Supplementation with Tomato-Based Products Increase Lycopene, Phytofluene, and Phytoene Levels in Human Serum and Protects Against UV-light-induced Erythema

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Abstract: Carotenoids are suitable photoprotectants, and β-carotene supplements are used for protection against ultraviolet (UV) light-induced erythema. Protective effects are also observed when carotenoids are provided with the diet. Here, we investigated the photoprotective effects of synthetic lycopene in comparison with a tomato extract (Lyc-o-Mato®) and a drink containing solubilized Lyc-o-Mato® (Lyc-o-Guard-Drink). With these different sources, the volunteers ingested similar amounts of lycopene (about 10 mg/day). After 12 weeks of supplementation, significant increases in lycopene serum levels and total skin carotenoids were observed in all groups. Significant increases in the serum levels of phytofluene and phytoene occurred in the Lyc-o-Mato and the Lyc-o-Guard-Drink group.

At weeks 0, 4, and 12 an erythema was induced with a solar light simulator. Dorsal skin of each subject was irradiated with 1.25 minimal erythema dose (MED). Reddening of the skin was evaluated before and 24 hours after irradiation by chromametry and expressed as positive *a*-values (red/green-axis). Δ a-values (difference of a-value before irradiation and after 24 hours) were used as an index of erythema intensity. A decrease in the Δ a-value from week 0 to week 12, indicating prevention of erythema formation, was observed in all groups. Compared to week 0, the Δ a-value at week 12 was 25% lower in the synthetic lycopene group. The protective effect was more pronounced in the Lyc-o-Mato (38%) and Lyc-o-Guard-Drink (48%) groups. In the two latter groups, phytofluene and phytoene may have contributed to protection. Both of these carotenoids exhibit absorption maxima at wavelengths of UV light. Absorption of UV light protects skin from photodamage and might explain the differences observed between groups.

Key words: Carotenoids, UV light, erythema, sun protection, skin

Abbrevations: HPLC, high-performance liquid chromatography; MED, minimal erythema dose.

Introduction

A primary response of skin to excessive exposure to ultraviolet-B (UVB) light is *erythema solare,* the most common acute sunlight-induced damage. Photo-oxidative processes are thought to be involved in the reaction of skin towards UV irradiation [1] leading to oxidative damage and interference with the regulation of gene expression [2]. UVB is especially erythematogenic, inducing DNA damage and promoting the development of skin cancer [3, 4]. There is increasing evidence that dietary antioxidants such as carotenoids, tocopherols, or ascorbate protect against photo-oxidative reactions and may be useful in the prevention of diseases related to photo-oxidative stress [5, 6].

Supplementation with β-carotene provides moderate protection against UV-induced erythema [7–10]. Other carotenoids such as lycopene or lutein are also efficient antioxidants scavenging singlet molecular oxygen or peroxyl radicals [11, 12]. Carotenoids are present in the human skin and contribute to UV protection. For sunburn prevention, β-carotene can be at least partially substituted by other carotenoids. Supplementing a combination of β-carotene, lutein, and lycopene, each compound at a dose of 8 mg/day, ameliorated UV-induced erythema in humans comparable to the treatment with 24 mg of β-carotene/day [13].

Fruits and vegetables are the major dietary sources of carotenoids, and selected dietary constituents or products are rich in particular carotenoids [14]. Xanthophylls like lutein and zeaxanthin are found in green leafy vegetables or corn; lycopene is the predominant carotenoid in the tomato. With a selected product or a diet rich in fruit and vegetables, up to 20 mg of carotenoids are ingested per day, and sun-protective effects may be achieved [15]. The consumption of tomato paste, providing about 16 mg/day of lycopene, leads to an increase in lycopene serum levels and total carotenoids in skin [15]. After dietary intervention for a period of ten weeks, protection against UV-induced erythema was observed. These protective effects were mainly attributed to biological activities of lycopene. However, tomatoes and tomato products also contain other carotenoids; e.g., carotenoid precursors like phytoene and phytofluene [16]. The amount of carotenoids present in the tomato varies depending on strain, agricultural conditions, and ripening state; phytoene and phytofluene contribute up to 30% of total carotenoids [17]. Both compounds are absorbed from the diet, and their plasma levels increase after consumption of tomato products [18].

