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Non-invasive Assessment of Carotenoid Status in vivo in the Human Skin

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Inhalt

1 Antioxidant Network

According to Darvin et al. [1] and Haag et al. [2] free radicals and reactive oxygen species (ROS), forming in the human body, are linked to external and internal factors as for example metabolic processes in mitochondria, where approximately 3% of oxygen is converted to oxygen free radicals, inflammatory processes [3] (often accompanied by serious diseases) or harmful external factors such as irradiation (UV [4], visible [5] and IR [6]) and environmental conditions [7] [8].

In case of signaling processes inside the living cell and between cellular systems [9] [10] as well as for the destruction of viruses and bacteria [11], free radicals are of great importance. However high levels of free radicals pose a constant threat to the human body and can cause oxidative cell damage, skin ageing and cancer [12] [13] [14].

Therefore the human organism has developed an effective defense system that is able to neutralize these threats efficiently [15]. The protection is mediated by antioxidant substances like carotenoids, vitamins (A, C and E), enzymes (superoxide dismutase, catalase, glutathione peroxidase), and other substances like flavonoids, lipoic acid, uric acid, selenium, coenzyme Q10, polyphenols etc [15] [1]. It is notable that carotenoids and vitamins A, C and E cannot be synthesized by the human organism as opposed to enzymes. Only nutritional supplementation or topical application can provide the body with those antioxidants [1]. This natural antioxidant protection system is suspected to act synergistically as a “protective chain” with substances protecting each other from the destructive action of free radicals and ROS during neutralization processes [16] [17] [15].

If the production of antioxidants cannot keep up with the formation of free radicals and reactive oxygen species, the human body cannot neutralize enough reactive molecules [10] and a chain reaction occurs leading to oxidative stress. Oxidative stress reflects an imbalance between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Hence, antioxidants are destroyed due to interaction with high amounts of ROS and free radicals [18] [19] and the antioxidant potential of the organism decreases leaving cells unprotected and giving rise to cell damage, ultimately leading to inflictions like cancer, infarcts, arteriosclerosis, arthritis, Alzheimer, Perkinsons and others [20, 21].

Skin, as the outermost barrier of the body, directly interacts with harmful oxidative environments and their negative influences. The epidermal antioxidant network consists of a complex defense system against oxidative stress in which the function of one antioxidant often supplements or regenerates another antioxidant [22]. The antioxidants are constantly being destroyed and restored and the resulting changes in the antioxidant levels of the human skin reflect these processes. Lademann et al. [23] state that a sufficient cutaneous level of antioxidants is of notable relevance as ROS formed by immunological or metabolic processes as well as irradiation may compromise the skin.

By comparing skin and blood samples it has been shown experimentally that the level of antioxidants in the skin mirrors theirs systemic concentration [24] [15].

2 Carotenoids

2.1 Physicochemical Details

It has been postulated that among the different dietary components of fruits and vegetables the so-called secondary plant constituents play a major role in disease prevention. Some of these phytochemicals are responsible for the bright colors of plants. Among the various natural pigments, carotenoids comprise an important group of more than 600 structurally different compounds [25].

Most carotenoids are composed of a central carbon chain of alternating single and double bonds and carry different cyclic or acyclic end groups. According to their chemical composition they are categorized as either carotenes (e.g. carotene, lycopene) or xanthophylls (e.g. zeaxanthin, lutein, cryptoxanthin, astaxanthin,…). Furthermore they can also be divided into provitamin A and non-provitamin A compounds [25]. The conjugated double bonds mainly exist in trans-configuration [26]. Carotenoids are lipophilic or amphiphilic molecules [27].

The balance between electron acceptance and donation forming radical anions and cations varies among the carotenoids and is important for their role in antioxidant networks. Electron acceptor and donor properties should be considered simultaneously, leading to a donator-acceptor map or two-dimensional antioxidant hierarchy as shown in Table 1. The role of carotenoids as both electron donor and acceptor seems unique and may explain their importance in antioxidant networks. [26]

Table 1: Donator-acceptor map or two-dimensional antioxidant hierarchy [26]

R_d/R_a	Low electron acceptor index (bad acceptors)	High electron acceptor index (good acceptors)
Low electron donation index (good donors)	Good radical scavengers Good antioxidants Example: Vit E	Best radical scavengers Example: β-carotene
High electron donation index (bad donors)	Poor radical scavengers Example: Vit C	Good radical scavengers Good “antireductants” Example: astaxanthin

In red palm oil, containing α- and β-carotene together with tocotrienols and tocopherols, the carotenes become depleted upon heating, apparently protecting the tocotrienols and tocopherols and thus suggesting the role of carotenes as first-line-of-defense antioxidants. [26]

Concerning the scavenging activity of oxidizing radicals, carotenoids show mainly three reaction pathways [26]:

$$\mathrm{Car} + \mathrm{R}^{\bullet} \rightarrow \mathrm{Car}^{\bullet+} + \mathrm{R}^{-} \ (\text{electron transfer},\ \mathrm{ET})$$

$$\mathrm{Car} + \mathrm{R}^{\bullet} \rightarrow [\mathrm{Car} \ldots \mathrm{R}]^{\bullet} \ (\text{radical adduct formation},\ \mathrm{RAF})$$

$$\mathrm{Car} + \mathrm{R}^{\bullet} \rightarrow \mathrm{Car}(-\mathrm{H})^{\bullet} + \mathrm{RH} \ (\text{hydrogen atom transfer},\ \mathrm{HAT})$$

Considering an antioxidant network including carotenoids and homologous tocopherols, ordering according to the tendency to reduce oxidizing radicals including one-electron-oxidized antioxidants can be shown. Figure 1 shows that the efficiency of lycopene, in its function as radical scavenger, is comparable to that of the tocopherols. [26]

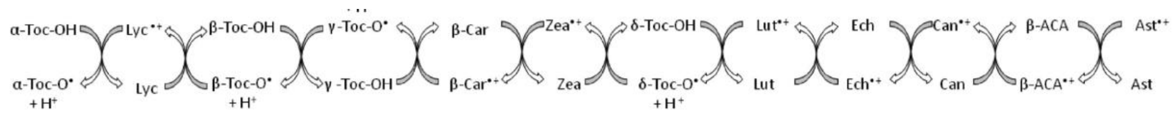

Figure 1: Antioxidant network including carotenoids and the homologous tocopherols according to their tendency to reduction [26]

Interactions between carotenoids and tocopherols are of special interest because of the role of carotenoids and compounds of the vitamin E family in the protection of membranes against oxidation. Ascorbate, being even more reducing than α-tocopherol, should regenerate β-carotene in case of contact at lipid/water interfaces. [26]

Due to their rigid backbone and proper length corresponding to the hydrophobic zone of membranes, lipophilic carotenoids reinforce cell membranes. Amphiphilic carotenoids with polar end groups such as xanthophylls are anchored in water-lipid-interfaces. [26] The lipophilicity/amphiphilictiy of carotenoids determines their subcellular distribution. They are mostly enriched in membranes and other lipophilic compartments [25].

