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Significant correlations of dermal total carotenoids and dermal lycopene with their respective plasma levels in healthy adults

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ABSTRACT

Carotenoids in skin have been known to play a role in photoprotection against UV radiation. We performed dermal biopsies of healthy humans ($N = 27$) and collected blood samples for pair-wise correlation analyses of total and individual carotenoid content by high performance liquid chromatography (HPLC). The hydrocarbon carotenoids (lycopene and beta-carotene) made up the majority of carotenoids in both skin and plasma, and skin was somewhat enriched in these carotenoids relative to plasma. Beta-cryptoxanthin, a monohydroxycarotenoid, was found in similar proportions in skin as in plasma. In contrast, the dihydroxycarotenoids, lutein and zeaxanthin, were relatively lacking in human skin in absolute and relative levels as compared to plasma. Total carotenoids were significantly correlated in skin and plasma ($r = 0.53$, $p < 0.01$). Our findings suggest that human skin is relatively enriched in lycopene and beta-carotene, compared to lutein and zeaxanthin, possibly reflecting a specific function of hydrocarbon carotenoids in human skin photoprotection.

Introduction

There is considerable interest in possible health effects of carotenoids in skin as recently reviewed by Goralczyk and Wertz [1]. Carotenoids are known to accumulate in human skin, with the levels of carotenoids reflecting dietary intake and bioavailability from the food source [2]. The most common carotenoids in the Western diet are alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin [3]. After absorption in the intestine, carotenoids are transported through the bloodstream by lipoproteins to various target tissues [4,5]. Recent evidence suggests that cholesterol transporters, such as scavenger receptor class B1 type 1 protein (SR-B1)[1] and Cluster of Differentiation 36 membrane protein (CD 36), facilitate absorption of carotenoids in the intestine [6]. There is suggestive evidence that these transporters may also facilitate carotenoid absorption in the epidermal layers of the skin [7]. Carotenoids are lipophilic molecules found in anatomical sites where the stratum corneum, the upper most skin layer, is thick [8]. Body sites highest in total carotenoid levels include the sole of the foot, forehead, and palm of the hand, which has a high lipid to protein ratio [9]. Adipose tissue is another main accumulation site for carotenoids due to the large volume in the human body [2]. The levels of carotenoids found in human adipose tissue are considered to be markers of usual intake because adipose tissue is a more stable repository for carotenoids compared with plasma, in which carotenoids have a shorter-half life [10].

Perhaps the best-studied potential health effect of carotenoids beyond their provitamin A activity is a promising role in photoprotection, that is, the protection against erythema and sunlight damage [11,12]. Beta-carotene has established efficacy in the treatment of erythropoietic protoporphyria, a photosensitivity disease [13,14]. In humans without this disease, there is also evidence from controlled studies that carotenoids such as beta-carotene have efficacy in the protection from sunburn [15], although the sun protection factor is modest (SPF approximately equal to 2). This meta-analysis observed a significant protective effect for beta-carotene supplementation vs. placebo on the development of a sunburn reaction. Carotenoids are known to quench singlet oxygen and other free radical species generated in the skin by exposure to UVA [13]. Finally, several recent studies have examined the potential protective effects of carotenoids against premature photoaging of the skin, marked by signs such as wrinkling,

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[1] *Abbreviations used:* SR-B1, scavenger receptor class B1 type 1 protein; CD 36, Cluster of Differentiation 36 membrane protein; SPF, sun protection factor; RRS, resonance Raman spectroscopy; SDS, sodium dodecyl sulfate; BHT, butylated hydroxytoluene; PDA, photodiode-array; SIM, single ion monitoring; APCI, atmospheric pressure chemical ionization; LDL, low-density lipoprotein.

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pigmentation, dryness, and inelasticity. There is suggestive evidence for a protective effect of beta-carotene on photoaging [16]. Evidence also suggests that higher levels of lycopene in the skin results in lower levels of skin roughness [17].

The variability in the photoprotective effect of carotenoids observed across human studies can be attributed to several factors. The bioavailability of the food source or supplementation affects the amount of carotenoids absorbed by the body and taken up by target tissues, including skin [2]. Additionally, greater UV exposure and skin sensitivity to UV radiation can decrease the photoprotective effect of these micronutrients [18,19]. Finally, lifestyle factors, notably smoking status [20], have also been associated with significantly lower dermal carotenoid levels in human skin, while genetics factors have been associated with lower plasma beta-carotene levels [21,22].

Arguably the most appropriate technique to examine the distribution and levels of individual carotenoids in human skin is HPLC analysis of dermal biopsies. Absorption spectroscopy has been used in some studies to estimate carotenoid content, but cannot differentiate between the various carotenes, xanthophylls, and their isomers. As noted by Goralczyk and Wertz, "Unfortunately, reports on carotenoid concentrations in skin of humans or laboratory animals are rare, many of them old and most referring to beta-carotene only" [1]. Goralczyk and Wertz note four studies that measured beta-carotene or total carotenoid levels in human skin using HPLC analysis to assess the photoprotective effects of moderate to high-dose carotenoid supplements [23–26]. Another study [27] from Peng et al. [23], examined the correlation between individual carotenoids measured by HPLC analysis of plasma samples and skin biopsies in a sample of adults ($N = 96$) in the context of a skin cancer chemoprevention trial. In this study, there was significant correlation between levels of the individual carotenoids measured in plasma and skin. Additionally, the levels of carotenoids in skin were lower in smokers and higher for supplement users, even after adjustment for potential covariates [27].

