

Raman spectroscopic analysis of the increase of the carotenoid antioxidant concentration in human skin after a 1-week diet with ecological eggs

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Abstract. Skin aging is mainly caused by the destructive action of free radicals, produced by the UV light of the sun. The human skin has developed a protection system against these highly reactive molecules in the form of the antioxidative potential. Carotenoids are one of the main components of the antioxidants of the human skin. From former studies, it is known that skin aging is reduced in individuals with high levels of carotenoids. Because most of the antioxidants cannot be produced by the human organism, they must be up taken by nutrition. Using noninvasive Raman spectroscopic measurements it is demonstrated that not only fruits and vegetables but also eggs contain high concentrations of antioxidants including carotenoids, which are even doubled in the case of ecological eggs. After a 1-week diet with ecological eggs performed by six volunteers, it is found that the concentration of the carotenoids in the skin of the volunteers increased by approx. 20%. Our study does not intend to recommend exorbitant egg consumption, as eggs also contain harmful cholesterol. But in the case of egg consumption, ecological eggs from hens kept on pasture should be preferred to also receive a benefit for the skin. © 2009 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3119257]

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1 Introduction

Striving to remain “forever young” is a basic characteristic of human beings. More than any other organ, the skin reflects our age to the environment. Even in ancient times, humans endeavored to reduce skin aging by the application of oils, plant extracts, and face masks from different types of soil.^{1,2}

Skin aging is determined by individual genetic factors^{3,4} and by the action of free radicals.⁵ It cannot be inhibited. Today, premature aging has increased due to the interaction of environmental influences on the skin.^{6,7} The most important environmental factor causing skin aging is the UV irradiation of the sun.⁸ UV light produces different types of free radicals in the human skin.⁹ These highly reactive molecules damage the skin on cellular and molecular levels. Sunburn, immunosuppression, premature skin aging, and also skin cancer can be the consequences.^{8,10–13} The human organism has developed a defence mechanism against the destructive action of free radicals in the form of antioxidant molecules.¹⁴ These molecules neutralize free radicals before they can damage the tissue. Typical cutaneous antioxidant substances are vitamins A, C, and E; carotenoids; and enzymes. Most of the antioxidants cannot be produced by the human organism, therefore

they must be uptaken by nutrition, e.g., by fruit and vegetables, which contain high amounts of antioxidants.¹⁵ Carotenoids are one of the most important antioxidants of the human skin.¹⁶

In a previous long-term study during 1 yr, 10 volunteers were investigated continuously concerning their antioxidative potential of the skin and their individual lifestyle.¹⁷ Volunteers who consumed high amounts of fruit and vegetables had higher carotenoid levels in their skin than other volunteers. Stress factors, such as nicotine and alcohol consumption, illness, and sleeplessness reduced the antioxidative potential. Additionally, the cutaneous antioxidative potential could be reduced by UV and IR irradiation.^{18,19} Comparing the antioxidative potential of volunteers of the same age, Darvin et al.²⁰ demonstrated that individuals with high concentrations of the carotenoid lycopene in the skin had fewer furrows and wrinkles, which meant, they looked younger for their age than those with low lycopene concentrations. Additionally, it was found that carotenoids can be considered as marker substances for the whole antioxidative potential of the skin,¹⁷ as different antioxidant substances protect each other from the destructive action of free radicals, forming a protection chain.¹⁴

Also, animal products such as the yolk of the hen's egg can be efficient sources of these essential substances.²¹ One

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marker for ecological husbandry of hens according to VO EWG 2092/91 (Decree of the European Commission of ecological agriculture and species-appropriate animal husbandry) is the “permanent access to grassland,” Hesterberg et al.²² observed a twofold concentration of carotenoid antioxidants in eggs laid by hens living with permanent access to grassland, in comparison to eggs laid by hens living in barns or batteries without access to green fodder. Furthermore, the carotenoids were not destroyed during boiling the eggs.²² In principle, the high concentration of antioxidants in egg yolk is not surprising, because it is uptaken by the chicken before it hatches and serves as nutrition and protection during the first days of its life.²³ Therefore, nature has developed a highly efficient protection system with the yolk of an egg.

Nevertheless, it was still unclear whether these positive properties of the egg remain during consumption by humans, and whether this leads to an increase in the antioxidative potential of the skin. Therefore, this study was carried out at the Center of Experimental and Applied Cutaneous Physiology, Charité–Universitätsmedizin Berlin, Germany, to investigate whether the uptake of eggs leads to an increase in the amount of carotenoids in the human skin.

