Influence on the Carotenoid Levels of Skin Arising from Age, Gender, Body Mass Index in Smoking/ Non-Smoking Individuals

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ABSTRACT

Introduction: Intakes of fruit and vegetables rich in carotenoids are associated with a lower risk for a variety of chronic diseases. Therefore, the carotenoid levels in the skin have been measured in various studies to investigate the effects of nutrition and lifestyle. However, statistically clear data regarding the influence of age, gender, body mass index, and smoking behaviour were not documented. **Methods:** Thus, non-invasive resonance Raman measurements were performed on 151 healthy volunteers in Berlin, Germany. **Results:** The investigations have shown significantly enhanced total carotenoid values in the skin for women and non-smokers; individuals with a BMI lower than 30 also showed a trend to higher values. In the case of lycopene, significantly enhanced values were found in people younger than 40 and non-smokers. **Conclusion:** Influences of gender, and smoking or non-smoking must be taken into account when carrying out a study with respect to the carotenoids. Age does not play any role for total carotenoids but for lycopene.

Keywords: lifestyle, lycopene, resonance Raman spectroscopy,

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INTRODUCTION

Intake of fruit and vegetables rich in carotenoids has been associated with a lower risk for a variety of chronic diseases[1-3] and a positive effect on the immune function of the skin.[4,5] This is explained by the powerful antioxidative action of carotenoids in the reactions of neutralization of free radicals in human skin.[6-10] Stimulated by the positive results of a diet rich in fruit and vegetables in the support of some medical treatments and in cosmetics, artificial and natural antioxidant substances have been broadly applied. As a result, not only positive but also strong negative results have been obtained by different authors.[11] The optimal dose and a natural combination of antioxidants should be supplied by nutrition. In order to acquire more information from volunteers, the carotenoid intake by means of a natural diet or supplements, in combination with the lifestyle and their relation to health problems, should be further investigated, as well as the bioavailability of the substances in the body. For this reason, non-invasive optical methods are an excellent requisite for fast measurements and follow-up investigations, including repetitive measurements.

The total carotenoids and lycopene have been measured non-invasively in the skin in different studies.[12-15] In a number of studies, it was shown that the nutrition influences the values,[16-21] whereby stress, in form of sun irradiation, illness or the lack of sleep, reduces the values.[20,22,23] However, up to now, no systematic study about the concentration and distribution of the carotenoids in a representative healthy group of the population has been performed. This is of interest when volunteers are selected for a study where the skin carotenoids are in focus. Therefore, the mean values and distribution in a healthy group should be established. Furthermore, the influence of gender, smoking, age and body mass index (BMI) could influence the results and has to be taken into account. So far, no statistically clear data are available. Thus, the measurements on 150 persons were performed in Berlin and the correlation to gender, age, body mass index and smoking behaviour was investigated.

MATERIALS AND METHODS

Volunteers

151 healthy volunteers were measured with the Raman spectrometer to determine their lycopene and ß-carotene concentration, non-inversely in their skin. The measurements were performed within clinical studies for screening volunteers, and at an exhibition on a Berlin market place, where we offered the measurement free of cost.

The results obtained should show roughly a representative cross section of the Berlin population measured over a period of one year.

The measurements were carried out on the palm of the volunteers. All volunteers had given their written informed consent and were questioned regarding their age, gender, weight, height and whether or not they were smokers. All volunteers stated that they were not suffering from serious health problems.

In Table 1, mean values of age, weight, height and BMI are summarized and the corresponding values sorted by gender.

The group included 32 smokers, 92 non-smokers and 27 persons, who had given no information as to whether they were smokers or not.