Absorption bands of important skin carotenoids are shown in Figure 2. The inlet shows a term scheme of carotenoid molecules, which feature an unusual even parity excited state. As a consequence, absorption transitions are electric-dipole allowed in these molecules but spontaneous emission is forbidden. The resulting absence of any strong fluorescence in carotenoids is the main reason for the possibility to use Resonance Raman Spectroscopy, shown as a solid, downward-pointing arrow (optical transition) in the inset, as a noninvasive means of carotenoid detection in human tissue. [28]

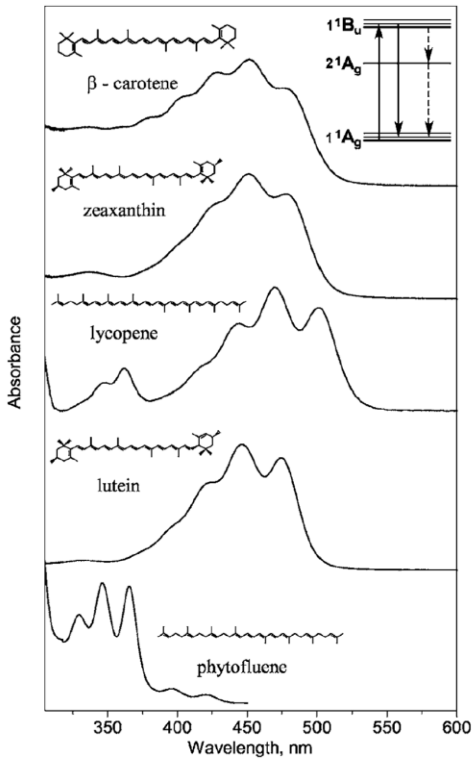

Figure 2: Absorption bands of important skin carotenoids; Inlet shows Carotenoid molecules, which feature an unusual even parity excited state [28]

The absorption characteristics of β-carotene radicals differs significantly from those of neutral carotenes. Figure 3 shows absorption spectra of β-carotene and its simple radicals.

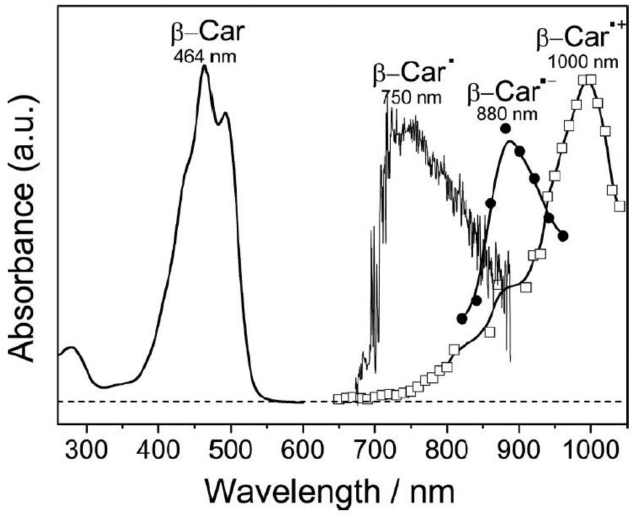

Figure 3: Absorption spectra of β-carotene and simple radicals of β-carotene [26]

2.2 Carotenoids in the Human Body

Carotenoids are powerful antioxidants and play an important role throughout the human body [29]. They are the most efficient natural scavengers of singlet molecular oxygen. The rate constants for the reaction of carotenoids with singlet oxygen are in the range of 10^9 M^{-1} s^{-1}, i.e. near diffusion control [25]. At low oxygen tension, carotenoids are also able to scavenge peroxyl radicals [30]. Moreover they are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen [25].

Studies show an inverse correlation between high dietary intake of carotenoids and the risk of various cancers, cardiovascular disease and degenerative diseases [29]. Lycopene and β-carotene are thought to play an important protective role in the protection of skin from UV and short-wavelength visible radiation. Lutein and lycopene may have protective functions for cardiovascular health and lycopene may play a role in the prevention of prostate cancer [28].

In the human skin the group of carotenoids includes α-, β-, γ- and σ-carotene, lutein, zeaxanthin, lycopene and their isomers [31]. Figure 4 shows the molecular structure of some selected carotenoids.

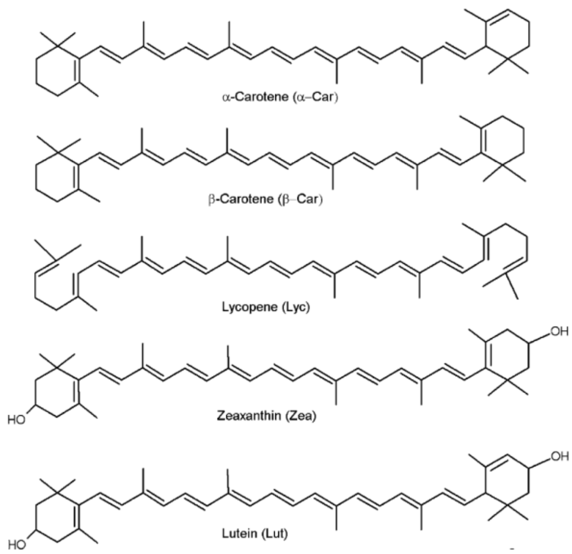

Figure 4: Molecular structures of selected carotenoids [26]

Of these, lycopene and the carotenes account for 60 – 70 % of the total carotenoid content [29].

The distribution of β-carotene in the human skin is shown in Figure 5.

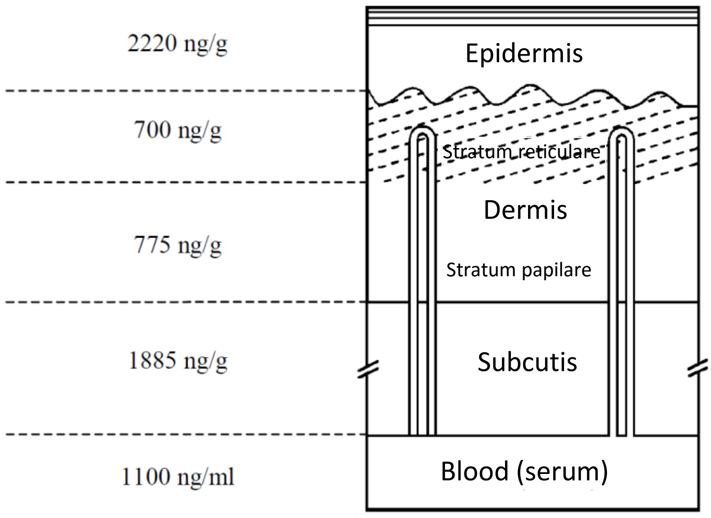

Figure 5: Schematic visualization of average β-carotene concentrations of different skin layers [32]

Since carotenoids are lipophilic molecules, they are well placed in the skin to act as chain-breaking antioxidants protecting epidermal polyunsaturated fatty acids from oxygen peroxidation. Other dermal antioxidants such as superoxide dismutase, glutathione peroxidase, α-tocopherol, ascorbic acid, and melanins work in collaboration with carotenoids to provide skin with a defensive mechanism against free radical attack and oxidative stress. Because these molecules work as a network, definitive measurement of a subset of these antioxidants provides an indication of the relative strength of the whole system. The effectiveness of this protective network can be diminished either by excessive generation of free radicals or by insufficient antioxidant molecules being supplied to the skin. [28] [33]

The accumulation of carotenoids in the human skin under oral supplementation is, compared to serum values, delayed by up to two weeks. Some individuals, however, also show accumulation without temporal delay. [34] Ribaya-Mercado et al. [19] remark that, after single oral administration of 120 mg β-carotene, the increase of dermal concentrations in the skin of healthy women amounted to 23% after 6 days. Stahl et al. [35] [36] showed that a steady-state of β-carotene concentrations in the human skin is not reached even after 12 weeks of oral supplementation. Accumulation occurs in all regions of the body. However, large inter-individual differences are noticeable. Darvin et al. [15] state that carotenoid levels can decrease quickly over the course of two hours whereas the recovery of the values took up to three days.

