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Effects of Controlled Exposure of Sunlight on Plasma and Skin Levels of β-carotene

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We conducted a randomized placebo-controlled double-blind study in 20 healthy young female students (skin type II + III, body mass index 18–22) in order to evaluate the efficacy of 10 weeks of moderate dose (30 mg/d) β-carotene (BC) on plasma and skin β-carotene levels during 12 days of time and intensity controlled sunlight exposure at sea level (30° latitude, Red Sea, Eilath, Israel). After 12 days of controlled sun exposure (total UV dose of about 10.000 J/cm^2), plasma β-carotene decreased in the placebo ($p<0.01$) and β-carotene group (not significant). In addition cutaneous β-carotene decreased significantly in both groups. Plasma α-tocopherol decreased significantly ($p<0.01$) during exposure time in both groups. In the supplemented group, however, the decrease of a-tocopherol was significantly greater ($p<0.01$) than in the placebo group. We conclude that sunlight influences the β-carotene and α-tocopherol content of blood and tissues.

Keywords: β-carotene, α-tocopherol, human skin, free radicals, UV-light

INTRODUCTION

Acute and chronic exposure to UV-light leads to a variety of changes in the epidermis, dermis, and the further compartments of the skin. The most significant of these alterations are a premature aging of the skin (photo-aging), UV-induced hyperkeratosis or atrophy, enhancement of skin diseases and precancerous lesions (actinic keratoses) and neoplasms of the skin, such as squamous cell carcinoma, basal cell carcinoma, and possibly malignant melanoma.[1,2,3] Because β-carotene may be involved in the prevention of carcinogen activation the plasma β-carotene level seems to be important. Indeed, low β-carotene plasma levels correlate with increased cancer risk.[4,5] The individual plasma level is influenced by different factors. Nutrition and seasonal changes alter plasma β-carotene and can therefore contribute to the individual risk.[6,7] A degradation of carotenoids in plasma due to prolonged artificial UV-A exposure[8] has also been demonstrated.

Previous studies have shown that the level of β-carotene in the blood and retinol in skin of rats fall after exposure to sunlight and artificial

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UV-light.[9] Thus the level of β-carotene in the epidermis and other tissues may not be sufficient in excessive exposure to sunlight. In the current study we explore whether natural sunlight may contribute to a decrease of plasma and skin β-carotene in humans and whether supplementation may be helpful to prevent the effects of sunlight on plasma and skin levels of β-carotene.

METHODS

45 female healthy students were selected from the medical and other faculties at the Free University of Berlin after being thoroughly instructed on the goals of the study. Inclusion criteria were skin type II/III, body mass index 18–23, age 20–25 years, non-smokers, agreement to biopsies, blood sampling, UV-exposure and regular use of a hormonal contraceptive. A general health examination was performed by a physician specialized in internal medicine. Blood and urine were checked for any pathological changes of the bloodcounts and blood corpuscles, liver and kidney, for diabetes, serum lipids, hepatitis B and HIV I + III infection. The skin condition was evaluated by an experienced dermatologist including history of sun exposure, solar trauma and examination of the total body skin as to pathological changes including increased numbers of sun freckles, lentigines or nevus-cell nevi and atypical nevi, solar elastosis and specific dermatoses as well as other exclusion criteria. The grading of skin type was performed by history, hair, eye and skin coloration and testing of the minimal erythemal dose (MED) by using increasing UV-B doses from 17.6–90 mJ/cm^2 at 297 nm delivered by a monochromator (Burleigh Instruments, UK). A lowered UV-A-erythemal dose was excluded by testing at 360 nm.

During the recruitment phase, 20 out of 45 volunteers fulfilled all requirements of inclusion criteria and were separated according to a randomized key to either the supplemented, β-carotene, or the placebo group. 18 out of 20 of the volunteers were medical students.

The design of the double-blind study was as follows: After recruitment of the volunteers (phase I), 2 × 3 capsules of 5 mg β-carotene/day (n = 10) or placebo (n = 10) were administered (phase II) over a ten weeks period (time point A1, A2) from the middle of December 1992 to the end of February 1993 (the β-carotene (Bella Carotin®) and placebos were kind gifts of 3M-Medica in Borken, Germany). The placebo capsules were visually identical to the verum capsules and their contents displayed the same color.

