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Antioxidant Supplements Improve Parameters Related to Skin Structure in Humans

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Key Words
Antioxidant supplements · Carotenoids · Skin structure · Skin surface

Abstract
In the present study we investigated the influence of two different antioxidant supplements composed of carotenoids, vitamin E and selenium on parameters related to skin health and skin aging. Thirty-nine volunteers with healthy, normal skin of skin type 2 were divided into 3 groups (n = 13) and supplemented for a period of 12 weeks. Group 1 received a mixture of lycopene (3 mg/day), lutein (3 mg/day), β-carotene (4.8 mg/day), α-tocopherol (10 mg/day) and selenium (75 μg/day). Group 2 was supplemented with a mixture of lycopene (6 mg/day), β-carotene (4.8 mg/day), α-tocopherol (10 mg/day) and selenium (75 μg/day). Group 3 was the placebo control. Upon supplementation serum levels of selected carotenoids increased in both verum groups. Skin density and thickness were determined by ultrasound measurements. A significant increase for both parameters was determined in the verum groups. Roughness, scaling, smoothness and wrinkling of the skin were determined by Surface Evaluation of Living Skin (Visioscan). Roughness and scaling were improved by the supplementation with antioxidant micronutrients. In the placebo group no changes were found for any of the parameters.

Introduction

Carotenoids are widely used as skin protectants and supplementation with carotenoids has been shown to protect against UV-induced erythema. However, little is known about other effects of carotenoids on skin health.

Considerable amounts of micronutrients such as antioxidant vitamins and carotenoids are present in the skin and are suggested to contribute to the maintenance of skin health [1]. There is convincing evidence that dietary antioxidants provide protection against skin damage from sunlight [2–7]. It has been shown that carotenoids, vitamin C and E prevent sunburn-associated erythema following UV exposure. UV radiation generates reactive oxygen species in the skin, leading to damaging reactions which have been associated with photocarcinogenesis, photosensitivity or premature skin ageing [8]. Thus, scavenging reactive oxygen species are thought to be one mechanism of action underlying skin protective effects of antioxidants [9, 10]. However, it has been shown that several dietary antioxidants exhibit biological properties not directly related to antioxidant activity. They influence cellular signaling pathways and may trigger cell cycle progression, cell growth and repair systems [11, 12].

Very little is known about the effects of carotenoids, vitamins and trace elements on skin texture and structure which is closely related to skin ageing [13–15]. Premature aging of the skin results in increased wrinkling and loss

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Table 1. Treatment allocation, dose/day in the three different treatment groups

Group 1 (n = 13) Formula A	3.0 mg lycopene 3.0 mg lutein 4.8 mg β-carotene 10.0 mg α-tocopherol 75.0 μg selenium
Group 2 (n = 13) Formula B	6.0 mg lycopene 4.8 mg β-carotene 10.0 mg α-tocopherol 75.0 μg selenium
Group 3 (n = 13) Placebo	soybean oil

of elasticity. In the present study we investigated whether the supplementation with dietary micronutrients modulates skin structure and texture contributing to the resistance of skin to environmental stress and improving general parameters indicative of skin health.

Methods

Study Design

The study was carried out as a monocentric, placebo-controlled supplementation study on the effects of antioxidant micronutrients on parameters of skin health and skin aging.

A total of 39 volunteers (10 males and 29 females, average age: 42 years) with healthy, normal skin of type 2 were included in the study. They were divided into 3 groups of 13 test subjects each (2 verum and 1 placebo group). Composition of the antioxidant supplements and dosing in the different treatment groups is summarized in table 1. The participants were asked not to change their dietary habits during the study.

The supplementation period lasted 12 weeks; skin parameters were determined before start of supplementation (week 0) and at week 6 and 12; blood samples for antioxidant analyses were taken at the same time points.

All test subjects received detailed information listing every single parameter relevant to the study. Every test subject had to submit a written declaration of consent for their participation in the study.

Inclusion Criteria

The inclusion criteria were as follows: age 18–65 years, nonsmoking, normal nutritional habits, not pregnant or lactating, BMI 18–25, no intake of vitamin supplements, no history of mal-absorption diseases, liver diseases or diseases of lipid metabolism, no history of photosensitizing disorders, no extensive sunbathing in the month before the study or during the study. Screening procedures checked the general health of the participants and included the medical history.

