Carotenoid Supplementation Reduces Erythema in Human Skin After Simulated Solar Radiation Exposure (44476)

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Abstract. **Excessive exposure to solar radiation, especially ultraviolet A (UVA: 320–400 nm) and ultraviolet B (UVB: 290–320 nm) radiation, may induce UV-carcinogenesis and erythema in the skin. Although the protective effects of carotenoids against skin lesions are still unclear, β-carotene has been proposed as an oral sun protectant. The purpose of this study was to determine the magnitude of the protective effects of oral α- and β-carotene supplementation for 24 weeks on UVA- and UVB-induced erythema in humans. While being exposed to UVA and UVB radiation, 22 subjects (11 men and 11 women) were supplemented with natural carotenoids for 24 weeks. Each day for the first 8 weeks, subjects were given 30 mg of natural carotenoids containing 29.4 mg of β-carotene, 0.36 mg of α-carotene, and traces of other carotenoids in vegetable oil. The natural carotenoid dose was progressively raised by 30-mg increments, at every 8 weeks, from 30 mg to 90 mg. Small areas (1 cm^2) of the skin were exposed to increasing doses of UV light (16–42 mJ/cm^2) to determine the minimal erythema dose (MED). MED was defined as a uniform pink color with well-defined borders. MED readings were obtained by visual inspection 24 hr postirradiation. Blood samples taken during supplementation were used to determine α- and β-carotene serum levels and for a lipid peroxidation analysis. During natural carotenoid supplementation, the MED of solar simulator radiation increased significantly ($P < 0.05$). After 24 weeks of supplementation, serum β-carotene levels were increased from 0.22 μg/ml (95% CI; 0.16–0.27) to 1.72 μg/ml (95% CI;1.61–1.83). Similarly, α-carotene serum levels increased from 0.07 μg/ml (95% CI;0.048–0.092) to 0.36 μg/ml (95% CI; 0.32–0.40). Serum lipid peroxidation was significantly ($P < 0.05$) inhibited in a dose-dependent manner during natural carotenoid supplementation. The present data suggest that supplementation with natural carotenoids may partially protect human skin from UVA- and UVB-induced erythema, although the magnitude of the protective effect is modest.**

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Most sun-induced erythema results from UVB (290–320 nm) and UVA (320–400 nm) irradiation (1). Susceptibility to solar erythema varies from individual to individual and within the same person from one skin site to another. The variable sensitivity of human skin to UV-induced erythema appears to correlate with the presence or absence of certain biochemicals that contribute to skin color, including carotenoids, oxyhemoglobin, reduced hemoglobin, and melanin. Thus, their amount, type, and distribution allow for the categorization of human skin types. Carotenoids are easily obtained from dietary sources, such as fruits and vegetables, and have been shown to possess antioxidant properties that may reduce oxidative stresses known to cause skin damage. Therefore, carotenoids might improve the resistance of human skin to sun-induced erythema.

The protective activity of β-carotene against photosensitization was first demonstrated in mice injected with hematoporphyrin (2). β-Carotene supplements have been used

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as oral sun protectants, and it is generally recommended that they be taken over a 4–6-week period prior to sun exposure (3). Recently, Garmyn *et al.* (4) reported that oral β-carotene supplementation (a single 120-mg dose or 90 mg daily for 23 days) is unlikely to modify the severity of sunburn in normal individuals to a clinically meaningful degree. However, it is worth noting that β-carotene may exert its effect on reducing the severity of sunburn only after chronic use, since its accumulation in skin takes several weeks (5). Thus, the aim of this study was to investigate the possible protective effect of oral α- and β-carotene, administered for 24 weeks, on UVA- and UVB-induced erythema in human skin.

