of a commercially available kit (OxisResearch, Portland, OR, USA) as described by Visioli et al. [31].

Urinary 8-iso-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) was quantified by double-extraction, followed by a mass spectrometry-validated enzyme immunoassay, according to Wang et al. [32] with modifications [33]. Acetylcholine esterase tracer was obtained from Cayman Chem (Ann Arbor, MI), and the specific antibody was kindly provided by the late Dr. J. Maclouf (Hopital Lariboisiere, Paris).

Statistical analysis

Results are expressed as means ± SD. Statistical analyses were performed using Statistica® software (StatSoft Inc., Tulsa, OK, USA). The effects of different treatments (run-in diet and tomato diet) on both plasma carotenoid concentrations and markers of lipid oxidation were evaluated by using one-way analysis of variance for repeated measures with the experimental times (T-7, T0, T21) as the dependent factor. Differences were considered significant if $P < 0.05$. Simple regression analysis was also used to determine the correlation between antioxidant concentrations and the different markers of lipid oxidation.

Results

Carotenoid plasma concentrations

One week controlled diet led to a significant decrease in plasma carotenoid concentrations ($P < 0.05$), except for α-carotene (Table 2). Furthermore, during the run-in period, the controlled diet led to a further decrease in the concentrations of lutein, zeaxanthin and β-cryptoxanthin ($P < 0.01$). The addition of tomato products to the diet produced a significant increase in plasma lycopene concentration ($P < 0.001$), which was sufficient to restore initial, basal values.

Antioxidant capacity of plasma

In Fig. 1, we report data on the antioxidant capacity of plasma, expressed as uric acid (reference compound) equivalents. Analyses of variance did not show significant modification of the antioxidant capacity of plasma during the whole experimental period.

Table 2 Carotenoid concentrations (mean ± SD) in plasma during the study

Carotenoids (µmol/L)	T-7	T0	T21
Lutein	0.64±0.06[a]	0.56±0.06	0.50±0.05[b]
Zeaxanthin	0.05±0.01[a]	0.04±0.01	0.03±0.00[b]
β-Cryptoxanthin	0.40±0.10[a]	0.32±0.08	0.23±0.05[b]
α-Carotene	0.10±0.01	0.07±0.01	0.07±0.01
β-Carotene	0.62±0.09[a]	0.44±0.06	0.38±0.06
Lycopene	0.54±0.06[a]	0.34±0.03	0.52±0.03[c]

[a] T0 vs T-7 $P < 0.05$; [b] T0 vs T21 $P < 0.01$; [c] T0 vs T21 $P < 0.001$

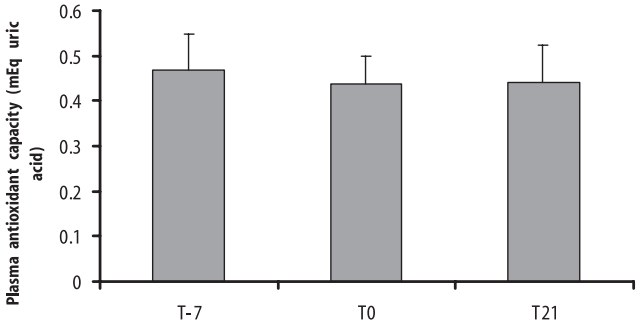

Fig. 1 Effect of different diets, controlled (from T-7 to T0) or added with (T21) determined amounts of carotenoids, on plasma antioxidant capacity

LDL oxidation

LDL oxidizability was significantly affected by the different diets. In particular, as shown in Fig. 2, the controlled diet caused a significant decrease in the time required to form conjugated dienes (lag phase, $P < 0.001$) whereas, after the run-in period, this time was significantly increased ($P < 0.01$). However, the values reached after supplementation were still lower than basal

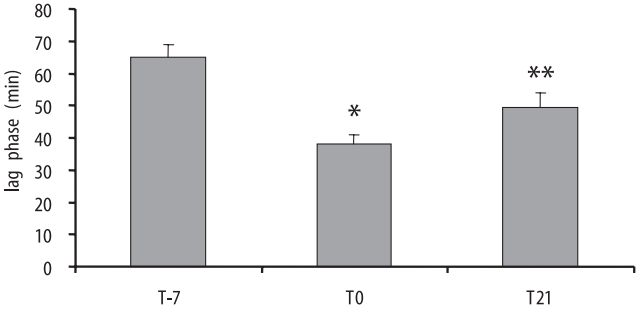

Fig. 2 Effect of different diets, controlled (from T-7 to T0) or added with (T21) determined amounts of carotenoids, on *ex vivo* copper sulfate-induced LDL oxidation. * $P < 0.001$ compared with T-7; ** $P < 0.01$ compared with T0

European Journal of Nutrition, Vol. 42, Number 4 (2003)

($P < 0.05$). Regression analyses performed between lag phase values and lycopene concentrations showed a significant and positive correlation ($r = 0.36$, $P < 0.05$).