Resonance Raman spectroscopy

Resonance Raman spectroscopy was used as a quick method for *in vivo* determination of the concentration of carotenoid antioxidant substances in human skin.[12] For the determination of cutaneous carotenoids, such as beta-carotene, lycopene and lutein, excitation wavelengths at 488 nm and 514.5 nm were applied. The carotenoids showed comparable absorption behaviour in the blue range of the spectrum. Therefore, blue light at 488 nm excites all the carotenoid substances with approximately the same Raman scattering efficiency.[24] Lycopene can be determined using an excitation wavelength of 514 nm.[24] In principle, the arbitrary units of the Raman signal can be transferred into absolute concentrations of β-carotene and lycopene, if the lutein concentration is negligible.[25] This was performed for the calculated mean values to give the opportunity to compare the results with other studies.

The reproducibility of the Raman measurements can be influenced by inhomogeneous pigmentation, roughness and the microstructure of the skin. The inhomogeneity of the skin pigmentation has a typical size of 1 to 2 mm. To reduce the influence of these factors, a laser spot with a diameter of 6.5 mm on the skin surface was used in the measuring device.[25] In this way, the spread of the Raman values did not exceed 10%. The measurements were performed on the palm in duplicate and mean values were calculated for further analysis.

Table 1. Statistical data of the 151 volunteers, (65 males and 86 females)

	Mean all ± SD	Mean (range)
Age [years]	38.2 ± 14.8	m 36.3 (18-69) f 39.7 (18-75)
Weight [kg]	72.4 ± 15.6	m 83.5 (57-140) f 64.7 (45-94)
Height [cm]	173.3 ± 9.1	m 179.4 (168-204) f 168.7 (152-186)
BMI [kg/m^2]	24.0 ± 4.1	m 25.6 (18.8-38.6) f 22.7 (17.2-34.5)

Statistical analysis

Diagrams were prepared using Microsoft Office EXCEL 2003. For statistical analysis, the SPSS 19.00 for Windows (SPSS Inc., Chicago, Illinois) software was used. An explorative data analysis was performed and for differences in mean values of the parametric independent data the t-test was used. The correlation coefficient was calculated in accordance with Pearson.

RESULTS

From the results in Table 2, it is evident that total carotenoids have a higher mean value compared to lycopene.

Higher values for total carotenoids were measured for all volunteers, but the ratio total carotenoids/lycopene changed in a broad range from 1.8 to 10, and some extreme values above 10 were measured up to 32. The correlation of the lycopene and total carotenoid values is shown in Figure 1. The Pearson correlation coefficient resulted in 0.714 with $p < 0.05$.

In Figure 2, the distribution of individual values of the carotenoids is presented dependent on age. From this diagram, no correlation was observed and the Pearson coefficient shows no significant correlation for total carotenoids; for lycopene a slight correlation was found ($r = 0.242$, $p < 0.01$). A calculation of the mean values below and above 40 years for all volunteers

Table 2. Mean ± SD values for total carotenoids and lycopene for all volunteers and sorted by gender, age, BMI and smoker/non-smoker

	Total carotenoids [10^{-4} a.u.]	Lycopene [10^{-4} a.u.]
Mean (n = 159)	5.36 ± 1.81	1.30 ± 0.65
Age < 40, n = 85	5.38 ± 1.77	1.40 ± 0.61
Age > 40, n = 62	5.37 ± 1.89	1.17 ± 0.69*
Male (n = 65)	4.91 ± 1.78*	1.20 ± 0.64
Female (n = 86)	5.69 ± 1.76	1.38 ± 0.65
Age < 40 Male (n = 39)	4,93 ± 1.84*	1.34 ± 0.64
Female (n = 46)	5.76 ± 1.65	1.45 ± 0.59
Age > 50 Male (n = 10)	4.30 ± 1.77	0.77 ± 0.49
Female (n = 23)	5.24 ± 1.74	1.13 ± 0.49
BMI < 30, n = 131	5.45 ± 1.84	1.34 ± 0.65
BMI ≥ 30, n = 20	4.76 ± 1.45(*)	1.07 ± 0.61(*)
Smoker (n = 28)	4.52 ± 1.77**	0.99 ± 0.50**
Non-smoker (n = 80)	5.69 ± 1.78	1.44 ± 0.69