Parameters of Skin Structure

Skin density and thickness were determined by means of ultrasound (B-scan), and skin surface was evaluated by image analysis (Surface Evaluation of Living Skin, SELS; Visioscan) at week 0, 6 and 12.

B-Scan. An ultrasound device with a frequency of 20 MHz (Derma Scan C, with 2-D configuration, Cortex Technology, Denmark) was used for noninvasive differentiation of individual tissue structures [16]. Two hundred and fifty-six randomly chosen colors are assigned to the different echo amplitudes which allow to determine even slight differences in the reflection behavior. Skin density is evaluated by the pixel density. Skin thickness (dermis and epidermis) is given in millimeters.

Surface Evaluation of Living Skin. Skin surface analysis according to the SELS method is based on the evaluation of an image of living skin taken under certain illumination; the picture is electronically processed for quantitative analyses. The skin surface is described by 4 different parameters: roughness, scaling, smoothness and wrinkling [17, 18]. The measuring device consists of a measuring head containing two contra-rotating metal halogen lamps evenly illuminating a 15 × 17 mm measuring field on the skin. The spectrum of the lamps and their intensity as well as their location allows to analyze the skin surface without interfering reflections from deeper layers. A CCD camera, located in the measuring head, records a picture of the skin, which is then transferred as graded grey values into a bitmap file (software: Skin-Visiometer, Courage & Khazaka, Cologne). The method allows to differentiate between 256 possible grey values. By means of the additional software (SELS) the skin-specific parameters are then calculated.

Antioxidants in Serum

Blood samples were taken after overnight fasting at week 0, 6 and 12; serum was prepared by centrifugation and stored at –80°C until analysis. Lycopene, β-carotene, α-carotene, lutein, zeaxanthin and cryptoxanthin were determined by HPLC as described by Stahl et al. [19]. Additionally phytoene, phytofluene, retinol, α-tocopherol and γ-tocopherol were determined. For phytofluene and phytoene analyses, 500 μl of serum were extracted with hexane/dichloromethane (5/1, v/v, stabilized with 0.1 % 2,6-di-tert-butyl-*p*-cresol) after protein precipitation with ethanol. The organic solvent was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 100 μl dichloromethane plus 100 μl acetonitrile.

The HPLC system consisted of a Merck-Hitachi L-7100 pump connected with a Merck-Hitachi UV/Vis detector and an integrator for data registration. HPLC was performed isocratically with an eluent consisting of acetonitrile/methanol (85/15, v/v) and a reversed-phase column (pKb-100, 250 × 4.6 mm, Supelco, Bellefonte, Pa., USA) protected by a guard column (4.6 × 4.6 mm) with the same stationary phase. The flow rate was set to 1 ml/min. A second UV/Vis detector was connected in series and set at 325 and 292 nm for quantitation of retinol, α-tocopherol and γ-tocopherol [20]. In a separate run under the same conditions phytofluene was detected at a wavelength of 348 nm and phytoene at 278 nm.

The concentrations were calculated from external calibration curves generated with original standard compounds.

Statistical Methods

For all parameters and all time points descriptive statistics were calculated; pre-post differences were calculated and analyzed descriptively. Within the three treatment groups each combination of