Materials and Methods

Study design

Thirty-six healthy adult volunteers took part in the study and were recruited independently by the Institute for Experimental Dermatology (University Witten-Herdecke). Criteria for inclusion were healthy condition, body mass index (BMI) of 18–25 kg/m^2, no pregnancy or lactation, no supplementation with vitamins, and no medication during the study. All volunteers were of skin-type II, which was evaluated based on the coloration of skin, hair, and eyes and the history of sensitivity to sun exposure [19]. The participants were randomly assigned to three groups of the same size (n = 12). Duration of the study was 12 weeks.

Volunteers in the lycopene group ingested two hard shell capsules per day that contained synthetic lycopene encapsulated as beadlets (5.1 mg/capsule).

In the Lyc-o-Mato group participants ingested two soft gel capsules per day containing a tomato extract (Lyc-o-Mato®, LycoRed, Natural Prod. Industr. Ltd., Beer-Sheva, Israel). The content of carotenoids in each capsule was: 4.9 mg lycopene, 0.4 mg phytofluene, 0.5 mg phytoene, and 0.2 mg β-carotene.

Participants assigned to the Lyc-o-Guard-Drink group consumed two times per day 250 mL of a solubilized Lyc-o-Mato®drink (Lyc-o-Guard-Drink, LycoRed, Israel). The content of carotenoids in 250 mL of the beverage was: 4.1 mg lycopene, 1.6 mg phytofluene, 2.3 mg phytoene, and 0.2 mg β-carotene.

The predominant isomer of lycopene was *all-trans* (> 95%) in both supplements and the Lyc-o-Mato®drink. Different isomers of phytoene and phytofluene were present in the tomato-based products. Due to the lack of appropriate standards they could not be assigned.

Capsules and the drink were taken twice a day with the main meal; the daily intake of lycopene, phytofluene, and phytoene in the different groups is summarized in Table I. Compliance was monitored by a questionnaire and by carotenoid serum analyses. The diet was not standardized during the study, but the participants were advised

Table I: Daily intake of carotenoids in the different groups

Carotenoid	Group		
	Lycopene	Lyc-o-Mato mg/day	Lyc-o-Guard-Drink
Lycopene	10.2	9.8	8.2
Phytofluene	–	0.8	3.2
Phytoene	–	1.0	4.6
β-Carotene	–	0.4	0.4

not to change their dietary habits. Written informed consent was obtained from each participant. The study design was approved by the ethics committee of the University of Witten-Herdecke, Germany.

Carotenoid analyses in serum and skin

Fasting blood samples were obtained on day 0, week 4 and week 12 of the study. Serum was prepared by centrifugation of clotted blood samples and stored at –80°C until analysis. Lycopene, β-carotene, α-carotene, lutein, zeaxanthin, and cryptoxanthin were determined by high-performance liquid chromatography (HPLC) as described [20]. The HPLC system consisted of a Merck-Hitachi L-7100 pump connected with a Merck-Hitachi UV/Vis detector and an integrator for data registration. For phytofluene and phytoene analyses, 500 µL of serum were extracted with hexane/dichloromethane (5/1, v/v, stabilized with 0.1% 2,6-di-*tert*-butyl-p-cresol) after protein precipitation with ethanol. The organic solvent was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 100 µL dichloromethane plus 100 µL acetonitrile.

HPLC was performed isocratically with an eluent consisting of acetonitrile/methanol (85/15, v/v) and a reversed phase column (pKb-100, 250 × 4.6 mm, Supelco, Bellefonte, PA) protected by a guard column (4.6 x 4.6 mm) with the same stationary phase. The flow rate was set to 1 mL/min; phytofluene was detected at a wavelength of 348 nm and phytoene at 278 nm. The concentrations were calculated from an external calibration curve. Phytofluene and phytoene standards were kindly provided by Dr. Zohar Nir, LycoRed Natural Prod. Industr. Ltd., Israel.