Materials and Methods

Subjects. Twenty-two fair-skinned volunteers (11 men and 11 women) of Fitzpatrick's skin types I–IV (6) were recruited from the University of Arizona. Ten subjects were of sun-reactive skin type I, six of type II, three of type III, and three of type IV. None of the subjects used commercial sun tanning beds or acquired a suntan during the study period. Since our subjects were relatively young, only a few reported that they were receiving nutritional supplementation. At the start of the experiment, one subject was taking calcium, one was taking vitamin E, and three were taking synthetic vitamin supplements. None were using β-carotene supplements. However, to eliminate the side effects from vitamin supplements, all subjects agreed not to take any vitamin pills during the study period. All subjects ceased supplementation 2 weeks prior to starting the study. However, since we measured the second baseline after 2 weeks, the total duration was 4 weeks before they actually began the supplementation protocol. Smokers were excluded from participation since cigarette smoking may influence the uptake of carotenoids by skin (1, 7). In addition, a dietary interview was performed by means of the Arizona Food Frequency Questionnaire (AFFQ) provided by the Arizona Board of Regents (version 5: 1/96). The study was approved by the University of Arizona's Institutional Review Board, and informed written consent was obtained prior to participation.

UV Source. The UV radiation source was a 150W Xenon lamp (Halogenics Inc., Amherst, NJ) emitting a continuous spectrum of radiation beginning at 240 nm through the infrared spectrum and maximally peaking at 360 nm. A liquid filter and 1-mm Schott WG 320 filter (Schott Glass, Duryea, PA) were used to reduce UVC and visible wavelengths. A dichroic filter was used to reflect the wavelengths shorter than 400 nm. Most spectral energy was between 310–400 nm, with very similar levels throughout (Solar Light Co., Philadelphia, PA). Spectroradiometric assessment of the lamp indicated that relative emission in the UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) wavebands was 32%, 61%, and 7%, respectively. The lamp was housed in a black plastic tube with six apertures, 1-cm in diameter. Relative irradiances at the six apertures were 16, 21, 27, 32, 37, and 42 mJ/cm^2. A 3D-600 meter (Solar Light Co.) was used to measure the irradiance.

Procedure. Subject MEDs were determined at baseline. Six sites of 1-cm in diameter were irradiated for 1 min in a horizontal row on mid-buttock, avoiding the central spinal area. New sites, on the other side of the mid-buttock, were used for each irradiation to be distinct from all previous sites. UV sensitivity of participants was assessed twice during the 2-week period prior to supplementation to determine the baseline. The natural carotenoids used in the present study were isolated from the sea algae *Dunaliella salina.* Natural-carotenoid pills were obtained from the Henkel Corporation (LaGrange, Illinois). Each pill had 15 mg of natural carotenoids: 14.7 mg of β-carotene, 0.18 mg of α-carotene, 0.036 mg of zeaxanthin, 0.042 mg of cryptoxanthin, and 0.027 mg of lutein in vegetable oil packaged in gelatin. The subjects were given 30 mg/day (2 pills/day) for the first 8 weeks. The concentration was increased in 30-mg increments (2 additional pills/day), at 8-week intervals, to a final dose of 90 mg/day. Supplements were taken with meals and consumed once daily. Blood samples were taken once a week for 2 weeks prior to supplementation. In addition, blood samples were taken three times during supplementation (one on each last day of the 30 mg, 60 mg, and 90 mg natural carotenoid supplementation). Serum α- and β-carotene levels were measured by HPLC, and samples were stored at −70°C until assayed. Duration of the study was 26 weeks for all subjects.

Determination of MED. MEDs were determined on previously non-sun-exposed mid-buttock skin by using six incremental exposures of 16–42 mJ/cm^2 through 1 × 1-cm portals. After 8 weeks of supplementation with natural carotenoids, six different doses of UV light were administered concurrently to the subjects. MED was defined as a uniform pink color with well-defined borders and determined by visual inspection 24 hr postirradiation.