A group of 14 out of 20 volunteers had been designated to take part in the controlled UV-exposure (phase III) at the Red Sea/Israel, the remaining volunteers constituted the reserve group. During the UV-phase, supplementation with verum and placebo was continued.

Nutrition was supervised by a dietician and a biochemist. Immediately after return from Eilath to Berlin all supplementation was stopped and a 4 weeks post-UV-washout phase (IV) completed the total study (time point D). The major characteristics of the study volunteers are given in Table 1.

Blood samples were taken in the morning before breakfast at the start of study (A1), directly before departure to the UV-exposure area (A2), daily from day 1 to 9 (2–11) during the stay at

TABLE 1 Information on Volunteers Participating in this Study

Characteristics	Placebo Group	β-Carotene group
Number	n=6	n=8
Mean age (range)	22.5 (21–25)	23.5 (21–22.4)
Body mass index	20.3 (18.7–22.0)	20.2 (18.9–22.4)
Skin type II	2	5
Skin type III	4	3
Eye color		
blue/grey	3	5
brown	3	3
Hair color		
fair	3	5
dark	3	3
MED mJ/cm^2 (297 nm)[†]	53.1	53.5
UV-cumulative dose*	10344 ± 644	10241 ± 945

[†] MED = minimal erythema dose at start of study
* H_{eff} mJ/cm^2 over days 1 to 13 of UV-exposure in Eilath

Eilath, at 12 hours after return to Berlin (12) and after the 4 week washout phase IV (D). Plasma was immediately separated and frozen at –70°C until determination. The transport of plasma samples was carried out on dry ice from Eilath to the analytical laboratory (University of Mainz). After return to Berlin a general health examination, including the skin, was performed.

6 mm punch biopsies for the assessment of β-carotene concentration were taken from the gluteal region at the start of the study (time point A1) (one biopsy), eight days before departure to Eilath and immediately after return from the UV-exposure area (time point 12) (2 biopsies).

The UV-exposure took place in March 1993 at sea level, 30° latitude (Red Sea, Eilath, Israel) where constant meteorological conditions could be expected. The volunteers all wore identical one-piece bathing suits which covered the same percentage of body surface. A 10 × 10 cm opaque film which prevented the penetration of UV rays was placed in the hip area of the bathing suits so that the underlying skin could serve as a reference field for skin complexion measurement. Two fields approximately 7.5 cm in diameter were cut out of the seat of each suit, one on each side of the gluteal cleft. The left field (No. 2) could be covered with a cloth flap. Depending on the particular volunteer's pretested individual MED (Mean Erythema Dose) this field was temporarily exposed to the UV-rays (so-called 'physiological exposure'). The right field (No. 3) was continuously exposed and both sites (No. 2 and No. 3) were not sunscreen-shielded. An area near the right shoulder blade served as field No. 4 to which sunscreen was regularly applied. The remaining body surface was divided into 20 fields (5a + b to 23a + b) and the development of erythema was monitored (data not shown). Every morning and afternoon the volunteers were then exposed to natural sunlight for an increasing length of time. They were not allowed to expose themselves to UV-rays except under supervision in the allowed period of exposure. In general, exposure was prohibited between 11.30 a.m. and 1.30 p.m. In non-UV-exposure times the students were engaged in creative games and supervised by a psychologist.

The use of UV-B/A-sunscreens was prescribed for both weeks in fields 4 to 23. The sunscreens were oil-in-water formulations containing Eusolex 4360 3%, Parsol 1789 1.8%, Eusolex 6900 1.2% and titanium dioxide 1.2%, resulting in an LPF (Light Protection Factor) of 11 (DIN-Norm) according to an US-SPF (Sun Protection Factor) of about 15 in the UV-B range and 7 in the UV-A range (DIN-Norm) at about 10 US-SPF. In the second week a LPF of 7 for UV-B (DIN) and UV-A 2 (DIN) was used.