The distribution of β-carotene in the human skin is far less homogenous after 4 weeks of chronic oral supplementation than after 6 weeks. This is due to the fact, that β-carotene starts to accumulate near the capillaries and only slowly starts to diffuse into the surrounding tissue. [37]

Peng et al. found a significant correlation of β-carotene concentrations between plasma and skin [38]. A linear supplementation only occurs, according to Biesalski et al. [18], due to oral supplementation. Correlation is especially high on the palms and the forehead. [36]

3 Invasive Quantification of Carotenoids in the Human Body

High pressure liquid chromatography (HPLC) is the standard method for the detection of antioxidants in tissue and blood samples. Major disadvantages of this method are long processing times, high costs and the fact that one needs to take biopsies or blood samples invasively from the human body [15]. Meinke et al. [39] show that the antioxidant status differs between in vivo and ex vivo skin samples and that results of both measurement forms must be critically compared.

4 Non-invasive Detection Methods for Carotenoids in Human Skin

4.1 Resonance Raman Spectroscopy (RRS)

RRS is a form of laser spectroscopy that detects the characteristic vibrational/rotational energy levels of a molecule by measuring inelastically scattered light. Carotenoids are particularly well suited to RRS as all have a conjugated carbon backbone molecular structure, strongly absorbing in the blue wavelength region and thus providing the basis for efficient resonant laser excitation of the molecules with visible laser lines [40].

In case of one-wavelength excitation, laser-light with a wavelength of 488 nm excites all cutaneous carotenoids simultaneously. Analyzing only the intensity of a subsequent Raman peak (provided by the inelastic scattering of light in the human skin) at 527.2 nm, the information about the concentration of all carotenoids existing in the skin is provided [31].

It is possible to detect lycopene and β-carotene individually using suited green and blue laser lines and taking advantage of differing Raman cross sectional profiles for the two molecules [41] [42] [43]. According to Darvin et al. [31] the main limitation of the two-wavelength excitation scheme is that other carotenoids such as lutein, zeaxanthin as well as carotenes can exert an influence on the Raman measurements because of the similar absorption spectra to β-carotene.

Strong correlations were obtained between plasma carotenoid values gathered by HPLC and resonance Raman spectroscopic measurements, reported by various working groups. [43] [44] [41] This renders RRS a valid non-invasive technological alternative to invasive blood measurements.

The advantage of the Raman spectroscopic method is its non-invasiveness, the measurement quickness and its high sensitivity which permits the detection of low concentrations of carotenoids in mammalian skin [31].

4.2 Reflection Spectroscopy (RS)

Reflection spectroscopy of carotenoids in human skin is based on the registration of the radiation reflected by the skin, irradiation occurring in the spectral range of maximal absorption of carotenoids (i.e. in the blue-green range of the spectrum) [31]. Niedorf et al. [32] presented reflection spectroscopy based determination of carotenoids with a strong correlation in comparison with HPLC plasma measurements. Their data showed a wide spread of the measured values. The reason for this could be the high penetration depth of the excitation light into the skin and the presence of cutaneous chromophores such as melanin, FAD, FADH2, deoxy-heamoglobin or oxy-haemoglobin and bilirubin which are inhomogeneously distributed in the skin. They absorb light in the blue-green range of the spectrum and thus potentially influence the final reflectance spectra [31].

It has been shown that it is possible to overcome or reduce the influence of chromophores which are mostly distributed in the basal level and in the dermis of the skin by detecting back-reflected light from depths not exceeding 200 µm, which corresponds with a depth of the epidermis on the thenal areas in humans [31] [45]. RRS can be used for the calibration of reflection spectroscopy devices because this method is well correlated with HPLC measurements. Strong correlations were obtained for human thenal skin areas in vivo and for bovine udder skin areas in vitro [31] [45].

The main advantages of reflection spectroscopy-based devices in comparison to Raman devices are the relatively low price, compact size and light weight. [31]

4.3 Other Non-invasive Detection Methods for Carotenoids in Human Skin

Pressure mediated reflection spectroscopy is a technique which enables the user to measure on different skin areas independent of the thickness of the epidermis [46] [31]. Applied external pressure temporarily squeezes blood out of the illuminated skin volume making epidermal carotenoids detectable in the range between 460 and 500 nm. The limitation of this method lies in the influence of melanin near the basal layer, which also absorbs in this optical range. [31] [47]

Raman Microscopy is a combination of in vivo confocal Raman spectroscopy and confocal microscopy of human skin. This method can be used for recording molecular concentration profiles in the skin. [31]

Skin color measurements are yet another possibility to detect carotenoids in the skin. Skin color is predominantly determined by the cutaneous pigments hemoglobin, bilirubin and melanin. Due to their absorption properties, epidermal carotenoids are also strongly contributing to the coloration of epidermis and as a result the total skin color. The measurements are based on spectro-photometric reflection measurements. The limitations of this method are similar to the limitations of reflection spectroscopy of carotenoids as melanin and blood significantly contribute to the total skin color. [31]

5 Carotenoids as Biomarkers for the Whole Antioxidant Network

In order to identify populations at particular risk for inadequate intake of fruits and vegetable intake, objective indicators of fruit and vegetable intake are critically needed.

The *2010 Dietary Guidelines for Americans* recommend increased consumption of fruit and vegetables, with specific guidelines for dark-green and red and orange vegetables [48]. Adherence to recommendations is low; 11% of the population meets the recommendation for vegetables, 20% for fruit and <7% for dark-green and orange vegetables [49].

The *National Academy of Sciences* states that “blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables” [50]. Blood concentrations of carotenoids can and have served as concentration biomarkers of fruit and vegetable intake and are used in nutritional surveillance, in observational research studies and as a marker of adherence relevant behavioral interventions. However there are major drawbacks to this method such as high costs, invasiveness, sample lability during processing and analysis and the relatively short half-life of carotenoids in blood [40]. Therefore carotenoid status measured non-invasively in the living skin has emerged as a promising new assessment method.

Blume-Peytavi et al. [51] remark that in contrast to blood, skin represents a storage reservoir for antioxidants for several weeks. Therefore fluctuation of carotenoid levels in skin is less sensitive or shows a slower response to oral supplementation as compared to plasma [52].

Lademann et al. [23] [53] and Darvin et al. [15] suggest that carotenoids can serve as a biomarker substance of the antioxidant network of the human skin. The total intake of antioxidants is significantly correlated with plasma carotenoids [54]. Haag et al [2] could provide indications for carotenoids as marker for the whole antioxidant capacity of the skin.

Jahns et al. [55] state that carotenoid compounds are attractive biomarkers of vegetable and fruit consumption because humans cannot synthesize them and they are readily deposited into body tissues. Mayne et al. [40] have done research relevant to develop this biomarker, including its variability, reproducibility, validity, feasibility for use in field settings and factors that affect the biomarker such as diet, smoking and adiposity.