On day 0, week 4, and week 12 of the study, lycopene levels of the skin (back, scapular region) were determined by means of reflection spectrophotometry as described [21].

Induction of erythema and determination of the skin color

For each participant the minimal erythema dose (MED) was determined before the study. The MED is defined as the individual UV dose that causes a minimal erythema (reddening of the skin) 24 hours after irradiation. Dorsal skin of each subject was irradiated with 1.25 MED using a solar light simulator (SOL 3 Hönle, Munich, Germany) on day 0, week 4, and week 12 of the study.

Skin color was evaluated before and 24 hours after irradiation by chromametry (Chromameter Minolta CR 200, Ahrensburg, Germany) using the three-dimensional color system (L, a, b-values). The L-value is a parameter for lightness of skin, and the b-value (blue/yellow axis) is indicative for pigmentation. A positive a-value (red/green-axis) represents redness of the skin. The erythema was quantified by measuring a-values before and 24 hours after irradiation; Δ a-values (difference of a-value before irradiation and after 24 hours) are an index of erythema intensity in response to UV exposure.

Statistics

Within the three treatment groups each combination of two time points was compared using the Wilcoxon signed-rank test. Each combination of two treatment groups was compared using the Wilcoxon rank-sum test. For all parameters descriptive statistics were calculated. Data are presented as mean values ± SD (n = 12).

Results

Serum and skin levels of carotenoids

The daily intake of carotenoids in the different groups is listed in Table I. Lycopene dose was highest (10.2 mg/day) in the lycopene group, but comparable amounts were supplemented with the Lyc-o-Mato capsules (9.8 mg/day) and the Lyc-o-Guard-Drink (8.2 mg/day). No other carotenoids were present in the supplement of the lycopene group, which was prepared from synthetic lycopene. Considerable amounts of the lycopene precursors, phytofluene (0.8 mg/day) and phytoene (1.0 mg/day), were ingested with the Lyc-o-Mato capsules. The daily doses of phytofluene and phytoene were even higher in the Lyc-o-Guard-Drink group, at 3.2 and 4.6 mg/day, respectively. The intake of β-carotene was 0.4 mg/day in both the Lyc-o-Mato- and the Lyc-o-Guard-Drink groups.

Serum levels of lycopene, phytofluene, phytoene, and β-carotene as well as the levels of total carotenoids in skin, determined in the three different groups, are shown in Table II. There are slight differences in the basal levels of carotenoids between groups, but these were not statistically significant. Basal levels of carotenoids in the skin were comparable in all groups and ranged from 0.16 to 0.18 nmol/g.

In the lycopene group, serum levels of lycopene increased from 0.28 (week 0) to 0.62 nmol/mL (week 12); total carotenoids in skin rose from 0.18 to 0.23 nmol/g. A slight decrease was observed for β-carotene, while no significant change was found for phytofluene and phytoene.

In the Lyc-o-Mato group, lycopene serum levels increased about two-fold during the study. The elevation of phytofluene and phytoene levels was in the same range. After 12 weeks of supplementation, 0.94 nmol phytofluene/mL were detected, exceeding the levels of all other carotenoids. A significant increase was also found for to-

Table II: Carotene levels in serum (nmol/mL) and total carotenoid levels in skin (nmol/g) at day 0, week 4, and week 12 of the study in the different supplementation groups (n = 9–12)