The volunteers wore a personal dosimeter during the day in order to measure the individual cumulative dose of UV-rays (H_{eff} in mJ/cm^2; Metec dosi, polysulphone type 2<320 nm, DK-UV-techniques, Hanau, Germany). In addition, UV-B and UV-A-irradiation from the sun were measured over the day from 8.30 a.m. to 4.30 p.m. using a Waldmann UV-A- and UV-B-meter device (Waldmann, Schwenningen, Germany) and a Robertson-Berger units device (Robertson + Berger, Philadelphia, Pa., USA). Measurement of the global irradiation and total UV-rays reaching the beach level at the Red Sea was performed by the Metereological Service Station of the Ministry of Transport, Israel. This station was located about 500 m away from the exposure area. In November, 1992, this study was approved by the Ethics Committee of the Faculty of the Steglitz Medical Centre, Free University of Berlin, Berlin.

Methods of Analysis

The determination of β-carotene, lycopene, retinol and α-tocopherol in human plasma and β-carotene skin samples was carried out according to a modified method.[10] The accuracy of the method was determined by repeated determination of human skin and plasma pool samples according to NIST standards. The HPLC was periodically calibrated with known amounts of each compound and the purity of the β-carotene,

lycopene, a-tocopherol and retinol standards was assessed by comparing the absorption spectrum with its known extinction coefficient. Briefly: 200 µl plasma samples were vortexed (15 s) with isopropanol (100 µl at pH 2). 850 µl n-hexane was added, vortexed (15 s) and shaken (5 min). After centrifugation (5000 rpm) the upper organic layer was pipetted off and 1 ml hexane added to the remaining pellet. After vortexing (15 s), shaking (5 min) and centrifugation the upper layer was pipetted off. Both organic layers were evaporated under nitrogen and redissolved with 200 µl mobile phase, including the internal standard (kind gift from Dr. J. Bausch, Hoffmann-La Roche AG, Basel, Switzerland) (Sudan II – for β-carotene –, lycopene and retinylacetate for vitamin A- derivatives were subsequently injected into the HPLC-system. Sudan II is a useful internal standard due to its absorption maximum (450 nm) and clear chromatographic separation from carotenoids. The 6 mm punch biopsies were minced and saponified in 1 ml of potassium hydroxide in methanol for 1 hour at 37°C. After homogenization, 0.5 µL mL of the homogenate was deproteinized with 50 µL of 5% perchloric acid and extracted by adding 0.6 mL of hexane. Butylated hydroxytoluene at a final concentration of 500 ppm was used as a preservative during the extraction procedure. The hexane extract was evaporated to dryness under nitrogen. The dried residue was dissolved in 150 µL of the HPLC mobile phase (n-hexane [98%], Isopropanol [2%]) and centrifuged again to remove any remaining microparticulate matter. One hundred microliters of supernatant was injected directly into the HPLC-system (Merck UV/VIS detector L4250; L 6200A pump. L5025 column-oven; Integrator D2500) and separated on a LiChrospher 100 CN column (250 mm × 4,6 mm; 5µ). The absorption of β-carotene and other carotenoids was monitored at 450 nm, α-tocopherol at 296 nm and retinol at 325 nm.

Statistical analysis

Descriptive statistics using representative parameters of the samples are given for all variables in the two treatment groups. Statistical analysis employs nonparametric methodology and the data were analysed for comparability between the two treatment groups at entry. A chi-square test was used for qualitative data and the Mann-Whitney test for quantitative data. P-values were set for all data at 0.2. We analysed quantitative serial samples within the groups with Friedman's ANOVA. Mann-Whitney tests were examined in order to compare representative characteristics of time serials (AUC, median, maximum etc.) between the treatment groups. All p-values are two-tailed and calculated at 0.05.[11]

Food questionnaire

The volunteers were asked to follow weekly an eight page nutritional protocol according to Nutrilog System (Frankfurt, Germany). The consumption of additional vitamin tablets or foods rich in β-carotene was prohibited. The dietary questionnaire was filled out at the beginning of the study, during the exposure time and at the end of the washout phase. The mean intake of β-carotene, α-tocopherol and preformed vitamin A was calculated by means of the EBIS program (Bundeslebensmittel-Schlüssel). Before starting the study the participants received detailed information on how to avoid high β-carotene intake. They were given a list of the fruits and vegetables to be avoided. During the exposure time the daily intake of β-carotene and vitamin E was calculated by a nutritionist.