5.1 Biomarker Validity

Validity of the correct assessment of skin carotenoid status is reported by Ermakov et al. [56] [28] as well as Mayne et al. [40] [44] by comparing the non-invasive Raman spectroscopic measurement with blood samples measured by HPLC. The resulting coefficients showed high correlation of data. In a non-invasive assessment of skin carotenoids as a biomarker of fruit and vegetables Aguilar [57] also notes that levels of skin and serum carotenoids are highly correlated. This is true both for adults as well as children.

Validity by comparison of RRS with skin biopsy was shown by [44] [56].

Comparing RRS data with reported vegetable and fruit intake validity was confirmed by [58] [44].

Jahns et al. [55] compared changes in skin carotenoid status with changes in plasma carotenoid concentrations during a controlled feeding intervention. Validity of skin carotenoid changes in response to increased vegetable and fruit consumption was shown.

5.2 Biomarker Variability

Intra-subject variability as well as inter-subject variability characteristics were investigated by Mayne et al. [40]. Ideal biomarkers would vary widely across different individuals within a population but be relatively constant over time within an individual. According to Mayne et al. [40] there is adequate inter-subject variation in skin carotenoid status as assessed by RRS. This has also been confirmed by [58], [44] and [29]. Intra-subject carotenoid levels were highly consistent over time within each body site, with the highest reproducibility shown for the palm [40].

5.3 Biomarker Reproducibility

High reproducibility of skin carotenoid concentrations over 6 months in the absence of intervention has been shown by Mayne et al. [44] as well as Scarmo et al. [59].

5.4 Feasibility

Mayne et al. [40] found it feasible to use a portable RRS device in out-of-lab-conditions by scanning a large number of children (nearly 400 total) in a relatively short time-frame with no difficulties in a preschool setting (e.g. upt to 60 children were assessed in a two-hour window of data collection).

6 Factors influencing Carotenoids as a Biomarker in the human Skin

Prior to using skin carotenoid status as a biomarker for human studies, a clear understanding of factors that affect the biomarker is needed. Some of these factors are known to be correlated with each other (for example, smokers are known to consume fewer fruits and vegetables that non-smokers). [40]

6.1 Oxidative Stress

Oxidative stress reflects an imbalance between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Radiation, smoking and bad diet can influence oxidative stress. Multiple studies show the relationship between these factors and carotenoid concentrations, values of antioxidant or oxidative status [60] [44]. But there may of course be other factors creating and mediating oxidative stress such as illness, psychological stress or fatigue.

When analyzed by a chemical assay based on urinary malondialdehyde excretion, an indicator of oxidative lipid damage, people with high oxidative stress had significantly lower skin carotenoid levels than people with low oxidative stress. [40] [61] These observations provide evidence that skin carotenoid readings might be useful as a surrogate marker for general antioxidant status. [28]

A study done by Haag et al. [2] used electron paramagnetic resonance spectroscopy to assess reduction of nitroxides as an indicator of antioxidant capacity of human skin and found that skin carotenoid status as assessed by RRS was correlated with the rate constant of nitroxide decrease. These observations suggest that skin carotenoid RRS scores might be useful as a surrogate marker for general antioxidant status.

General stress factors (such as fatigue, illness) have been suggested to influence skin carotenoid status but have not been quantitatively or systematically assessed in multivariate models controlling for other factors [40].

6.1.1 Sunlight and UV/VIS Radiation

Human skin is susceptible to free radical and ROS formation due to irradiation in the UV, visible and IR wavelength range [62]. With regard to the total sun wavelength spectrum, up to 50% of the radicals are not produced by wavelengths in the UV range [5]. According to Lademann et al. [23] sunscreens provide a double-track protection system against skin damage as they contain antioxidants in addition to UV filter substances. The antioxidants are also able to protect the skin in the visible and infrared spectral ranges [63].

Photoprotection of biological systems can be achieved by topical protection like protective clothing or endogenous pigments like melanine that scatter and absorb UV light or sunscreens. Endogenous photoprotection mechanisms work by increasing the barrier for UV light (e.g. UV-absorbing compounds), protecting target molecules while acting as scavengers (e.g. antioxidants), repairing UV-induced damage by induction of repair systems or suppressing cellular responses (e.g. anti-inflammatory agents).

Antioxidants, like carotenoids, tocopherols, flavonoids and other polyphenols as well as vitamin C, provide an additional beneficial effect on the skin when utilized as a filter against the UV region of solar radiation. The SPF (sun protection factor) of antioxidants is small (i.e. approximately equal to 2 [40]) and insufficient to provide protection against direct sunlight. It is enough though to reduce the unfavorable effects of indirect sunlight radiation. [1] Sies et al [30] state that while protection through individual dietary components in terms of SPF may be considerably lower than that achieved using topical sunscreens, an increased lifelong overall protection via dietary supply may contribute significantly to skin health.

According to Offord et al. [16] vitamin C, E and carnosic acid have the potential to be effective photoprotectors. The carotenoids β-carotene or lycopene must be delivered together with vitamin E to prevent formation of oxidative derivatives which may influence the cellular and molecular responses. Wrona et al. [17] showed that a combination of zeaxanthin and α-tocopherol offers synergistic protection against photosensitized lipid peroxidation in liposomal systems.

Sies et al. [30] state that most of the erythemal annual UV dose is encountered under non-vacation conditions when no sunscreen is applied. Dietary protection is provided by carotenoids, tocopherols, ascorbate, flavonoids or n-3 fatty acids, contributing to maintenance resistance as part of lifelong protection. While endogenous protection in terms of SPF may be low the cumulative effect receives increasing attention. Lifelong inadvertent sunlight exposure is important. It is noted that substances may act synergistically. Vitamin C for instance regenerates tocopherol, together they increase the sunburn threshold significantly. The single compounds provided moderate but statistically not significant protection. Mixtures of carotenoids (β-carotene, lutein and lycopene) ameliorate UV-induced erythema in humans whereas one-component supplementation of β-carotene proved less effective. Concerning retinoids, Vitamin A absorbs in the UVB range and participates in an adaptive response to UV exposure. Pholyphenols show antioxidant activity and at the same time act as enzyme inhibitors of inducers influencing anti-inflammatory pathways and affecting cell division. Fish oil, rich in omega-3-fatty acids, also show protective effects toward the susceptibility to UVB-induced erythema and epidermal lipid peroxidation. After six months of supplementation with about 10 g fish oil per day, the MED (minimal erythemal dose) was increased about 2.1-fold.

As noted in all studies so far carried out, there is a time of approximately 8 to 10 weeks until protection against erythema formation becomes significant [30].

By using EPR and RRS, Meinke et al. [39] showed that a decrease in the carotenoid concentration in vivo induced by UV irradiation is explainable by the formation of radicals in the skin.

Ermakov et al. [28] show a decrease of skin carotenoid levels with increased sun exposure (see Figure 6).

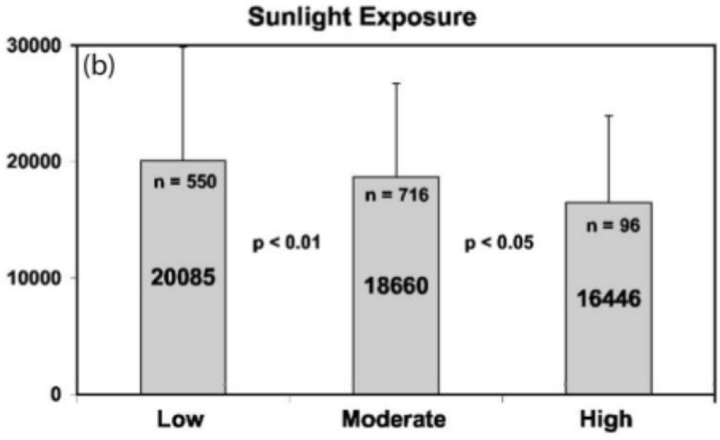

Figure 6: Resonance Raman intensity (counts) versus self-reported exposure of skin to sunlight, showing decrease of skin carotenoid levels with increased sun exposure

6.1.2 IR Radiation

Darvin et al. [64] and Akhalaya [65] show that utilization of the IR irradiation in medicine can have side effects. The carotenoids β-carotene and lycopene were decreased subsequent to IR irradiation of the skin.