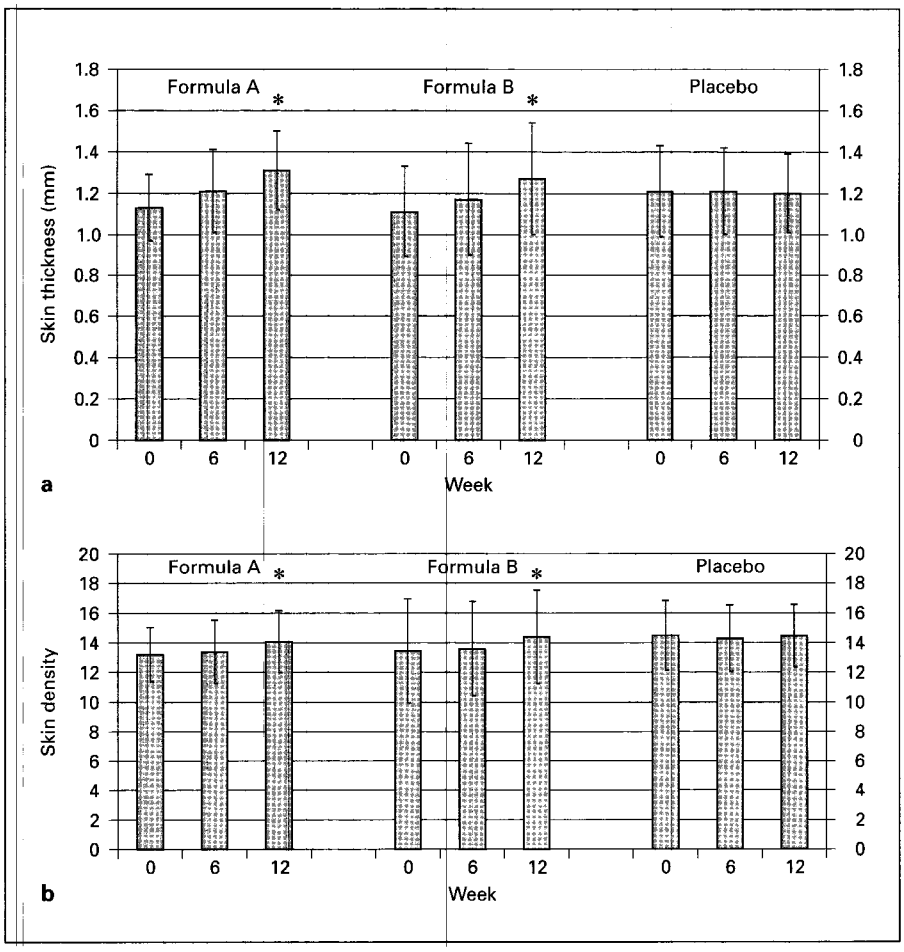

Fig. 1. Increase in skin thickness (der..... and epidermis; **a**) and density (**b**) during supplementation. Skin density and thickness were measured by means of ultrasound (B-scan).

two time points was compared using the Wilcoxon signed rank test. For the pre-post differences each combination of two treatment groups was compared using the Wilcoxon rank sum test. Percentual changes of all measured parameters were calculated and the p values were determined at all measuring points.

Results

Ultrasound Measurements (B-Scan)

In both treatment groups a statistically significant increase ($p < 0.05$) in skin density and skin thickness was observed after 6 and 12 weeks, compared to week 0 (fig. 1 and table 2). No statistically significant changes were observed in the placebo group. Skin density increased in groups 1 and 2 by about 7%, skin thickness by about 15%. No differences were found between treatment groups.

A typical ultrasound B-scan is presented in figure 2. It shows the density and thickness of the dermis before and after 12-week treatment of a volunteer from group 1.

Table 2. Results and statistical evaluation of the ultrasound measurements, skin density and skin thickness

Parameter and formula	Results	Change, %	p
Skin density (pixel density)			
Skin density formula A	increase	6.57	0.007*
Skin density formula B	increase	7.01	0.007*
Skin density placebo	no increase	0.27	n.s.
Skin thickness (dermis + epidermis)			
Skin thickness formula A	increase	15.99	0.000*
Skin thickness formula B	increase	14.09	0.000*
Skin thickness placebo	no increase	−1.37	n.s.

Skin density and thickness were measured by means of ultrasound (B-scan). Change implies change of means from week 0 to week 12. * $p < 0.05$, statistically significant.

