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Tomato Phytonutrients Balance UV Response: Results from a Double-Blind, Randomized, Placebo-Controlled Study

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Keywords
Carotenoids · Oral photoprotection · Erythema formation · UVB · Double-blind, randomized, placebo-controlled study

Abstract

Background: Our previous double-blinded, placebo-controlled cross-over study indicated that a nutritional supplement named lycopene-rich tomato nutrient complex (TNC) can protect from UVA1-induced (340–400 nm) and UVA- (320–400 nm)/UVB-induced (280–320 nm) upregulation of molecular markers associated with oxidative stress, inflammation, and ageing. Objectives: in the current double-blind, randomized, placebo-controlled multicenter study, we analyze whether a similar, synergistic carotenoid-rich TNC can protect from broadband UVB-induced threshold erythema formation assessed as increase in minimal erythemal dose (MED) reading, the intensity of erythema formation, and the upregulation of molecular markers associated with inflammation and immunosuppression, and whether this correlates with carotenoid blood levels. Methods: One hundred and forty-nine healthy volunteers were randomized to two groups and subjected to a 5-week washout phase, followed by a 12-week treatment phase receiving either 15 mg lycopene, 5.8 mg phytoene and phytofluene, 0.8 mg β-carotene, 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary extract per day or placebo made from medium-chain triglycerides. At the end of each phase, MED determination, UVB irradiation, chromametry, biopsies, and blood samples were undertaken. Results: The active supplement was well tolerated. Interestingly, no significant difference was seen in the MED between the active-supplement and placebo groups, as determined by visual grading by expert assessors. Of note, the carotenoid-containing supplement significantly protected against UVB-induced erythema formation measured as Δa☆ after the intervention minus Δa☆ after the washout phase as compared to the placebo. Moreover, intake of the active supplement significantly protected against UVB-induced upregulation of IL6 and TNFα as compared with the intake of placebo. Lastly, carotenoid plasma levels were significantly increased. Conclusion: This well-tolerated carotenoid-containing supplement significantly protected against UVB-induced erythema formation and upregulation of proinflammatory cytokines in healthy volunteers.
Introduction

The concept of oral photoprotection by antioxidant micronutrients gained very popular over the last decades [1, 2]. In case of topical sunscreens, the sun protection factor is a simple and noninvasive tool to quantify the photoprotective activity. The latter is defined as a quotient of the minimal erythema dose (MED) under treatment divided by the MED without intervention representing a measure for acute deleterious effects of UV irradiation [3]. An increased MED after intervention with a nutritional supplement can also therefore be used as an indicator of photoprotection in studies of potential orally ingested photoprotectants. A special focus was on β-carotene, which had been shown in a meta-analysis to provide significant protection against UV-induced erythema if taken at least over a period of 10 weeks [4]. Safety concerns after long-term intake of β-carotene at nonphysiological levels arose when adverse effects on the incidence of lung cancer in smokers and workers exposed to asbestos were found [5]. To circumvent this issue, other carotenoids such as lycopene or lutein came into focus. Recently, a double-blinded, placebo-controlled crossover study has shown that both these ingredients provided significant protection against UVA- or UVA/UVB-induced upregulation of UV-inducible markers such as heme oxygenase 1, intercellular adhesion molecule 1, and matrix-metalloproteinase 1 [6]. Some limitations of that study were (i) the relatively low number of volunteers included and (ii) the lack of showing an effect on the physiological erythema skin response after intervention with the active supplement. In order to complement and strengthen the results of that study, we therefore performed a double-blind, randomized, placebo-controlled, multicenter follow-up study to assess as primary objective the effect of carotenoid-rich tomato nutrient complex (TNC; containing and rosemary extract) on the acute UV radiation-mediated erythema skin response; i.e., (i) MED and (ii) intensity of erythema, compared with the placebo cohort following 12 weeks of twice daily oral supplement. As secondary objective, we wanted to evaluate the effect of carotenoid-rich TNC compared to that of placebo after 12 weeks of b.i.d. (twice a day) supplementation on skin biopsy biomarkers indicative for immunosuppression or inflammation. Analysis of UV-induced gene expression was very helpful to address photodamage to human skin in vivo, where it exerts profound immunosuppressive activities [9]. Proinflammatory cytokines such as TNFα and IL6 are induced upon UVB treatment in keratinocytes and in humans on mRNA and protein level [10, 11]. For a detailed review on UVB-induced cytokine production by keratinocytes or Langerhans cells, see [12]. The listed proinflammatory cytokines act in a cascade fashion to induce inflammation with initial release by keratinocytes or inflammatory cells in the skin and subsequent synergizing with UV-irradiated keratinocytes to further increase their cytokine production finally affecting local and systemic immunosurveillance [12, 13].