RESULTS

Plasma levels of β-carotene, retinol, lycopene, alpha-tocopherol

The mean values of the β-carotene plasma levels of the participants determined at different time points are given in Figure 1. The mean values of β-carotene and serum at baseline (A1) were not significantly different for the supplemented and the placebo group.

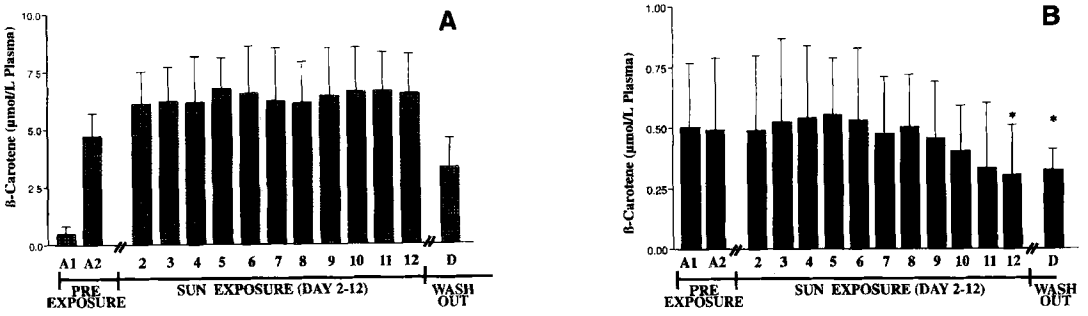

FIGURE 1 Plasma β-carotene values. Decrease of plasma β- carotene during sun exposure in the placebo group is highly significant ($p<0.01$) as calculated with the Friedman test. Data are given for the times: at start of the study (A1) after 10 weeks supplementation (A) or placebo (B) intake prior sun exposure (A2), during sun exposure (2–12) and after 4 weeks washout (D) in the supplemented (A) and placebo group (B).

After ten weeks of β-carotene supplementation (A2), β-carotene levels increased significantly ($p<0.01$) in the supplemented group but did not significantly deviate in the placebo group from that of the initial value (A1 vs A2). During the exposure time (day 2–12) there was no significant alteration of the plasma levels of the supplemented group (1A). In contrast the plasma levels of the placebo group (1B) decreased significantly between day 2 and day 12. (Friedman-Test, $p<0.01$) and remained low until the end of the washout time (D). The β-carotene plasma levels of the supplemented group dropped significantly due to the withdrawal of supplementation (day 12) from day 12 to D. In the placebo group the decline of β-carotene came to a halt (washout phase: D).

The retinol plasma level remained constant in both groups during the whole period of observation (Figure 2). In contrast, the alpha-tocopherol plasma level (lipid-adjusted) showed a substantial decrease during exposure to the sun (Figure 3). In the supplemented group (3A) the decrease of α-tocopherol in plasma was significantly higher (*$p<0,01$; ** $p<0,005$) between day 4 and 9 as compared to the placebo group (3B) despite equivalent initial values (A1, A2, day 2, day 3). Four weeks after cessation of supplementation (washout phase) and during low intensity of sun exposure (early spring in Berlin) the vitamin E plasma levels had reached the initial values in both groups. Lycopene remained nearly constant in both groups during the whole period of exposure (Figure 4). The mean intake of β- carotene prior to the sun exposure was estimated to be 0.8 ± 0.3 mg/day, mean vitamin E intake was 11.5 ±