Schröder et al. [66] [63] state that solar radiation damages human skin by causing premature skin ageing. Not only does this result from UV radiation but also from longer wavelengths, in particular near IR irradiation (IRA). IRA radiation has been demonstrated to alter the collagen equilibrium of the dermal extracellular matrix. Currently there are no specific chemical or physical filters available against IRA and existing compounds have not been shown to possess IRA-filtering capacity. Filters reflecting IRA (e.g. Titanium dioxide) might raise cosmetic issues. An alternative approach is the use of antioxidants, especially mitochondrially targeted antioxidants.

It has also been shown that topical application of β-carotene on skin can effectively neutralize free radicals which are produced on the skin surface due to IR irradiation [67]. Furthermore it was discovered that the topically applied antioxidant protects the natural antioxidant network of the skin.

6.1.3 Diet & Dietary Intervention

Concerning carotenoid supplementation trials, it is well known and accepted that carotenoid supplementation leads to measurable increases in carotenoids in human skin [53] [36] [68]. This obviously underlies much of the work done in the setting of using dietary sources of carotenoids for sun protection [69] [35] [24]. This also underlies the licensing of the RRS method to the nutritional supplement industry ("Biophotonic Scanner", NuSkin/Pharmanex Inc., Provo, Utah), based on the finding that carotenoid-containing vitamin supplements produced an increase in skin carotenoid values within a relatively short time frame. Meinke et al. [70] showed an increase in both skin and blood carotenoids by comparing the effects of an oil extract rich in various carotenoids to those of a placebo oil. All these studies clearly indicate that providing relatively bioavailable carotenoids through supplementation impacts skin carotenoid status. In another double blind placebo controlled study performed with 24 healthy volunteers Meinke et al. [62] supplemented the volunteer's diet with a natural carotenoid-rich product (BioActive Food GmbH, Bad Segeberg, Germany) containing curly kale extract, sea-buckthorn oil and olive oil.

Regarding food-based interventions Mayne et al. [40] report that an analysis of their early survey of 1375 healthy subjects indicated a pronounced cross-sectional relationship between self-reported fruit and vegetable intake (a source of carotenoids) and skin carotenoids RRS status [28] [61]. Subsequent cross-sectional studies are also quite consistent in showing significant associations between self-reported consumption of fruits/vegetables and/or dietary carotenoid intake and skin carotenoid status. This has been observed both in adults [44] [40] [15] [71] and in children [58]. It has been shown that skin carotenoid levels decreased during depletion and increased during high-carotenoid feeding, with skin carotenoid status tracking similarly to plasma carotenoids although the rates of decrease (during depletion) were faster in plasma versus skin. Skin carotenoids had not yet plateaued by 8 weeks post-intervention, suggesting that they reflect intake over at least the prior 2+ months. [40] Similar results were obtained by Jahns et al. [55], comparing RRS results with plasma HPLC analysis (see Figure 7). Twenty-nine participants consumed low-carotenoid diets (6 weeks, phases 1 and 3), a provided diet containing 6-cup equivalents (1046g/d) of vegetables and fruit (8 weeks, phase 2) and their usual diet (final 8 weeks, phase 4). Skin carotenoid status was measures by RRS >= 2 times per week during phases 1, 3 and 4 and 5 times per week during phase 2. At baseline, skin and plasma total carotenoid values were correlated (r=0.61, P<0.001). Skin and plasma carotenoid values decreased 36% and 30%, respectively, from baseline to the end of phase 1 and then increased by >200% at the end of phase 2. Plasma carotenoids returned to baseline concentrations by the middle of phase 3 and skin carotenoid concentrations by the middle of phase 4.

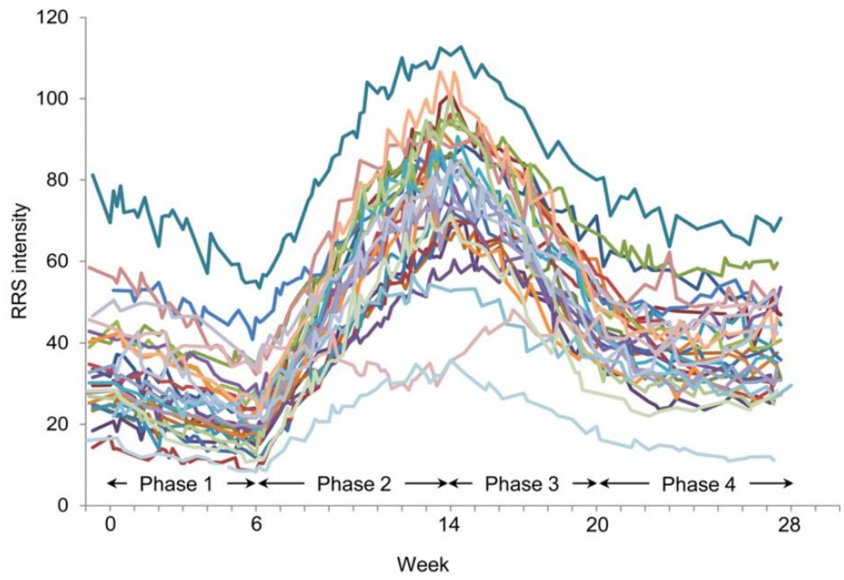

Figure 7: Individual responses of skin carotenoid status throughout the study of Jahns et al. [55]

Changes in skin carotenoid status closely followed changes in plasma across a broad range of intakes.

Ermakov et al. [28] show an increase of skin carotenoid concentrations with increased fruit and vegetable uptake (see Figure 8).

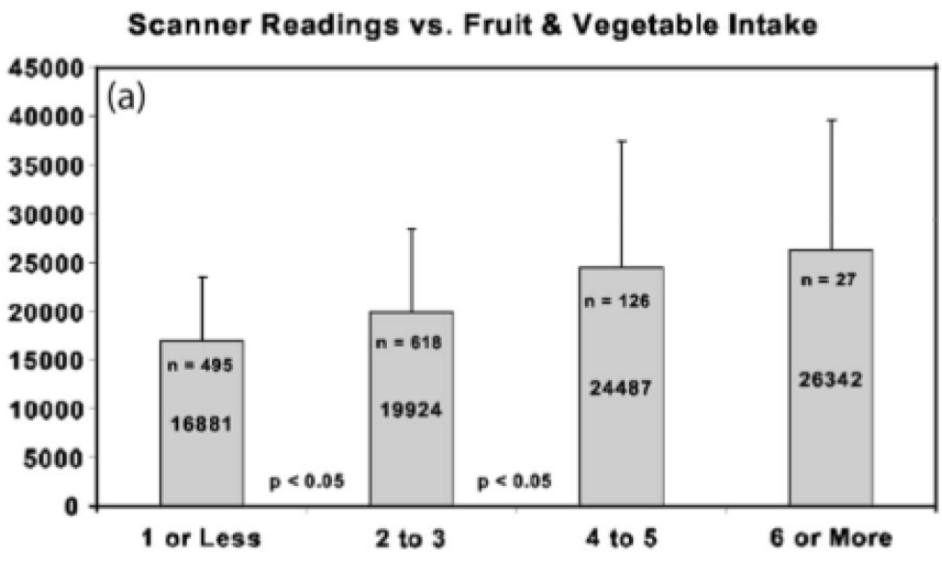

Figure 8: Resonance Raman intensity (counts) versus reported number of daily consumed servings of fruits and vegetables, demonstrating increase of skin carotenoid concentrations with increased fruit and vegetable uptake [28]