Serum α- and β-Carotene Levels. Serum levels of α- and β-carotene were measured by HPLC as described previously (8). Briefly, 250 μl of ethanol (containing 0.1% butylated hydroxytoluene [BHT] antioxidant) were added to a 250-μl aliquot of serum to precipitate proteins. After vortexing, samples were extracted into hexane, evaporated under nitrogen, and then redissolved in mobile phase (solvent A). Fifty μl of extractant were injected directly into the HPLC system. Separation was carried out with a 5-μm YMC C18 reversed-phase column (4.6 × 250 mm; YMC, Inc., Wilmington, NC) and detected at a wavelength of 452 nm, by use of the method of Xu *et al.* (8). The solvent system consisted of 95% solvent A and 5% solvent B and was delivered at a flow rate of 2.5 ml/min. Solvent A was acetonitrile (ACN)-tetrahydrofuran (THF) (85:15, v/v) with 250 ppm BHT and 0.05% triethylamine (TEA), and solvent B was 50 m*M* ammonium acetate in methanol with 0.05% TEA. The retention times for α- and β-carotene were 9.45 and 10.13 min, respectively. The total run time for a single analysis of sample was 13 min. Analytical quantitation was

performed by the external standard method. Extinction coefficients were used to validate spectrophotometrically the final solution concentrations. In hexane, extinction coefficients for α-carotene (at 444 nm) and β-carotene (at 452 nm) were 2800 and 2592 dl/g/cm, respectively. Standard reference material 968b (fat-soluble vitamins and cholesterol in human serum) supplied by the National Institute of Standards and Technology (NIST, Gaithersberg, MD) was used for assigning values to in-house control materials.

Lipid Peroxidation. Lipid peroxidation (LPO) in the serum was determined using K-Assay LPO-CC Assay Kit obtained from the Kamiya Biomedical Company (Seattle, WA). This method is more sensitive for measuring lipid peroxides (assay range: 2–300 nmol/ml) than conventional chemical analysis (9). Briefly, 20 μl of serum were added to 80 μl of oxidase and lipoprotein lipase solution. After a 10-min incubation at 30°C, 160 μl of MCDP (10-N-Methylcarbamoyl-3,7-dimethylamino-10H-phenothiazine) were added and incubated at 30°C for 30 min. Lipid peroxides were quantitated colormetrically at 675 nm and calculated by the equation provided by the Kamiya Biomedical Company. The LPO value was converted to percentage unit for illustration.

Statistics. Multiple comparisons were tested using the Wilcoxon rank-sum test with an adjusted significance level, 0.05 divided by the number of pairwise tests. The effect of natural carotenoid treatment on photosensitivity was assessed in this study using Student's two-tailed t test. Test statistics were considered significant at the $P < 0.05$ level and, for clarity, the 95% confidence interval for the mean value of each treatment was reported.

Results

Subjects. The average age of the 22 subjects was 26 years old for men (range 18–32) and 24 years old for women (range 20–29); there were ten subjects of sun-reactive skin type I, six of type II, three of type III, and three of type IV. Dietary analysis showed that 14 participants typically had fat-rich meals at least twice per week, 6 participants 4 times per week, and 2 participants 7 times per week. None of the subjects reported any side effects during the study period.

Determination of MED. Erythema responses from subjects of different skin types were compared using a Kruskal-Wallis test (data not shown). Minimal erythema dose responses are displayed in Table I. Prior to supplementation, 10 of 22 subjects had MED at 16 mJ/cm^2, 6 at 21 mJ/cm^2, 3 at 27 mJ/cm^2, and 3 at 32 mJ/cm^2, with a mean value of 20.9 mJ/cm^2 (95% CI = 18.9–22.8 mJ/cm^2; df = 21). No significant increase was seen after supplementation with 30 mg of natural carotenoids per day for 8 weeks. However, a slight trend was observed. Although its effect was small, there was a significant ($P < 0.05$) difference after supplementation with 60 mg natural carotenoids (mean MED = 25.3 mJ/cm^2, 95% CI = 23.1–27.4 mJ/cm^2; df = 21) compared with values obtained prior to supplementation (Table I). An increase in MED was also observed after supplementation with 90 mg natural carotenoids (mean MED = 31.7 mJ/cm^2, 95% CI = 29.6–33.7; df = 21) for 8 weeks. A dose-response relationship was noted between increasing levels of natural carotenoid administration and increasing doses of UV radiation required to achieve MED. There was a significant ($P < 0.05$) but modest difference in average MED with 90 mg natural carotenoid administration compared with that of baseline, 30 mg, and 60 mg natural carotenoid administration.