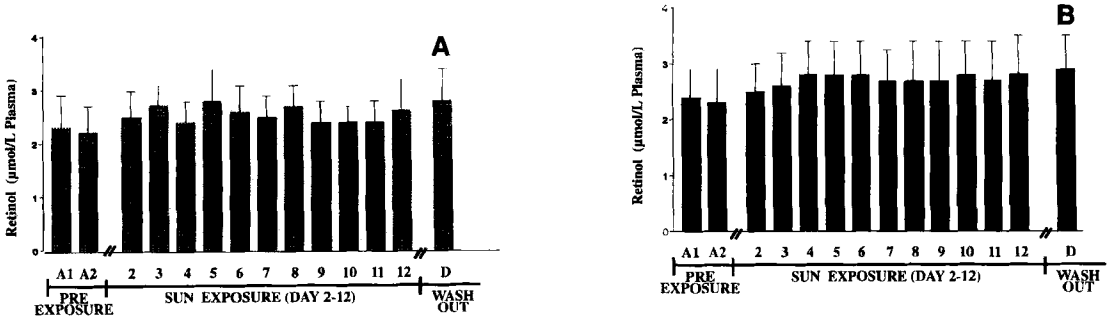

FIGURE 2 Plasma retinol concentration. Data are given for the times: at start of the study (A1) after 10 weeks supplementation (A) or placebo (B) intake prior sun exposure (A2), during sun exposure (2–12) and after 4 weeks washout (D).

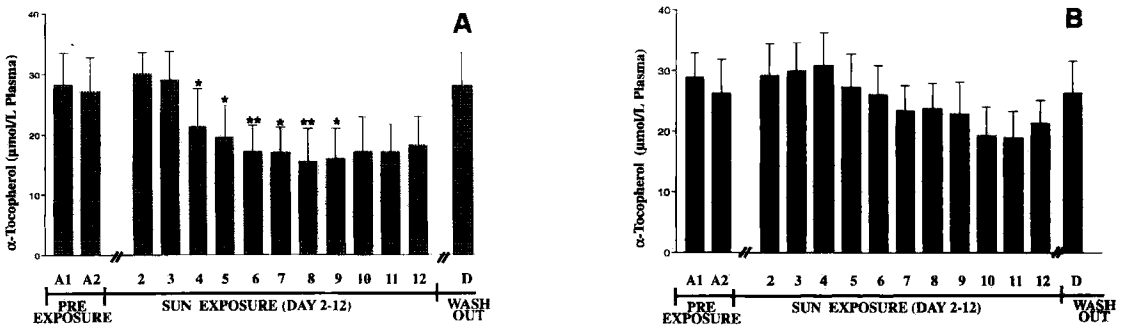

FIGURE 3 Plasma α-tocopherol concentration (lipid-adjusted). Data are given for the times: at start of the study (A1) after 10 weeks supplementation (A) or placebo (B) intake prior sun exposure (A2), during sun exposure (2–12) and after 4 weeks washout (D). Significance level $p<0.05$ (* significant); $p<0.001$ (** highly significant).

1.9 mg/day. During the exposure time the mean intake was calculated to be 0.9 ± 0.2 mg/day for β-carotene and 12.2 ± 2.3 mg/day for vitamin E.

β-carotene in the skin

Supplementation increases and sun exposure decreases the concentration of all-trans β-carotene in the skin (Figure 5). In the continuously exposed field (field 3) the decrease of β-carotene was significantly higher ($p<0.001$) than in the shortly exposed field (field 2) compared to the initial values. Between field 2 and 3 a highly significant decrease ($p < 0.005$) was evident. The correlation of plasma and skin concentration of β-carotene in both groups is shown in Table 2. The statistical analysis by two different tests shows that supplementation leads to a significant correlation ($p < 0,05$) of skin and plasma β-carotene.

Discussion

In our study, a significant decrease of β-carotene in serum and skin was shown following controlled sun exposure for 12 days. A significant decrease of plasma β-carotene after artificial UV-light exposure for 12 days (cross over design) was recently described.[8] In contrast, Berne *et al.*[12] did not observe a decrease of plasma carotene level after whole body artificial UV-exposure in 10 uremic patients and 5 healthy controls. The absence of an effect of UV-exposure on plasma carotenoids might be either due to the lower intensity of UV-exposure or to the fact that total carotenoids were