The determination of the carotenoids in eggs and in the human skin was performed by resonance Raman spectroscopic measurement.^{24–26}

2 Materials and Methods

2.1 Eggs

The eggs were obtained from free-range hens, bovans, black, nourished by green fodder, together with insects from the soil during the summer months.

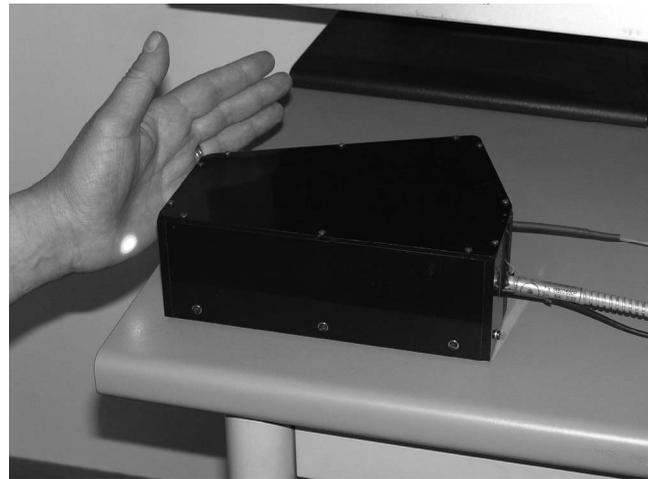
2.2 Volunteers

The investigations were performed on six healthy volunteers, aged between 35 and 50 yr. Raman spectroscopic measurements were performed on two body sites, i.e., on the palm and the forehead of volunteers. Approval of the experiments was obtained from the Ethics Committee of the Charité–Universitätsmedizin Berlin.

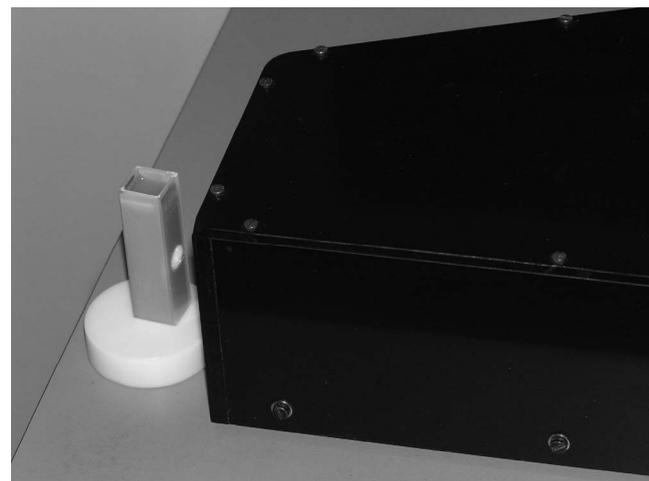
2.3 Application Protocol

Two eggs boiled for 4 min were consumed daily in the morning by all volunteers over a period of 5 days. The carotenoid concentration was measured daily at noon. The study investigators were aware of the fact that the intake of two eggs per day during a period of 5 days exceeds the mean daily egg consumption of the population, although an increased egg consumption for limited time periods such as Easter is an old European tradition, which has also been demonstrated by the statistics of egg consumption.²⁷ The volunteers were carefully enlightened about the risks of increased egg consumption, such as the accumulation of cholesterol levels, and signed informed written consent.

Moreover, volunteers were asked to undertake no changes in their usual nutritional habits during the study, regarding the supplementation of fruit, vegetables, and other foodstuffs, which are naturally rich in carotenoid antioxidants.



(a)



(b)

Fig. 1 Raman spectroscopic system for the noninvasive determination of the carotenoid concentration in (a) the human skin *in vivo* and (b) the yolks of eggs in a cuvette.

2.4 Determination of Carotenoids in the Human Skin

An argon laser with an excitation wavelength of 488 nm was used to determine the cutaneous carotenoids.²⁸ At 488 nm, the most prevalent carotenoid substances, beta-carotene, lutein, and lycopene have almost the same absorption coefficients.²⁵ The laser radiation was transferred by optical fibers to a hand piece containing the optical system. This hand piece can be utilized to measure the concentration of the carotenoids in the skin on all body sites²⁶ [see Fig. 1(a)] or even in the yolk of the egg in a cuvette [see Fig. 1(b)]. The corresponding resonance Raman signal produced by the excitation wavelengths was transferred by additional fibers from the skin surface to the basic station containing the spectrometer and the control unit. The time for one measurement was 5 s. The measurements were repeated three times on the same skin area to obtain an average value. Subsequently, the average cutaneous carotenoid concentration was determined. The standard deviation of these three measurements was always less than 10%.

