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Research paper

Influence of dietary carotenoids on radical scavenging capacity of the skin and skin lipids

M.C. Meinke [a,*], A. Friedrich [a], K. Tscherch [b], S.F. Haag [a], M.E. Darvin [a], H. Vollert [c], N. Groth [d], J. Lademann [a], S. Rohn [b]

[a] Center of Experimental Applied Cutaneous Physiology, Department of Dermatology and Allergology, Charité – Universitätsmedizin Berlin, Germany
[b] Institute of Food Chemistry, Hamburg School of Food Science, University of Hamburg, Germany
[c] BioActive Food GmbH, Bad Segeberg, Germany
[d] Privatinstitut Galenus GmbH, Berlin, Germany

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ABSTRACT

Nutrition rich in carotenoids is well known to prevent cell damage, premature skin aging, and skin cancer. Cutaneous carotenoids can be enriched in the skin by nutrition and topically applied antioxidants have shown an increase in radical protection after VIS/NIR irradiation. In this paper, it was investigated whether orally administered carotenoids increase the radical scavenging activity and the radical protection of the skin using *in vivo* electron paramagnetic resonance spectroscopy and the skin lipid profile was investigated applying HPTLC on skin lipid extracts. Furthermore, *in vivo* Raman resonance spectroscopy was used to measure the cutaneous carotenoid concentration. A double blind placebo controlled clinical study was performed with 24 healthy volunteers, who have shown a slow but significant and effective increase in cutaneous carotenoids in the verum group. The enhancement in carotenoids increases the radical scavenging activity of the skin and provides a significant protection against stress induced radical formation. Furthermore, the skin lipids in the verum group increased compared to the placebo group but only significantly for ceramide [NS]. These results indicate that a supplementation with dietary products containing carotenoids in physiological concentrations can protect the skin against reactive oxygen species and could avoid premature skin aging and other radical associated skin diseases.

1. Introduction

Cutaneous carotenoids are a good measure for the antioxidant protection system of the body [1]. Carotenoids, as most of the antioxidants, cannot be produced by the human organism, they must be taken up by nutrition which is rich in fruit and vegetables. In the past, the intake of carotenoids in a moderate dose in context with a natural food matrices has shown several health benefits, such as photoprotection against UV irradiation, increasing microcirculation, and prevention of accelerated skin aging [2–7]. The primary action of antioxidants is the scavenging of free radicals. This preventive mechanism can occur inside the cells to prevent cell damage, such as DNA oxidation or cell membrane damage which is correlated with lipid peroxidation [8,9]. In this context, sunlight is one of the most harmful environmental hazards for the skin, leading to premature skin aging, inflammation, and higher risk of skin cancer [10,11]. Recently, it was shown that not only UV-A and UV-B are responsible for radical production, but also visible and infrared light (VIS/NIR) are able to produce highly reactive radicals. With regard to the total sun wavelength spectrum, up to 50% of the radicals are not produced by wavelengths in the UV range [12]. Up to now, there are only two non-invasive methods available for determining the skin's antioxidant capacity *in vivo*. Cutaneous carotenoids were found to serve as a superior marker for the antioxidant capacity, which can be measured by Raman resonance spectroscopy. The uptake of carotenoids in the skin was clearly demonstrated by utilizing this method [13,14]. However, the appearance of carotenoids in the skin as a result of dietary intake is delayed and decreased only slightly after stopping the supplementation [13].

A further method for the determination of the complex antioxidant network *in vivo* is electron paramagnetic resonance (EPR) spectroscopy [15,16]. This method is based on topical application of a synthetic, semi-stable radical (2,2,6,6-tetramethyl-1-piperidinyloxy; TEMPO) prerequisite to be visualized by EPR spectroscopy [1]. TEMPO reacts with various reducing agents in the skin such as

* Corresponding author. Center of Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. Tel.: +49 30 450 518 244; fax: +49 30 450 518 918.

E-mail addresses: martina.meinke@charite.de (M.C. Meinke), annette.friedrich@charite.de (A. Friedrich), Kathrin.Tscherch@chemie.uni-hamburg.de (K. Tscherch), stefan.haag@charite.de (S.F. Haag), maxim.darvin@charite.de (M.E. Darvin), henning.vollert@t-online.de (H. Vollert), n.groth@galenus.com (N. Groth), juergen.lademann@charite.de (J. Lademann), rohn@chemie.uni-hamburg.de (S. Rohn).

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vitamin C and is reduced to the corresponding hydroxyl amine. However, it will not interact with carotenoids such as beta carotene and lycopene [17]. By means of *in vivo* EPR spectroscopy, the increase in cutaneous radical scavenging activity was shown as a result of supplementation with a water-soluble chokeberry extract and vitamin C [18].

The unique morphology of the *Stratum corneum* lipids is of special importance to ensure that the barrier function of the skin remains. The corneocytes of the *Stratum corneum* are surrounded by a lipid matrix, mainly consisting of ceramides, free fatty acids, and cholesterol [19]. Changes in this lipid profile have been linked to a disturbed skin barrier function [20] leading to skin diseases, for example, xerosis [21]. Decreased levels of *Stratum corneum* ceramides have been reported for patients suffering from atopic dermatitis [22]. Age dependent changes in ceramides and cholesterol have been reported by Rogers et al. [23] with higher lipid concentrations in young than in old skin. Furthermore, it is well known that aged skin becomes rougher. Previously, Darvin et al. found a correlation between the cutaneous carotenoid lycopene and the roughness of the skin. High lycopene levels were correlated with a less rough skin [24]. Therefore, an influence of carotenoids on the skin lipid profile is highly expected. For the analysis of the skin lipids, different methods can be applied [25–27].

