One-year study on the variation of carotenoid antioxidant substances in living human skin: influence of dietary supplementation and stress factors

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Abstract. Variation in the level of the carotenoid antioxidant substances beta-carotene and lycopene in the human skin of ten healthy volunteers was measured with resonance Raman spectroscopy in an *in vivo* experiment over the course of 12 months. Information on the lifestyle of the volunteers concerning dietary supplementation and stress factors was obtained daily by the completion of questionnaires. The results showed individual variations in the levels of carotenoid antioxidant substances in the skin of the volunteers, which strongly correlated to specific lifestyles, such as the intake of dietary supplementations rich in carotenoids, and the influence of stress factors. A carotenoid-rich nutrition, based on large amounts of fruit and vegetables, increased the measured carotenoid levels of skin, while stress factors such as fatigue, illness, smoking, and alcohol consumption gave rise to a decrease in carotenoid levels of the skin. These decreases occured relatively quickly over the course of one day, while the subsequent increases lasted for up to 3 days. During the summer and autumn months, an increase in the level of carotenoids in the skin was measured for all volunteers. The average "seasonal increase" of the carotenoid content in the skin was determined to be 1.26-fold.
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1 Introduction

The human organism has developed an effective defense system that neutralizes free radicals and reactive oxygen species (ROS).[1–4] These highly reactive substances are constantly produced in the organism as a result of physiological metabolic processes and harmful external factors, such as irradiations and environmental toxins. The mediators of this defense system include antioxidant substances such as carotenoids (beta-carotene, lycopene, and lutein/zeaxanthin), vitamins (A, C, D, and E), enzymes (superoxide dismutase, catalase, and glutathione peroxidase), as well as various other substances (flavonoids, lipoid acid, uric acid, selenium, coenzyme Q10, etc.).[1,5] Beta-carotene and lycopene represent the largest group of cutaneous antioxidants, constituting approximately 70% of the carotenoids in the human skin.[6] The antioxidant substances possess synergistic effects, and thus protect each other from direct destruction during neutralization of the free radicals and ROS.[7,8]

It is noteworthy that the human body can neither synthesize carotenoids nor the vitamins A, C, or E. These substances are taken up by the body via the diet, or can be applied to the skin as active ingredients in dermatological or cosmetic products.

For optimal defense and functioning of the organism, a balance must be maintained between the formation of free radicals and antioxidant production. If this balance is disturbed, and the amount of radicals formed is significantly increased, the defense mechanism of the body is not able to neutralize all these reactive molecules.[9] A chain reaction is initiated, and oxidative stress occurs. Antioxidants are destroyed as a result of the interaction with high amounts of free radicals and ROS.[10,11] The antioxidative potential of the organism is decreased, and free radicals react with the living cells in an uncontrolled manner, giving rise to cell damage. This may contribute to a number of serious inflictions such as cancer, infarcts, arteriosclerosis, arthritis, Alzheimer, Parkinsons, and others.[12–14]

During the course of a lifetime, the human skin is in contact with harmful environmental factors, which permanently reduce the level of antioxidants in the skin. Simultaneously, metabolic processes within the human organism also lead to changes in this level due to influences of free radicals and ROS. The antioxidants are thus constantly being destroyed and restored, and the resulting changes in the antioxidant levels of the human skin reflect these processes. Thus, the level

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of antioxidants in the skin mirrors their systemic concentration, which has been shown experimentally by comparing skin and blood samples.[15]

In the past, high pressure liquid chromatography (HPLC) was the standard method for detecting antioxidants in tissue and blood samples.[16] This method has the disadvantage that it is time-consuming and expensive. Additionally, it requires the invasive removal of biopsies or blood samples from volunteers. Subsequent to the introduction of the noninvasive online Raman spectroscopic measurements, it became possible to screen large groups of volunteers and to measure the kinetics of the antioxidative potential of the skin regarding the concentration of carotenoids *in vivo*, depending on the influence of external and internal factors.[17]

It could be demonstrated that there is a strong correlation between the level of antioxidants in the skin and skin aging. For example, people with a high carotenoid level in the skin are less susceptible to preliminary aging, which occurs as a result of the excessive action of free radicals in the skin. Recently obtained results demonstrated that a strong correlation exists between the appearance of the skin, i.e. furrows and wrinkles, and the level of lycopene in the skin.[18]

The distribution and concentration of antioxidants in the human skin strongly depends on the state of health, supplementation, lifestyle, skin type, body site, and the influence of stress factors. The differences remain significant after adjustments for age, gender, mode of living, and diet intake estimates.[19–22] Moreover, smokers usually have a lower level of carotenoids in the skin compared to nonsmokers.[17]

In the present study, resonance Raman spectroscopy was used for the noninvasive determination of the individual variations in the concentrations of carotenoids in the skin of ten healthy volunteers over the course of 12 months. The results were compared to the lifestyle of the volunteers based on their nutritional behavior and the influence of different stress factors.

