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Research paper

Cutaneous concentration of lycopene correlates significantly with the roughness of the skin

Maxim Darvin^a, Alexa Patzelt^a, Saskia Gehse^a, Sabine Schanzer^a, Christian Benderoth^b,
Wolfram Sterry^a, Juergen Lademann^{a,*}

^a Charité-Universitätsmedizin Berlin, Department of Dermatology, Berlin, Germany^b GFMesstechnik GmbH, Teltow/Berlin, Germany

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Abstract

Antioxidant substances in the skin are expected to slow down photo ageing. We therefore developed the hypothesis that high levels of antioxidant substances may be correlated to lower levels of skin roughness.

By utilizing modern optical non-invasive *in vivo* methods, the structures of the furrows and wrinkles as well as the concentration of lycopene were analyzed quantitatively on the forehead skin of 20 volunteers aged between 40 and 50 years.

In a first step, the age of the volunteers was correlated to their skin roughness. Here, no significant correlation was found. In a second step, a significant correlation was obtained between the skin roughness and the lycopene concentration ($R = 0.843$).

These findings indicate that higher levels of antioxidants in the skin effectively lead to lower levels of skin roughness, and therefore support our hypothesis.

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Keywords: Roughness; Furrows; Wrinkles; Lycopene; Free radicals; Skin ageing

1. Introduction

Recently, different authors have demonstrated that high doses of UV-radiation produce free radicals in the human skin, such as singlet oxygen (O_2), hydroxyl radicals (OH^\cdot), super-oxide-ions (O_2^\cdot) or peroxide radicals [1]. The processes of free radical and reactive oxygen species (ROS) formation are more efficient in the UVA spectral range than in the UVB part of the spectra [2,3]. The radicals and ROS not only damage the DNA, but also negatively influence the generation of connective tissue [3,4]. As a result, the renewal of elastin and collagen fibres is disturbed, and furrows and wrinkles appear on the skin [5,6].

Visual skin ageing is therefore in part the result of the accumulation of irreversible conformational changes and defects in the skin tissue, caused by the action of free radicals and ROS [7,8].

The human body has developed a natural protection mechanism against these reactive molecules. Antioxidant substances such as carotenoids, vitamins and others are stored in the skin, and are able to provide protection against free radicals and ROS before they interact with the living cells [9–11].

Carotenoids are one of the main antioxidant substances in the skin [12,13], which, however, cannot be synthesized by the human organism. Therefore, carotenoids must be taken up with the diet. The most important carotenoid antioxidants of the skin are β -carotene and lycopene. These substances effectively neutralize the oxidative activity of free radicals and other reactive species. The efficacy in neutralizing radicals is much higher for lycopene than for other carotenoids presented in the skin [14]. Several studies have

* Corresponding author. Charité-Universitätsmedizin Berlin, Department of Dermatology, Center of Experimental and Applied Cutaneous Physiology (CCP), Charitéplatz 1, 10117 Berlin, Germany. Tel.: +49 30 450 518 100; fax: +49 30 450 518 918.

E-mail address: juergen.lademann@charite.de (J. Lademann).

indicated that antioxidant substances can reduce skin damage by neutralizing the destructive action of free radicals and ROS [2,7,8,13]. Based on this assumption, different cosmetic products and nutritional foods are on the market, which promise an anti-ageing effect for the skin.

Taking the protective behaviour of antioxidant substances, in particular for the skin, into consideration, the hypothesis arises that a correlation exists between the cutaneous concentration of antioxidants and the visual appearance of the skin.

Therefore, the present study was developed to verify whether there is a correlation between the lycopene level in the skin and the skin surface structure (roughness), using modern non-invasive techniques. The development of non-invasive techniques for the measuring of the skin surface structures [15–17] and for the detection of carotenoid antioxidant substances in the skin by Raman spectroscopy [18–21] has substantially facilitated this effort.

2. Materials and methods

2.1. Volunteers

The experiments were performed on 15 female and 5 male volunteers, aged between 40 and 50 years (mean 44.85 ± 2.37 years). This age group was selected because the furrows and wrinkles of the skin are usually more pronounced in this age group compared with younger subjects. In addition, the narrow age range makes it possible to minimize the influence of age on the skin surface structure. Seventeen volunteers were of photo type II and 3 volunteers were of photo type III.