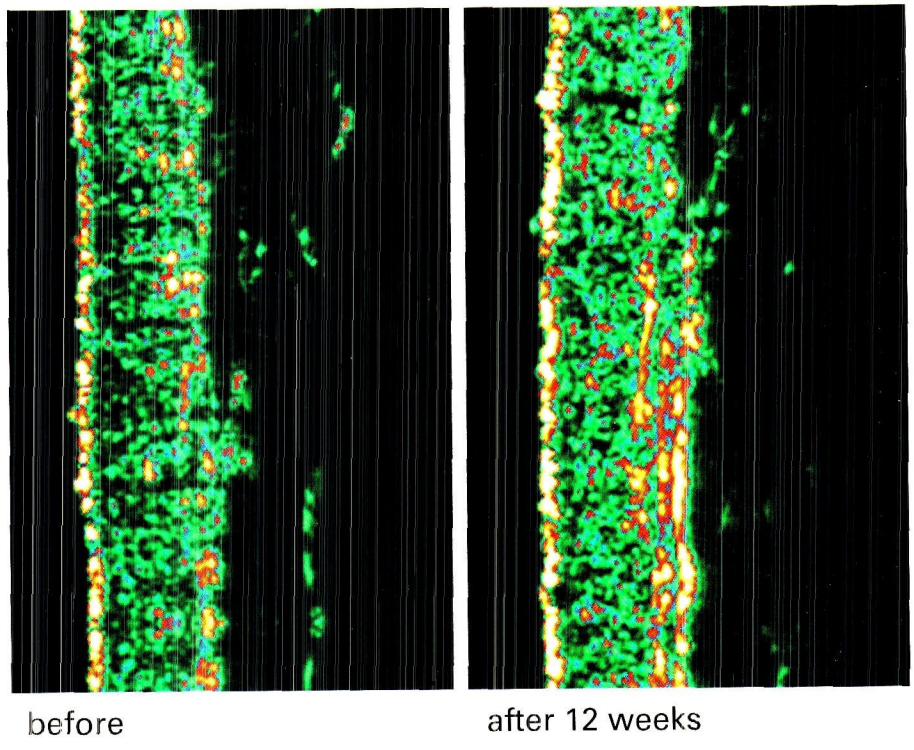

Fig. 2. Skin density before and after 12 weeks of treatment with formula A. A typical ultrasound B-scan shows the density and thickness of the dermis before and after 12 weeks of treatment; obtained from a volunteer of group 1.

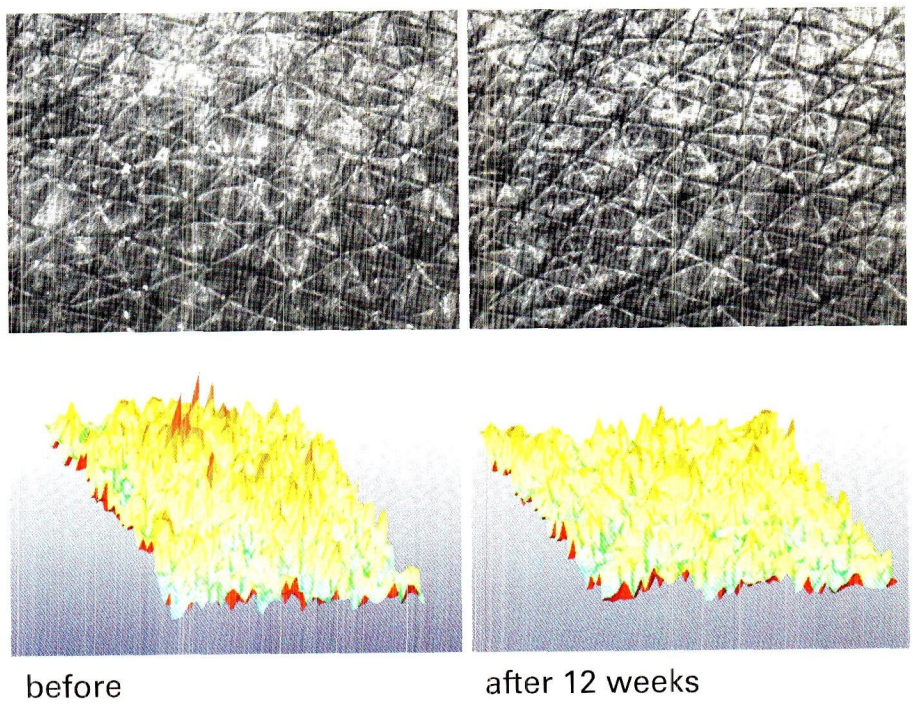

Fig. 3. Skin surface evaluation. Surface of the skin of a volunteer from group 2 (formula B) before and after 12 weeks of treatment; CCD picture and 3-D computerized image. Skin surface was evaluated by image analysis (SELS).

Surface Evaluation of Living Skin

SELS provides parameters related to the structure of the skin surface including roughness, scaling, smoothness and wrinkling (fig. 3, 4, table 3).

A typical SELS picture is presented in figure 3. It shows the surface of the skin of a volunteer from group 2 (formula B) before and after 12 weeks of treatment as well as a 3-D image.