Serum carotenoid (nmol/mL) Skin carotenoids (nmol/g)	Time (weeks) 0	4	12
Lycopene group			
Lycopene	0.28 ± 0.15	0.61 ± 0.29**	0.62 ± 0.17**
Phytofluene	0.33 ± 0.15	0.36 ± 0.16	0.31 ± 0.15
Phytoene	0.06 ± 0.04	0.05 ± 0.05	0.04 ± 0.04
β-Carotene	0.48 ± 0.34	0.38 ± 0.28	0.33 ± 0.22*
Skin carotenoids	0.18 ± 0.03	0.23 ± 0.06*	0.23 ± 0.06*
Lyc-o-Mato group			
Lycopene	0.28 ± 0.13	0.55 ± 0.20**	0.55 ± 0.16**
Phytofluene	0.44 ± 0.18	0.73 ± 0.25*	0.94 ± 0.52*
Phytoene	0.06 ± 0.04	0.12 ± 0.07*	0.14 ± 0.10*
β-Carotene	0.57 ± 0.31	0.60 ± 0.64	0.54 ± 0.41
Skin carotenoids	0.16 ± 0.08	0.18 ± 0.06	0.22 ± 0.04*
Lyc-o-Guard-Drink group			
Lycopene	0.37 ± 0.17	0.84 ± 0.26**	0.88 ± 0.26**
Phytofluene	0.27 ± 0.16	0.93 ± 0.34*	0.87 ± 0.39*
Phytoene	0.07 ± 0.06	0.18 ± 0.07*	0.18 ± 0.11*
β-Carotene	0.31 ± 0.13	0.44 ± 0.18*	0.44 ± 0.20
Skin carotenoids	0.16 ± 0.06	0.20 ± 0.05	0.21 ± 0.06*

Data represent mean values ± SD
* Significantly different to week 0; $p < 0.05$
** Significantly different to week 0; $p < 0.001$

tal carotenoids in skin, whereas the amount of β-carotene in serum hardly changed.

In the Lyc-o-Guard-Drink group an increase in lycopene serum levels from 0.37 (week 0) to 0.88 nmol/mL (week 12) was measured. Increases in phytofluene and phytoene serum levels were comparable to the Lyc-o-Mato group, although the doses applied with the drink were considerably higher. Compared to the basal level, the amount of β-carotene was also increased on week 4 and week 12 (statistically significant only on week 4). As in the other groups, total carotenoids in skin were elevated after four and 12 weeks of supplementation.

Evaluation of erythema intensity

A selected skin area of each subject was irradiated with 1.25 MED using a solar light simulator, and skin color was evaluated before and 24 hours after irradiation by chromametry. Chromametry a-values were determined at weeks 0, 4, and 12, indicating the redness of the skin (Table III). The a-values before irradiation were similar in all groups at all time points (0–12 weeks). The Δ a-value (a-value 24 hour after irradiation minus a-value before irradiation) is an index of erythema intensity; decreasing Δ a-values in comparison to week 0 reflect a protection against UV-induced erythema.

Upon treatment with synthetic lycopene (lycopene group), a slight decrease in the Δ a-value was observed between weeks 0 and 12. However, the difference was statistically not significant.

In the Lyc-o-Mato group the Δ a-values were lower at weeks 4 and 12 than at week 0. The differences from week 0 were statistically significant at week 12, indicating a UV-protective effect of supplementation.

The consumption of the Lyc-o-Guard-Drink also led to a decrease of the Δ a-values from week 0 to week 12. The difference in comparison to week 0 was statistically significant on week 12 of supplementation.

Upon comparison between groups, no statistically significant difference was observed for all Δ a-values evaluated in the Lyc-o-Mato- and Lyc-o-Guard-Drink groups. The preventive effect was more pronounced in the Lyc-o-Mato and Lyc-o-Guard-Drink groups than in the lycopene group.

Discussion

In the present study, three different sources were used to supply lycopene to volunteers and investigate UV-protective effects over a period of 12 weeks. The daily dose of lycopene was comparable between groups, ranging from 8.2 to 10.2 mg/day. One group received synthetic lycopene; no other carotenoid was present in this preparation. The other supplements were derived from tomato-based

Table III: Chromametric a-values of skin of volunteers (back) at day 0, week 4, and week 12 of the study in the different supplementation groups (n = 12)

a- value	Time (wk)		
	0	4	12
Lycopene group			
before irradiation	8.2 ± 1.7	8.2 ± 1.7	8.1 ± 2.0
24 hours after irradiation	11.7 ± 2.0	11.4 ± 1.6	10.7 ± 1.6
Δ *a*	3.5 ± 1.7	3.2 ± 2.0	2.6 ± 2.3
Lyc-o-Mato group			
Before irradiation	7.1 ± 2.1	7.2 ± 2.1	6.8 ± 2.2
24 hours after irradiation	12.3 ± 2.6	11.3 ± 2.1	10.0 ± 2.6**
Δ *a*	5.2 ± 0.8	4.1 ± 1.7	3.2 ± 1.4**
Lyc-o-Guard-Drink group			
before irradiation	6.9 ± 1.9	6.7 ± 1.7	6.9 ± 1.6
24 hours after irradiation	11.9 ± 1.6	11.1 ± 2.3	9.5 ± 2.1*
Δ *a*	5.0 ± 1.6	4.4 ± 2.7	2.6 ± 1.7**