2 Materials and Methods

2.1 *Volunteers*

The measurements of the carotenoid antioxidants in the skin were carried out over the course of 12 months on ten healthy volunteers (2 male, 8 female) aged between 23 and 50 years at the Center of Experimental and Applied Cutaneous Physiology (CCP) of the Department of Dermatology and Allergology, Charité—Universitätsmedizin Berlin. The measurements were performed daily at the same time, except for on weekends and holidays, and at the same position on the palm of the left hand. Before each measurement, volunteers were interviewed about their diets and the influence of possible stress factors, such as illness, smoking, alcohol consumption, fatigue, etc., using questionnaires. All volunteers had skin type 2.[23]

The volunteers were asked not to use dermatological or cosmetic products containing antioxidants during the course of the experiment.

Approval of the experiments had been obtained from the Ethics Committee of the Charité—Universitätsmedizin Berlin. The study was conducted according to the ethical rules stated in the Declaration of Helsinki Principles.

2.2 *Raman Spectroscopic Measurements*

Resonance Raman spectroscopy was used for the fast *in vivo* determination of the concentration of carotenoid antioxidant substances, such as beta-carotene and lycopene, in human skin.

The radiation of an Ar^+ laser, which operated at 488 nm, was filtered and focused onto the skin. The weak Raman scattered signal from the skin was filtered, collected by a lens system of an optical imaging system, and transferred into a fiber bundle to a spectrograph. The skin spectrum was recorded by a charge-coupled device (CCD) camera and visualized on a PC. The small Raman peak, which corresponded to the concentration of carotenoids in the skin, could easily be detected on a large fluorescence background. The software subtracted the fluorescence background and determined the intensity of the Raman peak, which corresponded directly to the concentration of carotenoids present in the skin. The obtained concentrations were measured in arbitrary units (a.u.) for all volunteers and were thus comparable between the subjects. The measured Raman peaks were usually more than ten-fold higher than the quantitative limit of the measurements, which was determined as a noise signal (averaged around 0.003 a.u.). One measurement, needed for determination of the concentration of carotenoids in the skin, lasted 5 s. This measurement was performed three times on the palm of the left hand to determine the scattering of the measured values of the cutaneous concentration of carotenoids. The average value was subsequently determined and analyzed. Thus the total time of the measurements was 15 s for one volunteer for each day.

The superficial penetration depth ($\sim$200 μm) of the blue laser light at 488 nm into the skin confined the Raman measurements to the epidermis.

The experimental arrangement for the measurements of carotenoid antioxidant substances have been described in detail by Darvin et al.[17]

2.3 *Statistical Analysis*

The average value and the standard deviation of the carotenoid concentrations measured daily were determined for ten volunteers. The confidence interval including approximately 68% of the measured values was evaluated as an average value $\pm$ standard deviation.

The Wilcoxon test (SPSS 13.0) was utilized for the statistical analysis of the seasonal differences regarding the content of carotenoids in the skin of the ten volunteers.

3 Results and Discussion

3.1 *Seasonal Influence on the Monthly Concentration of the Carotenoids in the Skin*

The average monthly values of the ten volunteers, which were obtained from the daily values measured over the period of 12 months, are shown in Figs. 1(a) and 1(b). The results show that in the summer and autumn months, the average values of cutaneous carotenoids were higher than during the spring and winter months.