Materials and Methods

Study Design

This study was approved by the local Ethics Committees of (i) the Heinrich Heine University, Düsseldorf, Germany (reference No. 4194), and (ii) the University of Dundee, Scotland, UK (No. SC015096). It was conducted at the IUF – Leibniz-Research Institute for Environmental Medicine, Düsseldorf, Germany, and at the Photobiology Unit, Department of Dermatology, Ninewells Hospital and Medical School, Dundee, UK, according to the ethical rules stated in the principles of the Declaration of Helsinki and the International Council for Harmanisation of Technical Requirements for Pharmaceuticals for Human Use. We conducted a randomized, double-blind, placebo-controlled, parallel-group, multicenter clinical trial in which each subject was randomized to receive carotenoid-rich TNC or placebo for a period of 12 weeks in a parallel-group design. A total of 149 subjects (female or male) were enrolled into the study, and 145 subjects completed the trial as planned. The treatment groups were stratified based on the age category, gender, and smoking to ensure equal distribution of such population within the two treatment arms (Table 1). The group taking the active supplement included 75 volunteers (56 female, 19 male), and the group taking placebo consisted of 74 volunteers (59 female, 15 male). The age distribution did not differ in both arms of the study (mean ages were 40.9 and 40.9 years). The age of the volunteers ranged from 20 to 50 years. Current smokers were very rare; in the carotenoid-rich TNC group, there were 5 smokers (7%), and in the placebo group 3 (4%). For ethical reasons, only two-thirds of the volunteers were asked for biopsies to be analyzed for expression of molecular markers. The subjects enrolled were distributed according to an online block randomization service by the sponsor; block size was 2, allocation ratio 1:1. Volunteers and investigators were unaware of the treatment.
Table 1. Baseline demographics and characteristics

<table>
<thead>
<tr>
<th>Carotenoid-rich Placebo</th>
<th>TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>75</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>56</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>19</td>
</tr>
<tr>
<td>Mean age (SD), years</td>
<td>40.9</td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>25.8</td>
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<tr>
<td>Mean initial lycopene level, nm</td>
<td>676</td>
</tr>
<tr>
<td>Median initial lycopene level, nm</td>
<td>646</td>
</tr>
<tr>
<td>Mean initial phytoene level, nm</td>
<td>65</td>
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<tr>
<td>Median initial phytoene level, nm</td>
<td>51</td>
</tr>
<tr>
<td>Mean initial phytofluene level, nm</td>
<td>54</td>
</tr>
<tr>
<td>Median initial phytofluene level, nm</td>
<td>50</td>
</tr>
</tbody>
</table>

All individuals were of good general health with a body mass index ≤ 30. Their Fitzpatrick skin type was type I–II. With regards to eating habits, a normal diet and the willingness to follow a lycopene- and antioxidant-restricted diet was requested. Exclusion criteria were sunbed use and pregnancy. The study was conducted between January 2014 and September 2016.

**Assessment of Blood Samples**

Blood samples for carotenoid determination were collected after a 5-week washout phase, at randomization, after 4 weeks of treatment, and at the end of the study in heparinized tubes and immediately centrifuged. The plasma was stored at –80 °C until analysis for carotenoids such as lycopene, α-, and β-carotene by high-performance liquid chromatography, as previously described [6, 17, 18]. In addition, the colorless carotenoids phytofluene and phytoene were measured because these precursors of carotenoids absorb radiation in the UV range [19, 20].

**Assessment of Gene Expression**

Biopsies were taken 24 h after chromametry using 1.25 MED at visits 4 and 8 (Fig. 1b), snap frozen in liquid nitrogen and stored at –80 °C until further analysis, as previously described [21–23]. For the evaluation of UV-induced gene expression, the 2−ΔΔCt method is used [24]. Gene expression is expressed as x-fold induction versus an unirradiated control. Primer pairs were as follows:

- IL1α (forward 5'-TGTATGGGATCCCAGATGAA-3', reverse 5'-CTAACCTGTGATGGTGTTTGTTGATC-3', NM_000575.4) [25]; IL6 (forward 5'-CTCTGACCCAGACGGCAAG-3', reverse 5'-AA-CTGAGCCGAGGGCG-CTTGTG-3', NM_000600.4) [26]; IL10 (forward 5'-AAGACCCAGACATCAAC-3', reverse 5'-AAATCGATGACAGCGCCGTAG-3', NM_000572.3) [27]; TNFa (forward 5'-GGAGAGGTTG-ACC-GACTCA-3', reverse 5'-GCCCAGACTGCGAAAGG-3', NM_000594.3) [28]; and 18S rRNA as housekeeping gene (forward 5'-GCCGCCTAGAGGTAATCTTG-3', reverse 5'-CATTCTTG-GCAAATGCTTTCG-3', X03205.1) [29].

