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

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Abstract

Background

Our previous double-blinded, placebo-controlled cross-over study indicated that nutritional supplement named lycopene-rich tomato nutrient complex (TNC) ca protect from UVA1-induced (340–400 nm) and UVA- (320–400 nm)/UVB-indu (280–320 nm) upregulation of molecular markers associated with oxidative stre inflammation, and ageing.

Objectives

in the current double-blind, randomized, placebo-controlled multicenter study, v 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, ar 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 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-ca 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary per day or placebo made from medium-chain triglycerides. At the end of each p MED determination, UVB irradiation, chromametry, biopsies, and blood sampl undertaken.

Results

The active supplement was well tolerated. Interestingly, no significant differenc seen in the MED between the active-supplement and placebo groups, as determi visual grading by expert assessors. Of note, the carotenoid-containing suppleme significantly protected against UVB-induced erythema formation measured as Δ after the intervention minus Δa* after the washout phase as compared to the pla Moreover, intake of the active supplement significantly protected against UVB- induced upregulation of IL6 and TNFα as compared with the intake of placebo. carotenoid plasma levels were significantly increased.

Conclusion

This well-tolerated carotenoid-containing supplement significantly protected ag UVB-induced erythema formation and upregulation of proinflammatory cytokir healthy volunteers.

Keywords: Carotenoids, Oral photoprotection, Erythema formation, UVB, Dou blind, randomized, placebo-controlled study

Introduction

The concept of oral photoprotection by antioxidant micronutrients gained very p over the last decades [1, 2]. In case of topical sunscreens, the sun protection fact simple and noninvasive tool to quantify the photoprotective activity. The latter i defined as a quotient of the minimal erythemal dose (MED) under treatment div by the MED without intervention representing a measure for acute deleterious e of UV irradiation [3]. An increased MED after intervention with a nutritional supplement can also therefore be used as an indicator of photoprotection in stud potential oral ly ingested photoprotectants. A special focus was on β-carotene, w 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 concern long-term intake of β-carotene at nonphysiological levels arose when adverse ef on the incidence of lung cancer in smokers and workers exposed to asbestos we found [5]. To circumvent this issue, other carotenoids such as lycopene or lutein into focus. Recently, a double-blinded, placebo-controlled cross-over study has that both these ingredients provided significant protection against UVA- or UVA/UVB-induced upregulation of UV-inducible markers such as heme oxyge intercellular adhesion molecule 1, and matrix-metalloproteinase 1 [6]. Some limitations of that study were (i) the relatively low number of volunteers include (ii) the lack of showing an effect on the physiological erythemal skin response a intervention with the active supplement. In order to complement and strengthen results of that study, we therefore performed a double-blind, randomized, placeb controlled, multicenter follow-up study to assess as primary objective the effect carotenoid-rich tomato nutrient complex (TNC; containing and rosemary extrac the acute UV radiation-mediated erythemal skin response; i.e., (i) MED and (ii) intensity of erythema, compared with the placebo cohort following 12 weeks of daily oral supplement. As secondary objective, we wanted to evaluate the effect carotenoid-rich TNC compared to that of placebo after 12 weeks of b.i.d. (twice supplementation on skin biopsy biomarkers indicative for immunosuppression c inflammation. Analysis of UV-induced gene expression was very helpful to add photodamage to human skin in vivo in the previous study [6]. With regards to U IL1α was shown to be upregulated in keratinocytes in vitro and in vivo [7, 8]. IL was shown to be induced in keratinocytes in vitro and in human skin in vivo, wh exerts profound immunosuppressive activities [9]. Proinflammatory cytokines s TNFα and IL6 are induced upon UVB treatment in keratinocytes and in humans

mRNA and protein level [10, 11]. For a detailed review on UVB-induced cytoki production by keratinocytes or Langerhans cells, see [12]. The listed proinflamn cytokines act in a cascade fashion to induce inflammation with initial release by keratinocytes or inflammatory cells in the skin and subsequent synergizing with irradiated keratinocytes to further increase their cytokine production finally affe local and systemic immunosurveillance [12, 13].

Materials and Methods

Materials

Carotenoid-rich TNC soft gel capsules contained 7.5 mg lycopene, 2.9 mg phyto and phytofluene, 0.4 mg β-carotene, 2.8 mg tocopherols from tomato extract, an carnosic acid from rosemary extract per capsule (2 capsules per daily dose). The placebo was packaged in an identically looking soft gel capsule and consisted o medium-chain triglycerides. Both treatments were obtained from Lycored Ltd., Sheva, Israel.