The volunteers were carefully interviewed about their lifestyle habits during the last 20 years with the help of a questionnaire, such as:

- private and occupational stress factors
- health conditions
- nutritional habits
- tobacco and alcohol abuse
- mean sun exposure.

This questioning was performed in order to exclude that lifestyle habits had changed significantly during the last 20 years. Substantial lifestyle changes as well as the regular application of skin care products or food supplements containing antioxidants led to an exclusion of the volunteers from the study.

Approval of the experiments had been obtained from the Ethics Committee of the Charité Hospital. The study was conducted according to the ethical rules stated in the Declaration of Helsinki Principles. The volunteers participating in the study had given their informed written consent.

Two measurements were carried out on the light-exposed skin area on the center of the forehead without any pre-treatment of the skin:

1. the determination of the skin surface structure
2. the determination of the relative concentration of lycopene in the skin, in order to evaluate whether both values show a significant correlation.

2.1.1. The determination of the skin surface structure (roughness)

The skin surface structure was analyzed in the non-contact mode using the 3D optical system Primos 4.0 (GF Messtechnik GmbH, Teltow, Germany) as described in detail by Jacobi et al. [16]. This system is based on the digital stripe projection technique, which is used as an optical measurement process. A parallel stripe pattern is projected onto the skin surface and depicted on the CCD chip of a camera through an optical system. The measurement system consists of a freely movable optical measurement head (with an integrated micro-mirror projector, a projection lens system, and a CCD recording camera), together with an evaluation computer. The 3D effect is achieved by the minute elevation differences on the skin surface, which deflect the parallel projection stripes. The measurements of these deflections represent qualitative and quantitative measurements of the skin profile [15–17]. The roughness, which is based on the depth and the density of the furrows and wrinkles of the skin, was determined using the software Primos system. The average roughness (R_a) was determined as a mean value of the roughness parameters measured along 24 selected lines. R_a is the mathematical average value of profile amounts within the total measuring length, and represents the roughness of the skin surface structure [22].

The same skin area was measured three times and the mean values and standard deviations were subsequently determined. High roughness values corresponded to deep furrows and wrinkles with a high density.

2.1.2. Determination of the relative concentration of lycopene in the skin

A non-invasive fast optical measuring method based on resonance Raman spectroscopy was used in this study. The experimental prototype of the Raman setup was specially developed and optimized for measurements of the carotenoid lycopene in the skin, as described in detail by Darvin et al. [20].

The radiation of an Ar⁺ laser at 514.5 nm is focused into an optical fibre which is connected to an optical imaging system, where the light is filtered and focused onto the skin. The Raman signal from the skin is collected by a lens system and transferred into a fibre bundle, connected to a spectrograph. The spectrum is recorded by a CCD camera and transferred to a personal computer.

The same skin area of each volunteer was measured three times, after which the average values and standard deviations were determined.

The relative concentration of lycopene was determined non-invasively by Raman spectroscopic measurements on

the same skin area where the skin surface structure had been analyzed. The measured skin area was 6 cm² for the determination of the skin surface structure and 0.33 cm² for the detection of lycopene. The lycopene measurements were performed in the centre of the area that was used for the skin structure measurements. In preparation of experiments, it could be shown that both measurements did not influence each other. The detection limit of lycopene is 0.002 arb. units.

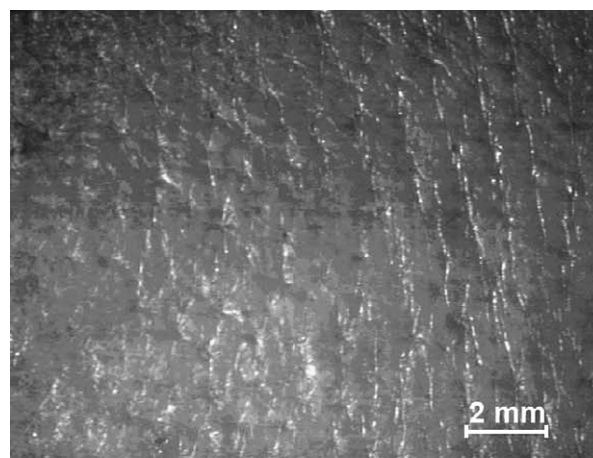
3. Results

For all volunteers, data for skin roughness and lycopene concentration were obtained. The data including mean values and standard deviations are summarized in Table 1. In a second step, correlation coefficients were calculated for skin roughness and age as well as for skin roughness and lycopene concentration.

