Orally administered mixed carotenoids protect human skin against ultraviolet A-induced skin pigmentation: A double-blind, placebo-controlled, randomized clinical trial

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Abstract
Background: Photoprotection of human skin is determined as the capacity of sunscreens to prevent ultraviolet (UV) B radiation-induced erythema and UVA radiation-induced pigmentation. It is unequivocal that, in addition to sunscreens, oral supplementation with carotenoids can protect human skin against UVB radiation-induced erythema. It is not known if this is also the case for UVA radiation-induced pigmentation.

Objective: To clinically evaluate the photoprotective effects of daily supplementation with carotenoids against UVA radiation-induced pigmentation.

Methods: In this double-blind, placebo-controlled trial, 60 subjects (Fitzpatrick types II-IV) were randomized to receive Nutrilite™ Multi Carotene supplement or placebo for 12 weeks. UVB-induced minimal erythemal dose (MED), UVA-induced minimal persistent pigmentation dose (MPPD) and skin carotenoid levels were measured at baseline, 4, 8, and 12 weeks of intervention. Skin color was evaluated by expert clinical graders and by colorimetry. Carotenoid levels in the skin were measured by the Biozoom® device.

Results: In the intervention group, a significant increase in comparison with the placebo group was observed in (a) skin carotenoid levels, (b) UVB-induced MED, and (c) UVA-induced MPPD values obtained by colorimetry.

Conclusion: Daily supplementation with carotenoids protects human skin against both UVB-induced erythema and UVA-induced pigmentation.

Keywords
carotenoids, oxidative stress, photoprotection, skin pigmentation, ultraviolet radiation
1 | INTRODUCTION

Both shorter wavelengths ultraviolet B (290-320 nm) as well as longer wavelengths UVA (320-400 nm) radiation can damage human skin and as a consequence it is now generally accepted that photoprotection of human skin must include protection against UVB as well as UVA rays. Accordingly, the efficacy of sunscreens is usually determined clinically as their capacity to protect against UVB-induced erythema and UVA radiation-induced skin pigmentation.1,2 In addition to topically applied sunscreens, dietary supplementation of carotenoids like β-carotene,3,4 lycopene,5–7 lutein,7,8 astaxanthin,9 and mixed carotenoids3,10 can provide photoprotection of human skin. Numerous studies have been conducted in the past to assess the capacity of oral carotenoid intake to reduce UVB-induced erythema. As a result, it is now unequivocal that dietary supplementation with carotenoids is effective in protecting human skin against UVB-induced erythema. However, very little is known about the capacity of orally administered carotenoids to protect human skin against UVA radiation. Although there is some directional evidence that the photoprotective effects of carotenoids extend from UVB to UVA radiation,7,11 to the best of our knowledge, the photoprotective effects of carotenoids against UVA-induced pigmentation have not yet been studied. In this study, we, therefore, intended to expand our understanding of the well-known photoprotective effect of mixed carotenoids against UVB to UVA radiation by using Nutrilite™ Multi Carotene softgel as a dietary intervention for 12 weeks.

2 | METHODS

2.1 | Study design

This randomized, double-blind, placebo-controlled, parallel group trial with rolling enrollment was designed to evaluate the photoprotective effects of an orally administered mixed carotenoid supplement (Nutrilite™ Multi Carotene) on UVA-induced pigmentation in healthy adults. In addition, we studied the effects of carotenoid intake on skin carotenoid levels as well as on UVB-induced erythema. This was a single-site study, and all data were collected at the IUF—Leibniz Research Institute for Environmental Medicine in Düsseldorf, Germany. The trial started in April 2018 and ended in December 2018. The study consisted of a 3-week run-in (washout) period followed by an intervention of oral supplementation for 12 weeks. Subjects visited the clinical site 10 times during the study. At each visit, subjects were asked not to change their regular diet and not to use food supplements or additives. This study was reviewed and approved by the local medical ethics committee (Ethikkommission der Med. Fakultät an der H.-Heine-Universität) and was conducted in compliance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects, guidelines of the ICH (International Conference on Harmonization) and national principles of Good Clinical Practice (GCP) for the protection of the rights and safety of the subjects.