Study Design

This study was approved by the local Ethics Committees of (i) the Heinrich Hei University, Düsseldorf, Germany (reference No. 4194), and (ii) the University o Dundee, Scotland, UK (No. SC015096). It was conducted at the IUF - Leibniz- Research Institute for Environmental Medicine, Düsseldorf, Germany, and at th Photobiology Unit, Department of Dermatology, Ninewells Hospital and Medic School, Dundee, UK, according to the ethical rules stated in the principles of the Declaration of Helsinki and the International Council for Harmonisation of Tech Requirements for Pharmaceuticals for Human Use. We conducted a randomized double-blind, placebo-controlled, parallel-group, multicenter clinical trial in wh each subject was randomized to receive carotenoid-rich TNC or placebo for a pe 12 weeks in a parallel-group design. A total of 149 subjects (female or male) we enrolled into the study, and 145 subjects completed the trial as planned. The trea groups were stratified based on the age category, gender, and smoking to ensure distribution of such population within the two treatment arms (Table 1). The gro taking the active supplement included 75 volunteers (56 female, 19 male), and t 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 4(

years). The age of the volunteers ranged from 20 to 50 years. Current smokers v very rare; in the carotenoid-rich TNC group, there were 5 smokers (7%), and in placebo group 3 (4%). For ethical reasons, only two-thirds of the volunteers wer asked for biopsies to be analyzed for expression of molecular markers. The subj enrolled were distributed according to an online block randomization service by sponsor; block size was 2, allocation ratio 1: 1. Volunteers and investigators we unaware of the treatment.

Table 1

Baseline demographics and characteristics

	Carotenoid-rich TNC	**Placebo**
Participants, n	75	74
Females, *n* (%)	56 (75)	59 (80)
Males, *n* (%)	19 (25)	15 (20)
Mean age (SD), years	40.9 (10.3)	40.9 (10.1)
Mean BMI (SD)	25.8 (2.8)	25.3 (3.0)
Mean initial lycopene level, n M	676	710
Median initial lycopene level, n M	646	670
Mean initial phytofluene level, n M	65	80
Median initial phytofluene level, n M	51	55
Mean initial phytoene level, n M	54	46
Median initial phytoene level, n M	50	29

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 a willingness to follow a lycopene- and antioxidant-restricted diet was requested. Exclusion criteria were sunbed use and pregnancy. The study was conducted be January 2014 and September 2016.

Determination

The MED was determined with a dermalight® 80 MED tester (Dr. Hönle, Gilch Germany) in the broadband UVB range on visits 2 and 7 on both sides of the bu by visual grading undertaken by expert assessors. The dermalight 80 MED teste serves for the definition of the MED according to the choice of the light source a skin type. The device contains a foil with 10 different test fields. The varying do in the test fields is caused by varying sizes of outlets and therefore varying UV irradiation.

Chromametry

MED was read 24 h after MED testing. Directly after the MED reading, a dose o MED was applied on an additional field (12 × 12 mm) of the buttock for skin co determination by chromametry (Chromameter CR400; Konica Minolta Sensing Europe B.V., Bremen, Germany) and for taking biopsies with a dermalight 80 M tester (Dr. Hönle). The erythema formation was measured as change of skin col which reflects the redness of the skin according to the Commission Internationa l'Eclairage, as evaluated in [14]. We followed the recommended guidelines for s color determination [15]. In this regard, color is expressed in a device-independ three-dimensional color space reflected by the variables L*, b*, and a*. The latt values represent green in the negative direction and red in the positive direction. dose of 1.25 MED was selected according to prior experience [16].

Assessment of Blood Samples

Blood samples for carotenoid determination were collected after a 5-week wash phase, at randomization, after 4 weeks of treatment, and at the end of the study i heparinized tubes and immediately centrifuged. The plasma was stored at −80°C analysis for carotenoids such as lycopene, α-, and β-carotene by high-performan

liquid chromatography, as previously described [6, 17, 18]. In addition, the colo
carotenoids phytofluene and phytoene were measured because these precursors
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 (F
1b), snap frozen in liquid nitrogen and stored at −80°C until further analysis as,
previously described [21, 22, 23]. For the evaluation of UV-induced gene expre
the $2^{(-\Delta\Delta c(t))}$ method is used [24]. Gene expression is indicated as x-fold inductic
versus an unirradiated control. Primer pairs were as follows:

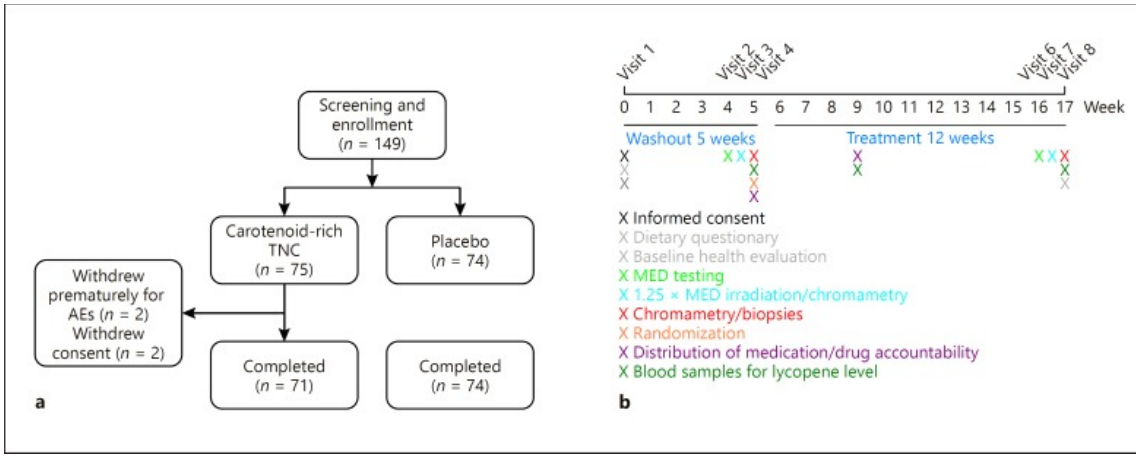

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Fig. 1

Design of the study. **a** Overall trial design giving the number of volunteers per protoc
and the corresponding dropouts. AEs, adverse events. **b** Details for both arms of the
double-blind, randomized, placebo-controlled, multicenter study as performed in eac
the centers. Shown are only those visits at which data were collected.

IL1α (forward 5′-TGTATGGACTGCCCAAGATGAA-3′, reverse 5′
-ACTACCTGTGATGGTTTTGGGTATC-3′, NM_ 000575.4) [25]; IL6 (forwa
-CCTCGAGCCCACCGGGAACG-3′, reverse 5′-AA-CTGGACCGAAGGCG-
CTTGTG-3′, NM_ 000600.4) [26]; IL10 (forward 5′-AAGACCCAGACATC-A

GGCG-3′, reverse 5′-AATCGATGACAGCGCCGTAG-3′, NM_ 000572.3) [27
TNFα (forward 5′-GGAGAAGGGTG-ACC GACTCA-3′, reverse 5′
-TGCCCAGACTCGGCAAAG-3′, NM_ 000594.3) [28]; and 18S rRNA as
housekeeping gene (forward 5′-GCCGCTAGAGGTGAAATTCTTG-3′, reverse
-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 formulations (Lyc-O-Mato and Lyc-O-Gua drink) for the primary endpoint "protection against ultraviolet (UV) light-induce erythema," such as [19, 30, 31]. The sample size corresponds to an effect size difference at 12 weeks between the two groups, with a power of 80% and a drop rate of 10%.

Data management and randomization were done by Medistat Ltd. (Tel-Aviv, Isr Additional studies were performed in-house with the help of SigmaPlot (version 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 Whitney rank sum tests were performed.

Results and Discussion

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

The treatment was safe and well tolerated, and no serious adverse events were observed within the two study populations. Only one severe adverse event diagn as pityriasis rosea occurred in the group with carotenoid-rich TNC intake. In add one volunteer in the same group discontinued the study due to mild eczema. Compliance was good in both arms, which was reflected by the carotenoid level determined at the end of the 5-week washout phase (visit 2), after 4 weeks of int (visit 5), and at the end of the 12 weeks of intake (visit 8) (Fig. 1b, 2a–c) from b samples. In detail, after 5 weeks of diet restriction, the starting median (25th per –75th percentile) lycopene level was 670 nM (480–919) for the group to take th placebo, and 646 nM (488–890) for the group to take the carotenoid-rich TNC. I 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 rea

final median level of 1,153 nM (847–1,551), whereas no such increase was seen placebo group. There, median levels of 690 nM (472–1,017) after 4 weeks and 7 (445–1,040) after 12 weeks were detected.