(*)Trend (p < 0.1); *Significant (p < 0.05); **Highly significant (p < 0.001)

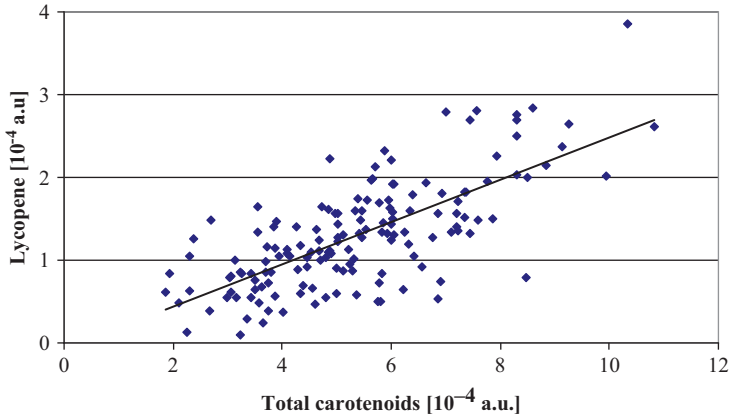

Figure 1. Correlation between measured values of lycopene and total carotenoids in the skin (n = 151)

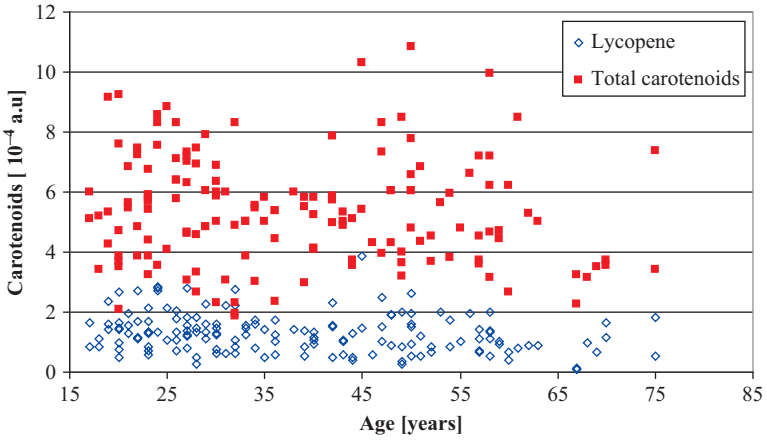

Figure 2. Correlation between the carotenoids in the skin and the age of the volunteers (n = 151)

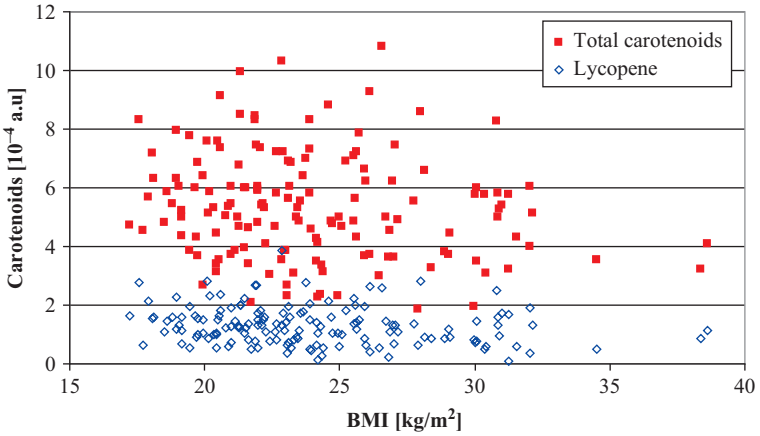

Figure 3. Correlation between the carotenoids in the skin and the body mass index (BMI) of the volunteers (n = 151)

(Table 2) showed no significant difference for total carotenoids, whereas lycopene showed significantly lower values in the group over 40 years of age.

Interestingly, the carotenoid values are influenced by gender (Table 2). Male volunteers exhibited significantly lower values than female volunteers ($p < 0.05$) of about 13%.