2.2 | Study population

Only generally healthy subjects were enrolled. To be eligible for the study, subjects had to meet all of the following inclusion criteria: age 20-40 years (inclusive), not pregnant or breastfeeding, Fitzpatrick skin types II-IV, no use of sunbeds for at least three months prior to recruitment into the study and willing to refrain from sunbathing or tanning for the entire duration of the study, normal eating habits (3 meals/d), no use of dietary supplements (eg herbal, vitamin) from the beginning of the study at the screening visit and throughout the study (see Table S1). Subjects following any special diet, including vegetarian, vegan, or high protein, were also excluded from the study.

2.3 | Recruitment and randomization of participants

A total of 64 subjects were screened for the study, and 60 subjects were enrolled. Informed consent of the subjects was obtained by the investigator prior to inclusion of the subject to the clinical investigation. Subjects were enrolled only if all inclusion criteria were met. After the 3-week run-in (washout) period, subjects were randomized to receive either the mixed carotenoid supplement or placebo capsule. Randomization was done by an independent entity. The subjects were randomized to one of two treatment groups (‘O’ or ‘E’) and the result recorded. Randomization was blocked, using random permuted blocks to ensure that the groups are balanced periodically. The chosen block size was four. The study was performed under complete double-blind conditions. To maintain blinding of the subject, study products were provided in standard treatment containers. The subject screening number was entered on the label at the time the study product was dispensed. Neither the subject nor the investigator/staff knew the composition of the dispensed product.

2.4 | Interventions

Study intervention was either the multi-carotenoid supplement or placebo. The multi-carotenoid supplement was taken three times daily and contained β-carotene 4.25 mg, α-carotene 1.10 mg, lutein 1.12 mg, and zeaxanthin 0.053 mg per softgel. Inert ingredients found in the formula include vegetable oil (olive oil, palm oil, and soybean oil), gelatin, glycerol, yellow beeswax, soy lecithin, sulfite ammonium caramel. The placebo composition was identical to Nutrilite™ Multi Carotene supplement but did not contain any active ingredients. The subjects were instructed to take the supplement with a meal with water each day.

2.5 | Outcome measurements

The primary outcomes investigated include measuring minimal persistent pigmentation dose (MPPD), Minimal Erythema Dose (MED),
and skin antioxidant levels. These outcomes were determined at baseline and after 4, 8, and 12 weeks of treatment. Skin color was evaluated 24 hours after irradiation exposure by colorimetry using the three-dimensional color system.

2.5.1 MPPD

The MPPD of each volunteer was assessed to determine the sensitivity to UVA irradiation. Significant increase in MPPD indicates increased protection against UVA-induced skin pigmentation. The minimum PPD dose is defined as the smallest UVA dose required to produce brown pigmentation with distinct borders. MPPD was determined by visual grading 20-24 hours after irradiation with a Dermalight 80 MPD Tester (Dr K. Hönle GmbH) and by colorimetry. The subjects were treated with scaled irradiation of UVA ranging from 1 to 20 J/cm² using the area from the mid to lower back. The peak wavelength for UVA irradiation was 370 nm. Skin pigmentation was measured by non-invasive technique with a Minolta CR-300 colorimeter. The individual typology angle (ITA°) was calculated using the following equation: ITA° = \[\text{arctan}\left(\frac{L^* - 50}{b^*}\right) \times 180/\pi\], where \(L^*\) is the luminance parameter (from dark to light) and \(b^*\) is chrominance parameter (blue-to-yellow spectrum). The ITA characterizes the melanic level of the skin and a decrease in ITA indicates increased pigmentation. For the analysis, to quantify color differences, ITA° was defined as the changes between the irradiated fields and a non-irradiated area.

2.5.2 MED

The MED of each volunteer was assessed to determine the sensitivity to UVB irradiation. MED is the minimal amount of energy required to induce visible erythema, which can be defined as a uniform, clearly demarcated redness at 16-24 hours after UV exposure. For this study, MED was determined by visual grading 20-24 hours after irradiation with a Dermalight 80 MED Tester (Dr K. Hönle GmbH) and by colorimetry using a Minolta CR-300 colorimeter. The subjects were treated with a 10-step UVB radiation scale using an area of the back between the scapula line and the waist. The peak wavelength for UVB irradiation was 311 nm (narrowband). \(\Delta a^*\) was defined as the changes between the irradiated fields and a non-irradiated area.