This "seasonal increase" of the cutaneous carotenoids, which characterizes an increase of the carotenoid concentration in the skin during the summer and autumn months com-

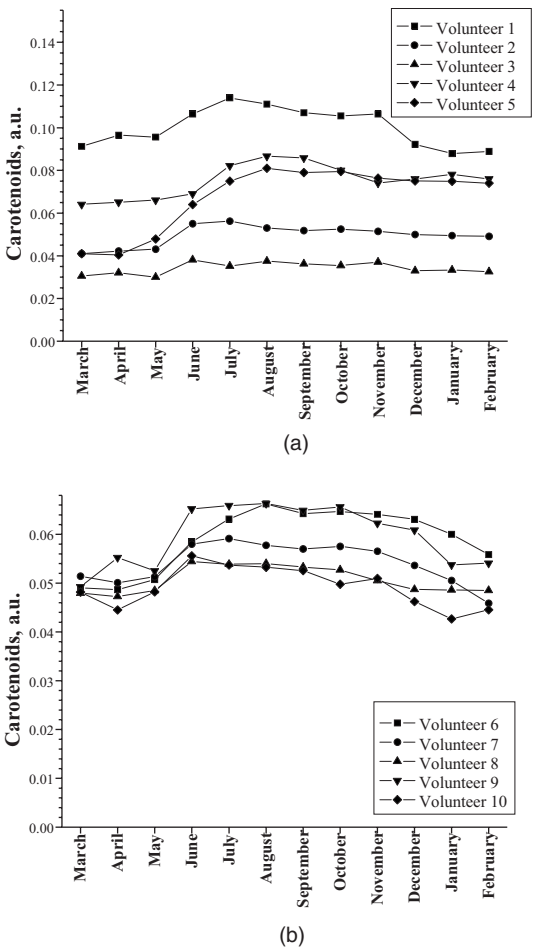

Fig. 1 Average monthly values of the carotenoid antioxidant substances in the skin of ten volunteers during a 12-month period. (a) Volunteers 1 to 5 and (b): volunteers 6 to 10.

pared with the winter and spring months, is summarized in Table 1 for each of the ten volunteers. The average "seasonal increase" of the carotenoid content in the skin for all volunteers was determined to be (1.26 ± 0.21)-fold. This increase was found to be statistically significant $(p=0.001)$.

The seasonal variation of the carotenoid antioxidant level in the skin can be explained by a higher consumption of fruit and vegetables, which are naturally rich in carotenoids, in the daily ration of the volunteers during the summer and autumn months, as well as by a decreased occurrence of diseases.

Different volunteers had different individual levels of carotenoid concentrations in their skin. The average values of the individual carotenoid levels in the skin are presented in Fig. 2 as an average value ± standard deviation.

The analysis of the questionnaires showed that, for example, volunteer 1 usually ate a very large amount of fruit and vegetables each day. Volunteers 2 and 3 ate small amounts of fruit and vegetables, while volunteer 3 was also a strong smoker. Their low cutaneous carotenoid levels can be explained by the consumption of only small amounts of carotenoid-rich food. The almost three-fold difference between the carotenoid levels of volunteers 1 and 3 strongly correlated with their lifestyle, in particular with their nutrition. There were almost no observed changes in the cutaneous concentration of the carotenoids during the one year for volunteer 3. The relatively constant level and very small "seasonal increase" (1.12 ± 0.04) of the carotenoid concentration for this volunteer suggests that the "seasonal increase" was mainly caused by nutrition, which included increased uptake of carotenoid-rich foodstuffs. The influence of external factors such as increased sun radiation, smoking, etc. gave rise to the temporary decrease in the level of carotenoids in the skin, which subsequently leveled.[22]

Volunteers 4 to 10 had moderate levels of carotenoids in the skin. Their diets remained relatively constant regarding the amount of fruit and vegetables in the daily ration, as did the amounts of influencing stress factors.

The volunteers, whose values are presented in Figs. 1 and 2, neither altered their diet nor their stress status significantly during the 12-month investigation period with the exception of volunteer 5. Their average level of carotenoids in the skin remained almost constant, excluding the presence of the "seasonal increase." Thus the recovery and degradation processes of the cutaneous carotenoids balanced each other under physiological conditions.

Volunteer 5 had a low initial concentration of carotenoids in the skin. Taking these low values into consideration, this volunteer started to increase his daily consumption of fruit and vegetables consistently for three months after the beginning of the measurements. The volunteer significantly minimized smoking and tried to exclude the influence of possible stress factors. The results presented in Fig. 1(a) show the average monthly concentration of carotenoids in the skin for this volunteer. As can be seen, the level of carotenoids increased approximately two-fold compared with the initial values as a result of the changes in lifestyle.

From the average monthly values presented in Fig. 1 and the average annual level presented in Fig. 2, it becomes clear that the individual carotenoid antioxidant level of the volunteers reflect their lifestyle. This became much more obvious when the daily measured values of the volunteers were analyzed over the course of 12 months.