Serum α- and β-Carotene Levels. Prior to supplementation, the mean of serum β-carotene value was 0.22 μg/ml (95% CI = 0.16–0.27 μg/ml; df = 21). After supplementation with 30 mg natural carotenoids per day for 8 weeks, serum β-carotene was markedly increased to 0.96 μg/ml (95% CI = 0.79–1.12 μg/ml; df = 21). Serum β-carotene levels achieved with the 90 mg/day supplementation protocol for 8 weeks reached 1.72 μg/ml (95% CI = 1.61–1.83 μg/ml; df = 21). Thus, β-carotene levels went

Table I. Effects of Natural-Carotenoid Concentration on Erythema Response in Buttock Skin

Solar exposure (mJ/cm^2)	Minimal erythema dose (MED) Natural carotenoid (mg/day) supplementation			
	0	30	60	90
16	10[a]	4	—	—
21	6	9	10	5
27	3	6	7	9
32	3	3	4	5
37	—	—	1	3
42	—	—	—	—
MED[b]	20.9 (18.9, 22.8)	23.9 (22.2, 25.5)	25.3 (23.1, 27.4)	31.7 (29.6, 33.7)

Note. MEDs were determined based on the erythema that occurred by simulated solar radiation (16–42 mJ/cm^2). Six sites of 1 cm in diameter were irradiated for 1 min at the same time in a horizontal row on mid-buttock, avoiding the central spinal area. Readings of the MED were visually performed 24 h following irradiation.

[a] Indicates the number of the subjects whose MED occurred at the designated erythemal dose of UVA and UVB irradiation. Natural carotenoids at 30 mg/day was consumed for 8 weeks. This was followed immediately by consumption of 60 mg/day for 8 weeks and then 90 mg/day for 8 weeks.

[b] Indicates mean value (95% CI) of MED determined by 0, 30, 60, and 90 mg of natural-carotenoid supplementation.

up significantly ($P < 0.05$) at each level of supplementation. In addition, natural carotenoid supplementation significantly ($P < 0.05$) increased serum α-carotene levels compared with the baseline value, 0.07 μg/ml (95% CI:0.048–0.092 μg/ml; df = 21) (Fig. 1). However, only the 90 mg/day treatment increased serum α-carotene levels above the previous supplementation doses.

Lipid Peroxidation. β-Carotene is well known as an antioxidant. As expected, serum lipid peroxidation was significantly ($P < 0.05$) inhibited by 60 and 90 mg natural-carotenoid supplementation per day by 32% and 38%, respectively, when compared with data from the unsupplemented subjects (Fig. 2). Increasing doses of natural carotenoids further decreased oxidation.

Discussion

The present data show that a small but significant difference in MED was observed as the supplementation of natural carotenoids was increased. This observation is consistent with the findings of Mathews-Roth (10) and Gollnick *et al.* (11) in which the threshold dose of sunlight required to produce MED was found to be slightly increased with natural carotenoid supplementation. Serum carotene levels were increased from 0.22 μg/ml (95% CI = 0.16–0.27 μg/ml; df = 21) to 1.72 μg/ml (95% CI = 1.61–1.83 μg/ml; df = 21) for β-carotene and from 0.07 μg/ml (95% CI = 0.048–0.092 μg/ml; df = 21) to 0.36 μg/ml (95% CI = 0.32 to 0.40 μg/ml; df = 21) for α-carotene after 24 weeks of supplementation. Interestingly, although a high serum value was detected, we found no significant increase in MED with 30 mg of natural carotenoid supplementation (serum β-carotene levels were increased by 436% after 30 mg natural carotenoid supplementation while MED was increased only 14.4%). This suggests that long-term supplementation with mixed carotenoids containing primarily α- and β-carotene is required to affect the severity of sunburns. Serum lipid peroxide levels were measured prior to, and post supplementation since oxidative stress due to UV exposure may alter the profile of lipids in the skin, resulting in skin damage. Serum lipid peroxidation was significantly ($P < 0.05$) inhibited in a dose-dependent manner during natural carotenoid-supplementation.