2.5.3 Skin carotenoid level

The skin carotenoid level of each volunteer was assessed using a manual reflectance spectroscopy-based device produced by Biozoom Services GmbH, on the thenar eminence of the palm of the hand. This device measures the presence of carotenoids in the skin. The Biozoom® scanner uses Multiple Spatially Resolved Reflection Spectroscopy (MSRRS) to precisely measure the carotenoid level, operating in the wavelength range from 440 to 490 nm. For the analysis, \(\Delta AL\) was defined as the changes within the visits before and after supplementation.

2.6 Sample size

A formal sample size calculation was conducted for this study using the designated software tool G*Power 3.1.9.2 for Windows. The statistical analysis for the study was planned to be done on the means of difference between two independent means (two groups) using the t test. For the sample size calculation, we used available outcome data from several previously concluded studies of similar layout. The sample size analysis was done for the study primary endpoint. 60 subjects (30 in each group) were required to meet the statistically determined power analysis.

2.7 Statistical analysis

To compare the assessment between the visits, the Student’s t test was used. All statistical tests were considered statistically significant if the P-value was less than or equal to .05. The differences between the visits were computed by subtracting point 2 (after baseline/supplementation) from point 1 (before baseline/supplementation).
RESULTS

The study was completed by all subjects (n = 29) in the placebo group whereas two subjects in the intervention group (n = 31) did not complete the study. Overall, 58 subjects completed the trial which still provided good statistical power for the study. In the intervention group, one subject withdrew prematurely due to adverse events before visit 5 (reason not related to intervention), and one subject withdrew due to pregnancy before visit 7. The study was deemed safe and was well tolerated by subjects. Figure 1 represents the CONSORT (Consolidated Standards of Reporting Trials) diagram which describes the enrollment of subjects, allocation of intervention, follow-up, and data analysis.

3.1 | Effect on minimal persistent pigmentation dose (MPPD)

The skin sensitivity to UVA irradiation is generally assessed in terms of MPPD. An increase in MPPD indicates increased skin protection against UVA radiation. The visual evaluation of MPPD for the placebo and intervention groups during the study period is summarized in Figure 2A. The mean values of MPPD for the intervention group directionally increased from baseline (15.19 ± 1.49) till the completion of study (15.50 ± 1.55) and decreased for the placebo group, but these changes were not statistically significant. Figure 2B summarizes the mean value of ΔITA° for the placebo and intervention groups during the study period. For the intervention group, the mean ΔITA° values showed significant increase after 8 weeks ($P = .000$) and 12 weeks ($P = .000$) relative to the placebo group and were also significantly higher at 12 weeks ($P = .000$) in comparison to the intra-group baseline before the start of the intervention. Thus, the quantitative assessment of skin pigmentation by chromametry indicated that the multi-carotenoid supplementation, but not the placebo, provided significant skin protection against UVA irradiation at 8 and 12 weeks.

3.2 | Effect on minimal erythemal dose (MED)

The skin sensitivity to UVB irradiation is usually measured in terms of MED. The visual evaluation of MED for the placebo and intervention groups during the study period is summarized in Figure 3A. For the placebo group, no statistically significant changes were observed between the start and completion, or between visits during the study. For the intervention group, significant differences were observed at 8 weeks ($P = .002$) and 12 weeks ($P = .000$) relative to the placebo group, and at 12 weeks ($P = .000$) relative to the baseline of the intervention group. Figure 3B shows the mean value of
Δα* for the placebo and intervention groups during the study period. For the placebo group, no statistically significant changes were observed between the start and completion, or between visits during the study. For the intervention group, significant decrease in the Δα* values were observed at 4 weeks (P = .000), 8 weeks (P = .001), and 12 weeks (P = .000) relative to the placebo group, and at 4 weeks (P = .000), 8 weeks (P = .000), and 12 weeks (P = .000) relative to the baseline of the intervention group. Since the Δα* values at the baseline showed significant difference between the placebo and intervention group, the values were normalized to zero for comparison which is represented by Figure 3C. In line with Figure 3B, significant differences were observed between the placebo and intervention group at 4, 8, and 12 weeks, indicating a significant increase in skin photoprotection against UVB irradiation.