3.2 *Relation between the Daily Carotenoid Concentration in the Skin and the Influence of Stress Factors*

As a typical example, Figs. 3(a) and 3(b) show the average daily values of the carotenoid concentration in the skin measured during a 12-month period for volunteer 6.

Two different areas marked "A" and "B" in Fig. 3(a) corresponded to the winter-spring months (area "A") and to the summer-autumn months (area "B"), where the minimum and maximum of measured values were obtained ("seasonal increase"). Each cross-hatched region in Fig. 3(a) corresponds to the confidence interval, which is equal to the average value ± standard deviation interval. The presented confidence interval contains approximately 68% of the measured values.

Questionnaires were used to find possible explanations for the appearance of untypically strong increased or decreased carotenoid values lying outside the confidence interval [see Fig. 3(a)]. These periods, when the daily values were significantly higher or lower than the average concentrations, were marked by numbered areas from "I" to "IV" in Fig. 3(b).

Table 1 The seasonal increase, confidence interval, and the quantity of strong variations in the content of carotenoids in the skin of ten volunteers (2 male, 8 female) are shown. Areas A and B correspond to the winter-spring and summer-autumn months, respectively.

Volunteer	Sex, age	Seasonal increase, [fold]	Measured region	Average value ± standard deviation	Quantitative amount of increases	Quantitative amount of decreases
1	F,		A	0.091±0.011	1	2
	45	1.20±0.06	B	0.109±0.008	2	1
2	F,		A	0.042±0.003	1	2
	23	1.31±0.06	B	0.055±0.005	2	1
3	F,		A	0.032±0.004	1	2
	45	1.12±0.04	B	0.036±0.003	2	2
4	F,		A	0.064±0.008	1	2
	32	1.33±0.05	B	0.085±0.009	3	1
5	F,		A	0.045±0.007	0	2
	34	1.95±0.06	B	0.087±0.009	3	1
6	F,		A	0.051±0.005	1	2
	48	1.31±0.05	B	0.067±0.006	1	2
7	M,		A	0.050±0.005	2	2
	28	1.16±0.05	B	0.058±0.005	3	2
8	F,		A	0.045±0.007	1	2
	27	1.15±0.08	B	0.052±0.006	2	1
9	F,		A	0.051±0.007	2	2
	27	1.27±0.09	B	0.065±0.008	1	1
10	M,		A	0.045±0.005	1	2
	50	1.18±0.09	B	0.053±0.006	2	1

During the evaluation of the questionnaires, it was found that these distinctive values correlated to strong stress factors, such as illness, fatigue, smoking, and alcohol consumption, to which the volunteers were exposed (when the values were less than average), or to the uptake of a high amount of food rich in carotenoids, as well as a reduced influence of stress factors (when the values were higher than average).

Table 1 shows the quantities of the strong increases/decreases in the level of cutaneous carotenoids for each of the ten volunteers, which were measured daily during the 12-month period. These specific events were directly correlated with the lifestyle changes of the volunteers, which had been noted in the questionnaire.

The periods of strong changes in the carotenoid concentration in the skin, marked by the numbered areas from "I" to "IV" in Fig. 3(b), are presented with a higher magnification in Fig. 4.

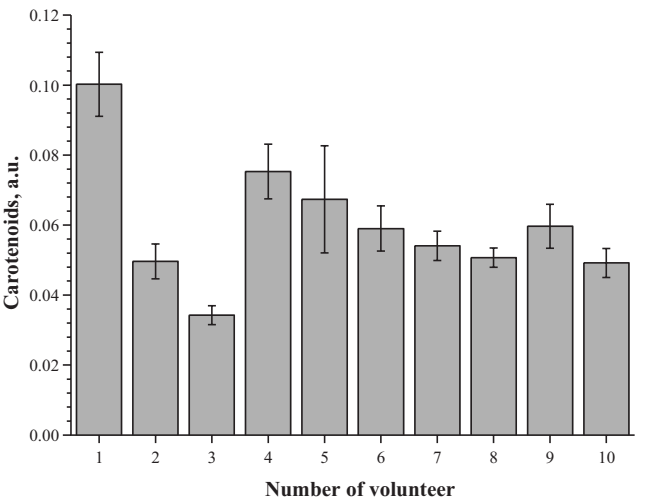

Fig. 2 Average annual values of the carotenoid concentrations in the skin of all volunteers.