Thus multiple lines of evidence now show that skin carotenoid status is responsive to carotenoid intervention involving supplements, vegetable oil extracts given as dietary supplements and carotenoid-rich fruits and vegetables as food-based interventions. [40]

6.1.4 Smoking

Meinke et al. [60] report that the carotenoid levels in skin of smokers are significantly lower, on average by 21% for total carotenoids, than in non-smokers. Mayne et al. [40] report that an analysis of their early survey of 1375 healthy subjects revealed that smokers had much lower levels of skin carotenoids than non-smokers [28] [61]. This has subsequently been observed in other studies [44]. More specifically, smokers had a 20-30% reduction in skin carotenoid levels when compared to non-smokers. This is due to the direct effects of smoking and the fact that smokers are additionally prone to lower intake of carotenoids. Scarmo et al. [58] showed that carotenoid levels in children, having a smoker in the home, were also associated with a modest reduction (7%). Ermakov et al. [28] show data of a 30% decrease of skin carotenoid levels in smokers compared to non-smokers (see Figure 9).

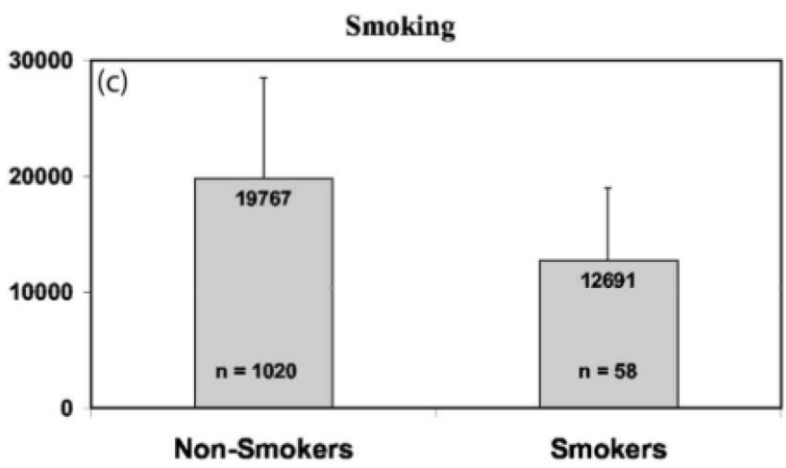

Figure 9: Resonance Raman intensity (counts) in nonsmokers and cigarette smokers, showing ~30% decrease of skin carotenoid levels in smokers [28]

6.2 Skin Pigmentation

Multivariate analysis performed by Mayne et al. [40] showed that skin tone was not significantly associated with skin carotenoid status. It is remarked though that more research is needed in diverse population, with quantitative measures of melanin content, to determine if skin pigmentation is a determinant of skin carotenoid status.

6.3 Genetic Factors

According to Mayne et al. [40] it appears that genetic variation is a significant determinant of individual carotenoid concentrations in blood. However, the impact on total carotenoids in blood would be expected to be less. Relevant to the skin carotenoid biomarker, they are unaware of any published studies examining genetic variation in relation to skin carotenoid status, although such studies are underway.

6.4 Age

Age does not appear to be an important determinant of skin carotenoid status in adults [60]. In babies and young children, however, those who are older on average have higher skin carotenoid status [40].

6.5 Gender

On average, women have higher skin carotenoid status than men, likely reflecting greater intake of fruits and vegetables in women along with smaller body size [40] [60] [44].

6.6 Body Mass Index

Meinke et al. [60] observed that obese subjects (BMI >=30) had 13% lower skin carotenoids, as measured by RRS, compared to normal/overweight subjects (BMI < 30). Rerksuppaphol et al. [71] also observed lower skin carotenoid status in obese subjects relative to normal weight subjects. Body mass index is, according to Mayne et al. [40], weakly and inconsistently associated with lower skin carotenoid status [60] [71]. Mayne remarks that it remains unclear whether lower carotenoid status in obese subjects reflects poorer diet, inflammation, a greater adipose tissue reservoir or multiple effects [40].

7 Pro- and Antioxidant Effects of Carotenoids

Analysis of a multitude of studies has shown beneficial effects of antioxidants on the organism and the positive results achieved with a balanced nutritional intake of food rich in fruit and vegetables concerning medical treatment of various diseases [1] [23]. All antioxidant substances act as a ‘protection chain’, which means that different antioxidant substances possess a synergic effect and protect each other from direct destruction in the processes of neutralization of the free radicals and other reactive species. Thus, they act effectively only in combination with the other types of antioxidant substances. This relation becomes disturbed when only one component is increased artificially by supplementation [1].

Supplementations with synthetic and extracted antioxidant substances show controversial results with no positive effects and sometimes even negative effects during and after medical therapies for some diseases [1] [23]. This was found for the systemic application of β-carotene and vitamins A and E. The negative results obtained by systemic application of antioxidant substances seem to have been caused by the application of a one-component antioxidant substance system during medical treatment. It can even happen that the antioxidants act, not as radical quenchers, but also as radical producers if their concentration is higher than the critical level [1]. This critical value depends on the concentration of the antioxidant substances and the combination of antioxidants in which they are applied [23].

8 Topical Versus Systemic Application of Antioxidants

Topically applied, antioxidants penetrated the skin without any loss due to metabolism. However, protection is only provided locally and the penetration into deeper skin layers almost negligible. Systemic application leads to an accumulation of the antioxidant in the whole organism. Both the skin surface and the deeper skin layers are enriched with the applied substance [23].

Additionally, topically applied carotenoids as a single antioxidant component are less stable than the carotenoids in the skin incorporated by nutrition and accumulated in a mixture with different antioxidant substances [67].

9 Carotenoid measurements in children

Aguilar [57] finds that the resonance Raman spectroscopy skin carotenoid concentrations may be valid and reliable indicators of serum concentrations of carotenoids and total fruit and vegetable intake among children. Skin carotenoid levels were positively correlated to serum carotenoid concentrations measured by HPLC among children. Similarly, skin carotenoid levels were positively correlated to total and carotenoid containing fruit and vegetable intake as assessed from both a FFQ and multiple 24HDR.

Skin carotenoids may provide a useful indicator of usual fruit and vegetable intake among children, a group for which it is difficult to obtain accurate information about usual dietary intake [57] [40] [72]. Parents and caregivers typically do not observe all meals consumed and young children lack the cognitive ability to self-report diet [40].

The magnitude of correlation observed for skin carotenoids measured from RRS and serum carotenoids measured with HPLC in children is similar to that observed among adults [57].

10 Skin Aging

Skin aging is a continuous process and results from intrinsic and extrinsic factors. A number of extrinsic factors often act together to prematurely age our skin. Most premature aging is caused by sun exposure and affects parameters of skin structure and surface [73].