3.3 | Effect on carotenoid levels in skin

The carotenoid levels in skin assessed after the washout period (baseline) and after 4, 8, and 12 weeks of placebo-controlled
The intervention are depicted in Figure 4. The ΔAL value, defined as the changes in the skin carotenoid levels before and after supplementation showed statistically significant increases at 4, 8, and 12 weeks for the treatment group, whereas there was no significant change in the placebo group.

### DISCUSSION

In this randomized, placebo-controlled, double-blinded study, we assessed the photoprotective effects of Nutrilite™ Multi Carotene supplementation against UVA radiation-induced skin pigmentation. We found that daily oral intake of a carotenoid mixture, but not of placebo, protected human skin against UVA radiation, because higher UVA doses were required to induce skin pigmentation as measured by determining MPPD. This increase in MPPD values was significant, if skin pigmentation was determined by chromametry. If MPPD was visually evaluated, this increase was seen by trend, indicating that for this purpose chromametry is a more sensitive method. Our results are in line with a previous study in which oral intake of carotenoids was found to decrease UVA radiation sensitivity of human skin at a molecular level. The current study corroborates and extends these observations and provides the first clinical evidence that oral intake of carotenoids can protect against UVA radiation.

In our study, oral intake of carotenoids also protected human skin against UVB radiation-induced erythema, thus confirming previous studies. Currently, it is not known if our intervention reduces UVB-induced DNA damage or if the reduction in MED is due to other carotenoid effects. The precise mechanisms which are responsible for photoprotection of human skin by orally administered carotenoids are currently not known. In this regard we here show that nutritional supplementation with carotenoids, but not with placebo, is associated with a significant increase in carotenoid levels in human skin. Given the fact that carotenoids are well-known antioxidants, we propose that oral intake of carotenoids causes an increase in skin carotenoid levels and thereby in the antioxidant capacity of human skin and that the latter might be at least partially responsible for the observed increase in photoprotection against UVB and UVA. In keeping with this concept both UVA radiation, and to a lesser extent UVB radiation, exert their biological effects on human skin through initiating oxidative stress responses.

The estimated intake of carotenoids can vary widely from 5.42 to 15.44 mg/d based on dietary habits, biological differences across individuals, regional & cultural differences, and seasonal or cultivar plant variations. It is also noteworthy to mention that the data collection of dietary intake information is complex due to method imprecision and discrepancies among food composition databases. Based on the epidemiological data and clinical studies, Toti et al. suggested that the recommended intake range for individual carotenoids are 2-4.8 mg/d for β-carotene, 10-20 mg/d for lutein, and 5.7-15 mg/d for lycopene. Thus, the recommended dose for total carotenoids (by calculation) fall in the range of 17.7-39.8 mg/d. The total dose of multi-carotenoids in this intervention study was 19.57 mg/d and falls within the recommend dose for total carotenoids.

In summary, we here provide the first clinical evidence that oral intake of carotenoids can protect human skin against UVA radiation. Notably, this effect was observed in healthy subjects who were not following an extensive dietary restriction, but instead were allowed to keep their normal dietary habits. We, therefore, believe that the carotenoid-based intervention described in this study might be of relevance for the general population. Also, increased skin pigmentation due to solar radiation exposure is a cosmetic concern to a large part of the world’s population. Future clinical work is needed to further address if and to what extent nutritional supplementation with carotenoids can mitigate or prevent clinical consequences of UVA irradiation beyond skin pigmentation, such as photoaging and photocarcinogenesis.

### CONFLICTS OF INTEREST

The authors SMB, AEK, JL, MM, and KG are employees of Amway Corporation with commercial offerings in nutrition and wellness.
space. JK serves as a consultant to Amway Corporation and IUF has received funding from Amway Corporation to carry out the clinical study described in this manuscript.

IRB APPROVAL STATUS
The study protocol was reviewed and approved by Local Ethical Committee in Düsseldorf (Study code 6220R).

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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