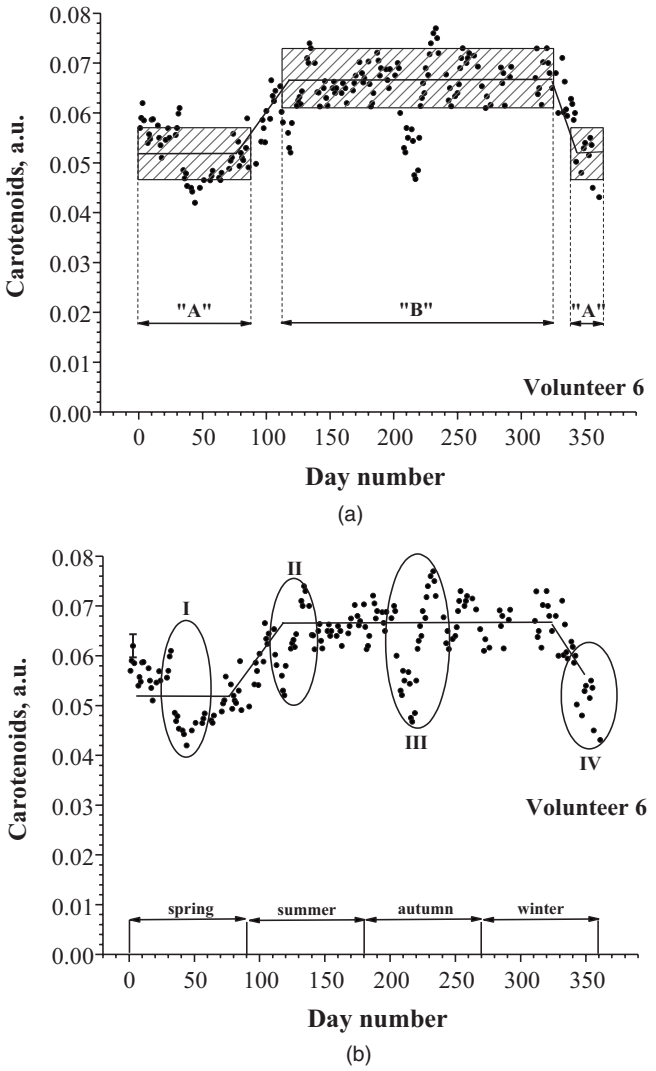

Fig. 3 Average daily values of the carotenoid concentrations in the skin during the course of one year (volunteer 6). (a) The average value ± standard deviation (confidence interval) is shown. (b) Areas lying outside the confidence interval are shown.

Additionally, in Table 2, extracts from the corresponding questionnaires are shown. The synopsis of the data reveals a distinct correlation between the state of health, a diet rich in fruit and vegetables, and the level of carotenoid antioxidants in the skin of volunteers. The current values of the concentration of carotenoids in the skin were compared with the previous values and then labeled with appropriate symbols (↔, ↑ or ↓), which are shown in the last column of Table 2. If there were no changes in the carotenoid concentration within the typical spread in values, this is marked with the symbol (↔). An increase is shown with the symbol (↑), while a decrease is shown with (↓). The paired symbols in the last column of Table 2 correspond to the group of events and indicate the slight increase [see for example I (1 to 4), I (8 to 10), and I (30 to 32)] or slight decrease [I (21 to 27), II (3), II (16 to 20), III (24 to 30), and III (4)] of the concentration of carotenoids in the skin. In all cases of illness and sickly feeling [see I (21 to 29), II (4 to 6), III (4 to 14), IV (10, 11) in Fig. 4 and in Table 2], the level of carotenoids in the skin strongly decreased compared with the initial level.

Additionally, other stress factors such as large amounts of alcohol and smoking reduced the level of carotenoids in the skin [see IV (3) in Fig. 4 and in Table 2].

The same effect was observed for stress in the workplace, such as long phases of physical and mental exertion without regular breaks [see II (2, 3) in Fig. 4 and in Table 2].

It was found that the level of carotenoid antioxidants in the skin of volunteers who had some periods of reduced stress conditions increased, even if their nutrition remained unchanged.

Stress is commonly accompanied by the production of high amounts of free radicals, which are immediately neutralized by the antioxidative system of the human body. This interaction gives rise to the neutralization of free radicals and, as a result, to the degradation of carotenoid antioxidants of the body.