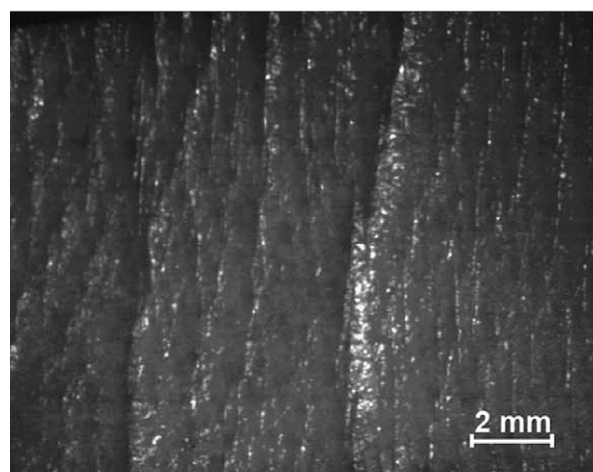
3.1. Correlation between the roughness of the skin and the age of the volunteers

Typical skin surface images and the corresponding skin surface profiles, representing a cross-section of the skin, are presented in Figs. 1 and 2, for two different volunteers, both 44 years old. The images were taken utilizing the integrated camera of the optical system Primos 4.0 (GFMess-technik GmbH, Teltow, Germany).

The structure of the furrows as well as the interindividual differences in the structure and depth of the furrows between the volunteers can be clearly seen. The corresponding roughness value R_a , which describes the depth and density of the furrows, can be utilized to objectify these interindividual differences. R_a was calculated as



Volunteer 1



Volunteer 2

Fig. 1. Skin surface images of two volunteers (a and b), both 44 years old, with a different skin surface structure and skin surface profile.

Table 1

Characterisation of volunteers, including sex, photo type, age, roughness and lycopene concentration

Volunteer No.	Sex m = male f = female	Photo type	Age	Roughness (μm)	Lycopene concentration (arb. units)
1	f	II	47	31	0.012353
2	f	II	45	24	0.013063
3	m	II	49	25	0.012
4	f	II	45	17	0.020783
5	f	II	45	18	0.0176
6	f	II	43	28	0.014457
7	f	II	46	21	0.01844
8	f	II	47	43	0.007948
9	f	II	42	29	0.011049
10	f	II	42	21	0.01693
11	f	II	46	19	0.020473
12	f	III	45	15	0.02102
13	m	III	41	22	0.016563
14	f	II	43	34	0.010657
15	m	II	45	42	0.0055
16	m	II	43	35	0.01259
17	f	III	46	22	0.02111
18	f	II	45	37	0.012545
19	f	II	50	33	0.013155
20	m	II	42	30	0.010504
Mean values and standard deviations			44.9 \pm 2.4	27.3 \pm 8.3	0.014437 \pm 0.004504

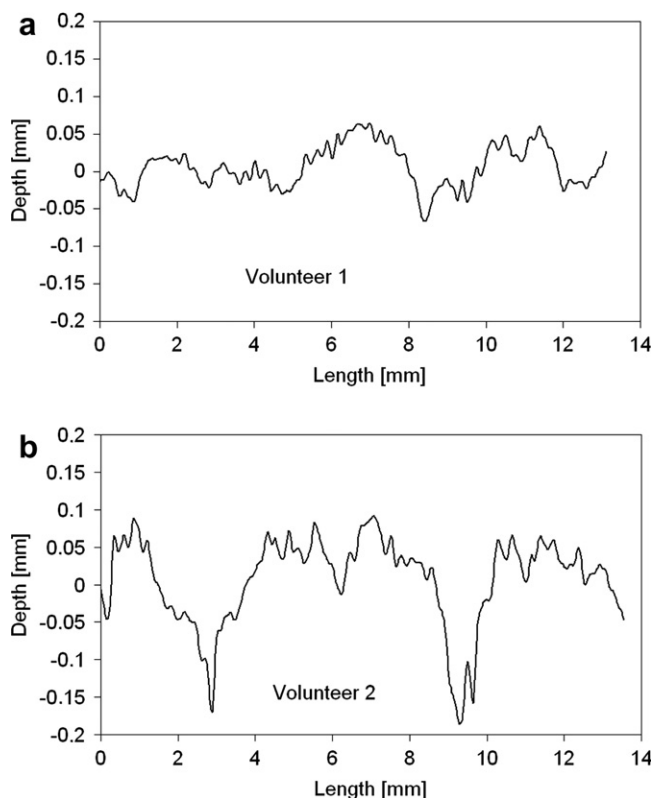


Fig. 2. (a) With a roughness of $R_a = 23.83$ (Volunteer 1); (b) with a roughness of $R_a = 35.65$ (Volunteer 2).