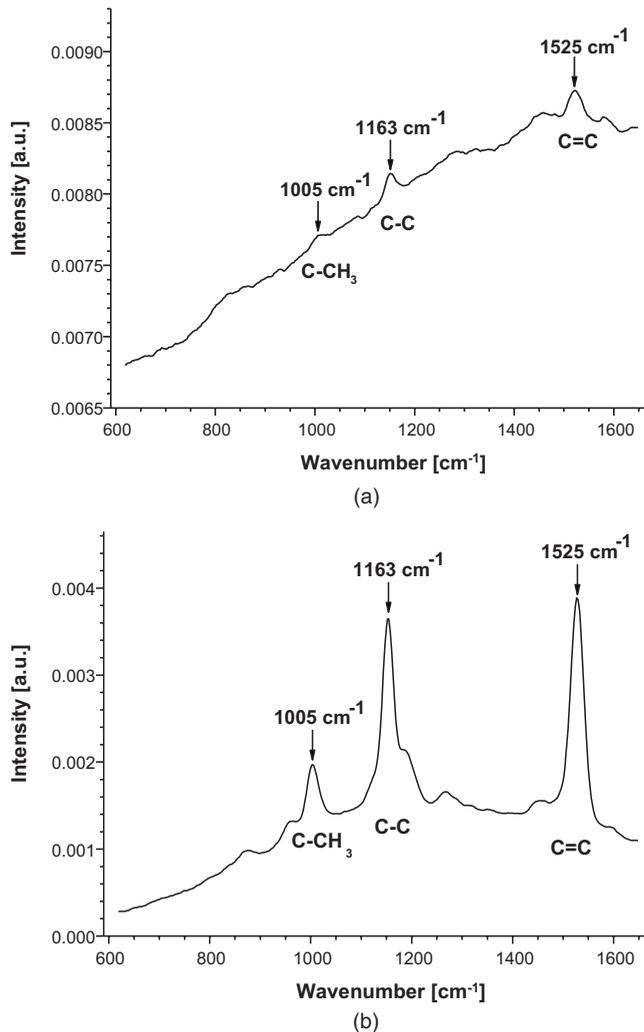


Fig. 2 Typical resonance Raman spectrum of the carotenoids in (a) human skin and (b) the yolks of eggs.

The measurements were performed on the palm and the forehead of the volunteers.

3 Results

Typical Raman spectra of the cutaneous carotenoids measured on the palm are presented in Fig. 2(a). Figure 2(b) presents Raman spectra obtained from the yolk of eggs. The Raman bands of the carotenoids at 1525, 1163, and 1005 cm^{-1} can be clearly recognized in both samples.

The volunteers were requested not to change their lifestyle during the period of investigation, excluding the uptake of two boiled eggs per day. Parameters of the volunteers were measured daily during a period of 8 days: the measurements started 3 days before the egg consumption and continued for the 5 days during egg uptake. The kinetics of the carotenoid concentration in the skin of the palm and the forehead during this period are presented in Figs. 3(a) and 3(b), correspondingly.

The volunteers started with different individual levels of carotenoids in their skin, which varied at a maximum by factor 3. The concentration of the carotenoids had already in-