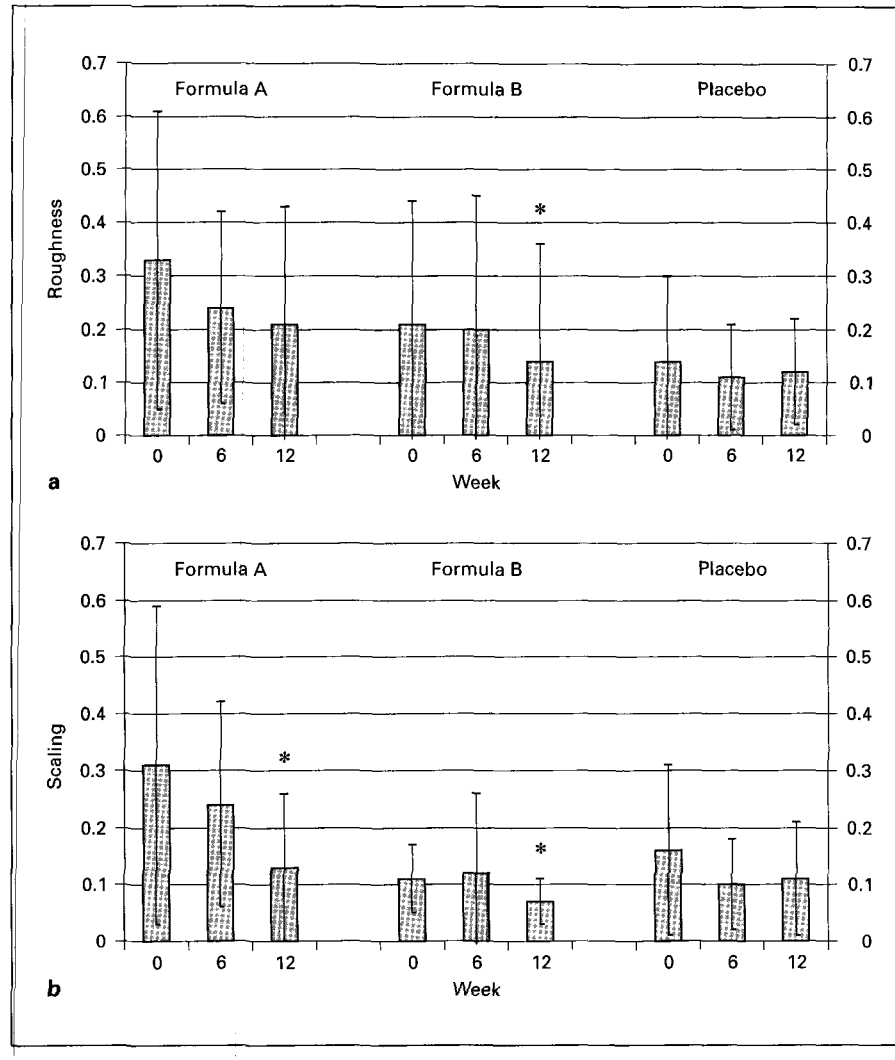

Fig. 4. Decrease in roughness (**a**) and scaling (**b**) during supplementation. Skin surface was evaluated by image analysis (SELS).

Table 3. Results and statistical evaluation of the skin surface evaluation

Parameter	Formula	Results	Change, %	p value
Roughness	A	decrease	−37.78	n.s.
Roughness	B	decrease	−32.71	0.005*
Roughness	placebo	decrease	−15.68	n.s.
Scaling	A	decrease	−57.99	0.007*
Scaling	B	decrease	−44.22	0.014*
Scaling	placebo	decrease	−30.14	n.s.

Skin surface was evaluated by image analysis (SELS). Change implies change of means from week 0 to week 12. * $p < 0.05$, statistically significant.

Following surface evaluation of living skin scaling was affected by supplementation with antioxidants. In both treatment groups a statistically significant decrease was observed after 12 weeks ($p < 0.05$). For the parameter roughness, only in the group 2, a statistically significant decrease was found ($p < 0.05$). Changes in scaling and roughness determined in the placebo group were statistically not significant.

Other SELS parameters, i.e. smoothness and wrinkling, were not affected by treatment and did not change in any of the groups (data not shown).