$R_a = 23.83 \mu\text{m}$ for the first volunteer (Fig. 2a), and as $R_a = 35.65 \mu\text{m}$ for the second volunteer (Fig. 2b). The roughness values determined for all 20 volunteers were correlated to the age of the volunteers by calculating the Spearman–Rho correlation coefficient, using the computer program SPSS[®]14.0. The correlation coefficient was 0.067 with a high significance level of 0.778, indicating that there is no significant correlation and that the two variables, age and skin roughness, do not correlate linearly.

3.2. Correlation between roughness and the concentration of lycopene in the skin

Furthermore, for each volunteer, the skin roughness was correlated to the concentration of lycopene in the skin. The Spearman–Rho correlation coefficient was -0.843 with a significance level of $p < 0.01$, which means the correlation is significant and that both variables, skin roughness and lycopene concentration, are linearly related.

4. Discussion

The present investigation was carried out on 20 healthy volunteers to investigate the hypothesis that higher levels of antioxidant substances might be related to lower levels of skin roughness. In total, three variables were determined for each volunteer: age, skin roughness and the concentra-

tion of lycopene in the skin. These variables were then investigated for their correlation.

The volunteers were aged between 40 and 50 years. This narrow age segment was chosen, as furrows and wrinkles are usually well pronounced and easily detectable on the skin at this age (see Figs. 1 and 2). The narrow age segment additionally reduced the influence of the age parameters on the skin surface structure. The skin roughness showed high interindividual differences (see Figs. 2a and b) of volunteers even of the same age. Therefore, it was not surprising that the two variables, age and skin roughness, showed no significant correlation. The results indicate that further influences apart from age might contribute to skin roughness.

Recently, different authors have demonstrated that oxidative stress, produced for example by irradiation with UV-light or by other environmental hazards, can damage the fibroblasts of the skin [1–6], which may contribute to premature skin ageing and the development of furrows and wrinkles. Moreover, it is known that antioxidant substances in the skin provide protection against free radicals and ROS [9], but little is known about whether high levels of antioxidants in the skin reduce the appearance of furrows and wrinkles. We therefore investigated the correlation between the level of the antioxidant substance lycopene as a representative of the carotenoids and the skin roughness. It is clear that the level of lycopene, measured on the day of the experiment, represents only a snapshot of the average level of lycopene of each volunteer, as the lycopene level is subject to fluctuations depending on nutrition and oxidative stress conditions. It is also well known that the development of furrows and wrinkles is a slow process, and that if our hypothesis is correct, a high long-term antioxidant level should be of relevance. Although it has been shown that different volunteers have different levels of lycopene in the skin [21,23], results from preliminary experiments suggest that the individual level of lycopene remains almost constant for each volunteer over a long period of time, provided that lifestyle habits have not changed significantly (unpublished results).

The calculation of the correlation between the lycopene level and the skin roughness revealed a correlation coefficient of -0.843 with a significance level of $p < 0.01$. This means that volunteers, who had high levels of lycopene exhibited lower levels of skin roughness.

These results support our hypothesis that lycopene, as well as other antioxidant substances, may be able to reduce the formation of furrows and wrinkles.

Our results correlate with the findings obtained by Heinrich et al., which showed that the roughness of the skin diminished after the supplementation with different antioxidant micronutrients, including lycopene [24]. Lycopene, in comparison to other types of carotenoids, is known to offer an increased protection efficiency in reactions with free radicals [14].

In conclusion, it can be assumed that skin roughness depends not only on age (when considering young and old people), but also on other factors. In the present study,

we provide indications that the level of antioxidant substances in the skin might play an important role in this context. As the level of antioxidant substances in the skin is strongly influenced by the oral or topical uptake of substances containing antioxidants, the appearance of the skin may reflect lifestyle conditions and nutritional habits. Lycopene can be assumed to represent an efficient protection system against the negative action of the free radicals in the skin. Similar results have to be expected also for other types of antioxidant substances, which are efficient radical quenchers, such as vitamins C and E.

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