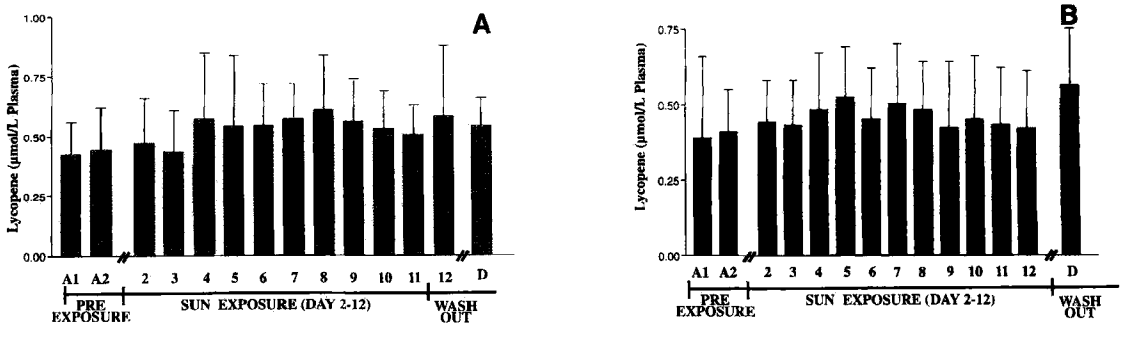

FIGURE 4 Plasma lycopene. Data are given for the times: at start of the study (A1) after 10 weeks supplementation (A) or placebo (B) intake prior sun exposure (A2), during sun exposure (2–12) and after 4 weeks washout (D).

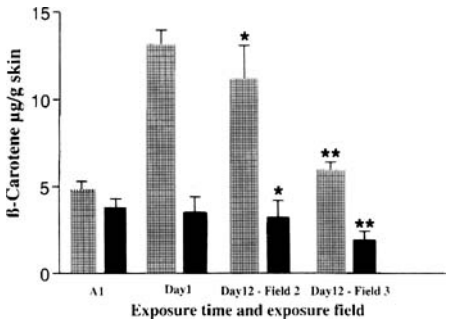

FIGURE 5 All trans β-carotene in skin. The difference in the β-carotene level within the groups is highly significant ($p<0.001$) between field 2 and 3; significant ($p<0.05$) between start (day 1) and end of sun exposure in field 2 and highly significant ($p < 0.001$) in field 3. Data are given for the times: at the beginning of the study (A1), at the beginning of sun exposure (day 1) and at the end of sun exposure (day 12) in field 2 (shortly exposed) and field 3 (continuously exposed). (⊞ supplemented group, ■ placebo group)

measured. Dietary effects (no control of intake of carotenoids[12]) may ameliorate the UV-effect on β-carotene. As our group of volunteers was strictly randomized and the dietary intake strictly controlled prior and during our study, plasma β-carotene variation as a result of differences in individual food intake or other factors (season, sex, alcohol intake) can be ruled out in our study. The controlled mean β-carotene intake through diet (0.7–1.1 mg/d) equals the calculated intake of the population of Germany.[13]

A decrease of β-carotene in skin from the beginning of the exposure to the end became evident in the supplemented and in the placebo group even at relatively low sunlight intensity and duration (field 2). The skin content of β-carotene was significantly decreased in field 3 (continuously exposed) as compared to field 2, and we assume that the degree of β-carotene depletion depends on duration of sun exposure.

TABLE 2 Correlation of Plasma and Skin β-carotene prior to supplementation (A1), at Day 1 and Day 12 of sun exposure field 2 and 3

	Placebo		β-carotene	
Time point	r	p	r	p
A1	0.48	n.s.	0.46	n.s.
Day 1	0.47	n.s.	0.75	<0.05
Day 12-Field 2	0.51	n.s.	0.40	n.s.
Day 12-Field 3	0.42	n.s.	0.79	<0.05

The decrease of plasma and skin β-carotene as a consequence of sun exposure may have resulted from a direct photodecomposition of β-carotene in the dermal cells and in the capillary bed of the papillary dermis. This photochemical transformation of β-carotene in vivo would be expected to resemble their absorption spectra in organic solvents. Although the major absorption band of carotenoids occur within the 400–500 nm range of the visible spectrum,[14] their absorption spectra extend into the UV-A range.