Antioxidants in Serum

The carotenoids, lycopene, β-carotene, lutein, zeaxanthin, α-carotene, cryptoxanthin, phytoene and phytofluene as well as α-tocopherol, γ-tocopherol and retinol were

Table 4. Antioxidants in serum (nmol) determined by HPLC analysis and statistical evaluation

Antioxidant	Formula A, group 1		Formula B, group 2		Placebo, group 3	
	week 0	week 12	week 0	week 12	week 0	week 12
β-Carotene	0.66	1.62*	0.68	1.33*	0.46	0.46
Lycopene total	0.46	0.76*	0.52	0.88*	0.49	0.60
Lycopene all-trans	0.25	0.43*	0.34	0.51*	0.24	0.30
Lutein	0.23	0.63*	0.26	0.22	0.24	0.28
Zeaxanthin	0.06	0.09*	0.07	0.07	0.05	0.06
Phytoene	0.09	0.13*	0.10	0.15*	0.07	0.10
Phytofluene	0.62	0.96*	0.77	1.29*	0.52	0.57
α-Tocopherol	30.34	32.98*	32.84	31.88	33.76	35.76

Increase in serum concentration during 12 week supplementation. Carotenoids were determined by HPLC according to reference [19]; α-tocopherol was measured following reference [20]. * $p < 0.05$, statistically significant changes.

analyzed in blood samples from all groups (table 4). The baseline levels of the micronutrients analyzed were comparable in all groups and are within the range of variance reported in the literature [21].

In group 1 lycopene and β-carotene levels increased to yield final concentrations (week 12) of 0.76 and 1.62 nmol/ml, respectively. Although the dose of lycopene was lower in this supplement, relative increases were comparable to those observed in group 2.

Phytoene and phytofluene serum concentrations were elevated after treatment; the values are comparable between both treatment groups. The supplement used in group 1 contained 1.5 mg lutein/capsule. Lutein levels increased from baseline 0.23 to 0.63 nmol/ml at the end of the study. Additionally, zeaxanthin increased. No changes in serum levels were found for cryptoxanthin, α-carotene and retinol (data not shown). None of these compounds was present in the supplement. α-Tocopherol levels were slightly elevated after treatment; γ-tocopherol remained unchanged.

In group 2 lycopene and β-carotene levels increased to yield final concentrations (week 12) of 0.88 and 1.33 nmol/ml, respectively. Both compounds were present in the supplement.

Increases in phytoene (level at week 12: 0.15 nmol/ml) and phytofluene (level at week 12: 1.29 nmol/ml) were also measured. Both carotenoids are precursors of lycopene and β-carotene and are present in carotenoid supplements which contain fruit or vegetable oleoresins. The increases are within the ranges from previous studies [2]. It should be noted that the bioavailability of carotenoids depends on several endogenous and exogenous factors leading to a wide range of variation in the individual uptake of single carotenoids. Serum responses vary between carotenoids and are not always linear with the dose.

Statistical Evaluation of the Serum Concentration of Antioxidants

For the treatment with formula A/group 1 statistically significant increases ($p < 0.05$) were evaluated for all antioxidants in serum after 12 weeks compared to week 0.

For the treatment with formula B/group 2 statistically significant increases ($p < 0.05$) for the antioxidants β-carotene, lycopene total, all-trans lycopene, phytoene and phytofluene were observed after 12 weeks compared to week 0. For lutein and α-tocopherol, no statistically significant changes were observed during the study. In the placebo group no statistically significant changes were observed.

Discussion

Skin structure and function is affected by endogenous and environmental factors with either beneficial or adverse effects on skin health. To optimize skin conditions a variety of skin care products is available; however, dietary constituents may also influence skin parameters including texture, color, moisture and other physiological properties. As any other tissue, skin requires an optimal supply with nutritive compounds including macronutrients such as lipids, amino acids or carbohydrates and micronutrients including vitamins and essential minerals [13].

Lipids and other nutritive compounds are applied topically in order to improve skin conditions. Less is known about the effects of endogenous supply of selected dietary components on skin tissue. Vitamins E and C as well as

carotenoids, like β-carotene and lycopene are used as nutritional supplements and photoprotective effects have been assigned to these compounds. Their efficacy in preventing UV-induced erythema has been shown in several intervention studies [2–7].

In the present study we demonstrated that a supplement mixture consisting of carotenoids, vitamin E and selenium increases skin density and thickness when ingested over a period of 12 weeks. Also skin surface parameters including scaling and roughness are improved upon supplementation.