There are still several questions to be considered: can orally taken carotenoids increase the radical scavenging activity in the skin? Can they protect against VIS/NIR-irradiation-induced radicals and do they influence the skin lipid profile?

In order to elucidate these questions, a double blind placebo controlled study was performed with 24 healthy volunteers, whose diet was supplemented with a natural carotenoid-rich curly kale extract or a placebo. First, the uptake of carotenoids in the skin was investigated by Raman resonance spectroscopy. Then, the antioxidant capacity was determined by *in vivo* EPR-spectroscopy using the test radical TEMPO. Although TEMPO does not react directly with the supplemented carotenoids, it was investigated whether carotenoids enhance the radical scavenging activity or so-called "antioxidant capacity" of the skin by internally interacting with the antioxidant network of the skin. Furthermore, photoprotection against radical formation induced by VIS/NIR irradiation was studied. In addition, the skin lipid profile was measured using high performance thin layer chromatography (HPTLC) after an extraction directly from the skin of the volunteers.

2. Materials and methods

2.1. Supplements

The supplements were provided as recommended by the manufacturer for a time period of 8 weeks. The verum capsules Lutex skin™ (BioActive Food GmbH, Bad Segeberg, Germany) consist of a curly kale extract (*Brassica oleracea* convar. *acephala* var. *sabellica* L.), sea-buckthorn oil (*Hippophae rhamnoides* L.) and olive oil, containing a high amount of carotenoids. The dosage for 1 day provided the following moderate amounts of carotenoids: 2200 μg lutein, 1000 μg beta carotin, 50 μg alpha carotin, 400 μg lycopin, 700 μg zeaxanthin, and100 μg cryptoxanthin. As a control, a placebo capsule containing no antioxidants was given.

2.2. Volunteers

The study protocol had been submitted and approved by the local ethics committee of the Charité – Universitätsmedizin Berlin in accordance with the *Declaration of Helsinki* as revised in 1983. 24 volunteers, after having given their written informed consent, were included in the study protocol, 11 female and 13 male, 10 smokers and 14 non-smokers, aged between 22 and 66 years. 12 volunteers, consisting of 5 smokers and 7 non-smokers, were included in each group. The volunteers were asked not to substantially change their living and eating habits during the study.

2.3. Questionnaire

All volunteers completed a questionnaire concerning skin type, sun exposure, physical exercise, stress/mood, frequency of common colds, infectious gastrointestinal diseases, consumption of preferred foodstuffs, alcohol consumption, and tobacco. Dietary intakes of fruit and vegetables were examined by self-assessment based on a food frequency questionnaire with two separate food lists containing 13 common fruits and 13 common vegetables that had to be marked in a frequency category ("rarely", "⩾once a month", "⩾once a week", "⩾4 times a week").

Furthermore, the baseline dietary uptake of carotenoids was estimated, in order to obtain an overview of the study population. This approximation is based on the information contained in the questionnaire, standard portion sizes that were assumed for all volunteers and food antioxidant content based on the nutrition tables of Souci-Fachmann-Kraut.[1]

3. Radical protection factor (RPF)

The radical protection factor (RPF) technology determines the radical scavenging activity of an antioxidant substance/product with EPR spectroscopy using the test radical DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma–Aldrich, Steinheim, Germany), which is reduced by the antioxidants in the investigated sample [28]. The number of reduced test radicals represents the radical scavenging activity normalized to 1 mg input of the antioxidant substance/product. The RPF is expressed by a positive number N with the measuring unit 10^{14} radicals/mg, which means: RPF = $N \cdot [10^{14}$ radicals/mg].

3.1. EPR spectroscopy

EPR spectroscopy is described by the resonant absorption of microwave energy by a molecule with an unpaired ("free") electron within a magnetic field. *In vivo* EPR measurements were performed using an L-band EPR-spectrometer (LBM MT 03; Magnettech, Berlin, Germany) with parameter settings as follows: microwave frequency (1.3 GHz), central magnetic field (46 mT), sweep width (8 mT), sweep time (10 s), and modulation amplitude (0.15 mT).

3.2. Determination of radical scavenging capacity

In this study, the skin antioxidant status of the volunteers was examined with a non-invasive EPR spectroscopy approach using the semi-stable nitroxide 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO; Sigma–Aldrich, Steinheim, Germany). TEMPO as an amphiphilic nitroxide and as one of the few nitroxides, which are allowed to be applied to the human skin *in vivo* [29,30], penetrates the skin well without the need of a special transport system. Yet, as soon as TEMPO begins to interact with the skin, it is reduced to the corresponding EPR-silent hydroxylamine, due to enzymatic and non-enzymatic reduction [31]. This decline in EPR signal intensity can be correlated with the antioxidant capacity of the skin. Experiments were performed as described previously [1]. Measurement areas were chosen on the sun-protected inner right forearm,

[1] Food Composition and Nutrition Tables, 2011 (Accessed June 22, 2012, at www.sfk-online.net).