Radicals can damage lipids, proteins, DNA as well as collagen and elastin fibres leading not only to skin aging but also to immune suppression and even skin cancer [23] [74]. According to Darvin et al. [74] there is a strong correlation between antioxidants found in the skin and skin aging. A high concentration of antioxidant substances in the skin is protective and associated with a reduction in skin wrinkling. Darvin et al. [75] measured the skin roughness by determining the depth and density of furrows and wrinkle reliefs [23] via profilometry and carotenoid values via RRS. They found a high correlation between skin surface structure and the concentration of carotenoids. Subjects of same age with high concentrations of carotenoids showed significantly less wrinkles than those with low carotenoid values.

Another study [73] investigated the improvement in the skin surface structure after systemic intake of antioxidants (carotenoids, tocopherol, selenium). Skin density and thickness, roughness, scaling, smoothness and wrinkling were determined. After 12 weeks the verum group showed an increase in skin density and thickness as well as improved values for roughness and scaling.

Schröder et al. [66] [63] state that solar radiation damages human skin by causing premature skin ageing. Not only does this result from UV radiation but also from longer wavelengths, in particular near IR irradiation (IRA).

It is not known yet if the process of premature aging of the skin can be modulated by dietary antioxidants. However, several studies show that dietary antioxidants provide photoprotective effects and improve skin structure when administered as food supplements [73].

11 Skin Lipid Profile

Meinke et al. [62] showed that there is an influence of carotenoids on the skin lipid profile.

12 Selected Studies

12.1 One Year Study on the Variation of Carotenoid in vivo: Influence of Dietary Supplementation and Stress Factors

Darvin et al [15] showed that carotenoid levels in the skin rise with higher intake of fruit and vegetables and absence of stress factors whereas negative factors like illness, fatigue, stress, alcohol consumption and smoking lead to a decline of carotenoid levels. Decreases occurred relatively quickly over the course of 2 hours while the recovery usually took up to 3 days. Figure 10 shows data of 10 volunteers participating in the one year study.

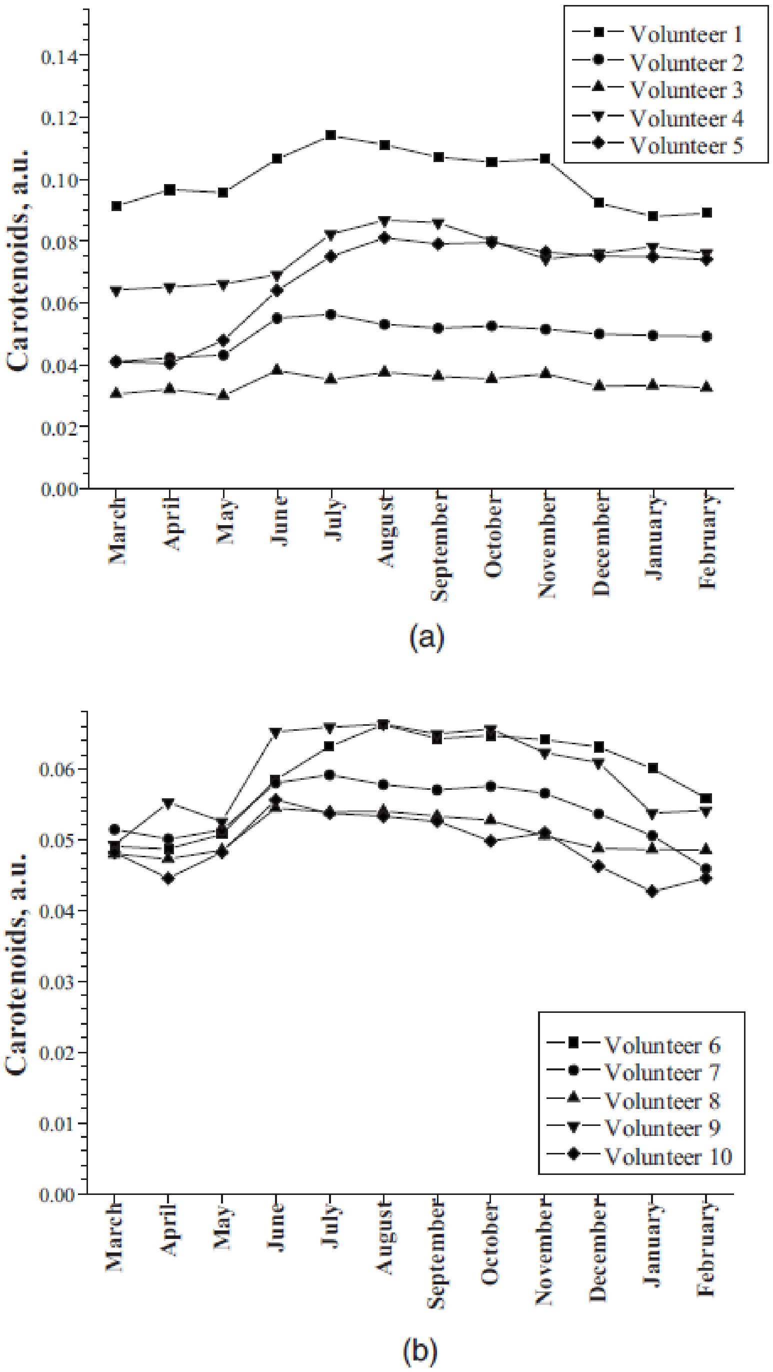

Figure 10: One-year study on the variation of carotenoid antioxidant substances in living human skin by Darvin et al [15]. Subfigure (a) shows carotenoid values of volunteers 1 to 5, subfigure (b) values of volunteers 6 to 10. The volunteer can be classified as follows:

Volunteer 1: very large amount of fruit and vegetables each day

Volunteer 2: small amounts of fruit and vegetables

Volunteer 3: small amounts of fruit and vegetables, strong smoker

Volunteer 4, 6-10: moderate levels of carotenoids in the skin, diets remained relatively constant as did amounts of stress factors

Volunteer 5: started to increase his daily consumption of fruit and vegetables consistently for 3 months after the beginning of the measurements; minimized smoking; tried to exclude influence of possible stress factors

The seasonal variation of carotenoid antioxidant levels in the skin can be explained by a higher consumption of fruit and vegetables during summer and autumn months. [15]

12.2 Spectoscopic biofeedback on cutaneous carotenoids as part of a prevention program could be effective to raise health awareness in adolescents

In a recent intervention study [76] conducted by the department of dermatology at the Charité, Berlin, and Opsolution GmbH in 2012, carotenoid status of 50 school kids, aged between 17 and 20 was assessed. Figure 3 shows the study design.