The biochemical mechanisms underlying the effects of such a mixture of micronutrients are poorly understood. All components of the supplement are either direct or indirect antioxidants. It has been suggested that under stress conditions topical and/or systemic application of antioxidants contribute to the maintenance of healthy skin barrier [22]. The antioxidant defense system of the organism is a complex network and comprises several antioxidants. Interactions between structurally different compounds with variable antioxidant activity may provide additional protection against increased oxidative stress. For example, there is evidence from in vitro studies, that β-carotene regenerates tocopherol from the tocopheroxyl radical. Scavenging reactive oxygen species may be one part of their biological activity. It should be noted that carotenoids and vitamin E additionally modulate signaling pathways independent of their antioxidant properties. Such modulating effects have been reported in cultured human cells [9, 10] and should be considered as further possible mechanisms underlying the effects of micronutrients on skin health and skin properties.

Skin aging is a continuous process and results from intrinsic and extrinsic factors. A number of extrinsic, or external, factors often act together to prematurely age our skin. Most premature aging is caused by sun exposure and affects parameters of skin structure and surface. It is not known yet if the process of premature aging of the skin can be modulated by dietary antioxidants. However, several studies, including the present one, show that dietary antioxidants provide photoprotective effects and improve skin structure when administered as food supplements [23, 24].

In the present study the efficacy of the ingested micronutrients and their influence on the structural changes were examined. All ultrasound and skin surface measurements were carried out on the forearm of the volunteers to avoid mimic influences during the test procedure. The accumulation of the tested formulations in skin and serum could be demonstrated as well as the efficacy of the supplementation in terms of skin structure, like skin density and skin surface.

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NUTRITIONAL SUPPLEMENTS POSITIVELY INFLUENCE SKIN AGING

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BACKGROUND

Carotenoids are useful skin protectants and supplementation with carotenoids protects against UV-induced erythema [1-5]. In this study the influence of skin ageing by nutritional supplements was examined. The objective of the study was an application test during the intake of different mixtures of carotenoid vs. placebo capsules.

METHODS

Serum samples of voluntary test subjects were examined by HPLC-analysis. Anti-ageing parameters were evaluated by measurements of the skin surface (SELS, Visioscan, C&K, Germany). Ultrasound measurements were carried out with a 20 MHz system (Derma-Scan, Denmark), to determine the thickness and density of the dermis.

STUDY DESIGN

The study was carried out as a monocentric, double-blind placebo-controlled application test. 39 healthy volunteers with skin of type II were included in the study. They were divided into 3 groups of 13 subjects (2 verum- and 1 placebo group). The study lasted for 12 weeks, dosage regimen being 2 capsules/day. Measuring points were before supplementation and after 6 and 12 weeks (Table 1 and 2).

The following supplementations were tested:		
1.	OE 02 08	1.5 mg Lycopene/capsule 1.5 mg Lutein/capsule 2.4 mg ß-Carotene/capsule 5 mg α-Tocopherol/capsule 37.5 µg Selenium/capsule
2.	OE 02 05 S	3 mg Lycopene/capsule 2.4 mg ß-Carotene/capsule 5 mg α-Tocopherol/capsule 37.5 µg Selenium/capsule
3.	Placebo	Soybean-Oil

Table 1

Treatment allocation	
Group 1	(13 subjects) consume OE 02 05 S (2 capsules/day)
Group 2	(13 subjects) consume OE 02 08 (2 capsules/day
Group 3	(13 subjects) consume Placebo (2 capsules/day)
12 weeks treatment. Measurements at day 0, week 6 and week 12.	

Table 2

STATISTICS

Descriptive statistics and pre-post differences were calculated. Within the treatment groups each combination of 2 time points was compared using the Wilcoxon signed-rank test. For the pre-post differences each combination of 2 groups was compared using the Wilcoxon rank sum test.

RESULTS

The following results were obtained in this study

1. Significant increase in skin thickness and density in both carotenoid groups (Table 3, Figs. 1-2).
2. Skin surface was positively influenced by ingestion, significant changes were found in "scaling" and "skin roughness" (Table 4, Figs. 3-4).
3. Analysis in the serum samples resulted in statistically significant increases of ß-Carotene, Lutein and Lycopene after 6 and 12 weeks (Table 5).