Approximately 35–50% of incident UV-A has been reported to penetrate to the dermis of Caucasians.[15] Exposure of epidermis to visible UV-A or UV-B results in reactions involving singlet oxygen and free radicals.[16,17] Reactions with peroxylradicals, generated by UV-light leads to a decoloration reaction of β-carotene.[18,19] Consequently, natural sunlight contributes to a decrease of β-carotene in blood and tissues. The decline of skin β-carotene levels makes the skin more sensitive towards sunlight-induced photodamage. Additionally, UV-light can influence the local and systemic immune response, resulting in a decrease of delayed type hypersensitivity and cutaneous hypersensitivity reactions or rejection of tumors transplanted in animals.[5,20–23]

In contrast to recent findings,[24,25] β-carotene supplementation did not seem to influence α-tocopherol plasma levels (A1–A2). However, plasma α-tocopherol decreased markedly in both groups during sun exposure (day 2 –day 12) and in the supplemented group the decrease of plasma α-tocopherol was significantly greater. Similar results were described by Xu and co-workers[25] who showed a significantly greater decrease of skin and plasma a-tocopherol in β-carotene-supplemented mice after UV-exposure as compared to unsupplemented mice. The transient decline of α-tocopherol in plasma during sun exposure

might explain the controversy whether β-carotene supplementation influences α-tocopherol plasma levels[25] or not.[26] If the blood sampling was carried out following a period of intensive exposure to sun or UV light, plasma α-tocopherol levels could be lower than during other periods of sampling. The decline of α-tocopherol in plasma during sun exposure might be a consequence of an increased demand for vitamin E in order to protect the skin against UV-induced formation of free radicals and subsequent damage of skin cells.[27] In the hairless mice an increased formation of lipid hydroperoxides[28] and a 50% decrease of the skin α-tocopherol content due to UV-exposure was described.[29]

The influence of β-carotene supplementation on the greater decrease of plasma α-tocopherol during sun exposure is rather puzzling. A direct influence of β-carotene on bioavailability of α-tocopherol seems not likely because the α-tocopherol plasma level in both groups were similar prior to sun exposure. Whether the β-carotene level in blood and tissue influences the recycling of the α-tocopheroxyl radical formed during sun exposure remains to be elucidated. Differences in the intake of vitamin E between the two groups can be ruled out. One reason for the greater decline of α-tocopherol in plasma in the β-carotene-supplemented group during sun exposure might be a reduced α-tocopherol content of the liver. As recently described, β-carotene supplementation resulted in diminished levels of α-tocopherol in the liver of rats.[24] In contrast, vitamin A-deficiency led to a marked increase in α-tocopherol (+69%) in the liver of rats.[30] From these reports we speculate that β-carotene supplied in higher doses may lead to a lower vitamin E level in the liver. As a consequence, the compensation of a sun-induced decrease of α-tocopherol plasma levels cannot be ensured to the same extent as in the unsupplemented group.

In our study, a significant decrease in serum and skin β-carotene levels was measured during the dose- and time-controlled UV-exposure of 12 days. The total UV dose was about 10.000 mJ/cm^2 which is approximately equivalent to the exposure received by a Caucasion during two weeks of sun bathing vacation. The dose, however, is markedly lower when compared to the UV dose in the study of Fuller *et al.*,[21] in which artificial UV-light was used but no significant decreases of β-carotene levels could be measured. At the end of the exposure time the placebo group was in the range of plasma values reported in epidemiological studies to be associated with the highest risk for the development of cancer at different body sites.[5,31,32] In contrast, the supplementation with 30 mg/d of β-carotene ensured post UV-exposure serum levels equivalent to or above those reported for the lowest cancer incidence. This finding is important since our study selected females and a time of the year with a possible seasonal (low vegetable intake) lower β-carotene level.[6,7]

The nature of UV-light-related pathological changes is complex but involves the production of free radicals in the skin.[33] Dimer formation and strand breaks of DNA occur[34] as well as cytoskeletal alterations[35] and release of inflammatory mediators.[36] Hence, an increase of agents capable of scavenging or quenching reactive oxygen species would be expected to decrease sun light-mediated skin damage.

On the basis of the combined result of the present study one may raise the question whether β-carotene supplementation prior to sun exposure may help to protect against sun-induced depletion of this provitamin in plasma and skin. An additional supplementation with α-tocopherol should be further investigated and at present it is up to the decision of the sun user.

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