Urinary excretion of 8-iso-$PGF_{2\alpha}$

The urinary concentration of F_2-isoprostanes at T-7, T0 and T21 is reported in Fig. 3. The enrichment of diet with tomato products significantly decreased 8-iso-$PGF_{2\alpha}$ urinary excretion ($P < 0.05$), whereas the first, one-week controlled diet did not affect its concentration. No correlation was observed with plasma lycopene concentrations.

Discussion

Tomato and its products are believed to possess disease-preventing properties [7], due to the presence of lycopene and other antioxidant compounds with the potential to scavenge radical species that damage cellular macromolecules [9, 10]. There is no current information on the amount of lycopene-containing products that should be ingested in order to increase plasma lycopene concentration and antioxidant capacity. The bioavailability of components of tomato products is high; however, bioavailability from raw products is lower than from processed ones [24, 25]. For this reason, we decided to use different lycopene sources in portions that could be easily incorporated into the habitual diet of the Italian population; such portions provided an average of eight mg lycopene/day. Furthermore, based on previous experience, we scheduled just one week of pre-treatment of a low-carotenoid diet in order to i) fully evaluate the effect of tomato-supplemented diet on the variables considered and to ii) concomitantly standardize plasma parameters among subjects. In fact, during longer periods of a low carotenoid diet, a continual decrease of carotenoid and other antioxidant concentrations leads to exaggerated effects on the subsequent treatments, which can strongly depend on the "extent of deprivation" [34].

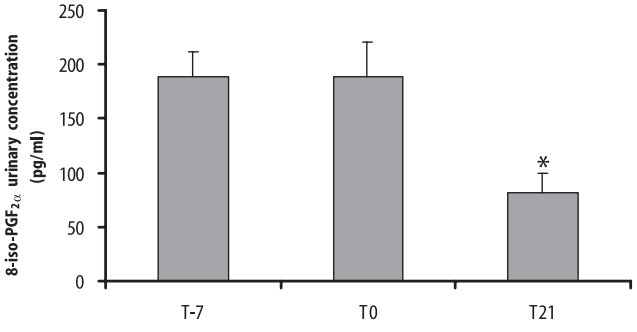

Fig. 3 Effect of different diets, controlled (from T-7 to T0) or added with (T21) determined amounts of carotenoids, on urinary excretion of 8-iso-$PGF_{2\alpha}$. * $P < 0.05$ compared with T0

It is noteworthy that plasma concentrations of carotenoids are exclusively dependent on dietary intake. Consequently, the decrease in lycopene concentrations (approximately 37 %) recorded after the first week of controlled diet was reverted by the subsequent three weeks of enrichment with tomato products (under conditions of habitual consumption), allowing a comparison between two experimental periods of different duration. While unequivocal data on the effects of diet enrichment with tomato on protection from DNA damage exist ([21, 34] and references therein), few studies on the potential role of tomato on lipid damage *ex vivo* have been conducted. *In vitro* data, obtained by enriching LDL samples with lycopene and other carotenoids, are still controversial. For example, Fuhrman et al. [35] found 65 % and 37 % reduction in LDL susceptibility to metal ion-dependent oxidation after *in vitro* supplementation with 3 μmol/L of lycopene and β-carotene, respectively. Conversely, other authors found lycopene and lutein to be non-effective, whereas β-carotene enrichment gave moderate protection from chemically-induced peroxidation [36]. An epidemiologic study did not find correlations between LDL carotenoid content and resistance to oxidation [37].

In our study, the addition of small daily portions of tomato products to the diet of a group of healthy subjects for three weeks was associated with a significant reduction (22 %) of LDL susceptibility to oxidation. In particular, variation in plasma lycopene concentration during tomato intervention (three weeks) was found to be significantly correlated ($r = 0.36$, $P = 0.033$) with the prolongation of lag phase, supporting the hypothesis of a potential role of this carotenoid in LDL protection.