**Sample Size and Statistical Evaluation**

Sample size calculation was based on previously conducted studies by Lycored employing different carotenoid-rich formula (Lyc-O-Mato and Lyc-O-Guard drink) for the primary endpoint “protection against ultraviolet (UV) light-induced erythema,” such as [19, 30, 31]. The sample size corresponds to an effect size difference at 2% between the two groups, with a power of 80% and a drop-out rate of 10%.

Data management and randomization were done by Medistat Ltd. (Tel-Aviv, Israel). Additional studies were performed in-house with the help of SigmaPlot (version 14.0). Normality of the data was tested using the Shapiro-Wilk test. For comparison of significant differences, the Kruskal-Wallis one-way ANOVA on ranks, t test, or Mann-Whitney rank sum tests were performed.

**Results and Discussion**

For the active arm of the study, 75 volunteers were recruited, and 71 completed the study. For the placebo arm, all 74 recruited volunteers also finished the trial (Fig. 1a).

The treatment was safe and well tolerated, and no serious adverse events were observed within the two study populations. Only one severe adverse event diagnosed as pityriasis rosea occurred in the group with carotenoid-rich TNC intake. In addition, one volunteer in the same population discontinued the study due to mild eczema. Compliance was good in both arms, which was reflected by the carotenoid levels determined at the end of the 5-week washout phase (visit 2), after 4 weeks of intake (visit 5), and at the end of the 12 weeks of intake (visit 8) (Fig. 1b, 2a–c) from blood samples. In detail, after 5 weeks of diet...
restriction, the starting median (25th percentile–75th percentile) lycopene level was 670 nM (480–919) for the group to take the placebo, and 646 nM (488–890) for the group to take the carotenoid-rich TNC. During the supplementation phase, the lycopene level significantly increased in the carotenoid-rich TNC-taking group to a median of 1,220 nM (832–1,520) and reached a final median level of 1,153 nM (847–1,551), whereas no such increase was seen in the placebo group. There, median levels of 690 nM (472–1,017) after 4 weeks and 720 nM (445–1,040) after 12 weeks were detected.

Similarly, the colorless carotenoid precursor phytofluene significantly increased in the carotenoid-rich TNC arm over time. Median phytofluene was 51 nM (18–94) at the end of the washout phase and increased to 178 nM (79–329) and to 156 nM (66–390) during the supplementation phase in the active arm. In contrast, in the placebo part of the study, phytofluene was 55 nM (18–108) in the depletion phase, which did not significantly change during the intake phase to 67 nM (20–117) and 55 nM (20–108) (Fig. 2b).

Finally, the colorless carotenoid precursor phytoene also significantly increased during the intake phase of the active arm (Fig. 2c). Median phytoene was 50 nM (18–90) at the end of the washout in the group supposed to take the active supplement and increased to 90 nM (78–174) and to 90 nM (66–154) over the supplementation phase. In the placebo group, the initial median phytoene was 29 nM (16–90). In the supplementation phase, these data did not change and were 52 nM (18–90) and 38 nM (18–90).

Whether the determination of the nutritional supplements such as lycopene from blood samples is a good indicator for compliance and/or bioavailability should be reconsidered because a significant increase in the corresponding plasma levels not always correlates to a significant photoprotection [19]. In this regard, it may be interesting that cutaneous lycopene can also be determined noninvasively by Raman spectroscopy [32], where gender-related differences in basal carotene and lycopene levels have recently been observed.

The first primary outcome, the MED reading, was identical in both intended treatment groups, given as a median (min, max) of 0.080 (0.035, 0.125) after the washout phase. After supplementation with carotenoid-rich TNC, the MED did not change significantly; we observed a median (min, max) of 0.080 (0.035, 0.147; data not shown). This negative result is in line with observations obtained in a human intervention study over 12 weeks, where the MED was compared in a group of 9 females with skin type II receiving 55 g tomato corresponding to 16 mg lycopene in tomato paste to a control group (n = 8) taking olive oil [33] and in a comparative 10-week study, where 10 females consumed either a pill or tomato paste corresponding to 16 mg lycopene [34].
Fig. 2. Compliance reflected by carotenoid blood levels. Lycopene (a), phytofluene (b), and phytoene (c) content in blood samples taken at the indicated time points was determined as described in Materials and Methods. Given are medians, 75th and 25th percentiles from n = 71 volunteers taking carotenoid-rich TNC and n = 74 volunteers taking placebo. Significance was determined by Kruskal-Wallis one-way ANOVA on ranks (Dunn’s) for each time point compared to the starting level at week 5, * p < 0.05 versus week 5 (beginning of supplementation).