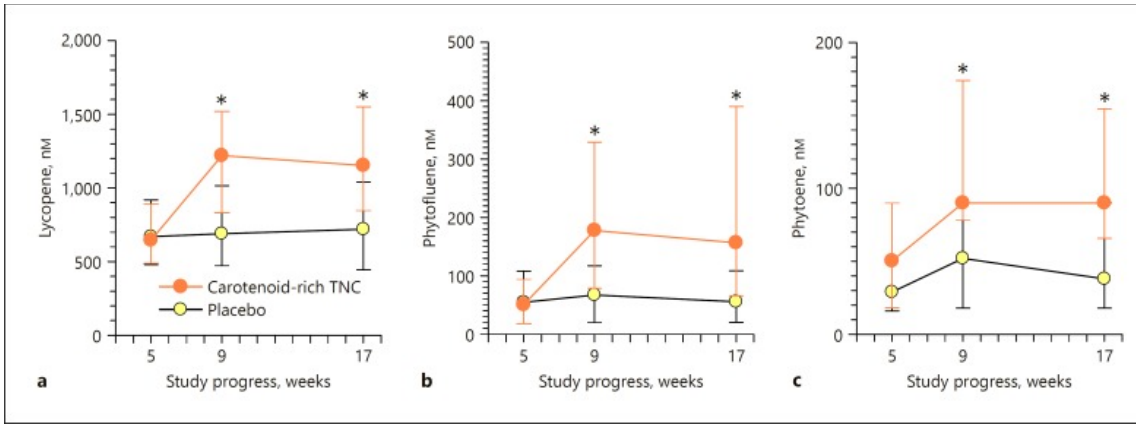

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Fig. 2

Compliance reflected by carotenoid blood levels. Lycopene (**a**) phytofluene (**b**), and phytoene (**c**) content in blood samples taken at the indicated time points was determi 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, * $p < 0.05$ vers week 5 (beginning of supplementation).

Similarly, the colorless carotenoid precursor phytofluene significantly increased carotenoid-rich TNC arm over time. Median phytofluene was 51 nM (18–94) at end of the washout phase and increased to 178 nM (79–329) and to 156 nM (66- during the supplementation phase in the active arm. In contrast, in the placebo p the study, phytofluene was 55 nM (18–108) in the depletion phase, which did nc significantly change during the intake phase to 67 nM (20–117) and 55 nM (20– (Fig. 2b).

Finally, the colorless carotenoid precursor phytoene also significantly increased the intake phase of the active arm (Fig. 2c). Median phytoene was 50 nM (18–9(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 p 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 : (18–90).

Whether the determination of the nutritional supplements such as lycopene from samples is a good indicator for compliance and/or bioavailability should be reconsidered because a significant increase in the corresponding plasma levels n always correlates to a significant photoprotection [19]. In this regard, it may be interesting that cutaneous lycopene can also be determined noninvasively by Ra spectroscopy [32], where gender-related differences in basal carotene and lycop levels have recently been observed.

The first primary outcome, the MED reading, was identical in both intended trea groups, given as a median (min, max) of 0.080 (0.035, 0.125) after the washout 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 lycoper tomato paste to a control group (n = 8) taking olive oil [33] and in a comparativ week study, where 10 females consumed either a pill or tomato paste correspon 16 mg lycopene [34]. In this regard, we must keep in mind that the MED was determined by expert grading in all three studies. Interestingly, Rizwan et al. [3 nevertheless observed a significant increase in the erythema index D_{30} in the tor paste-consuming group employing a reflectance instrument as compared to the s conditions.

The second primary outcome was the change of erythema Δa* determined chromametrically (Fig. 3a). Here, we observed a significant difference between two treatment groups (n = 71 for carotenoid-rich TNC, and n = 74 for placebo). expected, the change of erythema a* after supplementation with carotenoid-rich decreased less than after supplementation with placebo. This can be seen when the difference Δa* of the follow-up minus Δa* of the baseline. In case of the

carotenoid-rich TNC, the response under treatment is lower, and we get a differ
below zero. In case of the placebo, the erythema responses after supplementatio
not lower, and therefore the difference (follow-up minus baseline) is not below
These results corroborate a previous study where a group of nine volunteers eati
tomato paste corresponding to 16 mg lycopene, 0.5 mg β-carotene, and 0.1 mg l
over a 10-week period presented with a significantly decreased Δa* 24 h after 1
MED, while the placebo group of ten people having 10 g of olive oil did not [35
Similarly, comparing the efficacy of a 12-week intake of either synthetic lycope
tomato extract (Lyc-o-Mato), or a drink containing solubilized Lyc-o-Mato (Lyc
Guard-Drink) in a parallel-group design (n = 36) indicated a significantly decrea
Δa* 24 h after a 1.25 MED irradiation in the groups taking approximately 10 mg
natural lycopene [19].