Skin density by means of ultrasound before and after 12 weeks

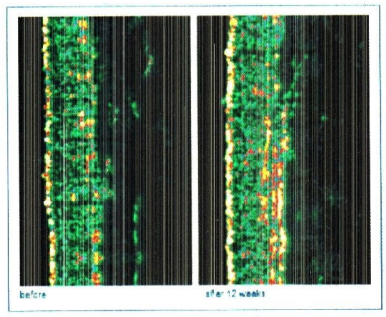

Figure 1

Increase of skin density during the supplementation

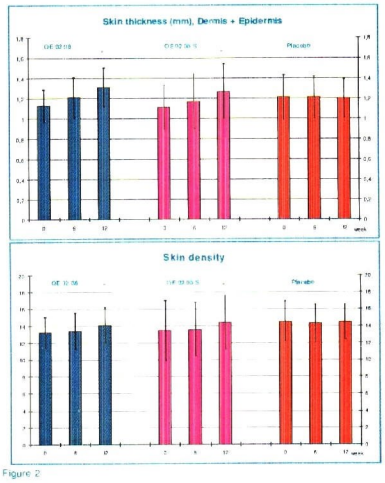

Figure 2

Skin density (pixel density)			
Skin density OE 02 08	increase	6.57 %	*p=0.007
Skin density OE 02 05 S	increase	7.01 %	*p=0.007
Skin density Placebo	no increase	0.27 %	n. s.
Skin thickness (mm), Dermis + Epidermis			
Skin thickness OE 02 08	increase	15.99 %	*p=0.000
Skin thickness OE 02 05S	increase	14.09 %	*p=0.000
Skin thickness Placebo	no increase	-1.37 %	
Percentual change of means from week 0 to week 12			

Table 3

Antioxidants in serum (increase of serum concentration during supplementation)			
	OE 02 08	OE 02 05 S	Placebo
ß-Carotene	*144%	95 %	0 %
Lycopene total	*65 %	*68 %	23%
Lycopene all-trans	*72 %	*49 %	27 %
Lutein	*169 %	-17 %	3 %
Zeaxanthin	*51 %	1 %	13 %
Phytoene	*40 %	*58 %	30 %
Phytofluene	*55 %	*67 %	9 %
α-Tocopherol	*8 %	-3 %	7 %
* Statistically significant changes (p<0.05)			

Table 5

Skin surface before and after 12 weeks

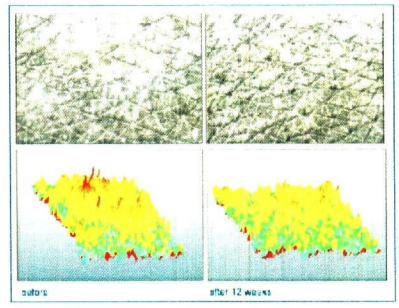

Figure 3

Decrease of roughness and scaling during the supplementation

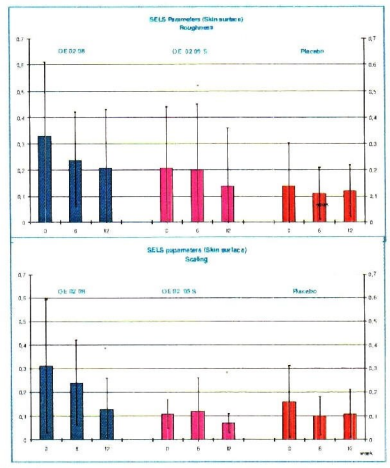

Figure 4

SELS parameters (Skin surface)				
Roughness	OE 02 08	decrease	- 37,78 %	n. s.
Roughness	OE 02 05 S	decrease	- 32,71 %	*p=0.005
Roughness	Placebo	decrease	- 15.68 %	n. s.
Scaling	OE 02 08	decrease	- 57.99 %	*p=0.007
Scaling	OE 02 05 S	decrease	- 44.22 %	*p=0.014
Scaling	Placebo	decrease	- 30.14 %	n. s.
Percentual change of means from week 0 to week 12				

Table 4

CONCLUSIONS

After 12 weeks of supplementation, it has been shown that a mix of carotenoids could positively influence skin ageing by increasing skin thickness and skin density as well as reducing roughness and scaling of the skin surface. Bioavailability of carotenoids was confirmed by significant increase of serum concentrations.

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