Fig. 3. Effect of carotenoid-rich TNC on erythema formation and gene expression. a Change of erythema (Δa*) formation was determined by chromametry, as described in Materials and Methods, from n = 71 volunteers taking carotenoid-rich TNC and n = 74 volunteers taking placebo. Δa* is defined as the difference between erythema development levels at 24 h following UV irradiation after supplementation (visit 8) and erythema development levels at 24 h following UV irradiation before supplementation (visit 4). Given are the differences by box plots with medians (solid line) and means (dashed line), dots represent outliers, and error bars represent the 95th and 5th percentiles. Significance between the treatment groups was determined by the t test; p < 0.05 and as indicated. b Gene expression analysis presented as quotient of gene induction after 12 weeks of supplementation with carotenoid-rich TNC or placebo (visit 8) divided by gene induction before supplementation (visit 4) from n = 46 volunteers taking carotenoid-rich TNC and n = 48 volunteers taking placebo. Given are the quotients as box plots with medians; dots represent outliers, and error bars represent the 95th and 5th percentiles. Significance between the treatment groups was determined by the Mann-Whitney rank sum test; p < 0.05 and as indicated. ns, not significant.
crease in the erythema index $D_{30}$ in the tomato paste-consum- 
mined by expert grading in all three studies. Interestingly, 
ingard, we must keep in mind that the MED was deter- 
we observed a significant difference between the two 
treatment groups ($n = 71$ for carotenoid-rich TNC, and 
As expected, the change of erythema $\Delta a^*$ after supplementation with carotenoid-rich TNC de- 
This can be seen when we use the difference $\Delta a^*$ of the 
baseline. In case of the carot- 
MED, while the placebo group of ten people having 10 g 
olyve oil did not [35]. Similarly, comparing the efficacy 
of a 12-week intake of either synthetic lycopene, a tomato 
e extract (Lyc-o-Mato), or a drink containing solubilized 
Lyc-o-Mato (Lyc-o-Guard-Drink) in a parallel-group de- 
n ($n = 36$) indicated a significantly decreased $\Delta a^*$ 24 h after a 1.25 
the placebo group of ten people having 10 g 
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n ($n = 36$) indicated a significantly decreased $\Delta a^*$ 24 h after a 1.25 
1 MED irradiation in the groups taking approxi- 
mately 10 mg/day natural lycopene [19]. 

The difference to MED determination where we failed 
in finding protection by the active supplement may be 
explained by (i) a higher UV dose applied (1.25 MED in- 
stead of 1 MED) and by (ii) a higher sensitivity due to an 
jective detection device, as already shown by Rizwan et 
al. [33]. 

A change of skin color, e.g., skin tanning evaluated as 
$\Delta L*$ or $\Delta ITA$, was not observed during the study, no mat- 
ter whether analyzing the whole population or stratified 
to seasonal enrollment. The effect of carotene 
take on skin color is still under debate because contra- 
dicting results were observed from having an impact only 
on skin yellowness $b^*$ [36, 37] and or redness $a^*$ [38]. 

The lycopene-rich TNC assessed in the current study 
contained 15 mg lycopene, 5.8 mg phytoene and phyto- 
fluene, 0.8 mg $\beta$-carotene, 5.6 mg tocopherols from to- 
mato extract, and 4 mg carnosic acid from rosemary ex- 
tact per day because previous in vitro studies/animal 

studies indicated a synergistic inhibition of LPS-induced 
NO production, TNF$\alpha$, superoxide and PGE$_2$ release in 
macrophages and in a mouse model of peritonitis by ly- 
copene, $\beta$-carotene, and the phenolic carnosic acid [39]. 

Carotenoid-rich TNC is a safe and well-tolerated nu- 
tritional supplement suited for significant protection 
from (i) UVB-induced erythema formation and (ii) UVB- 
induced upregulation of IL6 and TNFa. 

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analysis of the skin biopsies and the help of the study nurses Lydie 
Weiand and June Gardner is highly appreciated.

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Statement of Ethics

Subjects have given their written informed consent. This multicenter study was approved by the local committees of all participating centers. The study was conducted according to the Declaration of Helsinki principles (2013).

Disclosure Statement

Krutmann, Moseley, and Ferguson obtained funding. The other authors have no conflicts of interest to disclose.

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The costs of conducting this study were paid by Lycored Ltd. Be’er Sheva, Israel.

Author Contributions

Drs. Marini, Grether-Beck, Jaenicke, and Krutmann had full access to all data in the study and take the responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the study concept and design. Groten, Marini, Ibbotson, Grether-Beck, Jaenicke, Moseley, Ferguson, and Krutmann were responsible for the acquisition, analysis, and interpretation of the data. Krutmann, Grether-Beck, Marini, and Groten drafted the manuscript. Ferguson, Moseley, Ibbotson, and Jaenicke made a critical revision of the manuscript for important intellectual content. Groten, Grether-Beck, Jaenicke, and Marini made the statistical analysis. All authors provided administrative, technical, or material support. Krutmann had the supervision of the study.

References