Lycopene concentration decreased during the first week of the controlled diet, followed by a significant increase in LDL oxidizability. Yet, even though lycopene returned to its original values after tomato consumption, the degree of LDL protection relative to the unrestricted diet (T-7) was not reached. Although tomato products exert a protective effect, the effect may not be exclusively attributed to lycopene.

Furthermore, although the role of oxidized LDL in atherogenesis is yet to be fully understood [38, 39], our data support the hypothesis that tomato products might play cardioprotective roles through decreased LDL oxidizability. Similar conclusions were also drawn by a study that compared the effect of four weeks of supplementation with tomato juice, vitamin E or vitamin C on susceptibility of LDL to oxidation in type 2 diabetics at risk for CHD [40]. The authors found a three-fold increase in lycopene concentration and a 42 % increase in the lag time to LDL oxidation. Finally, both Bub et al. [22] and Agarwal and Rao [23] showed reduced LDL oxidiz-

ability after consumption of carotenoid-rich vegetables or lycopene-rich tomato products, respectively. The former proposed an enrichment of LDL in carotenoids (which we did not assess) following diet supplementation as an explanation for increased resistance to oxidation.

As a marker of endogenous oxidative stress we evaluated the urinary excretion of F_2-isoprostanes. Presently, F_2-isoprostanes are the most reliable and accepted *in vivo* marker of lipid peroxidation [2]. In addition, F2-isoprostanes are chemically stable end-products [41] and have been suggested as *in vivo* markers of LDL oxidation [41]. Urinary excretion of F_2-isoprostanes, namely 8-iso-$PGF_{2\alpha}$, is increased under conditions of organismal oxidative stress, such as that associated with diabetes [42, 43], hypercholesterolemia [44], coronary ischemia-reperfusion events [45, 46], and exposure to cigarette smoke [47, 48]. Nevertheless, the effect of lipid soluble antioxidants, namely vitamin E, on isoprostane formation are still equivocal [44]. For example, supplementation with high doses of α-tocopherol (up to 1200 mg/d for three weeks) did not decrease 8-iso-$PGF_{2\alpha}$ in heavy smokers [48, 49].

In our study, the daily intake of tomato products highly decreased (–53 %) excretion of 8-iso-$PGF_{2\alpha}$, probably indicating *in vivo* protection from oxidative damage. On the contrary, consumption of the carotenoid-controlled diet during the first week of experimentation did not significantly modify excretion of the marker with respect to its initial rate, in contrast to the observed LDL susceptibility to chemically-induced oxidation. The current lack of knowledge on the sites and kinetics of urinary isoprostanes production (not organ-specific markers of oxidative stress) makes it difficult to interpret this finding beyond speculation.

The lack of effect of tomato products consumption on serum antioxidant capacity (Fig. 1) is consistent with findings by Bub et al. [22]. These results might be due to the fact that carotenoids do not act as donor antioxidants (which would readily reduce metal ions) but, rather, as radical traps [50, 51]. Whether this parameter is truly an indicator of greater susceptibility to CHD is at present under debate [52].

Our data suggest that tomato products reduce lipid oxidation *in vivo*. The slight difference we observed in the response of the two markers of lipid oxidation selected may depend on the different approaches used, being in one case a model subjected to oxidative stress (*ex vivo* LDL oxidation) and in the second case the determination of an *in vivo* oxidation product (isoprostane). Use of both markers may help develop a better understanding of the role of antioxidants or the importance of antioxidant-rich foods. In the first case, an *ex vivo* model is used to measure the extent of protection against an insult. It is plausible that higher levels of stress induce a response that is strictly related to the exploitation of all the resources available. In the second case, we used a marker that should be sensitive to normal levels of stress. This may be the reason why sometimes there is a lack of an effect of antioxidant supplementation on lipid peroxidation in healthy subjects [53], a fact that could mean that only individuals at oxidative risk might benefit from antioxidant supplementation therapy. However, our data show that in healthy subjects the dietary supplementation with practical amounts of tomato products not only decreases LDL oxidizability but also F_2-isoprostane excretion, suggesting a protective *in vivo* role of carotenoids and other tomato components against lipid peroxidation.

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