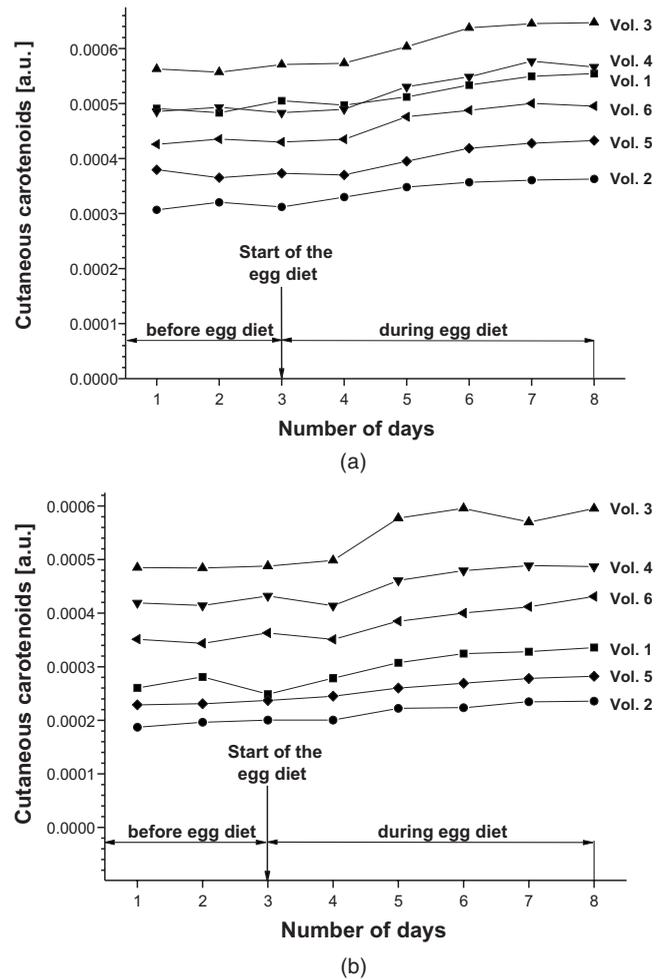


Fig. 3 Initial levels and kinetics of the carotenoid concentration in the skin on different body sites before and during the uptake of ecological eggs: (a) palm and (b) forehead.

creased on the second day after the start of the egg diet in both body regions. Table 1 summarizes the average concentration of the carotenoids in the palm of the volunteers during the 3 days before and during the last 3 days (days 6 to 8) of the egg diet. Table 2 summarizes the carotenoid values measured on the forehead of volunteers. For all volunteers, a significant increase $p < 0.05$ of the carotenoid level was observed. Days 4 and 5 were considered as transitional periods and were therefore not included in the calculation.

The average increase in the carotenoid concentration was $16 \pm 2\%$ for the palm and $21 \pm 2\%$ for the forehead region.

4 Discussion

As suggested by Darvin, et al.,²⁰ high concentrations of antioxidants in the human skin can be considered a good protection strategy against premature skin aging. Most of the relevant antioxidant substances have to be uptaken by nutrition. Fruit and vegetables are well-known sources for delivery of antioxidant substances.¹⁴ Hesterberg et al.²² demonstrated that also eggs, and in particular ecological eggs, contain high amounts of carotenoids. This is not surprising as the yolk of an egg represents the basis for the nutrition and protection

Table 1 Average carotenoid concentration in the skin of the palm of volunteers during the 3 days before and during the last 3 days (days 6 to 8) of the egg diet.

Volunter No.	Mean Carotenoid Concentration (a.u.)	
	Days 1 to 3 Before Egg Consumption	Days 6 to 8 During Egg Consumption
1	4.93±0.11	5.46±0.11
2	3.13±0.07	3.60±0.03
3	5.64±0.07	6.43±0.05
4	4.87±0.05	5.64±0.14
5	3.73±0.07	4.26±0.07
6	4.30±0.05	4.94±0.06
Increase in percent	16±2%	

system of the newly-hatched chicken during the first days of its life.²³ Carotenoids can be considered as marker substances for the complete antioxidative potential of the human organism.¹⁷

The results of this study demonstrate that after consumption of eggs, their carotenoids are transported to the skin, where they increase the antioxidative potential up to approx. 20%. Moreover, the present investigation shows that preparation does not necessarily decrease the antioxidants contained in foodstuffs. This is also known for tomato ketchup, where the lycopene concentration is considerably higher than in tomatoes themselves.²⁹ During the boiling process, the membranes of the cells are destroyed and carotenoids can be released, which simplifies their digestion.

Measurements were performed only with ecological eggs, whose initial concentration of carotenoids is two times higher than that of conventional eggs.

Table 2 Average carotenoid concentration in the skin of the forehead of volunteers during the 3 days before the last 3 days (days 6 to 8) of the egg diet.

Volunteer No.	Mean Carotenoid Concentration (a.u.)	
	Days 1 to 3 Before Egg Consumption	Days 6 to 8 during Egg Consumption
1	2.63±0.16	3.29±0.06
2	1.95±0.07	2.31±0.07
3	4.86±0.02	5.87±0.15
4	4.22±0.09	4.85±0.05
5	2.32±0.04	2.77±0.07
6	3.53±0.10	4.14±0.16
Increase in percent	21±2%	

On the other hand, it must be taken into consideration that eggs also contain harmful cholesterol, which can have a negative impact on the human organism³⁰ if levels are increased.

Therefore, the intention of this study was not to recommend exorbitant egg consumption to prevent skin aging but to demonstrate that the valuable ingredients of our nutrition are transported and accumulated in the skin. This means that in the case of egg uptake, ecological eggs from hens kept on pasture should be preferred, to also receive a benefit for the skin. In general, the uptake of considerable amounts of fruit and vegetables should be preferred to increase the antioxidative potential of the human organism and also of the skin. This can be considered a good protection strategy against skin aging.

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