A detailed analysis by gender at different ages is also shown in Table 2. Below 40 represents values for females before the menopause and values above 50 after the menopause. For the group over 50, no significant changes were found for total carotenoids and lycopene, whereby the number of male volunteers is only 10. Below 40 years of age, there is still a significant difference in total carotenoids.

Table 2 also offers the values for smokers and non-smokers. The results showed clearly that the values of the smokers are significantly lower, on average by 21% for total carotenoids.

Figure 3 presents the carotenoids versus the BMI. The correlation coefficient in accordance with Pearson resulted with $p < 0.05$ in –0.175 for total carotenoids and –0.196 for lycopene, respectively.

DISCUSSION

The absolute skin values and the relation of total carotenoids and lycopene depend on the nutrition as shown previously.[17,19-21] Nevertheless, a high correlation between the two measured values could be found..

The results have shown that the age of volunteers has no influence on the total carotenoid concentration in the skin; however, for lycopene, a low, but significant, correlation with age was found. Mayne et al. investigated 74 volunteers with a comparable method[19] and found lower values for total carotenoids over 33, though with no statistical significance. The Mayne study was limited to volunteers under the age of 65, with half the number of volunteers.

In the presented study, above and below the age of 40, the lycopene values exhibit 17% differences, which were not expected. Although acute or serious illness was not documented, it cannot be excluded that at a higher age, multiple diseases accumulate, which can play a role. Most impressive is the fact that the lycopene value is less than 40% for males over 50 years, compared to males below 40 years. In the case of females, the changes are less pronounced (22%). This leads us to observe the differences in gender.

The lower values for males are significant for total carotenoids, if all ages are considered. For males, the values are 14% lower, which is in accordance with Mayne et al., exhibiting 13% lower values for males, even if no statistical significance could be shown. Separating the ages of volunteers into groups below 40 and over 50, the younger group still remains significantly different. In the case of volunteers over 50 years, their number is much lower and this could be the reason for the lack of significant differences. However, the data could also give a hint as to hormonal influences. Plasma carotenoid levels also show differences in concentrations for men and women. In several studies, the differences in carotenoids for men and women are correlated with a different uptake of carotenoids from fruit and vegetables.[26-28] Nevertheless, the data are in contradiction to the findings of Yang et al.[29] where, in the population of the USA, the intake of carotenoids was found to be higher in men by using a questionnaire.

The lower levels of total carotenoids of about 20% in the skin of smokers are in accordance with the results of Mayne et al. and it is known that plasma carotenoid concentrations of smokers are lower than those of non-smokers[30,31] as well as lower in vitamin E, lymphocytes and platelets.[32] The present study supports the fact that smoking induces free radicals in the skin, which lead to a decrease in carotenoids in the skin to neutralize the radicals. The lower levels of lycopene in the skin of smokers could be correlated to an increased skin aging.[33]

The carotenoid values were found to be slightly negative when correlated with the body mass index. This is in accordance with other investigations.[16,19] Persons with a BMI between 20 and 25 have a more healthy nutrition than persons with a higher BMI.

The results of this study have demonstrated that the skin values show a comparable behaviour as the plasma values found in the literature. This finding was to be expected, because other studies have shown that the carotenoid skin values correlate with the carotenoid blood values.[14, 17]

CONCLUSION

In conclusion, the measurements showed significantly enhanced total carotenoid values for women and non-smokers, but no age dependency was found. In the case of lycopene, significantly enhanced values were found in people younger than 40 years of age and non-smokers. Reduced lycopene values for men were found, but these values are not significant. Individuals with a lower BMI than 30 exhibit a trend to higher values for total carotenoids as well as for lycopene. Therefore, these results should be borne in mind when comparable study groups are selected. These findings are in accordance with results obtained from blood plasma investigations, indicating that the non-invasive skin measurement of carotenoids provides reliable, fast and useful results, even when the skin is not in the focus of a study.

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