The uptake of high amounts of food rich in carotenoids, such as fruit and vegetables, increased the level of carotenoids in the skin [see I (19, 20), II (13, 15), and III (22, 23) in Fig. 4 and Table 2]. The kinetics of these findings are supported by results obtained previously, where the influence of carotenoid-rich nutrition on the cutaneous level of carotenoids was shown.[24,25] In Fig. 5, as an additional example, a long-term kinetic of the carotenoid concentration in the skin is shown for volunteer 7. Again, the values lying outside the confidence interval (marked areas in Fig. 5) corresponded to significant events, such as stress or antioxidant-rich supplementation, which were also interpreted in detail but not shown in this work. Similar tendencies were observed for all volunteers.

Analysis of the questionnaire showed correlations between the level of carotenoids in the skin and the influence of different stress factors, which were detected inside the confidence interval (see, for example, results presented for volunteer 6 in Figs. 3 and 4 and Table 2).

Significant decreases in the carotenoid antioxidant levels of the skin, which lay outside of the confidence interval, were found more often in the winter-spring months than in the summer-autumn months ($p<0.05$) for all volunteers. Additionally, significantly stronger increases were observed during the summer-autumn months ($p<0.05$) [see, for example, Figs. 3(a) and 5]. Figure 6 shows the difference between the quantitative amounts of increases and decreases for both areas A and B, which was averaged for ten volunteers. As can be seen, the obtained difference was positive for area B and negative for area A. These results demonstrate that the incidences of illnesses, which gave rise to the fall of carotenoids in the skin, occurred more often during the winter-spring months compared with summer-autumn months.

3.3 *Kinetics of Carotenoids in the Human Skin*

The increase and decrease of concentrations of carotenoids that were caused by different events showed different kinetics. The carotenoid decrease was detected immediately on the day after the volunteers were exposed to stress, while the increase needed up to three days to peak (see Fig. 4 and Table 2). The increase in the cutaneous carotenoid concentration due to an antioxidant-rich supplementation or the decrease as a result of stress occurred much more quickly than the leveling of the

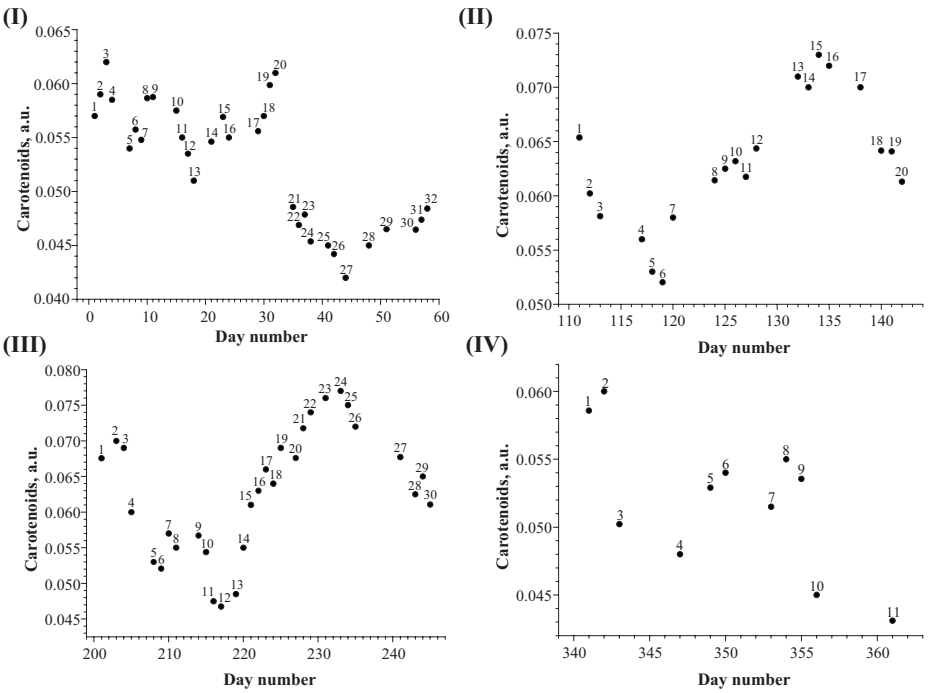

Fig. 4 Magnification of the numbered areas I to IV from Fig. 3(b) with strong variations in the carotenoid concentrations in the skin (volunteer 6).

carotenoid concentration back to the initial level subsequent to increasing or decreasing events.