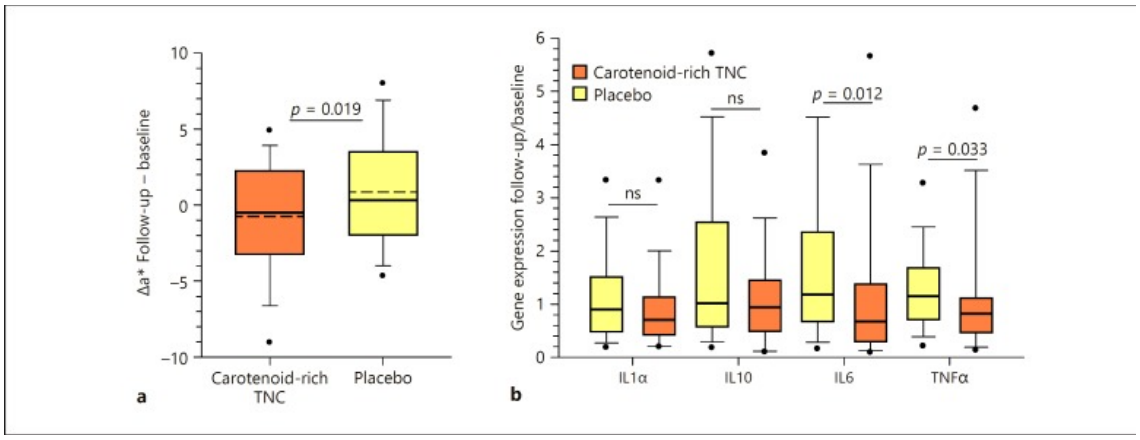

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Fig. 3

Effect of carotenoid-rich TNC on erythema formation and gene expression. **a** Change 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 level 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. 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.

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

A change of skin color, e.g., skin tanning evaluated as ΔL* or ΔITA, was not ob during the study, no matter whether analyzing the whole population or stratified according to seasonal enrollment. The effect of carotene intake on skin color is s under debate because contradicting results were observed from having an impac on skin yellowness b* [36, 37] and or redness a* [38].

The lycopene-rich TNC assessed in the current study contained 15 mg lycopene mg phytoene and phytofluene, 0.8 mg β-carotene, 5.6 mg tocopherols from toma extract, and 4 mg carnosic acid from rosemary extract per day because previous vitro studies/animal studies indicated a synergistic inhibition of LPS-induced NO production, TNFα-, superoxide and PGE_2 release in macrophages and in a mous model of peritonitis by lycopene, β-carotene, and the phenolic carnosic acid [39 Similarly, synergistic inhibition of oxidative stress in a mouse tumor model [40] inhibition of androgen signaling and PSA secretion by lycopene and tocopherol was determined. In addition, the synergistic effect of a TNC and carnosic acid (f rosemary) has recently been observed on inhibition of UVB-induced IL6 secreti keratinocytes as compared to the single interventions (Calniquer et al., unpublis data).

As secondary outcome, we determined UV-induced gene upregulation of marke associated with immunosuppression or inflammation in biopsies taken at visits 4 For ethical reasons, we did not take biopsies from all volunteers. Similarly, as fo erythema data Δa* (Fig. 2b), we further processed the relative gene expression c building a quotient of induction after supplementation divided by induction befo supplementation (Fig. 2c). For the markers IL-1α and IL10, we did not see a significant improvement by supplementation with carotenoid-rich TNC. In contr we saw a significant decrease in the UV response after carotenoid-rich TNC inta compared to the placebo treatment. Assessment of molecular markers might fos sensitivity of such studies as shown by a significantly decreased expression of U induced matrix metalloproteinase 1 postsupplementation in the lycopene-taking as compared to the olive oil-taking group [33] or in our previous study [6].

Finally, it should be noted that the daily amount of lycopene used in the current corresponds to 52 g canned tomato paste and that of β-carotene to 90 g [42]. Th amount of vitamin E would be obtained by a daily intake of 130 g of canned ton paste [42].

Conclusion

Carotenoid-rich TNC is a safe and well-tolerated nutritional supplement suited f significant protection from (i) UVB-induced erythema formation and (ii) UVB-induced upregulation of IL6 and TNFα.

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 cor 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.

Funding Sources

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

Author Contributions

Drs. Marini, Grether-Beck, Jaenicke, and Krutmann had full access to all data ir study and take the responsibility for the integrity of the data and the accuracy of data analysis.

All authors contributed to the study concept and design.

Groten, Marini, Ibbotson, Grether-Beck, Jaenicke, Moseley, Ferguson, and Krut 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 manu 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.

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