Data represent mean values ± SD
* Significantly different to week 0; $p < 0.05$
** Significantly different to week 0; $p < 0.001$

products. In addition to lycopene, also the carotenoids phytofluene and phytoene, both precursors of lycopene, were present. In the Lyco-o-Mato product the content of phytofluene and phytoene was lower than in the Lyc-o-Guard-Drink (Table I).

At the beginning of the study the carotenoid levels differed somewhat between groups but were in the range described in the literature [22, 23]. Upon supplementation, lycopene levels in serum increased in all three groups; the final concentration varied between 0.55 and 0.88 nmol/mL. Similar levels of lycopene have been reported in the literature after prolonged intake of tomato products or lycopene supplements [15, 24, 25]. Inter-individual differences related to absorption, transport, and metabolism of carotenoids determine variations in the maximal blood levels of individuals [26]. Thus, the higher levels of lycopene in the Lyc-o-Guard-Drink group at week 0 and week 12 may be assigned to individual differences of the volunteers. With the ingestion of the tomato products the levels of phytofluene and phytoene rose in serum. Phytofluene levels increased from 0.44 nmol/mL at baseline to 0.94 nmol/mL after 12 weeks in the Lyc-o-Mato group and from 0.27 nmol/mL to 0.87 nmol/mL in the Lyc-o-Guard-Drink group. Apparently, the bioavailability of phytofluene is high at low doses, and saturation of serum is obtained at even low doses over long treatment periods. The increases in phytoene were less pronounced, although similar amounts were present in the products. This might be due to physicochemical properties of the compound, instability *in vivo,* or pharmacokinetic parameters like poor absorption or rapid clearance. The final levels of phytofluene and phytoene in both groups are comparable, although the Lyc-o-Mato-Drink contained about four- to five-fold higher amounts of phytofluene and of phytoene. The amounts of phytofluene and phytoene in the serum described here are in accordance with data from the literature [18, 25].

On weeks 0, 4, and 12, skin was irradiated with a solar light simulator to induce an erythema that z was evaluated by chromametry. Δ a-values (difference of a-value before irradiation and 24 hours after irradiation) were used as an index of erythema intensity (Table III). A decrease in the Δ a-value from week 0 to week 12 indicates prevention against UV exposure and was observed in all groups. However, it was not statistically significant in the group that received the synthetic lycopene. The most pronounced protective effect was observed in the Lyc-o-Guard-Drink group followed by the Lyc-o-Mato group with decreased Δ a-values after 12 weeks of treatment, 48 and 38%, respectively. A similar protective effect was observed in a study where tomato paste was applied [15]. In that study, after ten weeks of treatment, the Δ a-value was about 40% lower compared to week 0. Supplementation with β-carotene at levels of 20–25 mg/day or mixtures of carotenoids provides similar protection [8–10].

The difference in the efficacy of the tomato products compared to synthetic lycopene might be due to the presence of phytofluene and phytoene. Increasing serum levels of both compounds are related to a more pronounced protection as compared to lycopene alone. Phytofluene and phytoene exhibit absorption maxima in the ultraviolet light at 348 nm (UVA) and 286 nm (UVB), respectively; synergistic effects of lycopene, phytofluene, and phytoene might be operative. However, a decrease in the β-carotene level was found in lycopene group, whereas no change or an increase was detected in the Lyc-o-Mato and

Lyc-o-Guard-Drink group. Thus, also β-carotene might have contributed to protection to some extent.

Endogenous protection associated with the ingestion of dietary carotenoids is not comparable to the use of a sunscreen with a high sun protection factor. However, increasing the basal protection systemically contributes to life-long defense against UV-dependent skin damage.

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