A decrease in carotenoids was caused by the influence of free radicals and ROS, which were produced in the skin as a result of the direct influence of stress factors, and could give rise to oxidative stress.[10,14,26] In this case, carotenoids in the skin decreased rapidly. This effect was also observed by *in vivo* analysis of the degradation kinetics of beta-carotene and lycopene during UV and IR irradiations of the skin.[22,27]

The increase in the level of carotenoids occurs relatively fast (1 to 3 days) in comparison to the slow process of stratum corneum renewal (2 to 3 weeks). Increases in the carotenoid levels seem to be caused by numerous processes, which act simultaneously and independently of each other. The first one is the diffusion of the carotenoids from the blood and the hypodermis and the dermis to the epidermis of the skin. This process dominated in our opinion. The second one is the transport of carotenoids from the blood and the hypodermis and the dermis into the sweat glands, and then with the sweat onto the skin surface. This hypothesis is supported by the results that the highest concentration of carotenoids in the skin was detected on body sites with a high density of sweat glands, i.e., on the forehead and the palm,[17] and by our measurements of concentration of carotenoid substances in human sweat (unpublished data).

This assumption is also supported by the measurements of lipophilic antioxidant vitamin E in the sebum and its delivery onto the skin surface by the exuded sebum.[28] A similar process can be assumed for lipophilic carotenoids, which are bound to the lipid structures that are present in sweat.

4 Conclusions

The level of carotenoid antioxidants in human skin is influenced by a multiplicity of different factors, which often superpose each other. On the one hand, it is well known that stress factors such as UV and IR irradiations cause a decrease in the carotenoid levels of the skin.[22,27] It can therefore be assumed that other stress factors, which are usually also accompanied by the production of free radicals and ROS, may also lead to a decrease in cutaneous carotenoid levels. On the other hand, increases can be expected in the carotenoid levels of the skin after the supplementation of carotenoid-rich products, such as fruit and vegetables.[25] The kinetics of these two processes are different. Decreases in the carotenoid levels in the skin occured relatively quickly over the course of 2 h,

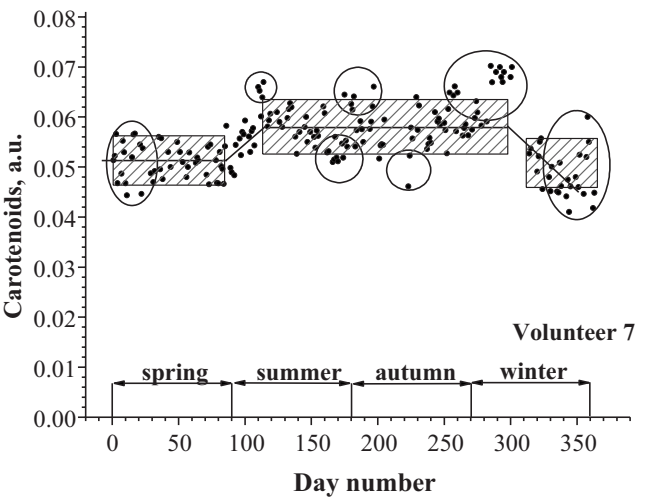

Fig. 5 Average daily values of the carotenoid concentrations in the skin of volunteer 7 over the course of 12 months. Confidence interval and areas with strong variations in the carotenoid concentrations in the skin are shown.

Table 2 Extracts from the completed questionnaire of volunteer 6, corresponding to the numbered areas I to IV shown in Figs. 3(b) and 4 (↔ is no changes, ↑ is an increase, and ↓ means decrease of the carotenoid concentration in the skin), are shown.

Event (numbered area)	Day number	Influenced factors (supplementation, stress)	Changes in carotenoid concentration
I	1 to 4	Normal lifestyle, after the leave	↔↑
	5 to 7	Normal lifestyle, working stress	↔
	8 to 10	Increased amount of fruit and vegetables in the daily ration	↑↔
	11, 12	Normal lifestyle	↔
	13	Fatigue, bad sleeping	↓
	14 to 18	Normal lifestyle	↔
	19, 20	Increased amount of fruit and vegetables in the daily ration, absence of stress	↑
	21 to 27	Illness (severe cold), sickly feeling, reduced amount of fruit and vegetables in the daily ration	↓↔
	28, 29	Sickly feeling, increased amount of fruit and vegetables in the daily ration	↔
	30 to 32	Normal feeling, increased amount of fruit and vegetables in the daily ration	↔↑
II	1	Normal lifestyle	↔
	2	Normal lifestyle, increased working stress	↓
	3	Working stress, reduced amount of fruit and vegetables in the daily ration	↓↔
	4 to 6	Food poisoning, sickly feeling, reduced amount of fruit and vegetables in the daily ration	↓
	7	Normal feeling, increased amount of fruit and vegetables in the daily ration	↑
	8 to 11	Normal lifestyle	↔
	12	Eating of pasta with a high amount of tomato sauce	↔
	13, 14	Normal lifestyle	↔
	15	Eating of a high amount of fruit and vegetables	↔
	16 to 20	Normal lifestyle	↔↓
III	1-3	Normal lifestyle	↔
	4	Sickly feeling, increased amount of fruit and vegetables in the daily ration	↓
	5 to 14	Illness, sickly feeling, reduced amount of fruit and vegetables in the daily ration	↔
	15 to 21	Normal feeling, increased amount of fruit and vegetables in the daily ration	↑
	22	Eating of pizza with a high amount of tomato sauce	↑
	23	Increased amount of tomatoes in the daily ration	↑