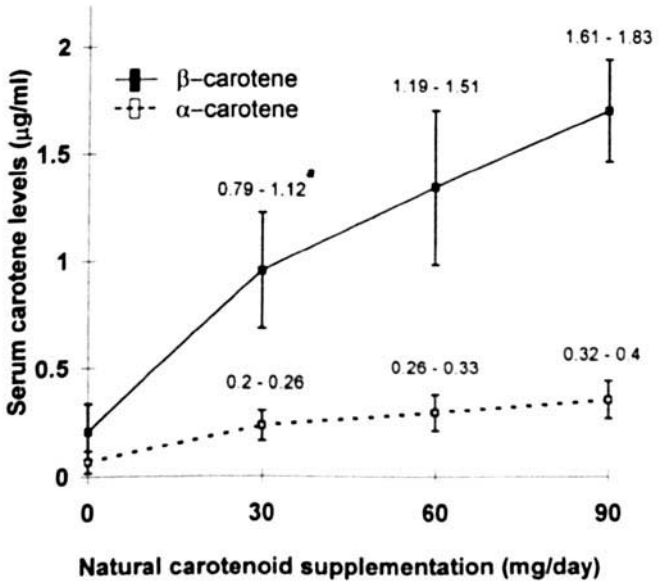

Figure 1. Serum carotene levels with dietary supplementation. α- and β-carotene levels in serum were measured by HPLC. The separation was carried out with a 5-μm YMC C18 reversed-phase column and detected at a wavelength of 444 nm for α-carotene and 452 nm for β-carotene. Data are presented as mean value and SD. *Indicates 95% CI, df = 21. The serum level at baseline (0 mg) was 0.07 μg/ml (95% CI:0.048–0.092 μg/ml) for α-carotene and 0.22 μg/ml (95% CI:0.16–0.27 μg/ml) for β-carotene. The data indicate that serum α- and β-carotene levels with supplementation were significantly increased with supplementation compared to each baseline.