We conducted a human study aimed at validating resonance Raman spectroscopy (RRS) for use in the noninvasive assessment of dermal carotenoids for epidemiologic research. As part of the validation of the RRS method, we needed to perform dermal biopsies of healthy humans, and analyze the total carotenoid content of these biopsies by HPLC. In that same study, we also collected blood samples, to examine how dermal carotenoid levels correlated with plasma carotenoids, with both assessed by HPLC, because plasma carotenoids have been the most commonly used measure of carotenoid status for epidemiologic research. This provided us with an opportunity to examine correlations for each of the major carotenoids found *in vivo* between paired blood and skin samples. The limited data estimating the accumulation of individual carotenoids, other than beta-carotene, in skin by HPLC underscores the need for the current research. If particular carotenoids preferentially accumulate in dermal tissue, it may suggest a specific function of these carotenoids in skin photoprotection. Below we describe the results of our analyses of carotenoid levels in paired skin and plasma samples from healthy humans.

Materials and methods

Subjects

Our goal was to recruit a total of 30 normal healthy adults between the ages of 21 and 65. Participants were part of a larger parent study examining resonance Raman spectroscopy (RRS) as an objective measure of carotenoid status and were not routine supplement users [28]. We attempted to recruit men and women, as well as smokers and nonsmokers (plasma carotenoid levels of smokers are known to be lower than those of nonsmokers [29]). For this portion of the research, subjects had to be willing to undergo phlebotomy and dermal biopsy. After obtaining signed informed consent, participants were interviewed to obtain demographic data, and then completed dermal biopsy and phlebotomy at the same clinic visit.

Dermal biopsies and phlebotomy

A dermatologic surgeon performed the dermal biopsy in the posterior hip area of each subject. Participants were given injected anesthetic (Lidocaine) at the biopsy site. Once the skin was numb, the dermatologic surgeon removed a 3 mm punch biopsy of skin. Residual adipose tissue was removed from the sample, prior to placing it into a cryovial. Biopsy samples were snap frozen immediately using liquid nitrogen. Biopsy sites were sutured in order to facilitate rapid healing.

Blood samples (10 ml) were obtained by venipuncture by a trained phlebotomist and collected into heparinized tubes. Tubes were protected from light, chilled but not frozen, and then centrifuged to obtain plasma. Plasma aliquots were obtained and stored at −70 °C prior to HPLC analysis.

Extraction and HPLC analyses

Extractions were carried out as described elsewhere [30]. Two hundred eighty microliters of phosphate buffered saline was added to the skin punch tissue. Thirty-five microliters of collagenase solution (50 mg/ml; Sigma Cat # E-1644) was added, vortex-mixed and incubated at 37 °C for 1 h. Tissues were homogenized on ice, and 35 μl of protease solution (20 mg/ml; Sigma catalog # 11360) was added, vortex-mixed, and incubated at 37 °C for 0.5 h. Four milliliters of sodium dodecyl sulfate (SDS)–ethanol–butylated hydroxytoluene (BHT) solution was added and vortexed for 60 s. Samples were then extracted twice with hexane (2 × 500 μl), and dried down prior to HPLC injection.

Plasma samples (100 μl) were treated with ethanol and hexane containing 0.1% (w/v) BHT, and centrifuged to remove the proteins. The proteins were re-extracted with hexane (3 × 300 μl), and the combined extract was evaporated to dryness under reduced pressure at below 40 °C. After evaporation of the solvent, the residue was reconstituted in the appropriate HPLC solvents and centrifuged at 2000*g* prior to analysis.

The chromatographic conditions for carotenoid separation and quantitation were similar to those reported earlier [31]. The mobile phase was an isocratic mixture of acetonitrile:isopropanol:ethyl acetate (50:40:10 v/v), at a flow rate of 0.7 ml/min. The analysis was performed on a reversed-phase Luna C18(2) analytical column [250 mm length × 4.6 mm id, (Phenomenex, Torrance, CA, USA); particle size 5 μm; pore size 100 Å]. The dried pigments (after extraction) were re-dissolved in 200 μl of HPLC mobile phase. The column was maintained at room temperature, and the HPLC detector was operated at 450 nm. Peak identities were confirmed by photodiode-array (PDA) spectra, mass spectra and by coelution with authentic standards as necessary. To avoid overloading of the mass spectrometer with eluted molecules, 50% of the eluant was directed to waste with the help of diverter valve. Lutein and zeaxanthin coeluted but were quantitated based on single ion monitoring (SIM) method [32].

Mass spectrometry equipment and analysis

MS analysis was performed using Thermo Electron MSQ single quadrupole mass spectrometer (San Jose, CA), equipped with an atmospheric pressure chemical ionization (APCI) source. The protonated precursor molecular ions were initially acquired in two full