Table 2 (*Continued.*)

Event (numbered area)	Day number	Influenced factors (supplementation, stress)	Changes in carotenoid concentration
	24 to 30	Normal lifestyle	↔↓
IV	1, 2	Normal lifestyle	↔
	3	Party at a night, smoking, alcohol consumption	↓
	4	Fatigue, reduced amount of fruit and vegetables in the daily ration	↔↓
	5 to 9	Normal lifestyle, reduced amount of fruit and vegetables in the daily ration	↔
	10, 11	Illness (strong inflammation), reduced amount of fruit and vegetables in the daily ration	↓

while the subsequent recovery usually took up to three days to level.

Despite the fact that it is difficult to measure stress directly, the possibility of measuring the influence of some stress factors on human skin is presented in this study. It is possible to correlate particular influencing factors with the changes in the antioxidative potential of the skin, regarding the concentration of cutaneous carotenoids. It is demonstrated that the consumption of carotenoid-rich food as well as the absence of stress factors increases the concentration of the carotenoids in living human skin, while negative factors such as illness, fatigue, stress, alcohol consumption, and smoking reduce it. The level and kinetics of the concentration of carotenoids in the skin are different and clearly reflect the lifestyle of the volunteer.

Our findings indicate that a well-balanced diet rich in fruit and vegetables, as well as the avoidance of stress, increases the concentration of carotenoids. A high level of these substances in human skin therefore may provide efficient protection against skin aging, as has been demonstrated previously.[18,22,27,29,30]

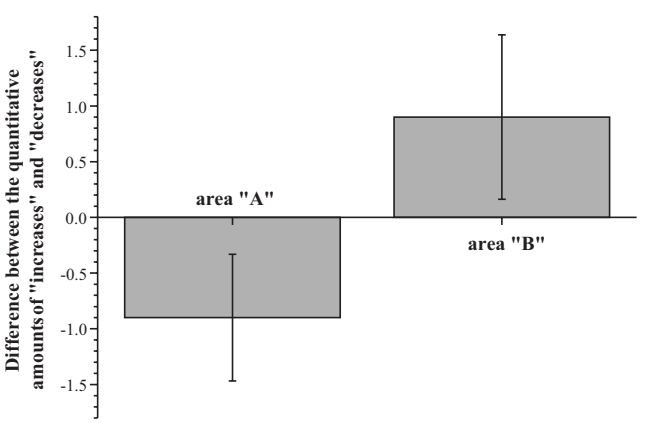

Fig. 6 Difference between the quantitative amounts of increases and decreases in the level of cutaneous carotenoids measured during winter-spring months (area A) and during summer-autumn months (area B), averaged for ten volunteers.

Thus, the concentration of cutaneous carotenoid substances can serve as a marker of increased/decreased oxidative processes in the organism, which occur under the influence of different stress factors, such as fatigue, illness, and different diseases.

The increase of carotenoid content in skin during the summer and autumn months compared with winter and spring months is determined to be 1.26-fold on average for all volunteers. The observed seasonal increase in the level of carotenoids in the skin is an important characteristic of the human skin, which allows a better understanding of the physiology of the skin. The seasonal increase provides a strengthened defense function of the skin during the months when sun irradiation can be dangerous.

Moreover, it is found that the quantity of decreases of the level of carotenoids in the skin is observed more often during the winter-spring months, compared with the summer-autumn months, which directly correlate with the incidences of illnesses during these months.

The Raman spectroscopy that is used in the present study is a perspective noninvasive method for screening the antioxidant potential based on carotenoid markers that can be applied for stress monitoring, and the evaluation of the protection efficacy of topically and systemically applied antioxidants on the skin.

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