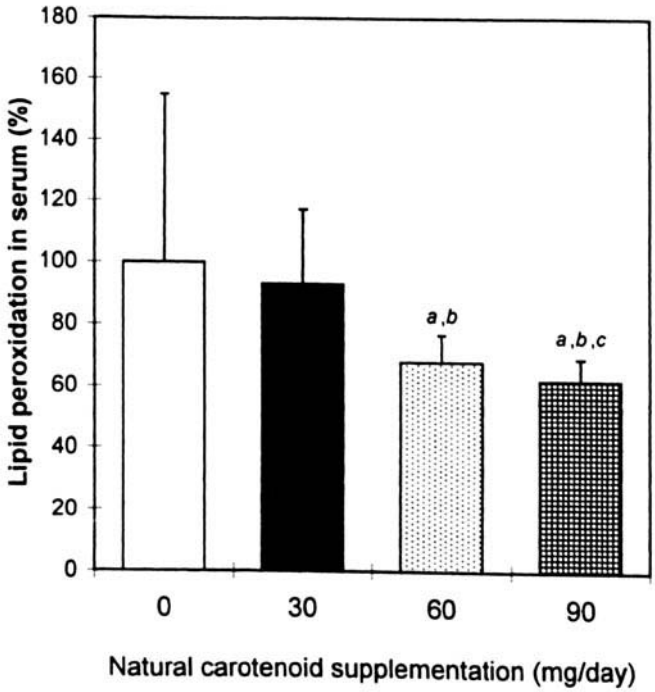

Figure 2. Lipid peroxide levels in serum after supplementation with 30–90 mg natural carotenoids for 24 weeks. The lipid peroxidation value at baseline was regarded as 100%. The concentration of lipid peroxides in serum after the 24-week trial period was divided by that at baseline to determine relative changes. [a,b,c]Indicate that mean values differ significantly from those in subjects in unsupplemented, 30-mg, and 60-mg supplement groups, respectively. The data show that daily supplementation with 60 and 90 mg natural carotenoids decreased serum levels of lipid peroxides.

Sunburn severity was reduced in subjects consuming vitamins C and E (12). Thus, antioxidants may protect against the adverse effects of UV exposure. When β-carotene was given to 30 subjects at a dose of 180 mg/day for 10 weeks, only a small increase in MED was observed (10). Our data show a significantly increased MED, which may be due to the increased 24-week duration of supplementation. This is particularly important as it takes several weeks for β-carotene to accumulate in tissues (5). Thus, serum levels achieved in the present study were higher due to either the increased length of supplementation or increased bioavailability of the natural carotenoids, primarily α- and β-carotene. In another study, 23 subjects were given 150 mg β-carotene for only 4 weeks. No reduction in

UV-induced erythema or in the production of reactive oxygen species was noted (13). Therefore, supplementation for only 4 weeks does not appear to be sufficient to induce notable changes. Furthermore, the length of supplementation may actually be more important than the increased dose to derive protective benefits. Although the present study mainly focused on the effects of higher doses of α- and β-carotene, it is possible that other carotenoids synergize with these compounds to protect the skin from UV irradiation.

Prior to supplementation, serum β-carotene levels were observed to be similar to those previously reported (3, 5, 14). Supplementation with natural carotenoids resulted in significant increases in serum α- and β-carotene levels, as previously noted (3, 5). These studies demonstrated that, with supplementation of 51–102 mg β-carotene/day, serum values corresponded to skin levels although there was a time lag of several weeks for β-carotene to accumulate in the skin (5). A similar correlation between serum and dermal levels of carotenoids was found with a natural carotenoid dose of 24 mg/day for 12 weeks (3). Therefore, it is likely that our subjects had increased concentrations of α- and β-carotene in their skin, thereby offering direct protection against UV exposure. No subjects reported any noticeable adverse changes in their skin appearance. However, upon examination of their nails and palms, a slight orange color was evident, again confirming the dermal and/or subcutaneous accumulation of these antioxidants.

Alterations in skin lipids due to exposure to UV radiation result in skin damage, leading to erythema. White *et al.* (15) first reported that UV irradiation could reduce plasma carotenoid levels in vivo. In a study of young-adult female subjects, plasma β-carotene levels were decreased after UV exposure (16). This appears to be in response to increased oxidative stress due to free radical generation initiated by UV irradiation. We found significant reductions in the amount of lipid peroxidation in serum with natural carotenoid supplementation, indicating that α- and β-carotene prevent the formation of reactive oxygen species. Therefore, it is possible to reduce or prevent erythema associated with free radicals by using β-carotene to inhibit lipid peroxidation.

Overall, long-term supplementation with high doses of a mixture of naturally occurring carotenoids high in β-carotene modestly raised MED in fair-skinned subjects and therefore may reduce the risk of sunburn. However, the degree of protection is small compared to that offered by conventional sunscreens. Although sunburn cannot be entirely prevented during intense exposure, regular natural carotenoid intake may afford additional protection. We believe the level of protection can be attributed to slight changes in skin pigmentation as well as to increased antioxidant activity preventing free radical damage to the skin. Natural carotenoids may, therefore, be an effective adjunct to sunscreens.

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