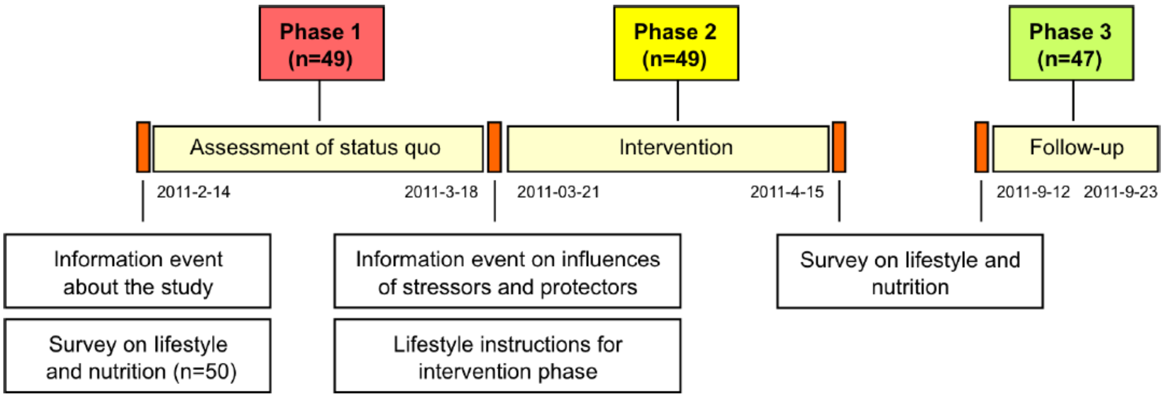

Figure 11: Study design

The carotenoid values measured by the spectroscopic device were shown on a scale from 1 to 12, with 12 corresponding to 1.2 nmol carotenoids per gram skin. The device was calibrated with a RRS system of the Center of Experimental & Applied Cutanoids Physiology of the Charité with which data from more than 10000 volunteers and patients had been investigated over a period of 10 years.

Health related lifestyle factors of the adolescents were assessed via questionnaires. However individual factors on their own are not correlated significantly with the measured carotenoid levels, even though a clear tendency was found. It was rather assumed that different factors have a synergetic effect on the antioxidant status as they have on health in general. Therefore the concentration of cutaneous carotenoids can be seen as a useful marker for general health.

A combination of several health promoting lifestyle habits such as healthy nutritional habits, non-smoking, low alcohol consumption, sufficient sleep and the absence of illness showed a positive effect on cutaneous antioxidant concentrations. Furthermore an association of high carotenoid concentration and good psychological status which was ascertained through questionnaires, could be found. The subjective view on the current overall quality of life correlates significantly with the measured antioxidant concentration. The decrease of the concentration of carotenoids in the skin can be as fast as within 2 hours after a stressful event, but the increase after the supplementation of carotenoid-rich products takes 1 to 3 days.

It was shown that the biofeedback given to the adolescents was a useful instrument to bring invisible physiological parameters to view and, consequently, to influence a subject's behavior. Biofeedback as part of an integrated health prevention program is able to help increasing the motivation to adopt a healthy lifestyle in high school students. In this particular study students stated that the measurement of their carotenoid status became some sort of competition among themselves. Carotenoid values were compared amongst the students. Even after the intervention study the student retained so of their newly acquired healthy lifestyle habits.

13 Pharmanex, Nuskin and the Biophotonic Scanner

NuSkin Enterprises (kurz NSE; NYSE: NUS) is an American network-marketing-cooperation currently active in more than 40 countries, selling cosmetics, anti-aging-products and nutrition supplements. In 1998 NuSkin acquired Pharmanex.

13.1 Biophotonic Scanner

The Biophotonic Scanner is based on Resonance Raman Spectroscopy. It has not been FDA approved. Pharmanex states that “the BioPhotonic Scanner has not been evaluated or approved by the FDA and is not intended to diagnose, predict, treat, cure or mitigate any disease.” Furthermore they say that “Pharmanex is the exclusive owner of the patented, BioPhotonic Scanner technology. The world’s first and only tool that measures carotenoid antioxidant levels non-invasively in living human tissue, validated by years of research, over 50 full-length peer-review articles, key studies and demonstrations presented at a number of prestigious scientific meetings and conferences all over the world. This technology is only available for use by the general public through the Pharmanex network of independent distributors."

The measured data is sent to an ipad mini (scanner works only with apple) via WLAN or bluetooth connection for display. In case of offline usage the data is saved locally on the ipad. Being online the data is uploaded to a server database in regular intervals.

The scanner has been built on experience and scientific data from the groups of Prof Gellermann and Prof Bernstein.

Concerning guaranteed results upon consumption/application of their products Pharmanex states:“Pharmanex guarantees that after 60 days of consuming a Guaranteed Product (or 90 days in the case of Eye Formula) at the recommended label dosage, your Skin Carotenoid Score, as determined by the Pharmanex BioPhotonic Scanner, will have increased. If you meet the criteria set forth below and your score has not increased from the time you were initially scanned and began taking the Guaranteed Product, you may submit a written request for a total refund of the purchase price of the approved purchased Guaranteed Product (less shipping costs).

In order to qualify for the BioPhotonic Scanner SCS Guarantee (the “Guarantee”), you must adhere to the following requirements:

The Guarantee applies ONLY to LifePak, LifePak Prime, LifePak Women, LifePak Nano, Jungamals SCS, g3 and Eye Formula (“Guaranteed Products”). LifePak Prenatal is NOT included in the Guarantee. If you are applying the Guarantee to Eye Formula, please read the Eye Formula section below as the rules and timetables for this product are different from the rules and timetables for the other Guaranteed Product.

You must receive your initial scan before you begin taking any of the guaranteed products. This Guarantee does not apply to people who have taken any of the Guaranteed Products within the past 3 months.

You must establish a monthly Auto-Delivery (ADR) order with the Guaranteed Product for which you intend to claim the Guarantee not later than 7 days following your initial scan."

Guarantee Usage Requirements:

You must begin taking 2 oz per day of g3 or the recommended dosage of LifePak, LifePak Prime, LifePak Women, LifePak Nano, or Jungamals SCS upon receipt of your product following your initial scan and continue taking the product for a total of 60 consecutive days before your subsequent scan."

14 Conclusion

According to the *National Academy of Sciences* "blood concentrations of carotenoids are the best biological markers for consumption of fruits and vegetables". It has been shown that carotenoids can even serve as a biomarker for the whole antioxidant network in the skin. Carotenoid levels in the skin correlate with plasma/blood levels and therefore mirror systemic concentrations.

Resonance Raman Spectroscopy (RRS) has been established as a quick, non-invasive and highly sensitive method to assess carotenoids in the human skin. RRS data correlate significantly with those of plasma HPLC methods. Reflectance spectroscopy is yet another non-invasive method for the assessment of carotenoids. The main advantages of reflection spectroscopy-based devices in comparison to Raman devices are the relatively low price, compact size and light weight. Reflection spectroscopy data correlate with RRS and plasma HPLC.

Validity of non-invasive measurements of carotenoids in human skin has been shown by several groups. Intra-subject variability as well as inter-subject variability of carotenoid measurements via RRS fulfill the requirements for carotenoids to be used as a biomarker. Feasibility and reproducibility of skin carotenoid measurements via RRS in non-lab-conditions has been acknowledged.

Assessed factors influencing carotenoids as a biomarker include irradiation (sunlight/UV/IR), diet and dietary intervention, smoking, gender, obesity (BMI), genetic factors and age (only relevant in children). Furthermore it has been shown that people with high oxidative stress had significantly lower skin carotenoid levels than people with low oxidative stress. It is also suggested that skin carotenoid RRS scores might be useful as a surrogate marker for general antioxidant status.

Skin aging is yet another topic of interest associated with skin carotenoid assessment. A high correlation between skin surface structure and the concentration of carotenoids has been found. Subjects of same age with high concentrations of carotenoids showed significantly less wrinkles than those with low carotenoid values.

Both food-based and supplementation based intervention correlate good with measurements. The composition of nutrition supplements needs to be well chosen.

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